

DOCTORAL THESIS



UCAM

UNIVERSIDAD CATÓLICA
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INTERNATIONAL DOCTORAL SCHOOL

Doctoral Programme in Health Science

*Evaluation of the effect of Antibiotic prophylaxis on an inflammatory
factor in salivary samples after third molars surgery*

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Murcia, January 2025

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THESIS SUPERVISORS' AUTHORISATION FOR THESIS SUBMISSION

Prof. Dr José Eduardo Maté Sánchez de Val and Prof. Dr Carlos Pérez-Albacete Martínez, as Supervisors of the Doctoral Thesis 'Evaluation of the effect of Antibiotic prophylaxis on an inflammatory factor in salivary samples after third molars surgery' by Ms Fatemeh Soleymani in the Doctorate Programme Health Sciences, **authorise(s) its submission**, given that it meets the required conditions for its defence.

Which I hereby sign in compliance with Spanish Royal Decree 99/2011, of 28 January, in Murcia, on 20th of January, 2025.

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ABSTRACT

Introduction: The extraction of third molars is a common dental procedure often associated with postoperative complications such as pain, swelling, and trismus. This study investigates the impact of a single preoperative dose of amoxicillin on the inflammatory response, specifically focusing on prostaglandin E2 (PGE2) levels in saliva, subjective and objective clinical outcomes (facial swelling, maximum mouth opening and pain) following mandibular third molar surgery.

Materials and Methods: This double-blind, randomized clinical trial included 18 healthy participants requiring third molar extraction. Participants were divided into two groups: the experimental group received 2g of amoxicillin one hour before surgery, while the placebo group received identical placebo capsules. Saliva samples were collected at baseline, 24 hours, and seven days post-surgery to measure PGE2 levels using ELISA. Clinical outcomes, including pain (measured by VAS), facial swelling, and maximum mouth opening (MMO), were assessed at multiple time points.

Results: The study found no significant differences in salivary PGE2 levels between the experimental and placebo groups at any time point. Pain levels, facial swelling, and MMO changes were also similar between the two groups. Correlation analyses revealed that longer surgery duration and performing osteotomy were associated with increased facial swelling and MMO changes. Higher baseline PGE2 levels were correlated with greater pain reported by participants.

Conclusion: A single preoperative dose of amoxicillin does not significantly impact salivary PGE2 levels or clinical outcomes following third molar extraction in healthy individuals. The findings suggest that routine use of prophylactic antibiotics in such cases may not be necessary, emphasizing the need to consider alternative strategies for managing postoperative complications.

RESUMEN

Introducción: La extracción de terceros molares es un procedimiento dental común que a menudo se asocia con complicaciones postoperatorias como dolor, hinchazón y trismo. Este estudio investiga el impacto de una dosis preoperatoria única de amoxicilina en la respuesta inflamatoria, centrándose específicamente en los niveles de prostaglandina E2 (PGE2) en saliva, y en los resultados clínicos subjetivos y objetivos (hinchazón facial, apertura máxima de la boca y dolor) tras la cirugía de extracción de terceros molares mandibulares.

Materiales y Métodos: Este ensayo clínico aleatorizado y doble ciego incluyó a 18 participantes sanos que necesitaban la extracción de terceros molares inferiores. Los participantes se dividieron en dos grupos: el grupo experimental recibió 2g de amoxicilina una hora antes de la cirugía, mientras que el grupo placebo recibió cápsulas de placebo idénticas. Se recogieron muestras de saliva en la línea de base, a las 24 horas y a los siete días después de la cirugía para medir los niveles de PGE2 utilizando ELISA. Los resultados clínicos, incluyendo el dolor (medido por escala analógica visual), la hinchazón facial y la apertura máxima de la boca (AMB), se evaluaron en múltiples puntos de tiempo.

Resultados: El estudio no encontró diferencias significativas en los niveles de PGE2 en saliva entre los grupos experimental y placebo en ningún momento. Los niveles de dolor, la hinchazón facial y los cambios en la AMB también fueron similares entre los dos grupos. Los análisis de correlación revelaron que una mayor duración de la cirugía y la realización de osteotomía se asociaron con un aumento de la hinchazón facial y los cambios en la AMB. Los niveles más altos de PGE2 en la línea de base se correlacionaron con un mayor dolor reportado por los participantes.

Conclusión: Una dosis preoperatoria única de amoxicilina no impacta significativamente los niveles de PGE2 en saliva ni los resultados clínicos tras la extracción de terceros molares en individuos sanos. Los hallazgos sugieren que el uso rutinario de antibióticos profilácticos en tales casos puede no ser necesario, enfatizando la necesidad de considerar estrategias alternativas para manejar las complicaciones postoperatorias.

KEY WORDS

Third Molar Surgery, Antibiotic Prophylaxis, Salivary PGE2, Postoperative Complications, Pain Assessment, Facial Swelling, Maximum Mouth Opening (MMO), Inflammatory Response, Randomized Clinical Trial, Amoxicillin

Palabras Claves

Antibióticos (241401), Bacterias (310801), Cirugía (321300), Cirugía Maxilofacial (321318)

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commitment, and determination that have brought me here, and I will carry these qualities forward in all my future endeavors.

‘The future belongs to those who believe in the beauty of
their dreams.’ Eleanor Roosevelt (1884-1962).

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ACRONYMS AND ABBREVIATIONS

ANS, Autonomic Nervous System
BIPP, Iodoform and Paraffin Paste
BPE, Basic Periodontal Examination
CBCT, Cone Beam Computed Tomography
CEJ, Cementoenamel Junction
CI, Confidence Interval
CNS, Central Nervous System
CRP, C-Reactive Protein
CT, Computed Tomography
DI, Difficulty Index
ECM, Extracellular Matrix
EPP, Exposure-Prone Procedures
FGF, Fibroblast Growth Factor
GCF, Gingival Crevicular Fluid
GB, Gingival Biotype
HVA, Homovanillic Acid
IASP, International Association for the Study of Pain
IgA, Immunoglobulin A
IGF, Insulin-like Growth Factor
IL, Interleukin
IV, Intravenous
IQR, Interquartile Range
MHPG, 3-Methoxy-4-Hydroxyphenylglycol
MMP, Matrix Metalloproteinase
MMO, Maximum Mouth Opening

NO, Nitric oxide

NRS, Numeric Rating Scale

NSAID, Nonsteroidal Anti-Inflammatory Drug

OAC, Oroantral Communication

OD, Optical Density

PAG, Periaqueductal Gray

PDGF, Platelet-Derived Growth Factor

PGE2, Prostaglandin E2

PGs, Prostaglandins

PPE, Personal Protective Equipment

PRF, Platelet-Rich Fibrin

SABC, Streptavidin Conjugate

SAPHO, Synovitis, Acne, Pustulosis, Hyperostosis, Osteitis

SSI, Surgical Site Infection

TGF- β , Transforming Growth Factor Beta

TNF- α , Tumor Necrosis Factor Alpha

UWS, Unstimulated Whole Saliva

VAS, Visual Analog Scale

VEGF, Vascular Endothelial Growth Factor

VRS, Verbal Rating Scale

WBC, White Blood Cells

WHO, World Health Organization

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I – INTRODUCTION

I - INTRODUCTION

1.1. TOOTH EXTRACTION

1.1.1. Indications

The reasons for tooth removal vary. A tooth may be extracted due to severe caries, periapical periodontitis, pericoronitis, abscesses, cysts, or tumours, or when they are misplaced, impacted, or over-retained. Additionally, extractions are commonly performed as part of orthodontic treatment, particularly for third molars and first premolars (Sengupta et al, 2022).

1.1.1.1. *Indications for third molar extraction*

There is no dispute over the benefits of extracting teeth linked to pathology, however, there has been debate on the propriety of extracting third molars without accompanying pathology in order to avoid the development of pathology (prophylactic removal). The following indications have been proposed for wisdom tooth extraction:

- pericoronitis
- unrestorable caries
- non-treatable pupal/periapical pathology
- cellulitis/abscess/osteomyelitis
- internal/external resorption of the tooth or adjacent teeth
- tooth fracture
- follicle disease such as cysts or tumors
- tooth impeding surgery or reconstructive jaw surgery
- tooth involved in tumor or in field of tumor resection (Sengupta et al, 2022).

The relative advantages and disadvantages, in light of the best available research data, professional expertise, and patient values, will determine whether or not wisdom teeth should be extracted. To make sure that no pathological process

develops, impacted third molar teeth that are not to be extracted should undoubtedly be monitored. Up to 30% of patients may develop caries in the second molar next to partially erupted impacted retained wisdom teeth. Additionally, cystic pathology might emerge. Therefore, in addition to general health and local circumstances, the choice will consider a number of criteria, including the caries risk (Sengupta et al, 2022).

In a study, after only a year of follow-up, it was discovered that 5.47% of patients needed extraction of their third molars (Fernandes et al., 2010). Nearly 40% of the wisdom teeth needed to be extracted for a number of reasons, including cavities and pericoronitis, according to different research that examined individuals with asymptomatic wisdom teeth four years after the first evaluation (von Wowern & Nielsen, 1989). According to the American Association of Oral and Maxillofacial Surgeons, 85% of wisdom teeth will ultimately need to be extracted (Rosemont, 2005).

The benefits of wisdom teeth removal most frequently include a reduction in the signs and symptoms of pericoronitis as well as any potential negative effects. Pain, poor taste, facial and peri-coronal tissue swelling, and limited mouth opening (trismus) are the signs of pericoronitis. The local infection may spread, causing malaise, pyrexia, and regional lymphadenopathy. In rare cases, swelling might endanger breathing and airway patency (Sengupta et al, 2022).

1.1.2. Necessary assessments before dental extraction

1.1.2.1. *Clinical assessment and medical history*

Naturally, a complete medical history is necessary before any surgery. Patients who might need extra preparation before even the most basic dental extraction will be identified in this way. These individuals may include, among many others, those who have received radiation therapy or are on anticoagulant medication. The information gathered will also dictate the setting and whether surgery should be performed by a specialist or a regular dentist (Sengupta et al, 2022).

Inquiries concerning prior dental and surgical experience as well as an evaluation of the patient's anxiety are also part of the history. The predicted ease

or difficulty of the extraction may be indicated by the patient's sex, general build, or other criteria. Afro-Caribbean patients may have more solid alveolar bones, older patients may have more brittle teeth, and children may have less cooperation and less intraoral access. For instance, extractions are considered to be more difficult in males who are powerfully built (Sengupta et al, 2022).

A note of the tooth's location and accessibility should be part of the intraoral examination. Endodontically treated teeth may be more brittle, and the crown of a highly repaired tooth is more prone to break during extraction (Sengupta et al, 2022).

1.1.2.2. *Radiographic assessment*

In most cases, radiographic evaluation is necessary to show root shape, anatomical linkages, or related disease. Pre-extraction radiographs should always be performed since mandibular and maxillary third molar teeth are known to have a broad range of root morphological variability (Sengupta et al, 2022). According to research comparing computed tomography (CT) with normal film radiography, CT is better at displaying tooth and root morphology, crown-root relationship, and dental inclination (Bodner et al., 2001). If extraction of the lower third molar is planned, it is equally crucial to understand the connection of the inferior alveolar canal. Cone beam CT (CBCT) imaging is becoming more widely available, which makes it a crucial diagnostic tool, especially in challenging situations (Eyrich et al., 2011). When a close association between the third molar and the inferior alveolar nerve canal is seen on plain film, cone beam computed tomography (CBCT) is recommended (Sengupta et al, 2022). However, research has repeatedly demonstrated that, when compared to panoramic imaging alone, CBCT's assessment of the third molar and inferior alveolar canal proximity does not always result in lower rates of long-term paresthesia (Eyrich et al., 2011; Ghaeminia et al., 2015; Korkmaz et al., 2017).

1.1.2.3. *Treatment planning*

The optimal course of action for the patient is developed using the data from history and examination. This will involve steps to ensure that the procedure is adequately prepared as well as the choice of anesthesia, such as general anesthesia,

conscious sedation with local anesthesia, or local anesthesia. It is preferable to talk about anticipated challenges with the patient prior to therapy rather than during it, when they can be interpreted as the consequence of poor preparation or experience (Sengupta et al, 2022).

1.1.3. Infection control

Like in dentistry, effective infection control is crucial in surgery to safeguard both patients and personnel. Procedures for instrument decontamination, disinfection, and sterilization, clinical waste disposal, environmental cleaning, personal protective equipment (PPE) use, staff immunization, and training will all be covered under the policy. Surgery frequently involves exposure-prone procedures (EPPs), which are invasive operations in which the patient's open tissues may get contaminated by the blood of the surgeon due to injuries from sharp objects, needles, or bone fragments (Sengupta et al, 2022).

1.1.4. Lower third molar extraction

1.1.4.1. *Determining Surgical Difficulty*

A crucial stage in the surgical process for extracting impacted teeth is the preoperative clinical and radiological assessment of the third molar. The range of variables that are known to make impaction surgery more or less challenging is something the surgeon pays close attention to. Numerous classification schemes have been created to help determine difficulty of the surgery, which are presented in Table 1 (Bhansali et al., 2021). The three most commonly utilized are the impacted tooth's angulation, its proximity to the ramus and second molar's anterior border, the depth of the impaction, and the kind of tissue that covers the affected tooth. The Pell and Gregory classification and the Winter classification are two common classification schemes that are used to explain these findings (Miloro, 2022).

The mesioangular impaction, which makes up around 45% of all impacted mandibular third molars, is often recognized as the easiest to extract. The most

difficult impaction is the distoangular one (5%), while the vertical impaction (40%) and horizontal impaction (10%) are intermediate in difficulty (Miloro, 2022).

Table 1. Chronological list of difficulty indices proposed and their specificity and sensitivity. Adapted from (Bhansali et al., 2021)

S. no.	Index	Year of publication	Specificity	Sensitivity
1	Winter's lines (WAR)	1926	Not accessed	
2	The Pell–Gregory classification	1933	62-88%	15-50%
3	Parant scale	1974	Not accessed	
4	WHARFE index	1976	Not accessed	
5	Pederson, modified Pederson	1988, 1994	45%, 89%	94.9%,20%
6	Koerner index	1994	Not accessed	
7	Modified Parant scale by Garcia et al.	2000	Not accessed	
8	Penarrocha et al. index	2000	Not accessed	
9	Yuasa index	2002	92%	85%
10	Gbotolorun index	2007	97%	80%
11	Carvalho et al. scale	2011	Not accessed	
12	Juodzbaly G (MRACBS) scale	2013	Not accessed	
13	Kharma scale	2014	68.40%	18.20%
14	Mario Vicente-Barrero (SPESD)	2014	Not accessed	
15	Roy et al. index	2015	Not accessed	
16	Conti scale	2015	Not accessed	
17	Pernambuco index	2017	87.90%	93.10%
18	Ribes Lainez N et al. index	2017	Not accessed	
19	Sammartino G et al. index	2017	Not accessed	
20	Zang et al. index	2019	Not accessed	

21	Kim JY et al. index	2019	Not accessed
22	Ku, Chang et al. index	2020	Not accessed

The space available for the tooth eruption and the intended extraction is reflected in the connection between the impacted tooth and the anterior edge of the ramus. The tooth is often easier to extract if the alveolar process is long enough anterior to the ramus's anterior border to permit tooth eruption. On the other hand, it is more difficult to extract teeth that are practically embedded in the mandibular ramus (Milorio, 2022).

Another principal factor in assessing the level of difficulty is the depth of impaction underneath the hard and soft tissues. While the Pell and Gregory classification assesses vertical position in relation to the occlusal plane and CEJ of the neighboring second molar, the most often used method for assessing difficulty considers the soft tissues and partial or total bony impaction. In addition to being the method needed to categorize and code impaction operations for all commercial insurance companies, this approach is extensively used since it may be the most accurate estimate of the amount of time needed for surgery. Surprisingly, the amount of time needed for surgery may not be much impacted by variables such as the angulation of impaction, the tooth's proximity to the ramus's front border, or the architecture of roots (Larsen et al., 1991).

It has been suggested that other variables contribute to the difficulty of the extraction procedure. Conical and fused roots or separate and divergent roots are the two types of roots; the latter is more challenging to control. A tooth with a big follicular sac surrounding its crown has greater access space and is thus easier to remove than one with virtually little space surrounding the tooth's crown (Milorio, 2022).

The patient's age is another significant factor that affects extraction difficulty. The procedure is nearly always easier to carry out when impacted teeth are extracted before the age of 20. Because the roots are typically not fully developed, less bone must be removed in order to retrieve the tooth. The tooth's follicle often creates a wider peri-coronal area, which offers more access for tooth extraction without removing bone. The impacted teeth roots are often isolated from the inferior alveolar nerve due to their inadequate formation (Milorio, 2022).

In contrast, it is nearly always more difficult to extract impacted teeth from individuals who are older. Since the roots are often fully developed, they are longer, requiring more bone removal, and they are also closer to the inferior alveolar canal, increasing the risk of paresthesia and postoperative anesthesia. Age-related follicular sac degeneration nearly invariably results in a narrower pericoronal gap, necessitating the removal of more bones to reach the tooth's crown. Ultimately, the bone is becoming less elastic and denser, requiring more bone excision to extract the tooth from its socket. The combination of these variables—Pell and Gregory Classification, Winter Classification, root features, patient age, and patient BMI—has produced an index that has demonstrated a high degree of accuracy in forecasting operation difficulty (de Carvalho & Vasconcelos, 2018). Since it has been demonstrated that even experienced surgeons make preoperative errors in assessing the difficulty of what become complex impacted extractions, the use of such objective predictors may be advantageous (Komerik et al., 2014).

Recovery from the surgery is a consequence of surgical difficulty. A more difficult and drawn-out surgical operation leads to a more difficult and drawn-out postoperative recovery. Surgery is more difficult to perform on elderly individuals, and their recovery after the treatment is more challenging (Miloró, 2022).

1.1.4.2. *Surgical techniques*

Generally, a well-designed and reflected soft tissue flap must provide the surgeon's approach with sufficient access to the tooth and surrounding bone. As little bone must be taken out and harmed as feasible during the removal process, which must be atraumatic, aseptic, and non-heat generating. After that, the tooth is separated into portions and delivered using elevators, using the least amount of force possible to avoid difficulties. In order to provide the ideal healing environment during the postoperative phase, the wound must be completely debrided manually and via irrigation (Miloró, 2022).

To extract the mandibular third molar, the area of bone that needs to be removed and the path of withdrawal of the tooth is related to the type of impaction (Figure 1) (Sengupta et al, 2022).

During the mandibular third molar removal surgery, to provide adequate access, a buccal mucoperiosteal flap is raised. The envelope flap, which extends from just the posterior to the location of the impacted tooth anteriorly to around the level of the first molar, is the most widely utilized flap. The envelope flap might not be adequate if the surgeon needs more access to extract a tooth that is significantly affected. In such instance, a three-cornered flap is created by performing a release incision on the anterior aspect of the incision (Miloró, 2022).

The lateral aspect of the anterior border of the mandibular ramus must be

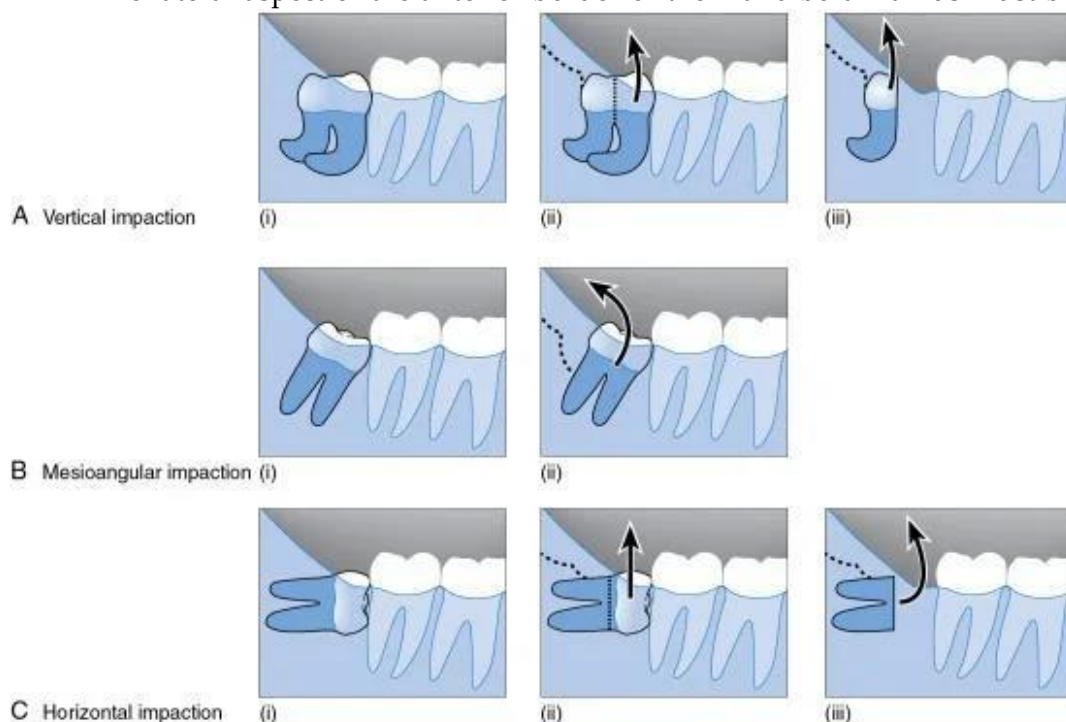


Figure 1. Examples of several kinds of third-molar impactions. (A) Bone removal and vertical sectioning are necessary due to vertical impaction with unfavorable root morphology. (B) Mesioangular impaction that necessitates the removal of bone and the elevation and upright removal of a mesial application point. (C) Horizontal impaction necessitates removing bone and sectioning the crown to allow for the gradual removal of the crown and roots. (D) In order to allow for elevation distally for removal without re-impaction, distoangular impaction necessitates a substantial amount of bone removal. Retrieved from (Sengupta, 2022)

reached by the posterior extension of the incision. The mandibular ramus diverges laterally; hence the incision should not continue posteriorly in a straight line. The lingual nerve might be harmed by the blade if the incision were made straight. Using high-resolution magnetic resonance imaging, Miloro showed that in 25% of

cases, the lingual nerve in the third molar area may be closely connected to the lingual cortical plate, and in 10% of cases, it may be above the lingual crest (Milorio et al., 1997). When removing distolingual bone for a lower third molar extraction is needed, it is crucial to raise a lingual flap and use a retractor instrument as a physical barrier to protect the lingual nerve from trauma caused by hand instruments or a surgical bur. Placing an instrument between the lingual nerve and the bone to be removed can prevent permanent nerve injury, though it may occasionally cause temporary nerve injury due to nerve stretching. Some surgeons prefer not to use an instrument for nerve protection to avoid potential temporary nerve injury by inexperienced operators, emphasizing the importance of only removing bone on the buccal aspect and not distal to the tooth (Sengupta et al, 2022)..

The removal of the bone surrounding the impacted tooth is the second key step (Figure 2). While some surgeons still prefer to use a chisel, the majority of surgeons utilize high-speed, high-torque air or nitrogen-driven handpieces; some now employ a piezoelectric handpiece. In order to prevent tissue emphysema or air embolism, the handpiece must exhaust the air pressure away from the surgical site and be fully sterilizable, often in a steam autoclave. Similar to nitrogen-driven tools, high-speed, high-torque electric drills have become widely used and significantly cut down on the amount of time needed for tooth sectioning and bone removal. The impacted tooth's occlusal, buccal, and (carefully) distal bones are removed all the way down to the cervical line. The depth of the impaction affects how much bone needs to be removed. Due to the possibility of harming the lingual nerve, it is best to avoid removing any bone from the lingual portion (Milorio, 2022). The lingual split technique, involving the removal of a segment of lingual bone plate with a chisel after protecting the nerve, is advocated by some as it may reduce permanent nerve damage compared to using burs. Bones can also be removed with piezoelectric ultrasound, though it takes longer (Sengupta et al, 2022).

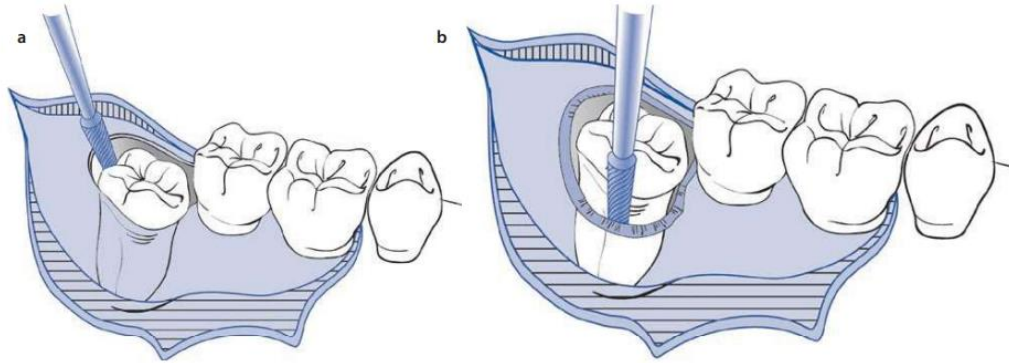


Figure 2. a, After the soft tissue has been reflected, the bone overlying the occlusal surface of tooth is removed with a fissure bur. b, The bone on the buccal and distal aspects of impacted tooth is then removed with bur. Retrieved from (Milorio, 2022)

The tooth may need to be divided before elevation and removal (Sengupta et al, 2022). The angulation of the impaction determines the direction of the divided impacted tooth. A bur is the most often used tool for tooth sectioning because it is effective and offers a more consistent plan of sectioning than a chisel or piezoelectric handpiece. Typically, a straight elevator or other such tool is used to split the tooth three-quarters of the way through to the lingual side and then split the remaining portion. By doing this, harm to the lingual cortical This lessens the chance of harming the lingual nerve and shields the lingual cortical plate from harm (Milorio, 2022).

The surgeon must be extremely careful to remove any specific bone chips and other debris from the site after the impacted tooth is extracted from the alveolar process. Using a periapical curette to manually debride the socket and the region beneath the flap is the most effective way to do this. Any sharp or jagged edges of the bone should be smoothed using a bone file. Any remaining tooth follicle is often delicately removed with a mosquito hemostat, being cautious not to grab tissue lingually too fervently because the residual lingual plate is frequently paper thin and close to the lingual nerve. Lastly, sterile water or saline should be used to completely irrigate the incision and socket (30 to 50 mL is ideal). The more irrigation utilized, within specified bounds, the lower the patient's risk of developing a dry socket, delayed healing, or other issues (Milorio, 2022).

Finally, the soft tissues are closed with appropriate suture material (Sengupta et al, 2022).

1.1.5. Perioperative Systemic Antibiotics

Preventing surgical-related postoperative infection is one of the surgeon's main objectives while performing any surgical treatment. In certain surgical procedures, prophylactic antibiotics are required to accomplish this purpose. The majority of these surgeries are classified as either clean or contaminated. In a clean operation, operator technique has a greater impact on the frequency of postoperative infections than does the use of preventative medicines. When performing any surgical procedure, one of the surgeon's primary goals is to prevent postoperative infection. Prophylactic antibiotics are necessary to achieve this goal in several surgical operations. Most of these procedures fall into one of two categories: polluted or clean. In a clean procedure, operator competence determines the frequency of postoperative infections more so than the use of prophylactic medicines. Surgery to remove the impacted third molars clearly fits under the clean-contaminated surgical category, even though the exact prevalence of postoperative infection is unknown precisely. Infection in the conventional sense of the word is probably a rare occurrence following third molar surgery. For the majority of surgeons, these infections are quite rare (Milorio, 2022). For all third molar surgeries, a skilled, experienced surgeon would typically anticipate an infection risk of 1% to 5% (Loukota, 1991).

Alveolar osteitis or dry socket is a more subdued kind of wound healing issue that develops following the surgical extraction of the impacted mandibular third tooth. Anaerobic bacteria and saliva are most likely the source of this disruption in wound healing. It is true that the frequency of dry socket is decreased when prophylactic antibiotics are used after third molar surgery. Additional methods that lessen bacterial contamination of the socket, such heavy irrigation, chlorhexidine preoperative rinses, and administering antibiotics in the extraction socket, also work well (Arteagoitia, Barbier, et al., 2016; Camps-Font et al., 2024; Cao et al., 2023).

1.1.6. Postoperative complications

About 10% of patients who have their impacted third molars surgically removed experience some degree of problems (Goldberg et al., 1985; Sayed et al., 2019). These side effects range from the more serious and long-term ones, including inferior alveolar nerve numbness and mandibular fracture, to the more anticipated and foreseeable ones, such edema, discomfort, stiffness, and moderate bleeding. The depth of impact, or whether there is a full bone impaction, and the patient's age are the two factors that are most intricately linked to the overall occurrence of problems and the severity of these consequences. Removing impacted teeth in an older patient is linked to a higher risk of postoperative sequelae, including alveolar osteitis, infections, mandibular fractures, and inferior alveolar nerve anesthesia, due to the previously mentioned causes. Additionally, linked to higher rates of postoperative pain and morbidity as well as an increased frequency of inferior alveolar nerve anesthesia is the removal of whole bone impactions (Miloró, 2022).

The relative expertise and experience of the surgeon is another factor that influences the likelihood of third molar surgical problems. Compared to a skilled, experienced surgeon, a less experienced surgeon would encounter a much greater frequency of problems (Larsen, 1991). Following the surgical extraction of an impacted third molar, certain typical physiological reactions take place. These include symptoms including discomfort, stiffness, edema, and slight bleeding. The patient perceives all of them as unpleasant, thus they should be reduced to the lowest feasible level (Miloró, 2022).

1.1.6.1. Postoperative pain

It is normal to have discomfort following dental extraction surgery, which can be reduced by taking an analgesic like paracetamol or a nonsteroidal anti-inflammatory medicine (NSAID) like ibuprofen. After a forceps dental extraction, severe discomfort is uncommon and might be a sign of another issue. Severe pain following tooth extraction surgery is common, though, and often peaks six hours after the procedure (Sengupta et al, 2022).

1.1.6.2. *Postoperative swelling*

A forceps dental extraction may result in mild inflammatory swelling, but unless bone removal was necessary, extensive edema is uncommon and typically denotes the presence of a hemorrhage or a postoperative infection. Drainage or systemic antibiotics may be necessary for infection management. It can be necessary to drain a big hemorrhage. When air is present in the tissue, surgical emphysema is less common but still possible. After a surgical tooth extraction that involves bone removal, swelling may peak in 36 hours and then decrease over the next few days (Sengupta et al, 2022).

1.1.6.3. *Trismus*

While trismus, or restricted mouth opening, following a forceps dental extraction is uncommon and probably of infectious origin, it is not uncommon following a surgical tooth extraction that involves bone removal, and it may take two to three weeks before full mouth opening recovers (Sengupta et al, 2022).

1.1.6.4. *Teeth fracture*

It is not uncommon for teeth to break during forceps extraction for a number of reasons. The existence of a massive restoration may cause the crown to shatter, but since the forceps are applied to the root, the extraction may still proceed. To see the root, a trans-alveolar approach will be required if the fracture happens subgingivally (Sengupta et al, 2022).

As long as it is not linked to an apical infection, a little (3 mm) root apex that remains after extraction may be kept in place. The decision to remove the apex must be documented and communicated to the patient in order to prevent the morbidity that comes with its surgical retrieval. It is necessary to administer antibiotics (Sengupta et al, 2022).

1.1.6.5. *Excessive bleeding*

Given that patients are typically worried, it might be challenging to determine the extent of the blood loss based only on their medical history. However, it is crucial to measure the patient's heart rate and blood pressure in order to determine whether or not they are shocked. To promote hemostasis, this

might be carried out as the patient bites down hard on a gauze swab which is the most effective way to achieve haemostasis (Miloró, 2022). The patient is usually in surgical shock and prompt volume replacement is required if the systolic pressure is less than 100 mmHg and the heart rate is greater than 100 beats per minute. This can be accomplished by injecting a crystalloid, such sodium chloride, or a plasma expander, like Gelofusine or Haemaccel, into a large peripheral vein. The patient should be taken right away to the hospital for this reason. The patient is more often not shocked and can be treated in the primary care context (Sengupta et al, 2022).

Investigating the source of the hemorrhage by collecting a history and doing an examination is the next stage of therapy. Known as "reactive hemorrhage," hemorrhage may be observed within the first few hours following surgery once the effects of the local anesthetic with adrenaline vasoconstrictor have worn off. "Subsequent hemorrhage," which is caused by a secondary infection of the clot, might happen a few days later (Sengupta et al, 2022).

1.1.6.6. *Dry socket (alveolar osteitis)*

A blood clot may not develop or dissolve properly under certain situations. Smoking, surgical trauma, the vasoconstrictor added to a local anesthetic solution, oral contraceptives, and a history of radiation therapy are all risk factors for osteitis. Touching the exposed bone causes excruciating agony (Sengupta et al, 2022).

Dry socket is controlled by:

- Confirming to the patient that the right tooth has been pulled
- Cleaning the socket with warm saline or mouthwash containing chlorhexidine to get rid of any debris
- Covering the socket with resorbable Alvogyl paste, an iodoform dressing, bismuth, iodoform and paraffin paste (BIPP), or lidocaine (lignocaine) gel on ribbon gauze to shield it from unpleasant stimuli; however, this must be taken off and put back on over a period of two or three weeks (Sengupta et al, 2022).

1.1.6.7. *Postoperative infection*

Sockets may occasionally develop a true infection, accompanied by pus, localized edema, and perhaps lymphadenopathy. Usually confined to the socket,

this can be treated similarly to a dry socket, however in certain cases, antibiotics could be required. To rule out the possibility of a sequestered bone or retained root, a radiograph should be conducted. The presence of such material in the socket suggests that the socket needs to be curetted (Sengupta et al, 2022).

1.1.6.8. *Osteomyelitis*

Radiological evidence of loss of the socket lamina dura and a rarefying osteitis in the surrounding bone, sometimes with scattered radio-opacities suggesting sequestra, can be used to identify osteomyelitis, despite its rarity (Sengupta et al, 2022).

1.1.6.9. *Soft tissue damage*

Soft tissues may sustain crush damage if a local or general anesthetic is administered and the patient does not react to the stimulation; in this case, the operator should be notified. When removing an upper tooth, this might happen to a lower anesthetized lip; if the lip is not twisted out of the way, it may get crushed between the teeth and the forceps (Sengupta et al, 2022).

1.1.6.10. *Nerve damage*

Although it is uncommon, damage to the lingual or inferior alveolar nerves during lower third molar extraction may cause temporary or permanent impaired feeling. These can be characterized as dysaesthesia, which is a painful changed feeling, paraesthesia, which is an aberrant sensation, mechanical allodynia, which is pain upon contact, and anesthesia, which is the total lack of sensation. Perception of taste may also be impacted by damage to the lingual nerve. Permanent nerve injuries are defined as those that have not healed within four to six months of the injury. Refer the patient to a specialist for treatment. When uncomfortable, systemic medications like gabapentin may be used, and topical lignocaine (lidocaine) patches may be applied to the skin of the chin or lip. Four to six months following damage, if symptoms have not improved on their own, surgical nerve exploration and repair may be explored. Although cognitive behavioral treatment (CBT) may not alleviate symptoms, it may help people deal better (Sengupta et al, 2022).

1.1.6.11. *The maxillary sinus opening*

When upper molar teeth are extracted, an oroantral communication (OAC) may be created between the maxillary sinus and the oral cavity (Sengupta et al, 2022).

1.1.6.12. *Loss of entire or fragments of tooth*

Sometimes, when a tooth is treated for displacement of a root fragment, the entire tooth may move into the maxillary sinus. Additionally, a tooth may fall into the tissue gaps around the jaws, or the infratemporal fossa, but this generally only happens when mucoperiosteal flaps are elevated (Sengupta et al, 2022).

Usually, when elevators are misused, a fragmented palatal root of an upper molar tooth is unintentionally forced into the maxillary sinus. A portion may occasionally be lost in other places, such the inferior alveolar canal (Sengupta et al, 2022).

1.1.6.13. *Jaw and maxillary tuberosity fracture*

The extraction of upper posterior molar teeth may cause a fracture of maxillary tuberosity (Sengupta et al, 2022).

A jaw fracture is an uncommon occurrence that is most likely caused by the uncontrolled application of high force. Small pieces of alveolar bone that may be connected to the tooth root are more frequently fractured. Additionally, any loose pieces must be completely removed (Sengupta et al, 2022).

1.1.6.14. *Jaw dislocation*

If the jaw is not sufficiently supported during the extraction of lower teeth, dislocation may result. It should be decreased right away because it is more prone to happen under general anesthesia (Sengupta et al, 2022).

1.1.6.15. *Displacement of a tooth into the airway*

When removing teeth from a supine patient, the airway is in danger. When the patient is receiving treatment under general anesthesia, it can be safeguarded; but, when the patient is aware or receiving treatment under conscious sedation, it

cannot. Therefore, it is crucial that an assistant be there and that a high-velocity suction and the right tool be accessible right once in order to remove any foreign bodies. If, after careful examination of the mouth and surroundings, including the floor and suction filter, a lost tooth cannot be located, a chest radiograph is necessary to rule out inhalation. To monitor transit through the gastrointestinal tract, an abdominal radiograph should also be taken into consideration. Additionally, this radiograph can be used to locate misplaced instruments and other foreign objects (Sengupta et al, 2022).

1.1.6.16. *Surgical emphysema*

When air gets into soft tissues, it can cause a distinctive cracking sound when touched. If a mucoperiosteal flap has not been raised, this is unlikely to occur. Because they have the potential to push air beneath soft tissue flaps, air-rotor dental drills should not be utilized during surgery. Antibiotics should be prescribed, the patient comforted, and a review scheduled (Sengupta et al, 2022).

1.2. WOUND HEALING AND INFLAMMATORY PROCESSES AFTER TOOTH EXTRACTION

An elaborate and meticulously planned series of cellular and molecular reactions aimed at regaining tissue integrity and functional ability after injury is manifested overtly in the healing wound. Even while orofacial healing usually ends without incident, several internal and external variables can either help or delay the process. The ability to comprehend wound healing at several levels, including biochemical, physiologic, cellular, and molecular, gives the surgeon a foundation upon which to make clinical judgments that maximize the healing response. Crucially, it enables the surgeon to assess and employ the expanding array of biologic strategies that aim to promote healing by positively altering the wound microenvironment (Milorio, 2022).

1.2.1. **Healing process**

Whether brought on by trauma or surgery, the repair of tissue integrity is a fundamental defensive mechanism that is phylogenetically primordial. The kind of tissue involved, and the type of tissue disruption are the main determinants of the

healing response. Regeneration occurs when tissue that is physically and functionally identical to natural tissue is used for restoration. Repair has been placed, nevertheless, if the development of scar tissue is the main factor in the restoration of tissue integrity (Miloró, 2022).

1.2.2. Wound Healing Response

A complicated sequence of coordinated and temporally overlapping activities aimed at repairing the integrity of the implicated tissue is initiated by any type of injury (Michael Miloró, 2022). The healing continuum of coagulation, inflammation, re-epithelialization, granulation tissue, and matrix and tissue remodeling is commonly divided into three separate overlapping phases: proliferative, remodeling, and inflammatory to make description easier (Sorg et al., 2017; Steed, 2003).

When a clean cut or surgical wound is mostly closed with sutures or another method, healing by first intention happens quickly, with little to no scarring and no dehiscence. In unfavorable circumstances, wound healing is more difficult and involves a lengthy filling of the tissue defect with connective and granulation tissue. This process, known as healing by second intention, is frequently linked to avulsion injuries, local infections, or insufficient wound closure. A tiered method that combines secondary healing with delayed primary closure may be used by the surgeon to try healing by third intention for more complicated wounds. After being debrided, the contaminated wound or avulsion is given five to seven days to granulate and heal on its own. After being debrided, the contaminated wound or avulsion is given five to seven days to granulate and heal on its own. The incision is sutured close to healing by first intention if sufficient granulation tissue has grown and the danger of infection seems low (Miloró, 2022).

1.2.2.1. *Inflammatory phase*

The inflammatory phase, which typically lasts three to five days, foreshadows the body's reparative reaction. An innate tissue response to halt bleeding is vasoconstriction of the damaged vasculature. Factor XII (Hageman factor), which is activated by tissue damage and local bleeding, starts the complement, plasminogen, kinin, and coagulation systems, among other effectors

of the healing cascade. At the site of damage, circulating platelets (thrombocytes) quickly clump together and stick to the exposed vascular subendothelial collagen to create a primary platelet plug arranged in a fibrin matrix. In addition to providing a temporary matrix for cell migration throughout the healing process, the clot ensures hemostasis. Furthermore, as active platelets degranulate, the clot acts as a reservoir for growth factors and cytokines (Figure 3). The bolus of released proteins, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor β (TGF- β), and interleukins, preserves the wound milieu and controls future healing (Miloró, 2022).

After hemostasis is achieved, a longer duration of vasodilation mediated by histamine, prostaglandins, kinins, and leukotrienes replaces the reactive vasoconstriction. By increasing vascular permeability, leucocytes, blood plasma, and other cellular healing agents can enter the extravascular region passing through the vessel walls. Pain, heat, redness, and swelling are examples of parallel clinical symptoms. The chemotactic signals that successively draw neutrophils and monocytes to the site of damage are supplied by cytokines secreted into the wound. Within minutes of damage, neutrophils often start to arrive at the wound site and quickly take over as the predominate cell type. The short-lived neutrophils migrate through the scaffolding that the fibrin-enriched clot provides, feeding the site with cytokines and proteases that aid in clearing the wound of devitalized tissue, contaminated bacteria, and broken-down matrix elements. Opsonic antibodies that seep into the wound from the altered vasculature enhance neutrophil activation. After a few days, neutrophil infiltration stops unless the site is very contaminated. But the pro-inflammatory cytokines (IL-1 α , IL-1 β) that dying neutrophils produce keep the inflammatory response going for a long time (Miloró, 2022; Werner & Grose, 2003).

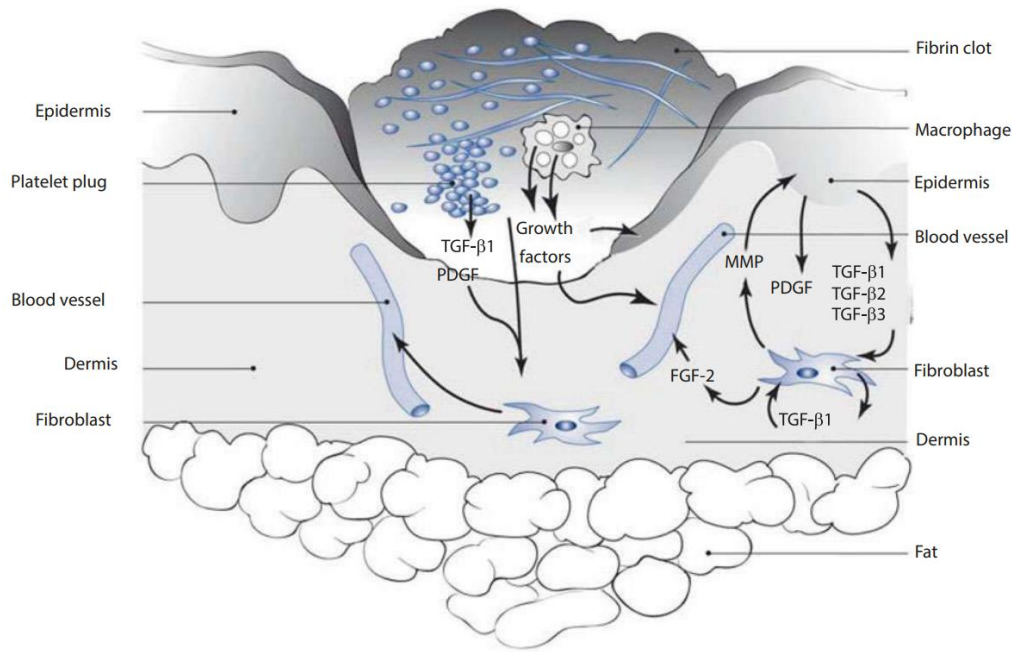


Figure 3. Platelets help a blood clot form right after injury, securing hemostasis and providing a transient matrix for cell migration. By breaking down extracellular matrix and encouraging the growth of new blood vessels, cytokines secreted by activated macrophages and fibroblasts start the production of granulation tissue. Reciprocal signaling between the epidermis and dermal fibroblasts via growth factors, MMPs, and TGF- β family members intensifies cellular connections. TGF- β transforming growth factor beta, PDGF platelet-derived growth factor, MMP matrix metalloproteinase, and FGF fibroblast growth factor Retrieved from (Miloro, 2022)

Over the course of the following 24 to 72 hours, the initial neutrophil counts start to decline as more blood-borne monocytes are sent to the injury site. Activated monocytes, which are now known as macrophages, carry on the neutrophil-initiated wound micro-debridement process. Collagenases and elastases are released by macrophages to degrade damaged tissue and phagocytose germs and cell debris. The macrophages are the main source of healing mediators in addition to their scavenging function (Miloro, 2022).

When macrophages are activated, they produce a variety of growth factors and cytokines near the wound site, including TGF- α , TGF- β 1, PDGF, insulin-like growth factor [IGF]-I and -II, TNF- α , and IL-1. This further intensifies and prolongs

the effects of the chemical and cellular mediators that were previously released by degranulating platelets and neutrophils. Through the regulation of local tissue remodeling by proteolytic enzymes (such as matrix metalloproteases and collagenases), the induction of new extracellular matrix, and the local synthesis of cytokines like thrombospondin-1 and IL-1b, macrophages influence every stage of early wound healing. The constant observation that animal wounds with reduced macrophage function exhibit reduced fibroplasia and poor repair highlights the critical role that macrophage function plays in early wound healing. Even while the macrophages' numbers and activity start to decline by the fifth post-injury day, they nevertheless influence the healing process of the wound until it is fully repaired (Miloró, 2022).

1.2.2.2. *Proliferative phase*

The proliferative phase of wound healing is characterized by the formation of granulation tissue and the establishment of a local microcirculation (Figure 4). This phase begins around day 3 after injury and can last for up to 3 weeks. A crucial step in this phase is the formation of new blood vessels (angiogenesis) from the damaged vasculature. This process is driven by wound hypoxia and growth factors such as VEGF, FGF-2, and TNF- β . These growth factors also attract fibroblasts to the wound site, where they begin synthesizing new extracellular matrix (ECM) and collagen. The collagen scaffold provides support for the newly formed blood vessels, while the ECM helps to restore the structural integrity of the wounded tissue (Gurtner et al., 2008; Miloró, 2022; Ridiandries et al., 2018).

As collagen and ECM accumulate, the tensile strength of the wound increases, reducing the need for external support. Simultaneously, epithelial cells from the wound margins proliferate and migrate to cover the exposed wound surface. This process, known as re-epithelialization, happens more rapidly in mucosal wounds compared to skin wounds. Once the epithelial edges meet, further lateral proliferation stops. Wound contraction, driven by myofibroblasts, helps to draw the wound margins closer together. The extent of wound contraction varies depending on the wound's depth and location (Miloró, 2022).

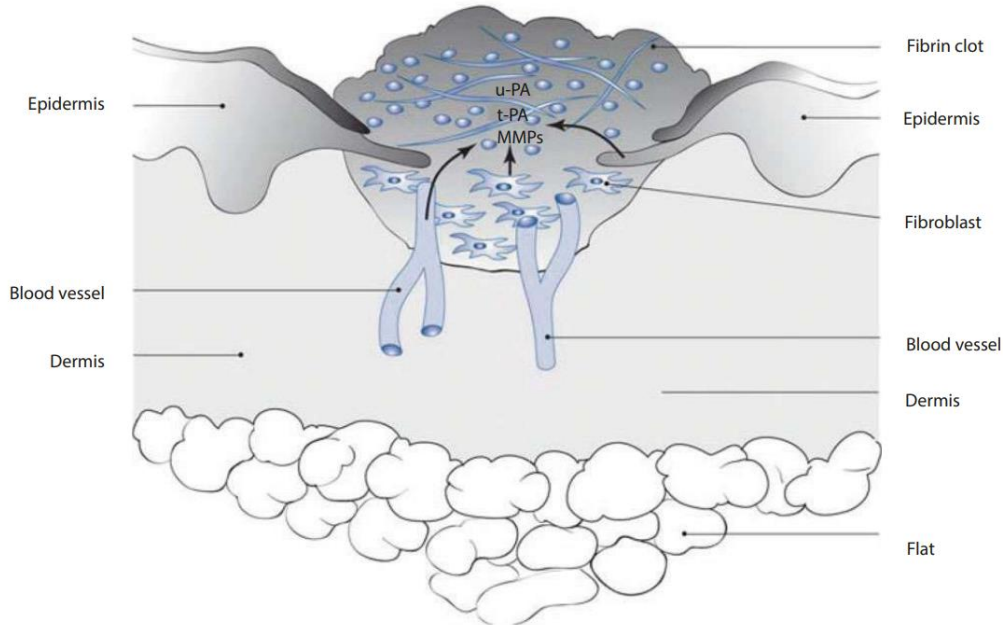


Figure 4. The succedent proliferative phase is mediated by the cytokine cascade. The development of local microcirculation, extracellular matrix, and immature collagen are characteristics of this phase. Granulation tissue starts to form beneath the epithelium, and epidermal cells move laterally beneath the fibrin clot. Matrix metalloproteinases MMPs, Urinary plasminogen activator (u-PA) and tissue plasminogen activator (t-PA). Retrieved from (Miloró, 2022)

1.2.2.3. **Remodeling Phase**

The remodeling phase is the final stage of wound healing, beginning around week 3 and potentially lasting for years. During this phase, the initial type III collagen is gradually replaced with stronger, more organized type I collagen, increasing the wound's tensile strength. While the wound may eventually achieve up to 80% of its original strength, the intricate basketweave pattern of collagen fibers found in normal skin is never fully replicated. As the remodeling progresses, the number of fibroblasts and blood vessels in the wound decreases, resulting in a less prominent scar. The final appearance of the scar depends on factors like wound size, depth, location, and individual healing capabilities (Miloró, 2022).

1.2.3. Extraction site healing

One specific instance of healing by second intention is the healing of an extraction socket (Duyck & Vandamme, 2014). Blood fills the extraction site as soon as the tooth is removed out of the socket. The clotting cascade's intrinsic and extrinsic routes are both triggered. The entrapped red blood cells in the resulting fibrin meshwork shut off the ripped blood arteries and minimize the size of the extraction incision. Inside the first 24 to 48 hours, the clot starts to organize with blood vessel engorgement and dilatation within the remains of the periodontal ligament, followed by leukocytic migration and the creation of a fibrin coating. During the first week, inflammatory cells move onto the temporary scaffold that the clot generates. The assembling clot's surface is covered in epithelium near the wound's edge. Active crestal resorption is facilitated by the accumulation of osteoclasts along the alveolar bone crest. In the remaining periodontal ligaments, angiogenesis continues. New blood vessels start to pierce the clot's core during the second week as the clot continues to form due to fibroplasia. Osteoclastic resorption of the alveolar socket's cortical edge is more pronounced, and osteoid trabeculae gradually extends from the alveolus into the clot. By the third week, the extraction socket is covered with granulation tissue, and the edges of the incision have weakly calcified bone. There is little to no scar and the wound's surface has fully re-epithelialized (Miloró, 2022).

For a few more weeks, there is active bone remodeling through deposition and resorption. Although the alveolar site may continue to reorganize and mature for up to a year following extraction, the majority of the clinically noticeable dimensions changes occur during the first three months (Huebsch & Hansen, 1969). Complete remodeling of the precursor braided bone into lamellar bone and bone marrow can take anywhere from a few months to years, and the rate of bone makeover varies greatly across people (Schropp et al., 2003). Localized alveolar osteitis can occasionally result from blood clot failure to form or dissolve. This results in a significant delay in healing and a progressive filling of the socket. The speed at which regenerate bone adheres to the existing alveolar bone is significantly slower when there is no good granulation tissue matrix present. The infected socket is left exposed or partly covered by hyperplastic epithelium for longer periods of time than a normal socket (Miloró, 2022).

1.3. SALIVA

1.3.1. Saliva and its roles

Saliva is a clear, seromucous exocrine secretion from different salivary glands (Fey et al., 2024). Several biomolecules with a range of activities are present in this cellular fluid. Saliva, for instance, provides a buffering ability, aids blood coagulation and wound healing, shields oral tissues from inflammation and infection, and keeps the hard and soft tissues from becoming dehydrated (Amerongen & Veerman, 2002; Fey et al., 2024; Llena-Puy, 2006).

Since saliva is an "ultra-filtrate" version of blood and an available source of clinical and pathological information, salivary biomarkers can almost exactly reflect the state of the body and a pathology, including bacterial, viral, cardiovascular, autoimmune, stress and cancerous conditions (Abdul Rehman et al., 2017; Bellagambi et al., 2020; Song et al., 2024; Zhang, Sun, & Wang, 2012). Saliva collection is useful for both children and adults since it is linked to less compliance issues and difficulties than other diagnostic matrices like blood or urine. Its primary benefits include being a straightforward, economical, non-invasive screening and/or diagnostic method. Additionally, there is no pain involved, and neither sterile equipment nor medically trained workers are needed (Fey et al., 2024; Punyadeera & Slowey, 2019). Saliva-based diagnostics can be used in personalized medicine to assess a patient's physiological state, track the course of a disease, and track how well treatments are working (Song et al., 2024; Zhang, Sun, Wang, & Wang, 2013). Saliva analysis is a possible blood alternative as a result of its non-invasive manner, and because it may be done by the patient at home or unskilled caretakers. This is especially true for long-term monitoring (such as therapeutic medication monitoring) or for screening many patients (Bellagambi et al., 2020; Ghimenti et al., 2011; Song et al., 2024).

1.3.2. Salivary production

With a baseline flow rate of around 0.5 mL/min, nominally healthy people normally produce 500–1500 mL of serous and mucinous saliva every day. The whole saliva is a combination of oral fluids rich in water (about 99%) and

endogenous chemicals such proteins, polypeptides, hormones, organic compounds (volatile, non-protein, and lipids), and inorganic compounds (Table 2) due to the secretions from both the main and small salivary glands (Aps & Martens, 2005; Humphrey & Williamson, 2001; Navazesh & Kumar, 2008). Saliva that has been activated has a different composition than unstimulated saliva, which is more like plasma. Despite significant individual variation, unstimulated salivary flow rates often exceed 0.1 mL/min, whereas stimulated saliva may reach up to approximately 4 mL/min (Bellagambi et al., 2020).

Table 2. Salivary and plasmatic amounts of inorganic substances are compared in this table adapted from (Bellagambi et al., 2020).

Inorganic compound	Concentration in unstimulated saliva [mmol/L]	Concentration in stimulated saliva [mmol/L]	Concentration in plasma [mmol/L]
Na ⁺	5	20-80	145
K ⁺	22	20	4
Cl ⁻	15	30-100	120
Ca ²⁺	14	14	2.2
HCO ₃ ⁻	5	1580	25
Mg ²⁺	0.2	0.2	1.2
NH ₃	6	3	0.05

Because saliva contains three buffer systems—bicarbonate, phosphate, and proteins—it has a small buffer capacity (Chiappin et al., 2007). Unstimulated saliva typically has a pH range of 6 to 7, but when the flow velocity varies, it can range from 5.3 to 7.8 (Aframian et al., 2006; Humphrey & Williamson, 2001). Since the concentration of bicarbonate ions in saliva is larger in stimulated saliva (from 2.4 ± 1.5 mM to 15 ± 7 mM), the pH rises (Aframian et al., 2006). Saliva tonicity is increased by high salivary flow rates because they raise salt and chloride concentrations while lowering potassium and phosphate concentrations (Jensdottir et al., 2005). Therefore, the buffering activity of saliva is more efficient at stimulated

high flow rates but is essentially worthless for unstimulated saliva. Basal unstimulated secretion is a little, constant salivary flow that occurs during rest and in the absence of external or pharmaceutical stimulus. The oral tissues are covered, lubricated, and moisturized by this flow (de Almeida Pdel et al., 2008).

1.3.3. Salivary composition

The autonomic nervous system (ANS) is primarily responsible for controlling salivary flow and composition:

- A parasympathetic stimulus results in a large flow of watery (less viscous) saliva with low quantities of organic and inorganic components (Nunes et al., 2015).
- Mucoïd (more viscous) saliva secretions are produced by sympathetic activation (Nunes et al., 2015).
- A low amount of saliva with a high protein content and a low mucin content is explained by α -adrenergic activation. Saliva has a low viscosity (Aps & Martens, 2005).
- Saliva with high protein and mucin content, high viscosity, and a foamy appearance can be explained by β -adrenergic activation (Aps & Martens, 2005; Chiappin et al., 2007).

Therefore, the volume and rate of salivary flow, as well as the concentrations of organic and inorganic substances, are altered by parasympathetic and sympathetic activation (Humphrey & Williamson, 2001). A list of inorganic chemicals and a comparison of their plasmatic and salivary levels are shown in Table 1. Therefore, endogenous, mechanical (such as high-frequency chewing and high biting force), gustatory (such as a strong acidic stimulation), and olfactory inputs all contribute to salivary production. Salivary secretion, for instance, may be increased by pregnancy-related hormonal changes, sympathomimetic and parasympathomimetic medications, and menopausal hormonal changes, stress, and/or pharmacological stimuli, such as anti-adrenergic and anticholinergic medications (Aps & Martens, 2005; Bellagambi et al., 2020; Humphrey & Williamson, 2001).

The primary components of entire saliva include glycoproteins (such mucins and proline-rich glycoproteins), enzymes (like α -amylase and carbonic anhydrase),

and a variety of peptides (including cystatins, statherin, histatins, and proline-rich proteins). The contralateral major (parotid, submandibular, and sublingual) and minor (von Ebner) salivary glands are the main producers of proteins in saliva, while oral tissues and microbes can also add to the salivary proteome. Numerous variables, including age, gender, nutrition, circadian rhythm, and physiological state, influence the quantity and kind of proteins in saliva (Battino et al., 2002; Bellagambi et al., 2020).

Gingival fold, oral mucosa transudate, intraoral bleeding (serum and cells), gingival crevicular fluid (GCF), expectorated bronchial and nasal secretions (such as mucous of the nasal cavity and throat), serum and blood derivatives from oral wounds, non-adherent oral bacteria, viruses, and fungi, desquamated epithelium and blood cells, and extrinsic materials (such as food debris) all contribute to the chemical makeup of saliva. Saliva may also include traces of chemicals or drugs, such as those in mouthwash and toothpaste (Bellagambi et al., 2020; Humphrey & Williamson, 2001).

1.3.4. Mechanisms of analyte transfer from blood to saliva

Compounds migrate from plasma to the oral cavity by a variety of mechanisms. Ultrafiltration, which only includes molecules with a molecular weight less than 1900 Da (such as water, ions, and hormones like catecholamines and steroids), is the most widely used transfer method from blood to oral fluid. Through the gap connections between the cells of secretory units (intercellular nexus), analytes can pass through the salivary glands during ultrafiltration. Transudation of plasma chemicals from crevicular fluid or straight from the oral mucosa into the oral cavity is another transfer method. Transudation is necessary for the presence of some plasmatic molecules, such as albumin, in saliva. High lipophilic substances (>5 , where is the partition coefficient), such steroid hormones and some medications, can also passively diffuse analytes across their salivary membranes. Drugs often diffuse passively across lipid membranes from the blood to the oral fluid. As a result, the concentration of medicines in saliva solely represents the amount of drug in plasma that are free (unbound). Drugs and metabolites can also be transferred from blood to oral fluid via active transport and ultrafiltration via cell membrane pores. Lipid solubility, drug molecular weight

and pKa, salivary flow rate and pH, and binding proteins in plasma all affect active transport (Bellagambi et al., 2020; Chiappin et al., 2007; Choo & Huestis, 2004; van den Elsen et al., 2018).

1.3.5. How to collect saliva samples?

Saliva sampling, however, might be difficult. The type of saliva sample, which can be the whole saliva or saliva generated by certain gland, and whether or not the sample is taken after stimulation affect the results (Bellagambi et al., 2020). Salivary composition dynamics are responsive to external influences. For example, the submandibular glands generate around two-thirds of the saliva that is unstimulated, while the parotid glands can enhance production to up to 70% of the overall amount when chewed or tasted. The sublingual glands produce a little amount during rest or stimulation. Therefore, the location of production and contribution of each gland may be identified by looking at the composition of saliva (Castagnola et al., 2011; Fey et al., 2024; Veerman et al., 1996). A distinct range of substances is secreted into the oral cavity by each gland. Saliva rich in alfa-amylase, for instance, is secreted by the parotid gland. On the other hand, saliva enriched with the glycoprotein MUC5B is secreted by the sublingual and submandibular glands (Veerman et al., 1996). Therefore, different types of chemicals that are expressed differently in specific gland secretions are secreted by the salivary glands (Fey et al., 2024). Therefore, to guarantee the accuracy and repeatability of clinical and research results, it is imperative to collect saliva using standardized techniques (Fey et al., 2024).

Regarding Shannon and Chauncey study, the best method for collecting saliva, both stimulated and unstimulated, is to sit upright, lean the head slightly forward, and keep the eyes open (Shannon & Chauncey, 1967).

The amount of required saliva and the device's capacity to retrieve the biomarkers determine which method and device is the best. Additionally, several factors, including age (salivary composition varies greatly in infants, children, and elderly people), flow rate, nutrition, temperature, and pH, can influence the content of saliva (de Almeida Pdel et al., 2008; Fey et al., 2024; Lomonaco et al., 2014).

The most common techniques for gathering saliva include the passive drooling method, spitting method, absorption method, and suction method. The unstimulated saliva is typically collected using the drooling method, while the stimulated saliva is sampled using the spitting, suction, and absorption methods (Priya & Prathibha, 2017). Chewing and gustatory stimulation, such as citric acid, can provide this stimulation (Fey et al., 2024).

1.3.5.1. *Unstimulated whole saliva sampling*

The combination of secretions that enter the oral cavity without the need for external stimulation is known as unstimulated whole saliva (UWS), and it is reliant on the daily baseline salivary flow rate in the oral cavity. Because it reduces analyte dilution, unstimulated saliva samples are frequently used. However, because the content of unstimulated saliva can be influenced by factors such as body posture, light exposure, medicines, circadian rhythm, head position during collection, and level of hydration, consistent sample protocols are required (Malamud, 2011; Priya & Prathibha, 2017).

1.3.5.1.1. Passive drooling and draining method

Because it is possible to rule out the impact of flow rate on saliva composition, the passive drooling of unstimulated whole saliva has been referred to as the gold standard for biological tests since 1934. To undertake passive drooling, the participant is often asked to "debur" (let the saliva fall) into plastic tubes (polypropylene tubes, for example, to prevent contamination or sample retention) (Bellagambi et al., 2020). According to Granger et al., passive drooling has several benefits, including a large sample volume and minimal impact from the materials and chemicals used to collect or induce salivary flow (Granger et al., 2007). However, those who are unable to cooperate effectively—such as extremely young toddlers, sleepers, and fragile old people—should not engage in passive drooling. It is better for these people to sample their saliva by absorption (Bellagambi et al., 2020).

Saliva is collected in the mouth and forced into the vial with the head bent forward using the Saliva Collection Aid (SCA, polypropylene), one of the devices suggested by salimetrics for passive drooling. Oasis Diagnostic® offers several

saliva collection tools, such as the UltraSal-2™ kit, which can sample up to 24 milliliters of saliva (Figure 5). This device divides saliva into two aliquots using two 12 mL tubes attached to a single buccal entering. Despite the supplier's recommendations to tilt or rotate the apparatus, it is challenging to guarantee aliquot uniformity (Bellagambi et al., 2020).

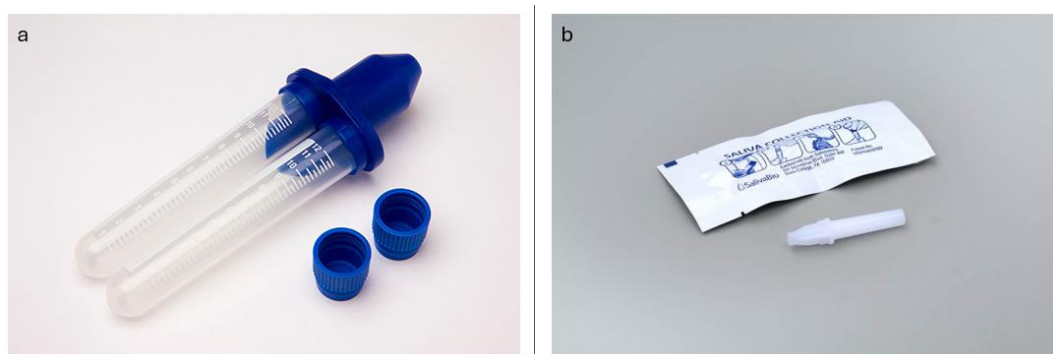


Figure 5. Introduced devices to collect saliva with drooling method. a) UltraSal-2™ kit, b) Saliva Collection Aid (SCA, polypropylene). Acquired at: <https://salimetrics.com/product/saliva-collection-aid-sca-50pk/> and <https://omniabiotech.com/ultrasal-2/>

1.3.5.1.2. Spitting

Spitting occurs when saliva builds up on the floor of the mouth and is then expelled into a graded or pre-weighed container, such as a funnel attached to a tube or container. This technique can be applied when the flow rate is extremely low and reduces the amount of saliva that evaporates during lengthy samplings; yet, it may have some stimulatory effects (Priya & Prathibha, 2017). Consequently, samples obtained by spitting cannot be regarded as authentic unstimulated samples since this sampling technique entails a degree of stimulation. Spitting should be followed by a homogenization step, such as filtering and recollection of the filtrate, due to the sample's poor homogeneity (Bellagambi et al., 2020).

1.3.5.1.3. Swab based sampling methods

Swabs or other absorbent materials can be placed in the mouth to extract unstimulated whole saliva (Figure 6). The subject's tolerability (dimensions, taste, and allergy) and the capacity to maintain the relevant analytes should guide the material selection. Salivary α -amylase is one example of an analyte whose retention

is so high that it has an inverse correlation with the amount of saliva that is adsorbed (Bosch et al., 2011).

Swab-based sampling is especially appropriate for less-or non-collaborative participants, such as those with disabilities, children, neonates, and the elderly, even if swallowing must be avoided and undesirable stimulation may exist. However, while taking a saliva sample, the swab must not be chewed or sucked (Bellagambi et al., 2020).

The salivary flow rate may be calculated by sample timing and weighing the swab both before and after the sampling, provided that the swab's maximal absorptive capacity is not exceeded (Bellagambi et al., 2020).



Figure 6. A selection of different swab-based devices for sampling saliva: (A) Super Sal™ (Oasis Diagnostic Corporation); (B) Versi Sal™ (Oasis Diagnostic Corporation); (C) Quantisal® (Immunalysis Corporation); (D) Intercept™ (Orasure Technologies Inc.); (E) Salivette™ (blue cap, Sarstedt); (F) SOS (Salimetrics); (H) Toothette-Plus swabs (Sage Products Inc.); (I) OraQuick Advance HIV-1/2 (Orasure Technologies Inc.); (J) BBL CultureSwab orange and white cap. Retrieved from (Bellagambi et al., 2020)

However, most manufacturers do not disclose information on analyte recovery or sample repeatability, even though a variety of swabs are commercially available, and some are sold for collecting certain salivary analytes (e.g., Salivette Cortisol is expressly intended for cortisol detection in saliva). Analyte recovery must thus be thoroughly examined to improve the sampling process (Bellagambi et al., 2020).

1.3.5.2. *Stimulated whole saliva collection*

Stimulated saliva is naturally produced in response to chewing or tasting food. The composition of stimulated saliva is affected by gland size, diet, smoking, gag reflex, and the type of stimulation used to produce the saliva. Researchers and clinicians can induce the production of stimulated saliva using a variety of methods. Masticatory stimulation is achieved by having subjects chew on substances such as paraffin wax, un-flavored chewing gum, cotton, or rubber bands. Gustatory stimulation involves the use of citric acid or sour candy. It is important to note that both the flow rate and composition of stimulated saliva are affected by the method of stimulation. While chewing has been shown to increase flow rate, protein output, and pH, gustatory stimuli appear to have an even more significant impact on the composition of stimulated saliva (Bellagambi et al., 2020).

A study conducted by Polland et al. examined the effects of chewing sugar-free gum on the flow rate and pH of saliva. The results revealed that both flow rate and pH remained elevated even after 90 minutes of intermittent chewing (Polland et al., 2003). Specifically, the average flow rate rose from 0.39 mL/min at rest to 2.7 mL/min during stimulation, while the pH increased from 6.7 to 7.35. Similar findings were reported by Dawes & Kubieniec, who observed that while flow rate reached a plateau after 35-40 minutes of chewing, pH continued to rise (Dawes & Kubieniec, 2004).

Mechanical, masticatory stimulation can also influence the concentration of specific compounds in saliva. For example, Dong et al. found that sucrose levels varied depending on chewing rate (Dong et al., 1995), while Higashi et al. reported a two-fold decrease in homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations due to increased salivary flow

caused by chewing gum (Higashi et al, 2012). These findings underscore the importance of standardizing saliva collection procedures to ensure the accuracy and reliability of saliva analysis. Researchers can enhance consistency by using standard-sized stimulants and controlling the chewing frequency with a metronome (Bellagambi et al., 2020).

1.3.6. Salivary biomarkers for clinical applications

1.3.6.1. Salivary steroids

Steroids are low molecular weight, lipophilic substances made from cholesterol. Because they affect both the central nervous system (CNS) and peripheral target tissues, steroids play important physiological functions. Steroids come in a vast range of physiologically active forms while having a considerably basic chemical composition. While unconjugated steroids are mostly attached to carrier proteins in plasma, conjugated steroids are often present in bodily fluids in the form of a hydrophilic moiety. The "free fraction" of steroids is the hormone that is immediately active and makes up 1–10% of the total plasma concentration (Bellagambi et al., 2020).

The method of transfer from plasma to saliva has been documented by Zolotukhin and Gröschl (Gröschl, 2009; Zolotukhin, 2013). Through the salivary glands acinar cells, the lipid-soluble hormones cortisol and aldosterone passively diffuse into saliva. Salivary concentrations of cortisol and aldosterone can be utilized to determine unbound levels in plasma since they are not influenced by salivary flow rate. Additionally, saliva can absorb steroids from blood or plasma through oral abrasions or directly from food through exogenous steroid contamination. In the late 1970s, the first measurement of steroids in saliva was made, and the levels of cortisol and testosterone were found (Lewis, 2006). Analyzing salivary hormones is used to test for Cushing's disease (cortisol), diagnose congenital adrenal hyperplasia (17 α -OH progesterone), evaluate ovarian function (progesterone and estradiol), and investigate adrenal function (Bellagambi et al., 2020).

1.3.6.2. *Peptides and proteins*

Saliva analysis has been more common in proteomics in recent years, particularly as a result of advancements in analytical methodologies (Hu et al., 2007). More than 2000 proteins and peptides, which are involved in several biological processes, are found in the saliva (Khurshid et al., 2016). For instance, matrix metalloproteinases (MMPs), interleukins (ILs), and tumor necrosis factor- α (TNF- α) are utilized as biomarkers in clinical practice and are linked to inflammation. A number of illnesses, including oral squamous cell carcinoma, oral leukoplakia, Sjögren's syndrome, chronic graft-versus-host disease, SAPHO (i.e., synovitis, acne, pustulosis, hyperostosis, and osteitis), schizophrenia, bipolar disorder, and genetic disorders including Down syndrome and Wilson disease, have recently been diagnosed and tracked using salivary proteomics. The impact of sampling techniques on the amounts of proteins and peptides, however, is rarely covered in the literature (Bellagambi et al., 2020).

1.3.6.3. *Prostaglandins*

It has been demonstrated that insufficient saliva can cause significant issues for oral health and postpone the healing of oral diseases. It has been confirmed that submandibulectomy causes a delay in the development of granulation tissue during the first 72 hours after tooth extraction. It also modifies the production of inflammatory mediators involved in wound healing, including prostaglandin E2 (PGE2), tumor necrosis factor- α (TNF), and nitric oxide (NO), which delays tissue regeneration in the early phases of the healing process (Mohn et al., 2015).

Inflammatory mechanisms that result in wound healing involve prostaglandins (PGs). PGE2 has been shown to be essential for the mechanisms that mend bone fractures. Because of their impact on osteoblasts and osteoclasts, PGs are regarded as significant local variables that regulate bone metabolism. Another important eicosanoid that osteoblasts make is PGE2. Numerous stimuli with varying effects on bone can produce PGE2. Because PGs act locally and transiently, are controlled at numerous levels, have multiple receptors, and might have conflicting effects depending on the test system, it can be difficult to understand their involvement in bone metabolism. PGE2's ability to engage other gas-activated signaling pathways and modify or integrate several signaling pathways appears to

be one of its primary functions. Target cells include fibroblasts, mesenchymal cells, and osteoblast and osteoclast precursors are localized targets of PGE₂, which mediates its tissue-specific pharmacological action through four distinct G protein-coupled receptor subtypes (Brochhausen et al., 2006; Mohn et al., 2018).

1.4. PAIN, PAIN CONTROL AND ASSESSMENT

1.4.1. Nociception and pain

1.4.1.1. *Nociception*

The process of identifying and communicating the existence of an unpleasant stimuli is known as nociception. Specialized sensory transducers called nociceptors, which are connected to A δ and C fibers, are activated during detection (Sengupta et al, 2022).

1.4.1.2. *Pain*

Pain is frequently referred to as the "fifth vital sign" (Sollami et al., 2015). An ancient Greek word meaning "penalty" and a Latin term meaning both "punishment" and "penalty" are the sources of the English word pain. In clinical dentistry and medicine, the word "pain" is used to refer to extreme discomfort (Nowak, 2019). "Unpleasant sensory and emotional experience connected with actual or potential harm or characterized in terms of such damage" is the definition of pain that has been approved by the International Association for the Study of Pain (IASP) (Merskey, 2012). Pain can happen without nociception and has both a sensory-discriminative and motivational-effective component (Sengupta et al, 2022).

It's critical to understand that pain serves an essential purpose, despite the suffering endured by the one experiencing it. Pain alerts the body to actual or perceived tissue damage, which motivates it to take action to lessen or eliminate discomfort. In this way, it is a desired experience for sustaining and directing an individual's daily activities (Nowak, 2019).

1.4.2. Pain system

It's important to realize that pain is more than just a feeling and an emotional reaction. Physical, chemical, humoral, affective (emotional), cognitive, psychological, behavioral, and social factors interact in a very intricate and multidimensional way. Undoubtedly, it is unclear what factors influence how a person perceives and responds to pain. Nonetheless, the corpus of information pertaining to the comprehension of pain has started to develop and is quickly becoming a valuable and scientific field. Comorbid symptoms, such as greater suffering, worse quality of life, and delayed recovery from the initial lesion, may be linked to the onset and persistence of pain (Liossi & Howard, 2016).

In a physiological context, pain is caused by brain impulses that go via several routes including neurons with unique sizes, shapes, chemistries, and spatial orientation. Numerous secondary reactions are triggered by these signals, and some of these responses may organize into a hierarchy of systems that involve parts of the central nervous system (CNS). Some of these reactions at the physiological level include the release of neurochemical and humoral active substances (such as endogenous opioids and γ aminobutyric acid) as well as additional neural signal transmission. Activating motor activity for intentional escape is one of the other mechanisms in this response hierarchy (Nowak, 2019).

Not every unpleasant stimulus that triggers nociceptors is necessarily felt as pain. Studies have indicated that the degree of pain awareness and, perhaps more significantly, the person's capacity to maintain self-control in the face of pain are significantly influenced by emotional and cognitive aspects of the pain process (Khera & Rangasamy, 2021). Pain, pricking, burning, aching, and stinging are examples of feelings that can be controlled, despite their urgent and basic nature. For instance, unpleasant inputs may cause far less discomfort than would be predicted under emergencies or crisis situations, or even when a person's focus is just elsewhere. It has been noted that in times of war, a person's dread of dying may cause them to numb the pain of an injury until they are out of direct danger on the front lines. On the other hand, postoperative discomfort may worsen if you are anxious about having elective surgery (Sengupta et al, 2022).

The fact that human pain varies indicates that there are brain processes that alter how pain signals are sent and alter how each person feels pain. Therefore,

pain is no longer thought of as a static process that occurs through exclusive channels from peripheral tissues to the brain via the spinal cord, but rather as signals that result from the interaction of excitatory and inhibitory neuronal networks at various levels of the CNS (Sengupta et al, 2022).

1.4.2.1. Central Nervous System Effects on Pain Perception and Control

A sophisticated endogenous opioid system that is extensively distributed throughout the mammalian central nervous system has been partially identified and characterized (Basbaum & Fields, 1978). Peptides called endogenous opioids are produced by the body spontaneously and have effects comparable to those of opiates, such as morphine. One of the strongest peptides is β -endorphin, which has the same N terminal as met-enkephalin, one of the first opioid peptides to be identified (Nowak, 2019).

The spinal cord is one of the several CNS opioid receptor sites where the active opioid peptides function after being broken down from bigger precursors. Each of the peptides may contribute to specific and specialized processes underlying the pain perception process, and while they are not all equally effective, they are all made inactive by the narcotic antagonist naloxone (Nowak, 2019).

Accordingly, nociceptin, ϵ , δ , and μ are the four opiate receptors that have been identified in the central nervous system. It is unclear how each contributes to the production of analgesia, though. Injecting opioid ligands that bind to μ receptors into the medulla's periaqueductal gray (PAG) region results in strong analgesia. When electrically or opiate-stimulated appropriately, other locations, such as the PAG, which stretch from the hypothalamus to the ventromedial medulla, induce analgesia (Nowak, 2019). It's interesting to note that nitrous oxide may directly activate opiate receptors or that its analgesic effects may be partially mediated by endogenous opioid ligands (Gad, 2014).

1.4.2.2. Cognitive Elements of Pain Perception

The intricate process of cognition leads to an understanding and frequently the following identification of possible outcomes as a result of "knowing." Perception, organizing, assessing, meaning, reasoning, and responding are just a few of the many processes that go into knowing (Nowak, 2019).

Being cognizant is being aware of how one's surroundings, both internal and external, affect oneself. Additionally, it suggests that actions may be made to take charge of those effects and utilize that power to change how one responds (e.g., coping). For example, by engaging in mental exercises, such as visualizing pleasurable experiences or counting the holes in a ceiling tile, one may be able to lessen the level of discomfort they are experiencing (Nowak, 2019).

Depending on how they view the issue, a person may manage a range of circumstances, including stressful situations. The results of coping methods depend on a number of factors, including the situation's timing, effects, and consequences, as well as personal resources. Techniques for hypnosis and relaxation, visualization, modeling, distraction, and reconceptualization are examples of coping mechanisms. Generally, therapeutic coping techniques for pain management have several components, such as (1) problem evaluation, (2) rethinking the patient's perspective, (3) learning useful skills (such breathing and relaxation), (4) applying and maintaining those skills to avoid relapse, and (5) measuring therapeutic progress (Turk & Meichenbaum, 2017).

According to some research, people who are given the impression that they have some influence over the suffering they may experience in the future are more tolerant of unpleasant stimuli (Khera & Rangasamy, 2021). Nonetheless, it appears that a strong conviction in their capacity to develop self-control plays a significant role in regulating the level of discomfort. People without this skill could "struggle less" when under the care of others (like doctors) and have greater faith in them (Nowak, 2019).

1.4.2.3. *Emotional Elements of Pain Perception*

Even while each individual feels pain and the expectation of unpleasant stimuli (such as anxiety) differently, most people have an awareness of the emotions that accompany these experiences. We are undoubtedly skilled at identifying another person's pain, and we may even be more sensitive to understanding someone else's expectation of difficulty (Nowak, 2019).

The way that emotional content is expressed during or before traumatic events is probably a complicated mix of an early-life phenomena that is largely inherited but also influenced by learning. As a newborn grows older, the way they

exhibit discomfort from vaccinations changes from a more dispersed, weeping, and reflexive reaction to one of anticipation, attention to the unpleasant thing, and even rage (Nowak, 2019).

Even while the emotional aspects of pain could be thought of as secondary to the actual pain, they can work together to modify unpleasant sensations. Certain pharmaceutical substances, such as benzodiazepines and nitrous oxide, may have effects on parts of the central nervous system that are in charge of emotional impact, according to indirect and anecdotal evidence. Although they are aware of the discomfort, they are not very irritated by it. On the other hand, it is well recognized that emotional distress in the face of discomfort lowers pain thresholds and increases responsiveness. Reducing anxiety and the level of sensitivity to unpleasant stimuli can be accomplished with cognitive techniques intended to evoke positive emotional states (Nowak, 2019).

1.4.3. Distinct types of pain

1.4.3.1. *Acute pain*

Acute pain is defined as pain that has just started and likely has a short duration. Usually, there is a causative relation with illness or damage. Long before the healing process is finished, patients cease reporting discomfort. This kind of discomfort would often occur after surgery or an injury (Sengupta et al, 2022).

1.4.3.2. *Chronic pain*

Long-lasting pain is sometimes referred to as chronic pain; nevertheless, the incapacity of the body to return physiological processes to normal homeostatic levels is what sets chronic pain apart from acute pain, not the length of the suffering. Chronic pain frequently lasts after an injury has healed, and its severity typically has little to do with the degree of tissue damage. In fact, there may not be a clear explanation (Sengupta et al, 2022).

1.4.3.3. *Inflammatory and neuropathic pain*

Clinical pain can have either a neuropathic or inflammatory origin; the former describes pain brought on by injury to peripheral tissues, such as that which

occurs during surgery, while the latter describes pain brought on by a nerve system lesion or illness, like postherpetic neuralgia or trigeminal neuralgia (Sengupta et al, 2022).

1.4.4. Pain control

Numerous publications, such as those from the Royal College of Surgeons and the College of Anaesthetists, have demonstrated that post-operative pain management in the UK has not been as good as it might be, and this problem is acknowledged worldwide (Rawal, 2016). According to a 2011 US National Institutes of Health report, less than 50% of patients receive appropriate pain treatment after surgery, and over 80% of patients have postoperative discomfort (Institute of Medicine (US) Committee on Advancing Pain Research, 2011). According to US surveys conducted in 1993, 2003, and 2012, postoperative pain is widespread and yet not adequately addressed, and the distribution and caliber of pain perception have essentially not altered (Gan et al., 2014). In addition, a European assessment including 746 institutions showed that postoperative pain treatment was not at its best. Among the issues noted in this 2008 research were that 34% of facilities did not measure pain, 56% did not document, and 75% did not have documented guidelines (Benhamou et al., 2008).

Among the causes include improper medication prescriptions and dosages, as well as a lack of awareness or assessment of pain (Sengupta et al, 2022).

1.4.5. Pain after dental surgery

In addition to considering possible side effects and the patient's overall health, analgesics should be recommended based on their efficacy and appropriateness for the patient's reported or expected postoperative pain severity (Table 3) (Sengupta et al, 2022).

Table 3. Systemic Pain Relief after Dental and Surgical Procedures. These adult protocols are based on evidence from postoperative pain systematic reviews adapted from (Sengupta et al, 2022).

Typical Pain Level	Type of Procedure	Protocol
Mild pain	Forceps extraction	Paracetamol 1 g every 6 hours regularly (maximum 4 g/24 h)
Moderate pain	Surgical removal of tooth	Ibuprofen 400 mg every 6 hours regularly (maximum of 2.4 g/24 h) and paracetamol 1 g every 6 hours as necessary (maximum of 4 g/24 h)
Severe pain	Surgical removal of tooth involving bone removal	Ibuprofen 400 mg every 6 hours regularly (maximum of 2.4 g/24 h) and paracetamol 1 g/codeine 60 mg combination every 6 hours regularly (maximum of 4 g paracetamol/24 h) When NSAIDs contraindicated: paracetamol 1 g/codeine 60 mg combination every 6 hours regularly (maximum of 4 g paracetamol/24 h)
Severe pain for inpatients	More difficult surgical removal of teeth or major surgery	Morphine by intravenous titration or intermittent intramuscular injection

1.4.6. Pain assessment

According to studies, medical professionals frequently underestimate the pain that patients perceived (Rawal, 2016). To give the practitioner a more objective way to evaluate and effectively manage pain in clinic, a pain assessment instrument, often known as a pain scale, should be used (Nowak, 2019).

Self-report, observational/behavioral, and physiological measurement are the three categories of pain assessment instruments (Nowak, 2019). For patients who are extremely young or have cognitive impairments, an observational/behavioral

evaluation method is utilized. The patient must be able to comprehend instructions and point to or articulate their response in order to use the self-report evaluation technique, which is optimal. It is believed that self-report of discomfort combined with certain physiological measurements—particularly heart rate—adds a crucial layer to the description of response specificity to painful stimuli (H. Breivik et al., 2008; Haefeli & Elfering, 2006; Lichtner et al., 2014).

1.4.6.1. *Visual analogue scale (VAS) and numeric rating scale (NRS)*

Identifying the location, temporal characteristics, and severity of acute pain—whether from trauma, surgery, or an acute medical disease—helps to describe the pain and assess the impact of treating its underlying cause (H. Breivik et al., 2008).

When evaluating acute pain following surgery, the widely used visual analogue scale (VAS) and numeric rating scale (NRS) both perform better than a four-point verbal category rating scale (VRS) and have good agreement and equal sensitivity (Figure 7). They work best for the patient's subjective perception of the current level of pain severity. They can be used for pain that has been the worst, least, or average during the past 24 hours or week. This has some limits since pain memories are not always precise and are frequently influenced by shifting contextual circumstances. They are also used to rate the impact of pain on function and determine how "unpleasant" the pain is. The VRS scale categories' specified ranges on the NRS are approximations, and there is substantial variance across patients and people at various times (E. K. Breivik et al., 2000; H. Breivik et al., 2008). According to a study that used simultaneous recordings of pain intensity on VAS, NRS, and VRS scales in many patients, the VAS and NRS are superior to VRS,. The power to identify a difference in pain intensity was higher with the NRS and VAS data than with the VRS data, according to computerized simulation research that randomly sampled 10,000 times from simultaneous observations of VAS, NRS, and VRS (E. K. Breivik et al., 2000).

While the NRS and VAS values typically agreed well, the verbal categories of mild, moderate, and severe pain may correspond to various VAS values in the same patient on different occasions (E. K. Breivik et al., 2000). Therefore, even in normal clinical evaluation, an NRS or VAS should be utilized for more precise pain

intensity measurement, and a categorical pain scale should only be used as a crude screening tool (H. Breivik et al., 2008).

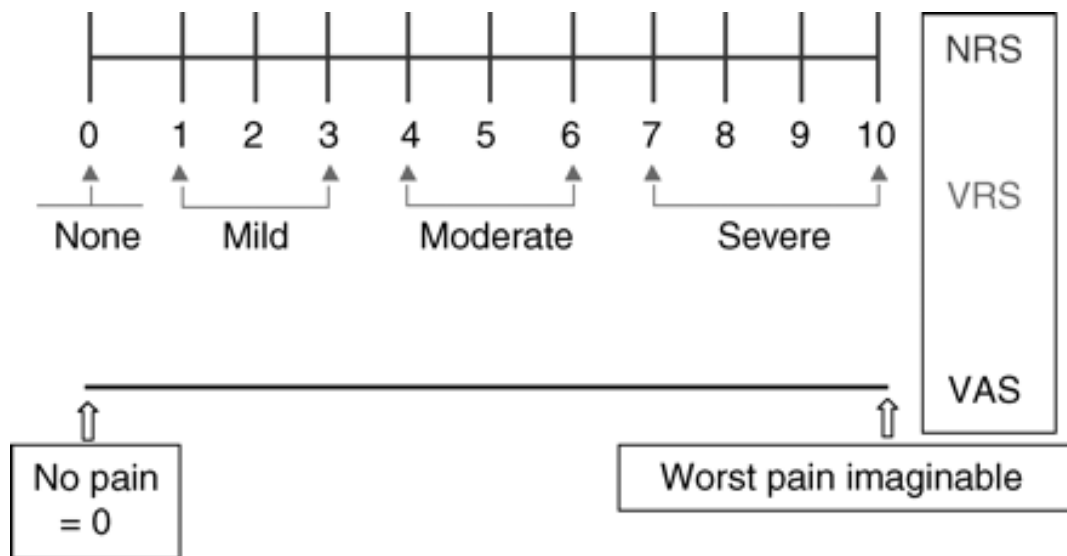


Figure 7. The 11-point NRS and the VAS, which range from no pain (=0) to the greatest suffering possible [=10 (or 100)], are two frequently used one-dimensional pain intensity measures, as well as the four-point verbal rating scale (VRS) with categories. Retrieved from (E. K. Breivik et al., 2000)

Compared to a VAS, an NRS that uses numbers ranging from 0 to 10 (from "no pain" to "worst pain imaginable") is more useful, simpler for most people to understand, and requires no paper, pen, dexterity, or sharp vision. A computerized telephone interview, a telephone interview, and the patient's direct entry of NRS data into a computer database utilizing a telephone keypad can all be used to precisely assess the level of pain. It has been demonstrated that the NRS and the VAS provide almost comparable results for the same patient at different points in time following surgery, but the four-point VRS appears to understate the most severe pain when compared to the VAS (E. K. Breivik et al., 2000; H. Breivik et al., 2008).

II – JUSTIFICATION

II - JUSTIFICATION

One of the most common surgeries in dentistry is the extraction or surgical removal of third molars. Clinical complications, pain and swelling, for instance, happen after these types of dental interventions that suggest a systemic inflammatory process occurs in the human body during and after third molars removal. Cei and colleagues (Cei et al., 2012) presented a model for blood inflammatory factors changes after third molar surgeries, which was followed by other research to complete this model (Graziani et al., 2017; Mehra et al., 2013; B. Sainz de Baranda et al., 2019). According to this model, the white blood cells count would meet a peak 24 hours after the third molar surgery: an acute increase in the Neutrophil count and a decrease in Lymphocyte count. These WBC differences would continue for a week. Another observed inflammatory adaptation that happens in blood after these surgeries are increasing the concentration of C-reactive protein (CRP) and fibrinogen, with a peak on the seventh day after surgical intervention. These alterations rebound to the baseline three months after the surgery (Cei et al., 2012; Graziani, et al., 2017).

Another body fluid that attracted the researchers' interest is saliva. There are plenty of detectable factors in the saliva, including inflammatory parameters such as prostaglandins. These biomarkers are valuable alternatives for diagnosis and monitoring several diseases, such as dental problems, gastrointestinal disorders, metabolic diseases, and respiratory conditions (Min et al., 2023). Furthermore, saliva samples are easy to acquire according to the on-site application possibility and its non-invasive method (Min et al., 2023). Studies utilized saliva as a source of biomarkers to diagnose several diseases in medical fields, such as systemic lupus erythematosus, COVID-19, diabetes, and chronic diseases (Abdullayev et al., 2022; Desai & Mathews, 2014; Prasad et al., 2016; Ruacho, et al., 2022; Shakeeb et al., 2022). In dentistry, saliva is a valuable source to assess biomarkers' transformations during dental caries, orthodontic movements, periodontitis, and dental treatments (Børsting et al., 2022; Isola, 2022; Kim & Kim, 2021; Veljovic et al., 2022). Jumaah and Al-Adili investigated the relationship between the postoperative level of Alpha-Amylase in saliva and the surgical difficulty of mandibular third molar

extraction. They showed a non-significant increased level of alpha-amylase 48 hours and seven days after surgery which was unrelated to the difficulty of the surgery (Jumaah & Al-Adili, 2022). However, Corrales and her team measured total protein, immunoglobulin A (IgA), and alpha amylase in saliva before extraction, immediately after extraction, two hours after the extraction, and seven days after surgery. They demonstrated a significant shift in salivary alpha-amylase after extraction of impacted mandibular third molars (Gutiérrez-Corrales et al., 2017). Chandini et al. said in their article that salivary alpha-amylase was not related to the inflammatory response after third molar surgery. By the way, they checked alpha-amylase concentration just immediately after the surgery (Chandini et al., 2022). Another Study tracked the prostaglandin E2 (PGE2) level in the saliva and urine of the patients who went under third molar surgery. The results showed a peak of PGE2 in the saliva after 24 hours and in urine after three days (Mehra et al., 2013).

Besides previous studies which attempted to find a timeline of inflammatory elements transformations, there are plenty of investigations that evaluated clinical infection and inflammation signs and symptoms after third molar surgeries. Trismus, swelling, alveolar osteitis (dry socket), surgical site infection (SSI), duration, and intensity of pain experienced by the patient were measured by the researchers to find out the effect of various treatments and surgical methods (Chuang et al., 2008; Kimbauer et al., 2022; Knezevic et al., 2018; Mehra et al., 2013; Stošić, Šarčev, Mirković, Bajkin, & Soldatović, 2022; Verma et al., 2022). Chuang and his team defined risk factors for surgical site infection and alveolar osteitis after third molars surgery. They introduced the impaction level of the tooth, pre-existing infection at the intervention site, and pathologies as the main risk factors (Chuang et al., 2008). Hence, researchers consider these factors in their inclusion and exclusion criteria in order to reduce errors in their analyses. However, Sainz de Baranda and her colleagues revealed that despite the difficulty of third molar extraction or surgery, inflammatory processes occurred in the blood and gingival tissue after the intervention. Their results presented a significant increase of Interleukin 6 (INL-6), C-reactive protein (CRP), and fibrinogen in the blood, as well as pain, bleeding, and swelling as clinical complications following the treatment, which were not related to the difficulty of the intervention (Sainz de Baranda et al., 2019).

Since pain, swelling, and trismus are common courses after third molars surgery, a dozen clinicians and researchers tested different drugs and methods to reduce them. Anti-inflammatory and palliative drugs are used as much as antibiotics to diminish complications following the surgical interventions, especially the risk of infection (Janas-Naze et al., 2022; Kirnbauer et al., 2022; Knezevic et al., 2018; Mehra et al., 2013; Stošić, Šarčev, Mirković, Bajkin, et al., 2022; Verma et al., 2022; Xue et al., 2015). Mehra et al. demonstrated that anti-inflammatory drugs such as Ibuprofen and dexamethasone significantly reduced pain and swelling after third molar surgery. They said a single preoperative dose of intravenous dexamethasone decreased complications transiently. However, the prescription of Ibuprofen for one week significantly enhanced the subjective and objective parameters of the study (Mehra et al., 2013).

Prophylactic antibiotic therapy before or after dental surgeries has been a controversial issue from the past to now (Curran et al., 1974; Kirnbauer et al., 2022). One of the biggest concerns of recent decades is antibiotic resistance of dangerous bacteria resulting from excessive consumption of antibiotics, which causes failures in the healing of infected sites. Stošić showed in their article that half of the patients were resistant to penicillin-class antibiotics (Stošić, Šarčev, et al., 2022). Moreover, the illogical use of antibiotics may lower the population of the advantageous microbiota of the body organs. In the long-term period, prescription antibiotics can provoke dysbiosis, which generates other situations in the body. For example, a published article proved that a long-term oral antibiotic develops gut dysbiosis that disturbs the microflora of the mouth and aggravates periodontitis and bone loss (Yuan et al., 2023). In view of the fact that antibiotic resistance is extremely crucial, recent studies underlined the aggressive effects of antibiotics prescribed for a prolonged period (Menon, Gopinath, et al., 2019; Soleymani et al., 2024; Stošić, Šarčev, et al., 2022). So, it seems reducing the number of antibiotic doses and using single-dose regimens are more beneficial.

Numerous published trials and systematic reviews showed different results and conclusions about antibiotic consumption to prevent dental surgery complications. While some trials prescribed antibiotics before or/and after the surgery (Janas-Naze et al., 2022; Kirnbauer et al., 2022; Maria del Mar Mariscal-Cazalla et al., 2021; Siddiqi et al., 2010; Stošić, Šarčev, Mirković, Bajkin, et al., 2022;

Stošić, Šarčev, et al., 2022; Xue et al., 2015; Yoshii et al., 2002), others assessed the effect of antibiotics as single-dose prophylaxis (Knezevic et al., 2018; Pasupathy & Alexander, 2011; Siddiqi et al., 2010). However, one study presented positive clinical results from a local antibiotic application in the surgery field of the third molar (Verma et al., 2022).

It seems the most common antibiotic used in the dentistry field and research is Amoxicillin in different dosages (Kirnbauer et al., 2022; Mariscal-Cazalla et al., 2021; Pasupathy & Alexander, 2011; Siddiqi et al., 2010; Sologova et al., 2022; Xue et al., 2015). Nonetheless, other antibiotics, such as Moxifloxacin (Stošić, Šarčev, Mirković, Bajkin, et al., 2022; Stošić, Šarčev, et al., 2022), Cefixime (Stošić, Šarčev, Mirković, Bajkin, et al., 2022; Stošić, Šarčev, et al., 2022), Metronidazole (Pasupathy & Alexander, 2011), Lenampicillin (Yoshii et al., 2002), Clindamycin (Janas-Naze et al., 2022), and Amoxicillin plus Clavulanic acid (Sainz de Baranda et al., 2019; Verma et al., 2022), are examined by researchers. The regimen of the antibiotics was also different in previous studies. The following paragraphs will present a summary of the recently published articles about the efficiency of different regimens and antibiotics in complications after third molars removal.

Xue et al. showed that prescribing amoxicillin one hour before the surgery continued for three days postoperatively did not reduce complications following impacted third molars removal (Xue et al., 2015). In addition, Siddiqi et al. indicated that amoxicillin (1gr, one hour preoperatively) alone or continued for two days did not benefit postoperative complications (Siddiqi et al., 2010). Both articles applied a split-mouth design in their studies with a three weeks' interval between the placebo and the experimental sessions. According to the inflammatory process after the surgeries, three weeks' interval is short for eliminating drugs and blood factors from the first session. Therefore, their results would be influenced by this issue (Menon, Gopinath, et al., 2019). Nonetheless, Mariscal-Cazalla 2021, published an article that evaluated the effect of amoxicillin (750mg) on lessening complications after mandibular third molar surgery. In that report, amoxicillin reduced pain, inflammation intensity, and the need for palliative drugs or other antibiotics compared to the placebo group (Mariscal-Cazalla et al., 2021).

Although in 2011, Pasupathy & Alexander presented no significant differences between the postoperative complications after the third molar surgery

between experimental and placebo groups (Pasupathy & Alexander, 2011), Knezevic et al. concluded that a single dose of amoxicillin (2g, one hour before surgery) decreased the swelling in the first 24 hours after the surgery (Knezevic et al., 2018). Moreover, Janas measured the concentration of Clindamycin in the saliva and evaluated its relation to the oral dose of the prescribed antibiotic, inflammation degree at the surgery site, and pain intensity experienced by the patient. She figured that the lowest amount of the antibiotic developed the best results and suggested considering lower doses and periods of antibiotics to prevent postoperative infections (Janas-Naze et al., 2022).

There are several systematic reviews and meta-analyses about antibiotic application for dental surgeries with controversial outcomes. Sologova et al. declared that amoxicillin with or without clavulanic acid was the most common antibiotic used to prevent complications after third molar removal. Furthermore, they found no significant differences between treatment groups according to the complications after interventions (Sologova et al., 2022). Nevertheless, Falci et al., in their network meta-analysis, showed that amoxicillin or metronidazole decreased the risk of infection and dry sockets (low-quality evidence) after third molar surgery.

They said postoperative amoxicillin with clavulanic acid and preoperative metronidazole was useful to prevent infection and dry sockets (Falci et al., 2022). In 2016, Marcussen et al. published a systematic review that proved single-dose preoperative antibiotics (Oral or IV administration) are adequate to decline the incidence of surgical site infection and alveolar osteitis (Marcussen et al., 2016).

According to the lack of information about the consequences of prophylactic antibiotic administration on inflammatory factors in saliva, to the best of our knowledge, no previous study has investigated the effect of prophylactic antibiotic therapy on salivary PGE2 levels, the principal aim of the following study was to evaluate the effect of a single dose of preoperative amoxicillin on PGE2 concentration in saliva, 24 hours and seven days after surgical removal of the third molar. The secondary aim was recording the effect of a single dose of 2gr amoxicillin on the signs and symptoms of the infection and inflammation, including pain, swelling, trismus, infection and dry socket. Then, we analysed the

data to find any relationship between salivary inflammatory parameters and clinical complications.

III – OBJECTIVES

III - OBJECTIVES

3.1. MAJOR OBJECTIVES

- Comparing postoperative concentrations of PGE2 in saliva of experimental and placebo group
- Comparing subjective pain assessment by VAS between experimental and placebo group

3.2. MINOR OBJECTIVES

- Estimating the difficulty index of the surgery and counting the time of the surgeries
- Comparing measurements of swelling and maximum mouth opening after surgery by the formula
- Measuring baseline concentration of PGE2 in saliva of experimental group
- Measuring baseline concentration of PGE2 in saliva of placebo group
- Measuring postoperative concentrations of PGE2 in saliva of experimental group, 24 hours after surgical removal of third molars
- Measuring postoperative concentrations of PGE2 in saliva of placebo group, 24 hours after surgical removal of third molars
- Measuring postoperative concentrations of PGE2 in saliva of experimental group, seven days after surgical removal of third molars
- Measuring postoperative concentrations of PGE2 in saliva of placebo group, seven days after surgical removal of third molars
- Correlation assessment between the pain and variables
- Correlation assessment between the facial swelling and variables
- Correlation assessment between the MMO changes and variables

- Correlation assessment between the salivary PGE2 concentration and variables
- Correlation assessment between the clinical outcomes (pain, facial swelling, and MMO changes) and laboratory outcome (salivary PGE2 concentration)

3.3. NULL HYPOTHESIS

Based on the findings of previous studies, the null hypothesis (H0) of this study posits that prophylactic antibiotics do not affect salivary PGE2 concentration or influence objective and subjective clinical outcomes.

IV – MATERIAL AND METHODS

IV -MATERIAL AND METHODS

4.1. PATIENTS

This double-blind, randomized clinical trial was performed in UCAM dental setting during 2023-24 on patients over 18-year-old who needed third molars extraction. Before the treatment process, complete medical and dental histories of the patients were recorded. Then, a full head and neck examination was performed by a dentist. The included patients were selected to enter to the investigation based on the following criteria:

- Healthy people (ASA I & II)
- Older than 18 years
- No smokers
- No history of viral or microbial diseases during the last four months
- No history of allergic reaction to penicillin-class antibiotics or other drugs
- No history of anti-inflammatory, antibiotics or contraceptive drugs taken during the last month
- Not being in pregnancy or nursing period for women
- Having at least one un-erupted mandibular wisdom tooth needs surgical intervention

Excluding criteria was:

- History of dental pain, inflammation, or abscess during the last month
- Taking thyroid hormone therapy
- ASA III, IV, V

4.2. ETHICS AND REGISTRATION

This investigation has been approved by the UCAM ethical committee under the CE032315 code.

According to the Declaration of Helsinki principles for medical research involving human subjects, the researcher explained the details of the study and the

content of the informed consent to the patients. Side effects, potential postoperative complications and possible risk of the surgery were presented to each patient orally and on a paper as an “informative form” and “consent form”. Every patient was allowed to leave the investigation at any time they want. The data of patients was protected. The outcome of the research released without the private information of patients.

The study has been registered retrospectively on www.clinicaltrials.gov with TRN: NCT06613776 on September 23, 2024.

4.3. SAMPLE SIZE

According to Branimir Stošić et al. (Stošić, Šarčev, Mirković, Bajkin, et al., 2022), the sample size of this study was calculated by the following formula, with $\alpha=5\%$, $\beta=10\%$ (power 90%):

$$n = [(Z\alpha/2 + Z\beta)^2 \times (2(\sigma)^2)] / (\mu_1 - \mu_2)^2 = 5.04=6$$

In accordance with dropout rate of 30%, the number of patients in each group was:

$$N=n/(1-d) =9$$

In total, eighteen patients based on the inclusion criteria were selected to be divided randomly into two groups.

4.4. DIFFICULTY INDEX

Before the surgery, a CBCT was administer for each included patient. The difficulty of surgical removal was analysed according to the Jeong-Kui Ku et al. (Ku et al., 2020) as following:

An axial cutting of the CBCT containing the middle of the third molar was produced using software (Sidexis4). Then, the difficulty score was calculated based on three categories:

a) Spatial relationship (1 to 5 points) (Figure 8, a): (1) mesioangular (11° to 79°), (2) horizontal (80° to 100°), (3) vertical (-10° to 10°), (4) distoangular (-11° to -79°), or (5) reverse where the crown of the third molar was more root-oriented than horizontal.

b) Depth (1 to 4 points) (Figure 8, b): levels A (1), more than half of the third molar crown located above the CEJ of the adjacent second molar

level B (2), less than half of the third molar crown was above the CEJ of the adjacent second molar

level C (3), the entire third molar crown was positioned below the CEJ of the adjacent second molar, and more than half of the third molar crown was above the mid-level of the adjacent second molar root

level D (4), the entire third molar crown was positioned below the CEJ of the adjacent second molar, and more than half of the third molar crown was below the mid-level of the adjacent second molar root

c) Ramus relationship/space (1 to 3 points) (Figure 8, c): class I (1), II (2), or III (3). Eruption space was the ratio of the distance between the distal side of the second molar to the ascending ramus (a) and the diameter of the third molar (b), as shown in Figure below. An eruption space (a/b) bigger than two-thirds the distance considered class I, between one-third and two-thirds, was class II, and less than one-third named class III.

The points were sum up, and the difficulty index categorized as follows:

- I (3-4 points)
- II (5-7 points)
- III (8-10 points)
- IV (11-12 points).

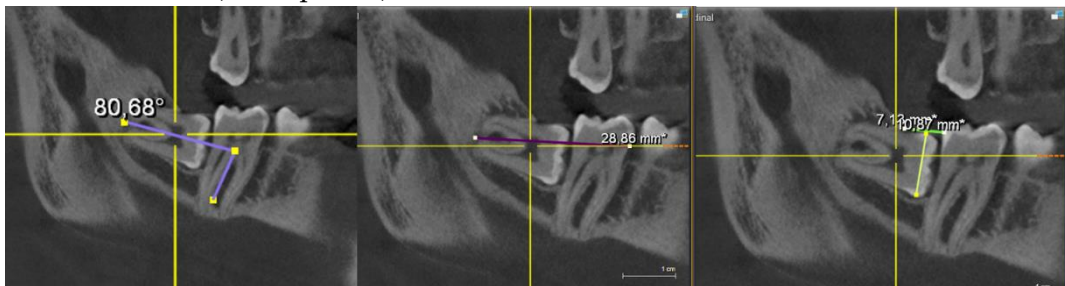


Figure 8. The difficulty index of the surgery was calculated by evaluating a) spatial relationship, b) depth, and c) ramus relationship/space of the third molar. Author's elaboration

4.5. GROUPS

Included patients divided into two groups randomly:

- Experimental group: Received four capsules containing 500mg amoxicillin (Amoxicilina Normon 500 mg cápsulas duras EFG, Laboratorios NORMON, S.A. Madrid, Spain, Figure 9), in total 2g, one hour before the surgery
- Placebo group: Took four placebo capsules (Empty gelatine shell capsules shaped like amoxicillin) with the same appearance one hour before the surgery



Figure 9. Ten patients in Experimental Group took 4 capsules containing 500mg Amoxicillin (Amoxicilina Normon 500 mg cápsulas duras EFG, Laboratorios NORMON, S.A. Madrid, Spain), in total 2g, one hour before the surgery. Ten patients in Control group took 4 empty capsules looked like this Amoxicillin.

Acquired from:
<https://www.normon.es/medicamenton/amoxicilina-normon-efg>

4.6. RANDOMIZATION AND BLINDNESS OF THE RESEARCHERS AND PATIENTS

All randomization and blinding processes were managed by a third person who was not involved in any other steps of the study. Initially, the experimental and control groups were randomly designated as “one” and “two” randomly using computer.

A computer-generated random set of twenty numbers with allocation ration of 1:1 (ten ones and ten twos) was created using (Figure 10):

<https://www.calculatorsoup.com/calculators/statistics/random-number-generator.php>.

The same person filled each of the twenty sealed packs with four capsules according to the random sequence and labelled them from 1 to 20 (Figure 11).

Patients received these labelled sealed packs sequentially based on their surgery dates: patient number one took the pack labelled 1, patient number 2 got the pack labelled 2, and patient number 18 took the pack labelled 18.

The details of the packs remained unknown to the investigator, patients, surgeon, and statistical analyst.

No one in the study knew the details of the packs until the analysis step had finished. After completing the statistical analysis, the third person who had prepared the sealed packs revealed the set of random numbers and the meaning of numbers one and two.

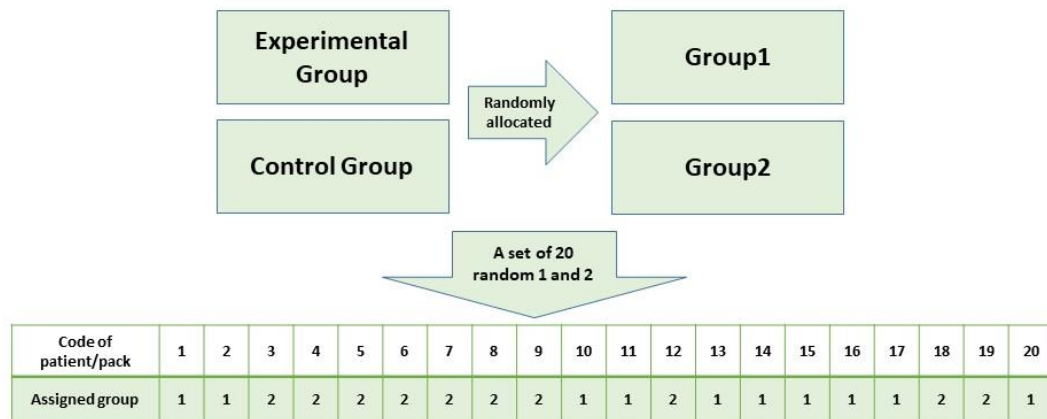


Figure 10. Details of group allocation and randomization. Nobody knew the meaning of 1 and 2 until the end of the statistical analysis step. Author's elaboration



Figure 11. Prepared labelled packs, each contains four capsules which could be Amoxicillin or placebo. No one knew the details until the end of the statistical analysis. Author's elaboration

4.7. PERIODONTAL ASSESSMENT

Basic Periodontal examination (BPE) and Gingival biotype assessment were used to evaluate the periodontal status of the participants.

4.7.1. Basic Periodontal examination

For recording BPE, all presented teeth in each sextant:

- Upper right (17 to 14)
- Lower right (47 to 44)
- Upper anterior (13 to 23)
- Lower anterior (43 to 33)
- Upper left (24 to 27)
- Lower left (34 to 37)

were examined using a World Health Organisation (WHO) BPE probe (Figure 12). The probe walked through every tooth in each sextant to record the highest score based on the released Guideline of BPE by British Society of Periodontology (Dietrich et al., 2019). Scores were as follow:

0 : This indicates that there are no pockets >3.5mm (i.e. the black band is completely visible), no bleeding and no calculus or plaque traps (e.g. overhanging restorations)

1 : This indicates that there are no pockets >3.5mm (i.e. the black band is completely visible), no calculus or plaque traps (e.g. overhanging restorations) but there is bleeding after probing

2 : This indicates that there are no pockets >3.5mm (i.e. the black band is completely visible) but there is calculus or plaque traps present

3 : This indicates that probing depths between 3.5mm and 5.5mm have been found (i.e. black band is partially visible)

4: This indicates a probing depth of >5.5mm (i.e. black band is completely hidden in pocket)

* Indicates furcation involvement

→ Scoring Codes



Figure 12. Basic Periodontal examination (BPE) scores based on the released Guideline of BPE by British Society of Periodontology. Retrieved from (Dietrich et al., 2019)

4.7.2. Gingival Biotype

To investigate Gingival Biotype (GB) a method which introduced by De Rouck et al. (De Rouck et al., 2009) was used to distinguish thin and thick gingival biotypes utilizing a periodontal probe through the gingival margin (Figure 13). This visual method is based on the visibility of the periodontal probe through the free gingiva while probing the buccal sulcus at the midfacial aspect of both central maxillary incisors. When the outline of the underlying periodontal probe can be seen through the gingiva, the gingival biotype is considered thin. The gingival biotype is thick in the other case.



Figure 13. Gingival Biotype (GB) recording by WHO probe. A) Thick: Probe is not visible through the gingiva, B) Thin: Probe is visible through the gingival sulcus. Retrieved from (Abraham et al., 2014)

4.8. TAKING SALIVA SAMPLE

Five millilitres of unstimulated whole saliva were collected using cold sterile 50 ml tubes (Figure 14) in each appointment to determine the concentration of PGE₂. In total, three samples were collected from every patient in three sessions:

1. Before taking the capsules (Baseline sample)
2. 24 hours after the surgery
3. Seven days after the surgery

Participants were asked not to eat or drink one hour before collecting saliva samples. They put the cold sterile tube in their mouth and inclined while sitting to

let the unstimulated saliva get out steadily without spitting. The process continued until the saliva reached the 5 ml marker on the tube.

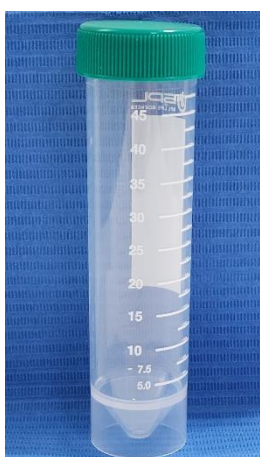


Figure 14. 50ml Sterile tubes were used to collect saliva in each appointment. Patients were asked not to eat or drink one hour before collecting saliva samples. They put the sterile tube in their mouth and inclined while sitting to let the unstimulated saliva get out steadily without spitting. The process continued until the saliva reached the 5ml marker on the tube. Author's elaboration

Then, the researcher put the tube in a cool box and transferred it to the lab, as soon as possible, to start the freezing process (Figure 15). One millilitre of each sample was transferred into one Eppendorf using disposable tips and micropipette to avoid multiple freeze-thaw cycles. Five Eppendorf tubes with a label including the code number of the patient and the number of the session were prepared.



Figure 15. Eppendorf tubes with a label including the code number of the patient and the number of the session were prepared to store in the Vertical Deep Freezer. Author's elaboration

All prepared samples were stored at -80 centigrade degrees in a vertical deep freezer (Infrico Medcare, Cordoba, Spain, Figure 16) until the step of ELISA analysis started.



Figure 16. Vertical Deep Freezer (Infrico Medcare, Cordoba, Spain) was used to freeze saliva samples at -80 centigrade degrees until performing ELISA test. Author's elaboration

4.9. SURGERY PROCEDURE:

At the surgery appointment, all patients, after receiving another explanation of the study, a new facial and oral examination, and signing the informed consent, were asked to collect the saliva sample, take the capsules, and then wait one hour to start the extraction procedure. Meanwhile, the first set of photos, which are explained in the Facial swelling part, were taken.

1. Patients were asked to rinse their mouths with 0.12% chlorhexidine mouthwash for 30 seconds before the surgery to reduce the number of salivary microorganisms, and then proceeded to the local anaesthesia with Ultracain epinephrine (articaine with 4% epinephrine) and slow induction. Aspirating was done before

infiltrating, to make sure that we were not in a blood vessel. All surgeries performed by a unique surgeon under local anaesthesia (Inferior alveolar block injection).

2. Clean and firm incision at full thickness was done. Once the incision was made, we provided a constant aspiration, in order to have good visibility from the beginning of the intervention.
3. The surgeon opened the flap, which was wide enough and a full thickness mucoperiosteal flap lifting without tears was made.
4. We moved on to the osteotomy and/or tooth section, which was done with abundant irrigation with 0.9% saline solution, by means of a turbine at high-speed using a Lindemann type odontosection drill.
5. Luxation with the botators, luxators and / or winters was done.
6. Removing tooth by means of forceps of the sectioned fragments.
7. Cleaning of the surgical site.
8. 4/0 synthetic monofilament suture.

The details of each surgery, including bone removal, tooth sectioning, and number and type of sutures, were recorded as the duration of surgery for each patient. The duration of surgery was recorded by a chronometer from the start of the incision to the moment of removing the last part of the tooth.

4.10. AFTER SURGERY

The researcher demonstrated precise postoperative instruction to the patients after surgery and provide them with a prepared pamphlet (Figure 17) containing the following instructions:

- Routine post-surgery instructions such as tooth brushing and mouthwash using
- Analgesic drugs: Paracetamol 650mg every 8 hours for seven days or Zaldiar (37.5mg/325 mg every 8 hours for seven days)
- Not using any other pills for a week after surgery (especially antibiotics and anti-inflammatory ones)

- A recording sheet to report experienced pain immediately after the surgery, 6 hours after the surgery, 12 hours after surgery, 24 hours after surgery, every day until seven days after surgery at the 17:00 by using a Visual Analogue Scale (VAS) and 11-point pain scale (Figure 19).

Instrucciones postoperatorias



Muerda con presión firme la compresa de gasa durante al menos 60 minutos



Aplique compresas de hielo inmediatamente en todas las zonas donde se haya realizado la cirugía. Coloque las compresas de hielo durante 30 minutos y luego retírelas durante 30 minutos. Continúe durante al menos 24-48 horas.



No tome otras pastillas, especialmente antibióticos o antiinflamatorios.



No beba con pajita y evite las bebidas alcohólicas o carbonatadas y fumar durante 7 días.



Toma Paracetamol 500 cada 8h durante 5 días



La dieta debe limitarse a alimentos frescos y blandos y líquidos sin gas durante al menos 48 horas.

Figure 17. Post-operative instructions pamphlet. The investigator explained all details of this pamphlet to each patient and every patient had the printed version of it to review at home. Author's elaboration

The following appointments were set for 24 hours and seven days after the surgery. A contact number introduced to the patient to call if any problem occurred.

4.11. FOLLOW-UP SESSIONS

In the two follow-up sessions, probable swelling, inflammation, infection, and dry socket of the surgical site were recorded by the investigator.

4.11.1. First follow-up appointment

At the second appointment (24 hours after the surgery), after examining the patient, taking required measurements, and the saliva sample, if the patient had magnificent pain and swelling, the surgeon prescribed them antibiotic (Augmentine 875 mg/125 mg, every 8 hours, for seven days) to prevent any infection and insuring the patient.

4.11.2. Second follow-up appointment

In the last appointment, which was seven days after the surgery, another saliva test was performed in order to measure the final amount of PGE2 before removing the sutures. Moreover, in this appointment, measurements were done and recorded again.

4.12. PAIN RECORDS

As mentioned before, the pain after surgery recorded by the patients at different times using Visual Analogue Scale (VAS) and a 11-point system: 0 (no pain) to 10 (worst pain experienced) (Figure 18 and 19).

The time for recording pain was as follow:

- immediately after the surgery,
- six hours after the surgery
- 12 hours after surgery
- 24 hours after surgery
- every day until seven days after surgery at 17:00.

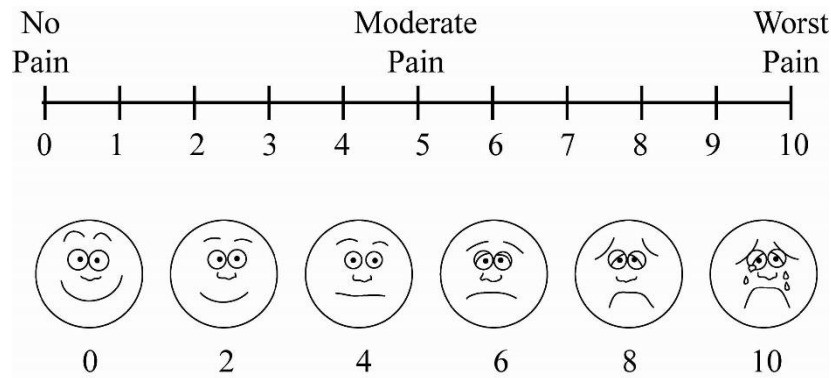


Figure 18. Visual Analog Scale (VAS) and a 11-point system to determine subjective pain of patients during the days after surgery: 0 (no pain) to 10 (worst pain experienced). Acquired at: <https://assessment-module.yale.edu/im-palliative/visual-analogue-scale>

Historial de dolor

Nombre y Apellido:

Fecha de la cirugía:

Código de grupo:

después de la cirugía	primer día después de la cirugía a las 17:00	cuarto día a las 17:00
6 horas después de la cirugía	segundo día a las 17:00	quinto día a las 17:00
12 horas después de la cirugía	tercer día a las 17:00	sexto día a las 17:00

Figure 19. The recording sheet to record the subjective pain history of the patients after surgery. After explaining the VAS and 11-point system to the patient, they were asked to mark their pain on the printed paper exactly after the surgery, 6 hours after the surgery, 12 hours after the surgery, 24 hours after surgery, and every day at 17:00 until the last follow-up appointment. Author's elaboration

4.13. FACIAL SWELLING MEASUREMENT:

According to Macedo Costa et al. (Costa et al., 2022), facial swelling was computed using a tape-measuring method (Figure 20). The patient sat straight at a 90° angle and put their mandible at rest. Three facial lines, including the tragus distance to the corner of the mouth as line A, the tragus distance to soft tissue pogonion as line B, and the distance of the outer corner of the eye to the angle of the mandible as line C, were measured.

The facial measurement formula is $(A + B + C)/3$, and facial swelling (%) was calculated as: $[(\text{postoperative measurement on T2} - \text{preoperative measurement}) / \text{preoperative measurement}] * 100$.

Facial measurements were done by the researcher before the surgery and at every follow-up appointment in order to evaluate facial swelling. Moreover, a set of photos were taken from the patients' faces to record the measurements before surgery, the day after the surgery and seven days after the surgery.

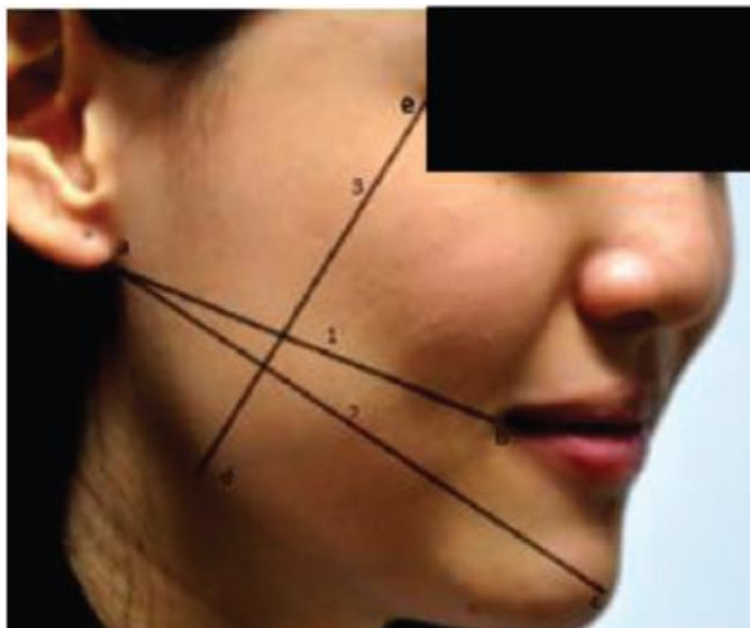


Figure 20. The measurements of the face to evaluate the percentage of the swelling after the surgery. A: Lateral canthus of the eye, B: Midline of the tragus, C: Most inferior point on the angle of mandible, D: Soft tissue pogonion, which is most prominent point at midline on chin, E: Most lateral point on corner of mouth. Retrieved from (Arindra & Indrapradana, 2018; Costa et al., 2022).

4.14. MAXIMUM MOUTH OPENING (MMO)

This parameter was recorded as follow: measuring the maximum distance between the maxillary incisor's incisal edge to the lower incisor (mm) using a metal ruler. Maximum mouth opening was evaluated by the researcher before the surgery, exactly after finishing the surgery, and at every follow up appointment. The difference between the first MMO and other measurements was used to analyse.

4.15. PGE2 MEASUREMENT:

An Eppendorf containing the saliva thawed rapidly in a bath water at 20°C to start the PGE2 measurement process in the HiTech office of UCAM. The samples were centrifuged for two minutes at 10,000 x g at 2 -8°C. Then, the supernatant was collected to perform the assay immediately.

Enzyme-linked immunosorbent assay (ELISA) testing using the double-antibody sandwich technique was used for the optical density (OD) measurement of each well in the sample plates. PGE2 ELISA kit (Human PGE2 ELISA kit, FineTest®, Wuhan Fine Biotech Co., China) was utilized to calculate the PGE2 level in saliva samples (Figure 21).



Figure 21. Human PGE2 ELISA kit, FineTest®, Wuhan Fine Biotech Co., China. Used to evaluate the PGE2 amount in the saliva samples of the patients. (Acquired at the manufacture website.)

All steps of this assay are explained by details in the following:

4.15.1. Reagent preparation and storage

All reagents and samples were brought to the room temperature for 20 minutes before use.

4.15.1.1. Wash Buffer:

We warmed the Wash buffer with 40°C water bath and mixed gently to dissolve the crystals of the washing buffer completely. Then, the solution cooled to room temperature before use.

Investigator diluted 30ml Concentrated Wash Buffer to 750ml Wash Buffer with distilled water.

4.15.1.2. Standards:

1) Standards tube was centrifuged for one minute at 10000xg. One milliliter of Sample Dilution Buffer was added into one Standard tube (labeled as zero tube), the tube was kept at room temperature for 10 minutes and was mixed thoroughly.

2) Seven EP tubes were labeled with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank, respectively. 0.3ml of the Sample Dilution Buffer was added into each tube. 0.3ml of the above Standard solution (from zero tube) was added into 1st tube and was mixed thoroughly. 0.3ml from the 1st tube was transferred to 2nd tube and was mixed thoroughly. 0.3ml from 2nd tube was transferred to 3rd tube and was mixed thoroughly, and so on till 1/64 tube. Sample Dilution Buffer was used for the blank control which contained only 0.3ml sample dilution buffer. The standard concentration from zero tube to blank tube was 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.25pg/ml, 0pg/ml (Figure 22).

- Standard Solutions were used within 2 hours.

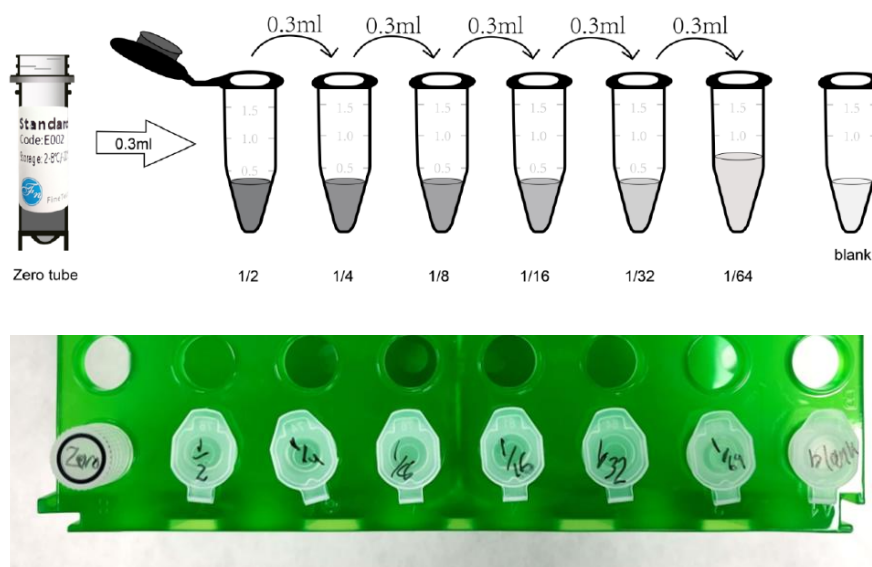


Figure 22. Preparing Standards. Seven EP tubes were labeled with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank, respectively. 0.3ml of the Sample Dilution Buffer was added into each tube. 0.3ml of the above Standard solution (from zero tube) was added into 1st tube and was mixed thoroughly. 0.3ml from 1st tube was transferred to 2nd tube and was mixed thoroughly. 0.3ml from 2nd tube was transferred to 3rd tube and was mix thoroughly, and so on till 1/64 tube. Sample Dilution Buffer was used for the blank control which contained only 0.3ml sample dilution buffer. The standard concentration from zero tube to blank tube was 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.25pg/ml, 0pg/ml. Retrieved from the manufacture's instruction and Author's elaboration

4.15.1.3. Preparation of Biotin-labeled Antibody Working Solution:

Biotin-labeled Antibody Working Solution was prepared within one hour before experiment.

1) Dissolve: Centrifuged for one minute at 2000xg and bring down the concentrated biotin-labelled antibody to the bottom of tube. 70µl purified water was added into tube and was mixed thoroughly, after the biotin-labeled antibody was dissolved, it was stored at 2-8°C.

2) Required total volume of the working solution was calculated: 50µl /well x quantity of wells. (Plus 0.1-0.2ml more than the total volume.)

3) The Biotin-detection antibody was diluted with Antibody Dilution Buffer at 1:100 and were mixed thoroughly. (i.e., one μ l Biotin-labeled antibody was added into 99 μ l Antibody Dilution Buffer.)

4.15.1.4. *Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution:*

It was prepared within 30 minutes before the experiment.

- 1) Total volume required for the working solution was calculated: 0.1 ml/well x quantity of wells. (Plus 0.1-0.2 ml more than the total volume.)
- 2) Centrifuged for one minute at 1000xg in low speed and brought down the concentrated SABC to the bottom of tube.
- 3) The SABC was diluted with SABC Dilution Buffer at 1:100 and were mixed thoroughly. (i.e., one μ l of SABC was added into 99 μ l of SABC Dilution Buffer.)

4.15.2. **Assay Procedure**

Before adding TMB into wells, TMB Substrate equilibrated for 30 minutes at 37°C.

1. Standard, test samples, control (blank) wells were set on the pre-coated plate respectively, and then, their positions were recorded. Each standard and sample was measured duplicate. Plate was washed two times before adding standard, sample and control (blank) wells.
2. Adding Sample and Biotin-labelled Antibody: 50 μ l of Standard, Blank, or Sample were added per well. The blank well was added with Sample/Standard Dilution Buffer. 50 μ l Biotin-labelled Antibody Working Solution was added into each well, immediately (Figure 23). The plate was covered with the provided Plate sealer. The plate was tapped gently to ensure thorough mixing. Then, it was incubated for 45 minutes at 37°C.

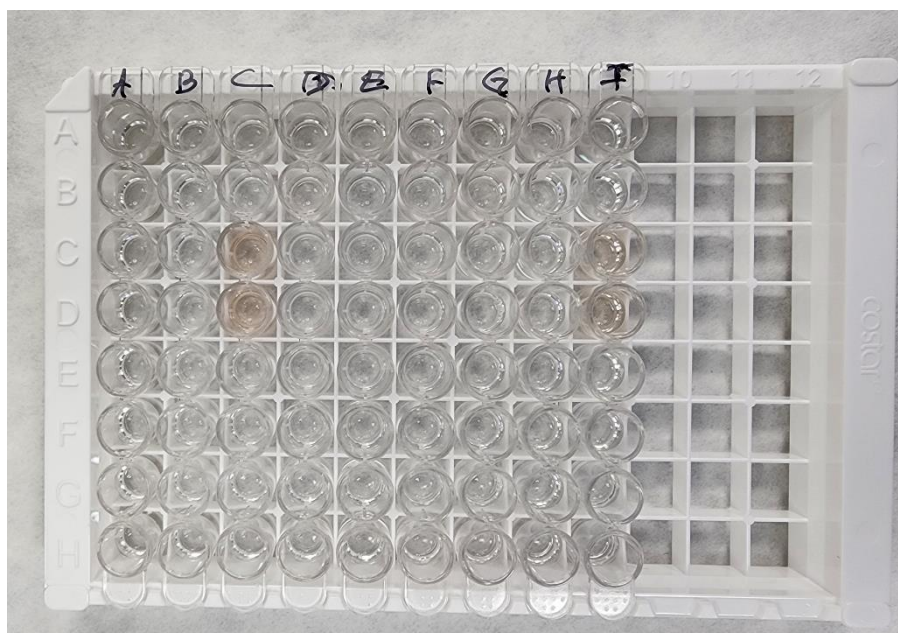


Figure 23. Adding Sample and Biotin-labelled Antibody: 50 μ l of Standard, Blank, or Sample were added per well. The blank well was added with Sample/Standard Dilution Buffer. 50 μ l Biotin-labelled Antibody Working Solution was added into each well, immediately. The plate was covered with the provided Plate sealer to be incubated for 45 minutes at 37°C. Author's elaboration

3. Wash: The cover was removed to wash the plate three times with Wash Buffer, and the wash buffer stayed in the wells for one minute each time. After the last wash, any remaining Wash Buffer by decanting was removed.
4. HRP-Streptavidin Conjugate (SABC): 100 μ l SABC Working Solution was added into each well. It was covered with a new Plate sealer (Figure 24). Then, it was incubated for 30 minutes at 37°C.

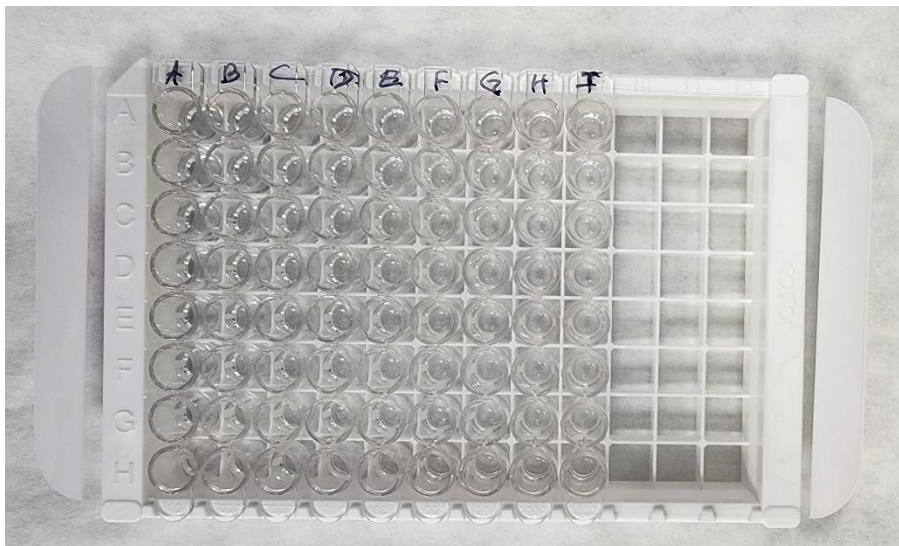


Figure 24. HRP-Streptavidin Conjugate (SABC): 100 μ l SABC Working Solution was added into each well. It was covered with a new Plate sealer. Then, it was incubated for 30 minutes at 37°C. Author's elaboration

5. Wash: The cover was removed to wash plate 5 times with Wash Buffer. The wash buffer stayed in the wells for 1-2 minutes each time.
6. TMB Substrate: 90 μ l TMB Substrate was added into each well, the plate was covered and incubated at 37°C in dark within 10-20 minutes. (Before adding TMB into wells, TMB Substrate was equilibrated for 30 minutes at 37°C.).
7. Stop: 50 μ l Stop Solution was added into each well. The color turned yellow immediately after adding this solution into the plate (Figure 25). The added order of Stop Solution was the same as the TMB Substrate Solution.

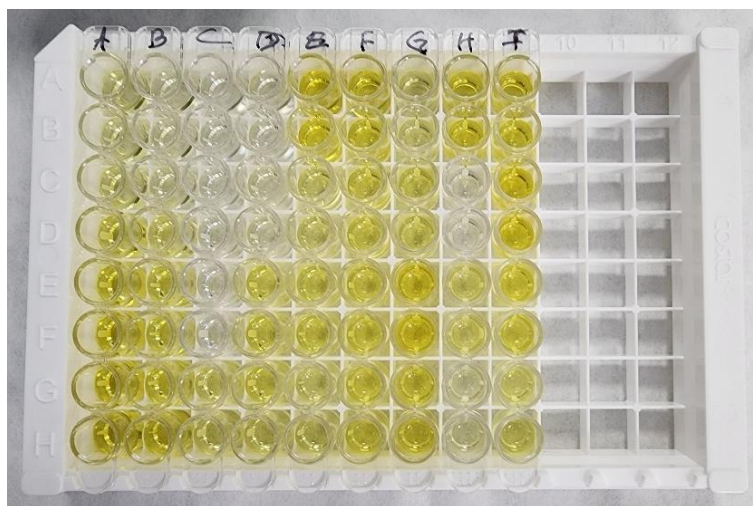


Figure 25. TMB and Stop Substrate: 90 μ l TMB Substrate was added into each well, the plate was covered and incubated at 37°C in dark within 10-20 minutes, Then, 50 μ l Stop Solution was added into each well. The colour was turned yellow immediately after adding this solution into the plate. The added order of Stop Solution was as the same as the TMB Substrate Solution. Author's elaboration

8. OD Measurement: The O.D. absorbance at 450nm was read in Microplate Reader (a spectrometer) immediately after adding the stop solution.

Figure 26 presents the summary of the ELISA steps.

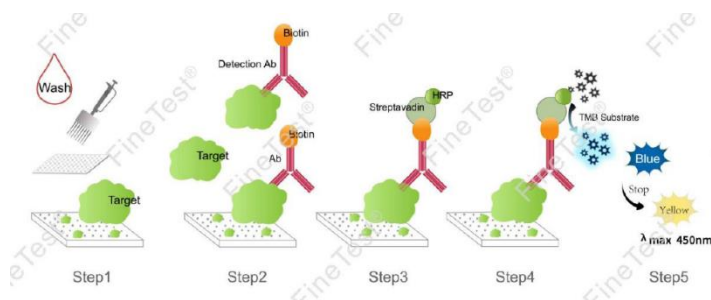


Figure 26. Step1: plate was washed 2 times before adding Standard, Sample and Control (blank) wells! Step2: 50 μ l Standard or Sample was added into each well. Immediately 50 μ l Biotin-labelled Antibody was added into each well, the plate was taped for 1min gently to ensure thorough mixing then static incubated for 45 minutes at 37°C. Washing: The plate was washed three times and immersed for 1min each time. Step 3: 100 μ l SABC working solution was added into each well, the plate was sealed and static incubated for 30 minutes at 37°C. Washing: The plate was washed five times and immersed for 1min each time. Step 4: 90 μ l TMB substrate solution was added, the plate was sealed and static incubated for 10-20 minutes at 37°C. Step 5: 50 μ l stop solution was added. Read at 450nm immediately and calculated. Retrieved from manufacture's instruction

4.15.3. Standard plot

Excel Microsoft software was used to draft the Standard curve. The standard curve was plotted as the O.D. 450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples was interpolated from the standard curve using a formula derived from the Standard curve.

Each specimen was tested two times to reduce laboratory error, and the average value was used in statistical analysis.

Since the surgeries for 18 patients took 10 months to complete, we performed the ELISA twice to reduce the freezing time of the primary samples: once in January and once in July. Consequently, we generated two standard curves and two derived formulas for each date, which will be discussed in detail in the results section.

4.16. STATISTICAL ANALYSIS:

4.16.1. Data gathering

All data recorded in MS Excell and was added to the IBM SPSS Statistics (ver. 26.0; IBM, Armonk, NY, USA) according to group assignments and patient numbers.

4.16.2. Descriptive statistics

Tables and graphs were used to demonstrate the mean $\pm$ standard deviation (SD) or number of each variable and outcome in each group.

4.16.3. Normal distribution

The samples were checked for normality using the Shapiro-Wilk Test.

4.16.4. Statistical tests

The Chi-square and Independent Samples t-tests were used to evaluate the baseline data similarity between the two groups.

The primary clinical outcome (patient-reported pain at various time points) and secondary clinical outcomes (MMO change and facial swelling) were compared between the two groups using the Friedman test, Independent Samples T test and Mann-Whitney U tests.

PGE2 level after 24 hours and after seven days, were compared between the two groups using repeated measures ANOVA test and Independent Sample T test.

4.16.5. Correlation tests

Point-biserial correlation, Pearson correlation coefficient, and Spearman's rank-order correlation were employed to assess the relationships between age, gender, surgery duration, osteotomy, tooth section, DI, number of sutures, and clinical outcomes (pain, MMO change and facial swelling percentage).

Point-biserial correlation, Pearson correlation, and Spearman's correlation were employed to assess the correlations between age, gender, surgery duration, osteotomy, tooth section, DI, number of sutures, and PGE2 concentration changes.

The correlation between PGE2 level and clinical outcomes (facial swelling, reported pain, and MMO changes) was evaluated using Spearman's correlation and Pearson correlation tests.

4.16.6. Groups revealing

After completing the data analysis, the third person revealed the group assignments.

4.16.7. Winsorizing

To address the issue of extreme outliers in the PGE2 concentration data, we applied the Winsorize process. Specifically, values below the 5th percentile and above the 95th percentile in each group at each time point were replaced with the

values at the 5th and 95th percentiles, respectively. The exact values used for Winsorizing were as follows: for Experimental group, PGE2 base values below 9.98 pg/ml were capped at 9.98pg/ml, and values above 1378pg/ml were capped at 1378 pg/ml; PGE2 at 24 hours after surgery values below 46.1 pg/ml were capped at 46.1 pg/ml, and values above 1329 pg/ml were capped at 1329 pg/ml; PGE2 at seven days after surgery values below 24.5 were capped at 24.5, and values above 1157 pg/ml were capped at 1157 pg/ml. For Placebo group, PGE2 base values below 42.1 pg/ml were capped at 42.1 pg/ml, and values above 1298 were capped at 1298; PGE2 at 24 hours after surgery values below 70.5 pg/ml were capped at 70.5, and values above 1367 pg/ml were capped at 1367; PGE2 at seven days after surgery values below 8.24 pg/ml were capped at 8.24 pg/ml, and values above 1314 pg/ml were capped at 1314 pg/ml. The Winsorized data were then used for all subsequent statistical analyses.

4.16.8. Significance level

A P-value less than 0.05 indicated significance and less than 0.01 considered as very significant.

4.16.9. Study power analysis

Post hoc power analysis was conducted using the *pwr* package in R (version 4.1.1).

V – RESULTS

V - RESULTS

5.1. GENERAL RESULTS

5.1.1. Included and excluded participants

The investigators evaluated 1,116 archived and new patients who visited the UCAM dental clinic during 2023 and 2024. Of these, 1,045 patients did not meet the inclusion criteria, and 37 were excluded due to specific exclusion criteria explained in the CONSORT diagram presented in Figure 27. Sixteen patients were referred to a maxillofacial surgeon because their CBCT examinations indicated a high risk of mandibular nerve damage. Ultimately, eighteen participants were randomly

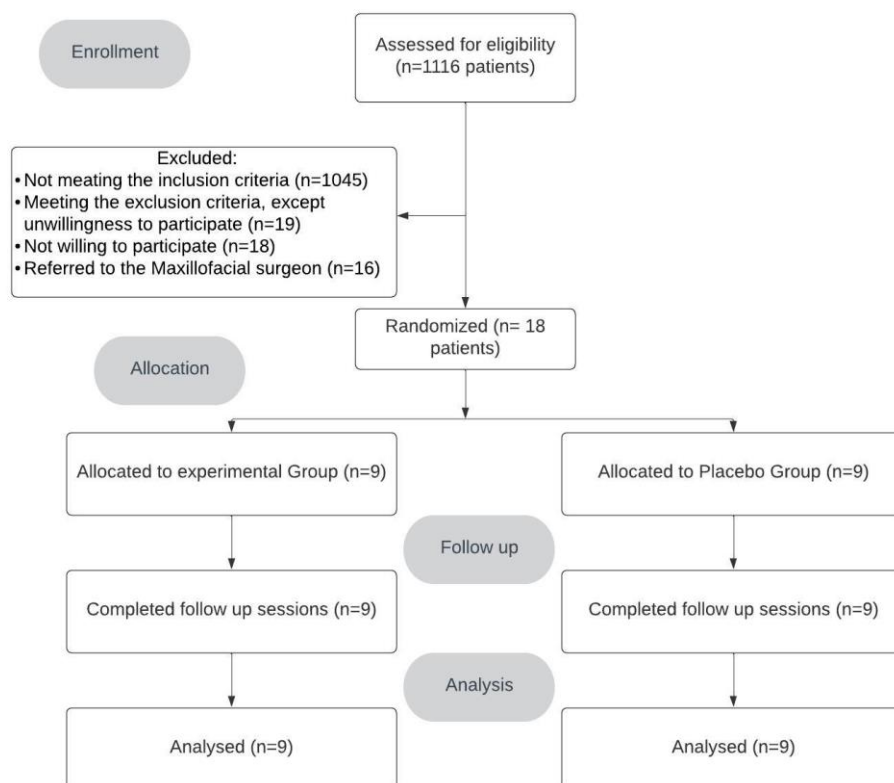


Figure 27. CONSORT diagram presenting the details of excluded and included participants.

divided into two groups of nine for the study and all of them completed the follow-up sessions.

5.1.2. Basic information of the participants

Table 4 presents gender and age distribution of participants in experimental and placebo groups. A total of 18 participants were involved in the study, comprising five women and thirteen men, with a mean age of 24.94 ± 4.15 years old. The Chi-Square test indicated no significant gender differences between the two groups ($p = 0.114$).

Due to the Shapiro-Wilk test revealing a non-normal distribution of age in the experimental group ($p = 0.034$), the Mann-Whitney U test was employed to assess age differences between the groups, which showed no significant difference ($p = 0.155$).

Table 4. Gender and age distribution in experimental and placebo group.

		Experimental	Placebo	Total	p
Gender	Male	5	8	13	0.114
	Female	4	1	5	
Age, Mean $\pm$ SD		24.11 $\pm$ 4.4	25.78 $\pm$ 3.962	18	0.155

5.1.3. Details of surgeries

5.1.3.1. Difficulty index

The difficulty index of each surgery was evaluated as described in the methodology by examining the CBCT of each participant. All surgeries were categorized into level one and level two, with no surgeries at level three or four (Table 5). Given the two levels of difficulty index, the Chi-Square test was used to compare this variable between the two groups. The p-value of 0.016 indicated that the difficulty index of the surgeries in the placebo group was significantly higher than in the experimental group.

Table 5. Surgeries' details in experimental and placebo groups.

		Experimental	Placebo	Total	p
Difficulty index (DI)	I	8	3	11	0.016
	II	1	6	7	
	III	0	0	0	
	IV	0	0	0	
Surgery duration (in seconds), Mean $\pm$ SD		1605.33 $\pm$ 1478.584	1205.11 $\pm$ 964.153	18	0.056
Osteotomy	Yes	5	5	10	1
	No	4	4	8	
Tooth section	Yes	6	3	9	0.599
	No	7	2	9	
Number of tooth sections, Mean $\pm$ SD		3.22 $\pm$ 2.99	2.72 $\pm$ 3	9	0.443
Number of sutures, Mean $\pm$ SD		3.44 $\pm$ 1.01	3.11 $\pm$ 0.96	18	0.208

5.1.3.2. *Duration of the surgery*

As described in the methodology, the duration of each surgery was calculated using a chronometer, measuring from the first incision to the removal of the last part of the tooth in seconds. The mean surgery duration was 1405 $\pm$ 1228 seconds, approximately 23 $\pm$ 20 minutes (Table 5).

The Shapiro-Wilk test was used to assess the normal distribution in both the experimental and placebo groups, with both groups showing normal distribution ($p = 0.119$ and $p = 0.222$, respectively). An Independent Samples t-test was conducted to compare the surgery durations between the two groups, revealing no significant difference ($p = 0.506$) (Table 5).

5.1.3.3. *Osteotomy*

Osteotomy was performed in a total of 10 surgeries. The Chi-Square test indicated no significant difference between the two groups regarding this variable ($p = 1.000$) (Table 5).

5.1.3.4. *Tooth section*

Tooth sectioning was performed in nine surgeries, with a mean of 2.72 ± 3 sections per surgery.

The Shapiro-Wilk test showed a non-normal distribution of tooth section's numbers in the placebo group ($p = 0.009$). Consequently, the Mann-Whitney U test was used to compare the two groups, revealing no significant difference ($p = 0.443$) (Table 5).

5.1.3.5. *Sutures after surgery*

All surgeries were finished with simple sutures, with a mean of 3.11 ± 0.963 sutures per surgery.

The normality test results indicated that the number of sutures in each group was not normally distributed ($p < 0.05$). The Mann-Whitney U test was used to examine the difference between the two groups, showing no significant difference ($p = 0.208$) (Table 5).

5.1.4. **Comparison of the two groups based on their basic and surgery data**

Regarding the results of the basic and surgery evaluation, the two groups were similar in all aspects, except for the difficulty of surgery (Table 4 and 5).

5.2. CLINICAL OUTCOMES

In the follow-up sessions, antibiotics were given to four participants in the experimental group and five in the placebo group because of significant pain and swelling. However, both groups were similar regarding the number of patients who received antibiotic after the surgery (Chi Square test p -value= 0.629). As the second measurements were conducted 24 hours after surgery and before the

initiation of antibiotic treatment, these participants continued in the study and not excluded.

Notably, there were no occurrences of dry socket, and only one case of surgical site infection was reported in the placebo group, which was not statistically significant ($p = 0.303$).

5.2.1. Subjective clinical outcome, reported pain by patients

As explained in the methodology, to assess participants' pain levels, the Visual Analog Scale (VAS) and a 11-point pain scale were utilized. Pain was recorded at multiple time points: immediately post-surgery, at 6 hours, 12 hours, 24 hours, and daily until the 6th day post-surgery, which corresponds to the day before the final follow-up session. Figure 28 shows the history of pain completed by one participant in the study.

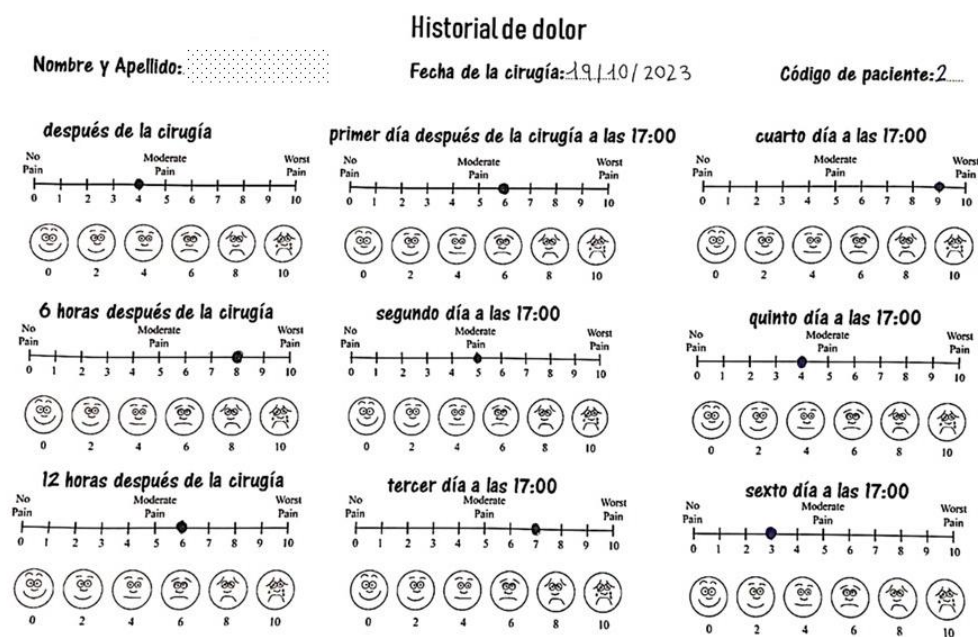


Figure 28. The history of pain completed by number 2. Author's elaboration

A normality test indicated that the pain levels reported at each time point, both overall and within each group, did not follow a normal distribution (p-value < 0.05).

The Friedman test was employed to evaluate differences in pain levels across the various time points. The test yielded a p-value of 0, indicating significant differences. Consequently, a post hoc analysis using the Wilcoxon signed-rank test was conducted to compare pain levels at each time point with those recorded immediately post-surgery. To mitigate the risk of Type I error, the Bonferroni correction was applied, adjusting the significance level to 0.00625 (0.05/8).

The results demonstrated that pain levels reported by participants up to 48-hour post-surgery were significantly higher than those reported immediately after surgery (Table 6).

Table 6. Wilcoxon signed-rank test results to evaluate the pain level reported in each time-point by participants in both groups paired with the pain immediately after the surgery.

Pair	Pain after 6 hours	Pain after 12 hours	Pain after 24 hours	Pain after 2 days	Pain after 3 days	Pain after 4 days	Pain after 5 days	Pain after 6 days
p	0.000	0.001	0.001	0.002	0.011	0.063	0.157	0.280

Table 7 and Figure 29 present the median pain scores and interquartile ranges (IQR) for patients in both experimental and placebo groups at various time points after surgery. Mann Whitney U test was used to compare the pain in two groups in each time point. Initially, the experimental group reported a median pain score of 1 (IQR: 3) compared to 0 (IQR: 1.5) in the placebo group, with an effect size of 0.275 and a p-value of 0.244, indicating no significant difference. At 6 hours post-surgery, the experimental group had a median pain score of 6 (IQR: 2) versus 4 (IQR: 5) in the placebo group, with an effect size of 0.317 and a p-value of 0.179, again showing no significant difference. By 12 hours, the scores were 5 (IQR: 3) for the experimental group and 4 (IQR: 7.5) for the placebo group, with an exceedingly small effect size of 0.011 and a p-value of 0.964, indicating no significant difference. Over the next few days, pain scores generally decreased in both groups, with the experimental group consistently showing slightly lower median pain scores, but

none of the differences reached statistical significance, as indicated by the p-values all being greater than 0.05. This suggests that the antibiotic therapy before the third molar extraction did not significantly reduce pain compared to the placebo at any measured time point.

Table 7. The reported pain level and their differences between the two groups and their effect sizes.

Time Point	Median $\pm$ IQR		Effect Size	P
	Experimental	Placebo		
Pain after surgery	1 $\pm$ 3	0 $\pm$ 1.5	0.275	0.244
Pain after 6 hours	6 $\pm$ 2	4 $\pm$ 5	0.317	0.179
Pain after 12 hours	5 $\pm$ 3	4 $\pm$ 7.5	0.011	0.964
Pain after 24 hours	5 $\pm$ 1.5	3 $\pm$ 8.5	0.189	0.424
Pain after 2 days	4 $\pm$ 3.5	2 $\pm$ 7.5	0.000	1.000
Pain after 3 days	2 $\pm$ 4.5	2 $\pm$ 7	0.054	0.820
Pain after 4 days	1 $\pm$ 4	4 $\pm$ 5.5	0.128	0.587
Pain after 5 days	1 $\pm$ 3	2 $\pm$ 3	0.329	0.163
Pain after 6 days	1 $\pm$ 3	1 $\pm$ 3.5	0.313	0.184

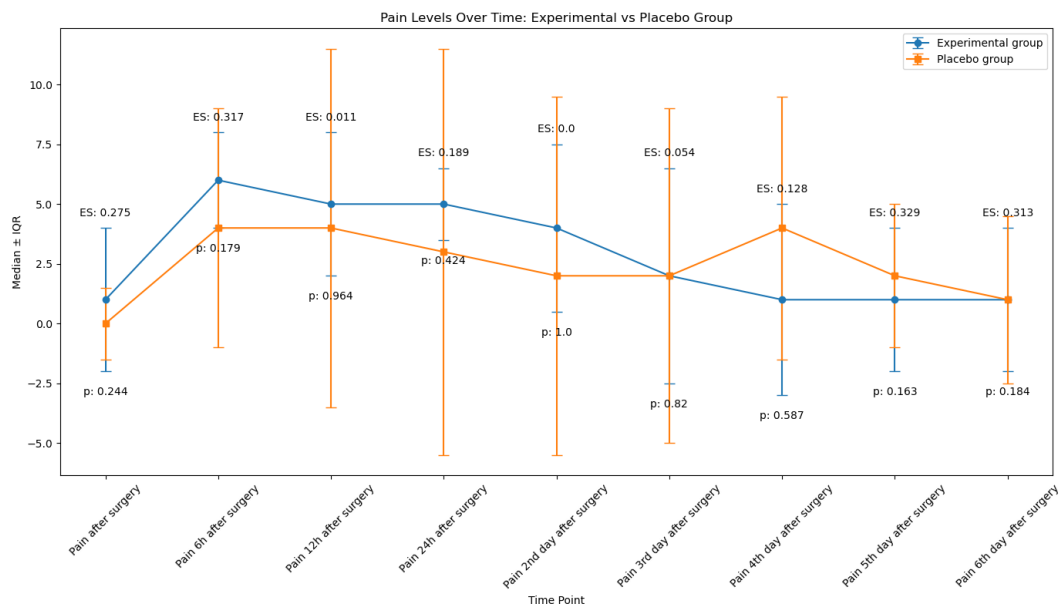


Figure 29. The reported pain level in each group through the time. ES: effect size, p: p-value.

5.2.2. Objective clinical outcomes

All participants had a thick gingival biotype and a BPE index of less than 2 in all teeth examined before the surgery, indicating healthy gingival conditions.

To evaluate post-surgical inflammation in the clinic, we assessed facial swelling and changes in maximum mouth opening (MMO).

5.2.2.1. Facial swelling

As described in the methodology, facial swelling was assessed by measuring facial dimensions at three time points: before surgery, 24 hours after surgery, and seven days post-surgery. These measurements, illustrated in Figure 30, were then used to calculate the percentage of changes in facial dimensions at 24 hours and seven days post-surgery compared to the pre-surgery baseline.

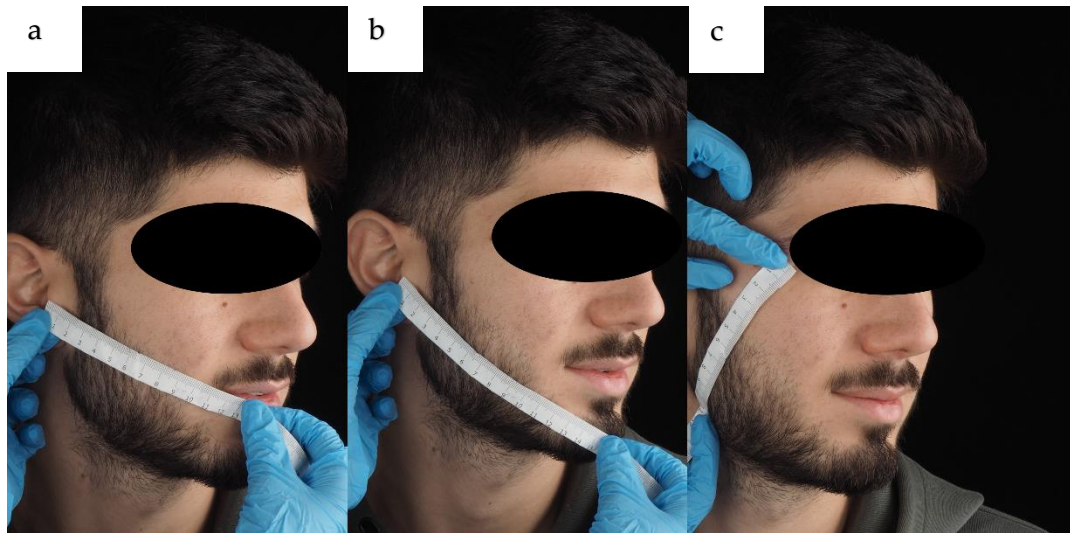


Figure 30. A paper ruler was used to measure facial dimensions: a) the tragus distance to the corner of the mouth as line A, b) the tragus distance to soft tissue pogonion as line B, and c) the distance of the outer corner of the eye to the angle of the mandible. Author's elaboration

Since the results of Shapiro Wilk test showed normal distribution of this variable in all times in both groups ($p\text{-value} > 0.05$), the difference between two groups was evaluated using independent samples t-test.

Table 8 and Figure 31 present a comparison of facial swelling between the experimental and placebo groups at two time points: 24 hours and seven days

post-surgery. At 24 hours, the experimental group exhibited a mean facial swelling of 4.43% (± 1.84552), while the placebo group showed a mean swelling of 3.54% (± 2.62599). The effect size for this time point was 0.39, with a 95% confidence interval (CI) ranging from -1.38 to 3.16, and a p-value of 0.417, indicating no statistically significant difference. After seven days, the mean facial swelling in the experimental group decreased to 1.33% (± 1.01505), compared to 1.09% (± 1.09478) in the placebo group. The effect size at this time point was 0.23, with a 95% CI of -0.81 to 1.30, and a p-value of 0.628, again showing no significant difference between the groups.

Table 8. Comparison of Facial Swelling in Experimental and Placebo Groups at 24 Hours and seven days post-surgery

	(Mean $\pm$ SD)		Effect Size (95% CI)	p
	Experimental	Placebo		
Facial Swelling after 24 hours (%)	4.4318 $\pm$ 1.84552	3.5403 $\pm$ 2.62599	0.39 (-1.38 to 3.16)	0.417
Facial Swelling after 7 days (%)	1.3320 $\pm$ 1.01505	1.0864 $\pm$ 1.09478	0.23 (-0.81 to 1.30)	0.628

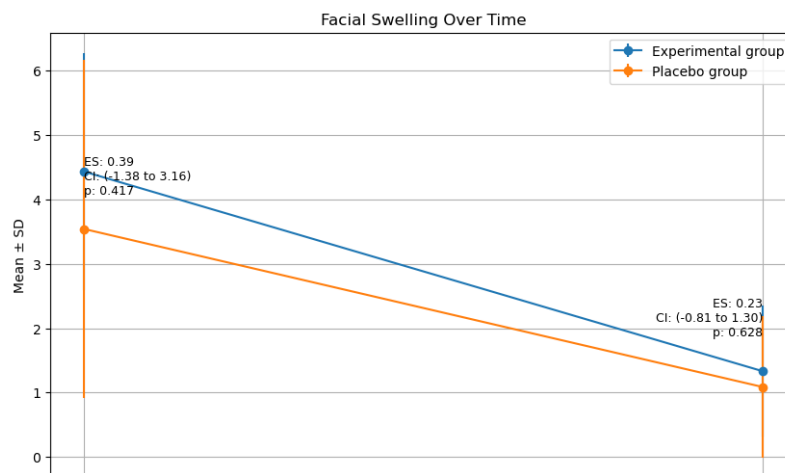


Figure 31. Comparison of Facial Swelling in Experimental and Placebo Groups at 24 Hours and seven days post-surgery. ES: effect size, CI: confidence interval, p: p-value.

5.2.2.2. *Maximum Mouth Opening (MMO) changes*

As described in the methodology, maximum mouth opening (MMO) was assessed by measuring the distance between the incisal edges of the upper and lower central incisors at four time points: before surgery, immediately after surgery, 24 hours post-surgery, and seven days post-surgery. To calculate the MMO change, the second, third, and fourth measurements were compared to the first one.

Due to one missing data point in the second measurement for the experimental group, all analyses of MMO changes immediately after surgery were performed on 17 records (8 in the experimental group and 9 in the placebo group).

The Shapiro-Wilk test indicated a normal distribution for all MMO change measurements in both groups, except for the MMO change immediately after surgery in the placebo group. Independent T-tests and Mann-Whitney U tests were conducted to evaluate the differences in MMO changes between the two groups depending on the normal distribution of them.

Table 9 and Figure 32 compare the changes in maximum mouth opening between the experimental and placebo groups at three different time points:

- **Immediately after surgery**, the experimental group had a mean MMO change of 4.13 mm (± 3.441), while the placebo group had a mean change of 4.22 mm (± 2.108). The effect size was -0.03, with a 95% confidence interval (CI) ranging from -12.823 to 11.490, and a p-value of 0.658, indicating no significant difference between the groups.
- **24 hours post-surgery**, the experimental group showed a mean MMO change of 17.89 mm (± 12.654), compared to 18.56 mm (± 11.642) in the placebo group. The effect size was -0.06, with a 95% CI of -12.823 to 11.490, and a p-value of 0.909, again showing no significant difference.
- **seven days post-surgery**, the experimental group had a mean MMO change of 13.00 mm (± 11.023), while the placebo group had a mean change of 9.44 mm (± 9.501). The effect size was 0.35, with a 95% CI of -6.746 to 13.857, and a p-value of 0.474, indicating no significant difference between the groups.

Overall, the data suggests that there were no statistically significant differences in MMO changes between the experimental and placebo groups at any of the measured time-points

Table 9. Comparison of Maximum Mouth Opening (MMO) Changes Between Experimental and Placebo Groups at Different Time Points

	(Mean $\pm$ SD)		Effect Size (95% CI)	P
	Experimental	Placebo		
MMO change after surgery (mm)	4.13 $\pm$ 3.441	4.22 $\pm$ 2.108	-0.03 (-12.823 to 11.490)	0.658
MMO change after 24 hours (mm)	17.89 $\pm$ 12.654	18.56 $\pm$ 11.642	-0.06 (-12.823 to 11.490)	0.909
MMO change after 7 days (mm)	13.00 $\pm$ 11.023	9.44 $\pm$ 9.501	0.35 (-6.746 to 13.857)	0.474

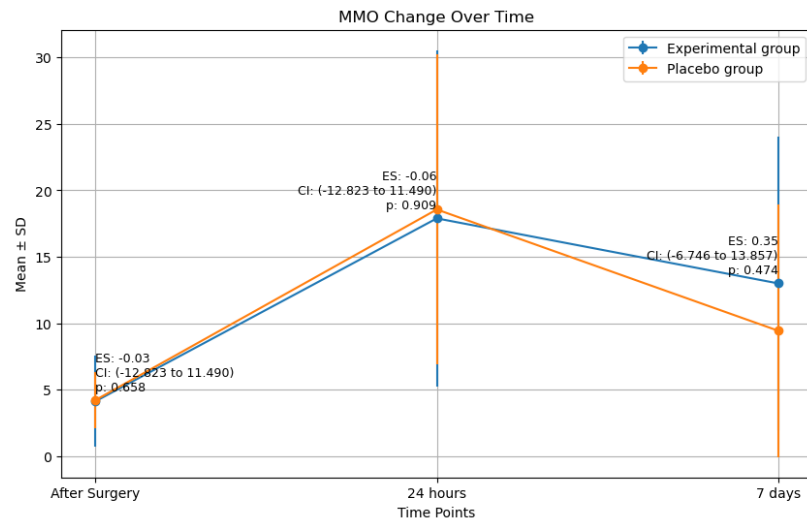


Figure 32. Comparison of Maximum Mouth Opening (MMO) Changes Between Experimental and Placebo Groups at Different Time Points. ES: effect size, CI: confidence interval, p: p-value

5.3. LABORATORY OUTCOME, PGE2 CONCENTRATION IN SALIVA

As described in the methodology, ELISA kit was used to assess the PGE2 concentrations of the saliva samples which were taken under the specific condition before the surgery, 24 hours and seven days after the surgery.

5.3.1. Standard plots

To avoid long-term freezing of saliva samples, ELISA was performed at two different times: once for the saliva samples of the first 10 patients on January 31st, 2024, and once for the remaining samples on July 24th, 2024. Consequently, two standard plots and equations were utilized to calculate the PGE2 concentration of each sample based on OD absorptions (Figure 33 and 34). The OD value used to calculate the PGE2 concentration of each saliva sample was the mean of duplicate OD measurements for each sample.

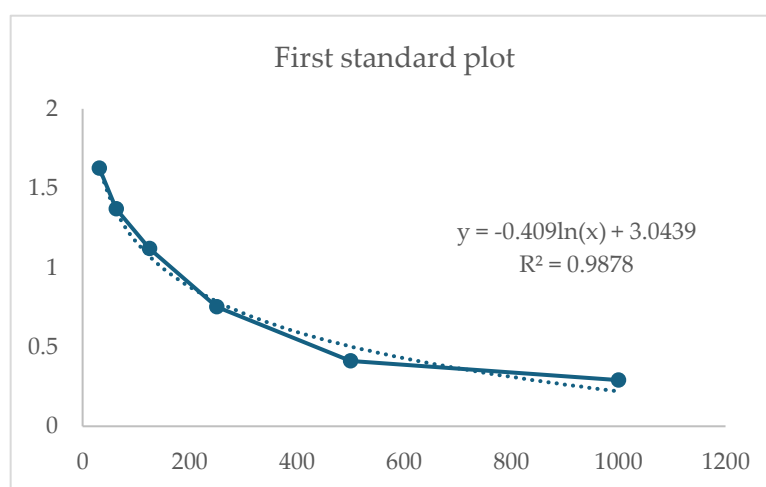


Figure 33. The standard plot and the derived equation based on the OD absorptions of the standard solutions on 31st of January. Y axis is the OD and X axis is the concentration of the PGE2.

Regarding the standard plot and the equation, the concentration of PGE2 in each saliva sample for the first 10 patients was calculated using the following formula:

$$\text{PGE2 concentration} = \exp ((\text{OD mean} - 3.0439) / -0.409)$$

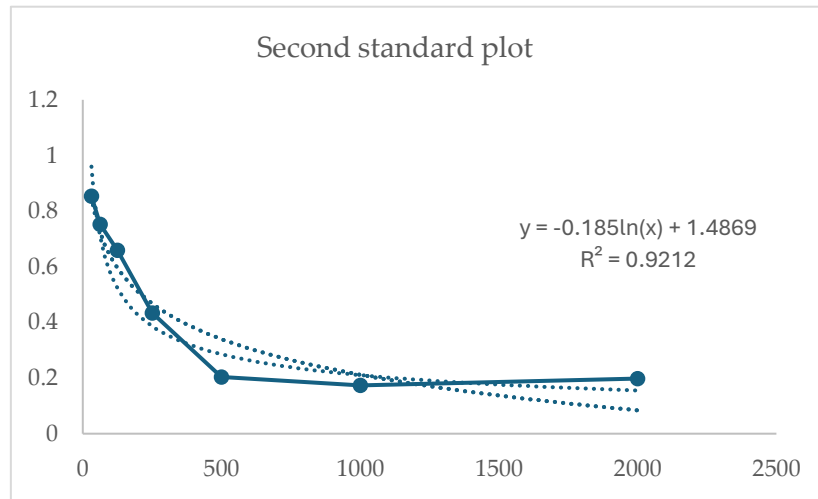


Figure 34. The standard plot and the derived equation based on the OD absorptions of the standard solutions on 24th of July. Y axis is the OD and X axis is the concentration of the PGE2.

Regarding the standard plot and the equation, the concentration of PGE2 in each saliva sample was calculated using the following formula for patients 11 to 18:

$$\text{PGE2 concentration} = \exp((\text{OD mean} - 1.4869) / -0.185)$$

Subsequently, the Winsorize process was applied to replace extreme outliers, as detailed in the methodology section.

5.3.2. Salivary PGE2 concentration

The Shapiro-Wilk test confirmed a normal distribution of PGE2 concentrations at all time points in both groups (p-values > 0.05).

Table 10 presents the mean $\pm$ SD of PGE2 concentrations (pg/ml) for the experimental and placebo groups at three different time points: baseline, 24 hours, and seven days post-surgery.

- **Baseline:** The experimental group had a mean PGE2 concentration of 422.54 ± 436.83 pg/ml, while the placebo group had a higher mean of 687.93 ± 497.07 pg/ml.
- **24 Hours:** The experimental group's mean increased to 487.79 ± 453.30 pg/ml, whereas the placebo group's mean rose to 760.31 ± 461.28 pg/ml.

- **Seven Days:** The experimental group's mean PGE2 concentration further increased to 652.32 ± 513.77 pg/ml, and the placebo group's mean slightly decreased to 657.62 ± 445.81 pg/ml.

However, the repeated measures ANOVA test revealed no significant difference in the changes in PGE2 concentrations over time between the two groups ($p = 0.244$). Additionally, the p-value for the between-subject effects was 0.355, indicating no significant overall difference between the two groups. An independent samples t-test was used to evaluate each time point separately, and the results are presented in Table 10 which indicate that the difference on each time points between two groups was not significant. The lack of significant differences between the two groups at baseline indicates their initial similarity. Additionally, the insignificant differences in PGE2 concentrations between the groups at 24 hours and seven days post-surgery suggest that the prophylactic antibiotic administered before surgery did not impact the PGE2 concentration in the saliva of the participants.

Table 10. Salivary PGE2 concentrations in different time points in total and in each group.

	Group	Mean $\pm$ SD (pg/ml)	Effect size	P-value
Baseline PGE2 concentration	Experimental	422.5357 $\pm$ 436.8328	-0.57	0.246
	Placebo	687.9257 $\pm$ 497.0744		
	Total	555.2307 $\pm$ 474.0434		
PGE2 concentration after 24 hours	Experimental	487.7884 $\pm$ 453.2987	-0.63	0.202
	Placebo	760.3132 $\pm$ 461.2822		
	Total	619.5508 $\pm$ 458.5644		
PGE2 concentration after 7 days	Experimental	652.3224 $\pm$ 513.7675	-0.01	0.982
	Placebo	657.6175 $\pm$ 445.8147		
	Total	654.9700 $\pm$ 466.6393		

Figure 35 presents the salivary PGE2 concentrations in two groups through time.

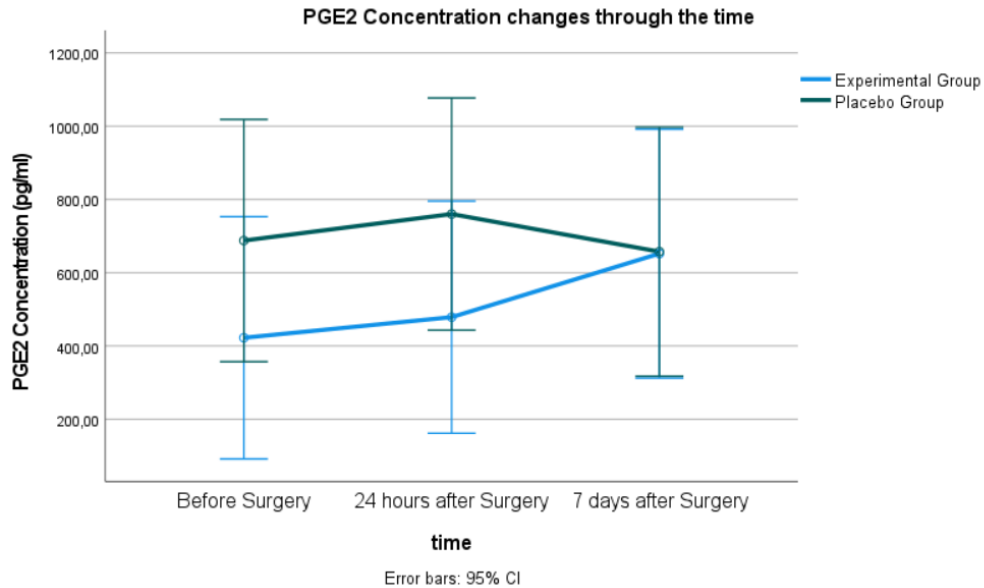


Figure 35. Salivary PGE2 concentration changes through time in each group.

5.4. CORRELATION BETWEEN OUTCOMES AND BASIC/SURGERY VARIABLES

Since the results showed no correlation between the prophylactic antibiotic and clinical or laboratory outcomes, we performed analyses to assess the correlation between the basic or surgical variables and the outcomes.

In correlation assessments, correlation coefficients indicate the strength and direction of the relationship between a variable and an outcome:

- **0 to 0.1:** Negligible correlation
- **0.1 to 0.39:** Weak correlation
- **0.4 to 0.69:** Moderate correlation
- **0.7 to 0.89:** Strong correlation
- **0.9 to 1.0:** Very strong correlation

Positive correlations indicate that as the variable increases, the outcome also increases. Conversely, negative correlations indicate that as the variable increases, the outcome decreases.

P-value of the correlation tests shows the significance of the relationship.

5.4.1. Correlations between pain and basic/surgery variables

Correlation analysis was performed to assess the relationship between the pain reported by participants at each time point and other variables.

- Spearman's rank-order correlation test was used to examine the correlation between age, surgery duration, number of sutures, number of sections, and post-surgery pain at different time-points.
- Point-biserial correlation was utilized to assess the correlation between gender, difficulty index (DI), tooth section, osteotomy, and pain at different time-points.

The results (Table 11) showed a significant moderate correlation between gender and pain on the 2nd day after surgery, indicating that women reported more pain on this day in this study. Additionally, a significant moderate negative correlation was found between the age of the participants and the pain recorded immediately after surgery, with younger participants reporting higher levels of pain.

A significant moderate positive correlation was observed between the number of sutures and pain on the day of surgery (6 hours and 12 hours post-surgery). This finding, combined with the lack of correlation between other surgical data and pain on the surgery day, suggests that a smaller number of sutures may help patients experience less pain on that day.

Furthermore, a very significant moderate positive correlation was found between performing osteotomy and the pain reported on the 6th day after third molar extraction.

The results presented no correlation between other variables and the pain recorded at different time points.

Table 11. Correlation Analyses between pain recorded on different time points and the basic or surgery variables.

		Pain after surgery	Pain after 6 hours	Pain after 12 hours	Pain after 24 hours	Pain after 2 days	Pain after 3 days	Pain after 4 days	Pain after 5 days	Pain after 6 days
Gender	CC	-0.105	-0.291	-0.339	-0.373	- 0.495*	- 0.431	- 0.306	- 0.134	-0.050
	p	0.678	0.241	0.169	0.128	0.037	0.074	0.216	0.596	0.845
Age	CC	-0.562*	0.126	-0.064	-0.037	-0.045	- 0.172	- 0.246	- 0.162	-0.310
	p	0.015	0.618	0.802	0.885	0.861	0.496	0.325	0.522	0.210
DI	CC	0.000	-0.279	0.178	0.022	0.000	0.226	0.281	0.415	0.570*
	p	1.000	0.263	0.480	0.931	1.000	0.367	0.258	0.087	0.014
Tooth section	CC	0.000	-0.036	0.012	-0.024	-0.169	- 0.197	- 0.110	- 0.134	-0.050
	p	1.000	0.886	0.962	0.925	0.503	0.433	0.663	0.596	0.845
Number of tooth sections	CC	0.218	-0.059	-0.061	-0.056	-0.360	- 0.331	- 0.283	- 0.348	-0.092
	p	0.384	0.815	0.810	0.825	0.142	0.179	0.256	0.157	0.717
Number of sutures	CC	0.132	0.557*	0.572*	0.311	0.395	0.214	0.143	0.044	0.118
	p	0.601	0.016	0.013	0.210	0.105	0.393	0.572	0.861	0.642
Surgery duration (S)	CC	0.204	-0.108	0.052	-0.015	-0.245	- 0.159	- 0.124	- 0.053	0.260
	p	0.416	0.670	0.837	0.954	0.327	0.527	0.625	0.836	0.297
Osteotomy	CC	0.047	-0.022	0.229	0.195	0.163	0.266	0.221	0.385	0.615**
	p	0.852	0.931	0.360	0.438	0.518	0.285	0.378	0.115	0.007

CC: Correlation Coefficient, p: p-value, * significant, ** very significant

5.4.2. Correlations between facial swelling and basic/surgery variables

Correlation analysis was performed to assess the relationship between the facial swelling, measured after 24 hours and seven days and other variables.

- The Pearson correlation coefficient test was performed to evaluate the correlation between age, surgery duration and facial swelling at two different time-points.
- Spearman's rank-order correlation test was used to examine the correlation between number of sutures, number of sections, and post-surgery facial swelling after 24 hours and seven days.
- Point-biserial correlation was utilized to assess the correlation between gender, difficulty index (DI), tooth section, osteotomy, and facial swelling 24 hours and seven days after surgery.

The results (Table 12) showed a significant moderate positive correlation between surgery duration and performing osteotomy with facial swelling after 24 hours. No other significant correlations were found.

Table 12. Correlation analyses between the facial swelling in different time points and basic or surgery variables

		24-hour Facial swelling	7-day Facial swelling
Gender	CC	-0.299	-0.324
	P	0.228	0.189
Age	PC	-0.440	-0.260
	P	0.068	0.297
Group allocated	PC	-0.204	-0.122
	P	0.417	0.628
DI	CC	0.213	0.199
	P	0.396	0.429
Tooth section	PC	0.137	-0.025
	P	0.589	0.921
Number of sections	CC	0.383	0.160
	P	0.117	0.526
Number of sutures	CC	0.275	0.242
	P	0.269	0.333
Surgery duration (S)	PC	0.515*	0.113
	P	0.029	0.655
Osteotomy	PC	0.507*	0.075
	P	0.032	0.768

CC: Correlation Coefficient, PC: Pearson Correlation, p: p-value, * significant

5.4.3. Correlations between MMO changes and basic/surgery variables

Correlation analysis was performed to assess the relationship between the MMO changes measured immediately after surgery, 24 hours and seven days post-surgery and other variables.

- The Pearson correlation coefficient test was performed to evaluate the correlation between age, surgery duration and MMO change at three different time-points.
- Spearman's rank-order correlation test was used to examine the correlation between number of sutures, number of sections, and post-surgery MMO change immediately after surgery, after 24 hours and seven days.
- Point-biserial correlation was utilized to assess the correlation between gender, difficulty index (DI), tooth section, osteotomy, and MMO changes immediately post-surgery, 24 hours and seven days after surgery.

The results, which are presented in Table 13, showed a very significant moderate positive correlation between the difficulty index and the 24-hour MMO change, indicating that higher difficulty indices caused more difficulty for patients in opening their mouths 24 hours after surgery. Additionally, a positive moderate correlation was found between surgery duration and MMO changes immediately and 24 hours after surgery, which were very significant and significant, respectively. Furthermore, very significant moderate positive correlations were found between performing osteotomy and MMO changes at 24 hours and seven days post-surgery. No other significant correlations were found. In summary, longer surgery duration and higher difficulty index were related to short-term difficulties in mouth opening, while performing osteotomy was related to longer-term difficulties.

Table 13. Correlation Analyses between MMO changes recorded on different time points and the basic or surgery variables.

		After surgery MMO change	24-hour MMO change	7-day MMO change
Gender	CC	0.361	0.084	-0.241
	P	0.154	0.740	0.335
Age	PC	-0.116	-0.449	-0.293
	P	0.656	0.062	0.238
Group allocated	PC	0.018	0.029	-0.180
	P	0.944	0.909	0.474
DI	CC	0.274	0.629**	0.299
	P	0.287	0.005	0.228
Tooth section	PC	0.195	0.207	0.115
	P	0.454	0.411	0.651
Number of sections	CC	0.228	0.310	0.133
	P	0.378	0.211	0.598
Number of sutures	CC	0.243	0.330	0.381
	P	0.347	0.180	0.119
Surgery duration (S)	PC	0.669**	0.553*	0.356
	P	0.003	0.017	0.146
Osteotomy	PC	0.463	0.768**	0.678**
	P	0.061	0.000	0.002

CC: Correlation Coefficient, PC: Pearson Correlation, p: p-value, * significant, ** very significant

5.4.4. Correlations between PGE2 concentrations and other variables

Correlation analysis was performed to assess the relationship between the salivary PGE2 concentration before, 24 hours and seven days post-surgery and other variables.

- The Pearson correlation coefficient test was performed to evaluate the correlation between age, surgery duration and PGE2 concentration at three different time-points.
- Spearman's rank-order correlation test was used to examine the correlation between number of sutures, number of sections, and PGE2 concentration in various times.
- Point-biserial correlation was utilized to assess the correlation between gender, difficulty index (DI), tooth section, osteotomy, and PGE2 concentration in various times.

The results are presented in Table 14 below:

Table 14. Correlation between salivary PGE2 concentrations and basic or surgery variables.

		Baseline PGE2 concentration	PGE2 concentration after 24 hours	PGE2 concentration after 7 days
Gender	PC	0.173	-0.187	-0.243
	p	0.492	0.458	0.332
Age	PC	0.155	-0.241	-0.233
	P	0.540	0.335	0.352
DI	CC	0.077	0.033	-0.143
	P	0.762	0.897	0.572
Surgery duration (S)	PC	-0.321	-0.444	-0.517*
	P	0.193	0.065	0.028
Osteotomy	PC	-0.004	-0.142	-0.200
	p	0.987	0.574	0.426
Tooth section	PC	-0.051	-0.494*	-0.287
	p	0.840	0.037	0.247
Number of tooth sections	PC	0.325	0.159	0.250
	p	0.188	0.530	0.317
Number of sutures	PC	0.325	0.159	0.250
	p	0.188	0.530	0.317

CC: Correlation Coefficient, PC: Pearson Correlation, p: p-value, * significant, ** very significant

As seen in the Table 14, a significant moderate negative correlation was found between the duration of the surgery and salivary PGE2 concentration after seven days. Additionally, the same correlation was observed between performing a tooth section procedure during the surgery to remove the third molars and PGE2 levels in the saliva after 24 hours. These results indicate that longer surgery duration and performing a tooth section procedure cause a lower increase in PGE2 concentration in saliva.

5.5. CORRELATION BETWEEN CLINICAL AND LABORATORY OUTCOMES

5.5.1. Correlation between subjective clinical outcome and other outcomes

To evaluate the correlation between the pain reported by participants at different time points and objective clinical outcomes or salivary PGE2 concentrations, we conducted the following correlation assessment.

5.5.1.1. *Correlation between pain and other clinical outcomes*

Spearman's rank correlation coefficient was used to assess the relationship between recorded pain levels and facial swelling, as well as MMO changes, at different time points.

The results are presented in Table 15.

Table 15. Correlation analyses between pain and objective clinical outcomes.

		Pain after surger y	Pain after 6 hour s	Pain after 12 hours	Pain after 24 hour s	Pain after 2 days	Pain after 3 days	Pain after 4 days	Pain after 5 days	Pain after 6 days
After surgery MMO change	C	0.307	0.073	0.129	-0.060	-0.056	0.039	0.083	0.285	0.323
	p	0.231	0.782	0.621	0.818	0.832	0.882	0.751	0.267	0.207
24-hour MMO change	C	0.087	0.062	0.377	0.186	0.197	0.292	0.323	0.417	0.520 *
	p	0.732	0.806	0.123	0.459	0.432	0.239	0.191	0.085	0.027
7-day MMO change	C	0.135	0.323	0.440	0.421	0.527 *	0.605* *	0.611* *	0.598* *	0.531 *
	p	0.593	0.191	0.068	0.082	0.025	0.008	0.007	0.009	0.023
24-hour Facial swellin g	C	0.427	0.382	0.528 *	0.426	0.304	0.356	0.339	0.264	0.390
	p	0.077	0.117	0.024	0.078	0.219	0.147	0.169	0.289	0.110
7-day Facial swellin g	C	0.402	0.323	0.262	0.251	0.362	0.375	0.339	0.043	-0.012
	p	0.098	0.191	0.294	0.316	0.139	0.125	0.169	0.866	0.961

CC: Correlation Coefficient, p: p-value, * significant, ** very significant

5.5.1.1.1. Correlation between pain and MMO changes

Significant to very significant moderate positive correlations were observed between the pain reported between the 2nd and 6th days post-surgery and the MMO change after seven days. This suggests that participants may have limited mouth opening due to the pain they experienced. On the other hand, the MMO change after 24 hours was correlated with the pain reported on the 6th day post-surgery, but this appears to be clinically insignificant.

No other correlations were found between pain and MMO changes (Table 15).

5.5.1.1.2. Correlation between pain and Facial swelling

A significant moderate positive correlation was found between the pain reported 12 hours after surgery and the facial swelling observed the following day. This finding suggests that patients should take inflammation control measures more seriously if they experience higher levels of pain on the day of surgery.

5.5.1.2. *Correlation between pain and laboratory outcome*

Spearman's rank correlation coefficient was used to assess the relationship between recorded pain levels and salivary PGE2 concentrations before surgery, 24 hours post-surgery, and seven days post-surgery. The results are presented in Table 16 below:

Table 16. Correlation assessment between pain and salivary PGE2 concentrations.

		Baseline PGE2 concentration	PGE2 concentration after 24 hours	PGE2 concentration after 7 days
Pain after surgery	CC	-0.109	-0.027	0.036
	P	0.667	0.914	0.886
Pain after 6 hours	CC	0.433	0.192	0.284
	P	0.073	0.446	0.253
Pain after 12 hours	CC	0.315	0.433	0.182
	P	0.204	0.073	0.470
Pain after 24 hours	CC	0.310	0.303	0.254
	P	0.210	0.221	0.308
Pain after 2 days	CC	0.486*	0.505*	0.514*
	P	0.041	0.032	0.029
Pain after 3 days	CC	0.481*	0.481*	0.526*
	P	0.044	0.044	0.025
Pain after 4 days	CC	0.585*	0.563*	0.626**
	P	0.011	0.015	0.005
Pain after 5 days	CC	0.509*	0.555*	0.494*
	P	0.031	0.017	0.037
Pain after 6 days	CC	0.107	0.341	0.050
	P	0.672	0.167	0.843

CC: Correlation Coefficient, p: p-value, * significant, ** very significant

Significant to very significant moderate positive correlations were found between salivary PGE2 concentrations before surgery, at 24 hours, and at seven days post-surgery, and the pain experienced by participants on the 2nd to 5th day after surgery. This finding indicates that higher salivary PGE2 levels, even before surgery, can lead to increased pain after surgery.

5.5.2. Correlation between objective clinical outcomes and PGE2 concentrations

The Pearson Correlation Coefficient test was used to evaluate the correlation between facial swelling, MMO changes, and salivary PGE2 concentrations.

5.5.2.1. *Correlation between objective clinical outcomes*

The results of the Pearson Correlation Coefficient test showed a very significant, positive, and moderate correlation between facial swelling at 24 hours and MMO changes at both 24 hours and seven days. This indicates that greater facial swelling at 24 hours was associated with more significant MMO changes at these time points. Additionally, a significant, moderate, and positive correlation was found between facial swelling and MMO changes at seven days, which was expected (Table 17).

Furthermore, a very significant correlation was observed between MMO changes at different time points, which was obvious.

Table 17. Correlation assessment between facial swelling and MMO changes.

		After surgery MMO change	24-hour MMO change	7-day MMO change	24-hour Facial swelling	7-day Facial swelling
After surgery MMO change	PC	1	0.616**	0.377	0.478	-0.004
	P		0.008	0.135	0.053	0.987
24-hour MMO change	PC	0.616**	1	0.849**	0.661**	0.376
	P	0.008		0.000	0.003	0.124
7-day MMO change	PC	0.377	0.849**	1	0.634**	0.510*
	P	0.135	0.000		0.005	0.031

PC: Pearson Correlation, p: p-value, * significant, ** very significant

5.5.2.2. Correlation between objective clinical outcomes and salivary PGE2 concentrations

The results of Pearson Correlation Coefficient test, which is presented in table 18, showed no correlations between PGE2 concentrations and facial swelling or MMO changes at different time points.

Table 18. Correlations between objective clinical outcomes and salivary PGE concentrations.

		Baseline PGE2 concentration	PGE2 concentration after 24 hours	PGE2 concentration after 7 days
Facial Swelling 24 hours	PC	-0.150	-0.074	-0.076
	p	0.554	0.770	0.765
Facial Swelling 7days	PC	0.247	0.068	0.219
	p	0.323	0.789	0.383
MMO change after surgery	PC	-0.120	-0.159	-0.255
	p	0.647	0.543	0.323
MMO change after 24 hours	PC	0.206	-0.025	0.127
	p	0.413	0.921	0.614
MMO change after 7 days	PC	0.307	-0.028	0.232
	p	0.215	0.914	0.353

PC: Pearson Correlation, p: p-value

5.6. STUDY POWER ASSESSMENT

5.6.1. Clinical study power

A post hoc power analysis, utilizing an effect size of 0.39 in facial swelling after 24 hours, a pooled standard deviation of 2.27, a sample size of nine, and a significance level of 0.05, revealed a study power of 12.18% in clinical part.

5.6.2. Laboratory study power

A post hoc power analysis, utilizing an effect size of -0.63 in salivary PGE2 concentration after 24 hours, a pooled standard deviation of 457.34, a sample size of nine, and a significance level of 0.05, revealed a study power of 20.43% in laboratory part.

VI – DISCUSSION

VI -DISCUSSION

In dental clinics, third molar extraction surgery is a common procedure. Antibiotic prescriptions are sadly more frequent than necessary in an attempt to reassure patients and avoid potential infections. A network meta-analysis has shown that even when antibiotic therapy effectively prevents infections following third molar surgeries, the number of patients needed to treat to achieve this benefit is high (Camps-Font et al., 2024). They claimed that antibiotics could prevent infection in every 18 patients undergoing mandibular third molar surgery and can prevent one dry socket incidence in every 25 patients (Camps-Font et al., 2024). Despite a downward trend in antibiotic prescriptions in some countries (Soleymani et al., 2024), according to questionnaire-based studies, many dentists still prescribe antibiotics for all third molar surgeries (Arteagoitia, Ramos, et al., 2016; Choi, 2020). This practice, which frequently ignores the underlying causes of infection or alternative preventive measures, is intended to reassure patients and is motivated by their fear of possible post-surgery complications and infections (Arteagoitia, Ramos, et al., 2016; Choi, 2020).

We live in a time when no new antibiotics are being developed or found to be introduced to the market (Hansson & Brenthel, 2022). Furthermore, we must acknowledge that current medications will become less effective or possibly ineffective in the near future due to bacterial resistance to antibiotics (Hansson & Brenthel, 2022). In 1995, Lewis et al. reported that 73% of aspirated pus from acute oral infections had at least one β -lactamase-producing strain and 55% had at least one penicillin-resistant strain (Lewis et al., 1995). Now, almost 20 years later, these figures are probably higher, and the situation is more complicated. In addition, the World Health Organization (WHO) report presented a significant threat to public health worldwide caused by resistance rates among prevalent bacterial pathogens (*Global antimicrobial resistance and use surveillance system (GLASS) report 2022. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO.*). Reports indicated that, in 2019, antimicrobial-resistant bacteria were estimated to be directly responsible for approximately 1.27 million deaths globally ("Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis," 2022). We know,

if current trends continue, this number could rise to 10 million deaths annually by 2050 (de Kraker et al., 2016). Therefore, to avoid a close bacterial and infection catastrophe in the upcoming decades, all health sectors, including dental settings, must be aware of the effects of antibiotic resistance and the appropriate remedies. Reducing the overuse of antibiotics in professions like dentistry is then essential.

Our study assessed the laboratory and clinical aspects of an infection prevention strategy—prophylactic antibiotic prescription prior to third molar extraction surgery—to determine whether it is sufficiently effective to be used in clinical settings going forward. Following lower third molar surgery, this double-blind, randomized, parallel two-armed clinical trial showed that giving 2g of pre-operative amoxicillin had no discernible effect on objective or subjective clinical outcomes or laboratory results. Assessments of nine participants in each group—one receiving amoxicillin capsules and the other receiving placebo capsules—showed no significant differences in salivary PGE2 concentration, maximum mouth opening (MMO) changes, facial swelling, or reported pain over a one-week period.

Administering prophylactic antibiotics or pre-operative antibiotics can ensure high concentrations of antibiotics in the bloodstream and adequate levels at the surgical site before, during, and shortly after the procedure. This approach reduces the risk of bacterial invasions by common oral surgical infection pathogens such as *Staphylococcus spp.*, *Streptococcus spp.*, and anaerobic Gram-positive and Gram-negative rods (Kuriyama et al., 2002; Torof et al., 2023; Xue et al., 2015). Oppelaar et al. reviewed the efficacy of short-course (≤ 24 hours) versus extended-course (≥ 72 hours) antibiotic prophylaxis and found no significant difference in infection risk (Oppelaar et al., 2019). They recommended short-term antibiotics for ear, nose, throat, and oral and maxillofacial surgeries to minimize side effects like antibiotic resistance and disruption of the healthy microbiome, which can increase the risk of future infections and complications (Camps-Font et al., 2024; Menon, Gopinath, et al, 2019; Oppelaar et al., 2019; Wang & Liu, 2022; Yuan et al., 2023). Furthermore, a meta-analysis by Lupi et al. showed that pre-operative antibiotic administration is just as beneficial as both pre- and post-operative prophylaxis in preventing surgical wound infections for lower third molar extractions, especially when osteotomy is used (Lupi et al., 2021). Given this context, we decided to

evaluate the effect of pre-operative antibiotic therapy on post-operative surgical complications.

We selected amoxicillin as a pre-operative antibiotic to evaluate in our study because amoxicillin has been using by dentists traditionally to reduce the risk of dry socket and infection after surgical treatments (Soleymani et al., 2024). This antibiotic, with or without clavulanic acid, is the most common one which is prescribed by dentists to prevent postoperative infection after third mandibular molar surgery (Menon, Gopinath, et al., 2019). Clindamycin, doxycycline, erythromycin, and metronidazole are other common antibiotics for this purpose (Lodi et al., 2021). A systematic review also demonstrated that a single pre-operative dose of 2g of amoxicillin significantly reduces the risk of surgical site infections (Marcussen et al., 2016).

6.1. CLINICAL PART

According to correlation analyses of the findings of a recent study, more sutures were linked to more pain on the day of surgery, and higher pain levels six days after surgery were linked to a higher surgical difficulty rating. Furthermore, it was discovered that the use of osteotomy, a longer surgical length, and a higher difficulty index all contributed to more noticeable maximum mouth opening alterations. On the other hand, longer surgical times and osteotomy results were associated with more facial swelling. The findings suggested that greater facial edema is linked to a decreased capacity to open the mouth because they showed a correlation between maximum mouth opening alterations and facial swelling and higher degrees of pain. Additionally, there was a correlation between increased face swelling 24 hours after surgery and higher pain levels 12 hours following surgery.

Our study's findings are comparable to those reported by Mendes et al. in their 2023 publication (Mendes et al., 2023). By measuring bleeding, the state of the tissue around the socket, suppuration, tissue color, and tissue firmness, they assessed the soft tissue healing process besides face edema and variations in mouth opening. Additionally, they measured the patient's pain using the VAS instrument. All assessments were done on days 3, 7, 14, and 30 following the surgery. They demonstrated that these factors were comparable between the groups that took one

gram of amoxicillin an hour prior to the operation to remove the third molar and the groups that did not take any medication before or after the procedure. Their approach was similar to ours in that they used fewer preventive antibiotic doses. They also assessed the facial swelling using two lines of three measurements, which we also employed. The distance between Pogonion and Tragus was the missing measurement. However, because the comparisons are more concise, the three measures technique is prioritized.

Stošić et al., on the other hand, discovered that antibiotics helped to lessen inflammatory problems following surgery for the third molar (Stošić, Šarčev, Mirković, Bajkin, et al., 2022). The reasons for this contradictory outcome can be seen by looking at the specifics of their investigation. In Stošić's double-blind randomized clinical trial, there were three groups: a placebo and two test groups. One group took 400 mg of cefixime, and another group took 400 mg of moxifloxacin once daily for five days after surgery. Post-operative problems were lower in the moxifloxacin group, according to Stošić's published results. As is well known, this cephalosporin has anti-inflammatory properties in addition to its antibacterial ones, which may account for these results (Dalhoff & Shalit, 2003). Limeres et al. also found moxifloxacin useful to reduce the post-operative recovery time by minimizing the pain and shortening the period of oral function impairments (Limeres et al., 2009). In our study, we gave either a placebo capsule or a dose of 2 grams of amoxicillin an hour before surgery. To examine how well amoxicillin and moxifloxacin or cefixime work for post-surgical problems, it is a promising idea to compare them. By the way, it appears to be difficult or impossible to do the study with patients and researchers blinded because the treatment regimens for these antibiotics differ (amoxicillin should be given three times day, while the other two should be taken once daily). Nevertheless, research comparing their efficacy as a pre-operative drug with blinding patients might be proposed.

In addition to the primary distinctions between Stošić's and our study—namely, the antibiotics and their regimen in two studies—there are other variations in the methodology. To manage the pain following surgery, Stošić et al. prescribed 200 mg of ibuprofen plus 325 mg of paracetamol (Stošić, Šarčev, Mirković, Bajkin, et al., 2022). They noted how many analgesics patients had taken over the course of a week as a measure of their pain. But at the same period of time, we used the

VAS tool to record the self-reported pain. Of course, even in the absence of pain, people can use analgesics to avoid any potential discomfort. In their study, they demonstrated that participants in the moxifloxacin group used fewer analgesics and reported less pain, in the first and second days after the surgery, than those in the other two groups, as well as comparing cefixime group with a placebo. However, we found no difference between two groups at every time points.

The technique used to quantify face edema or swelling is another difference between these two investigations. Stošić et al. evaluated one dimension, from chin tip to tragus, to assess this variable (Stošić, Šarčev, Mirković, Bajkin, et al., 2022), however we used three dimensions of the face in our study to compute more accurate measures. As previously stated, the three-dimensional approach is more accurate and is advised for determining face edema and swelling in various investigations (Costa et al., 2022; Kirnbauer et al., 2022). They did, however, compute the mouth opening ability using the same methodology. Their results showed that, in the first- and second days following surgery, the moxifloxacin group had less trismus and facial edema than the other two groups. On the other hand, Stošić et al. demonstrated that the length of surgery is not a significant factor in the post-operative inflammatory problems, even though our study established a positive correlation between the surgery interval and the face edema 24 hours after the surgery. Once more, the methodology showed a difference. Less than 20 minutes, between 20 and 40 minutes, and more than 40 minutes were the three categories into which they separated the surgical intervals. We analyzed the operation length using parametric statistical tests, which are more trustworthy than non-parametric tests, which are often used to evaluate ordinal variables like the three groups of surgery duration in the Stošić et al. research. This is because we saw the surgery duration as a continuous variable in our study.

The Stošić et al. study found no infection incidence in the placebo group, despite the fact that all of the advantages of moxifloxacin were validated (Stošić, Šarčev, Mirković, Bajkin, et al., 2022). Additionally, trismus, reported pain, and lower facial edema were only seen in the first two days following surgery, while there were no discernible differences between the three groups seven days later. On the other hand, we may believe that using anti-inflammatory drugs and sufficient post-operative self-care would achieve the same outcomes. For instance,

Momesso et al. stated in their study, non-steroidal anti-inflammatory drugs (NSAIDs) are a good choice to control the post-operative pain after third molar surgery (Momesso et al., 2021). Therefore, it is best to avoid considering moxifloxacin or other antibiotics in healthy individuals with sound teeth and gingiva following regular extraction, even if moxifloxacin can help reduce post-operative challenges.

Another investigation by Siddiqi et al. came to the same conclusions as ours (Siddiqi et al., 2010). After the third molar surgery, they attempted to determine the impact of one gram of preoperative amoxicillin and preoperative plus two days postoperative amoxicillin (500 mg, three times per day) on the following symptoms: pain (assessed by VAS for two weeks), facial swelling (visual technique), infection, trismus, and body temperature. Unlike our study, they recruited subjects with four third molars and employed the split mouth approach. Since patients used placebo capsules in place of amoxicillin for one surgery, each patient functioned as their own control. Since it takes two months for the inflammatory alterations in the blood following third molar surgery to return to normal, the three-week gap between each participant's two procedures – which was considered in Siddiqi's study – is too small to assess the inflammatory changes independently. We didn't employ the split mouth approach in our study to avoid this consequence. The technique used to quantify facial swelling as a continuous variable rather than using a categorized visual system was another top aspect for our investigation. In order to minimize errors, their study, like ours, used a single surgeon for all procedures and a single operator for all measures. They discovered that there is no noticeable difference between taking amoxicillin once before surgery and continuing to do so for two days after the procedure, and that antibiotics had no effect on post-operative problems following third molar surgery.

Kirnbauer et al.'s publication in 2022 is another research that employed a split mouth design, similar to that of Seddiqi et al. (Siddiqi et al., 2010) but took into account a three-month gap between two procedures in order to eliminate the previously described alterations in blood inflammatory factors (Kirnbauer et al., 2022). To determine if there was a difference between the two groups in terms of surgical site infection, swelling (three dimensions measured on the face before, 24 hours, and 7 days after the operation and by obtaining digital face scans and

superimposing method), trismus (MMO changes), and pain (VAS tool and analgesics intake as yes/no), they gave two grams of amoxicillin an hour before surgery and 1.5 grams for three days after surgery in their double-blind, randomized, placebo control trial. Three surgeons performed the procedures using a standard technique on patients who had four impacted or partially impacted third molars. Similar to our findings, they demonstrated that antibiotics had no effect on post-operative problems; however, their study's infection incidence rate was 11%, whereas ours was 5% (one infection in 18 cases). The digital approach they employed as a supplement to the analog facial measurements, which can reduce human and random errors by using computer-based software and computations, is one advantageous feature of their study.

In summary, clinical results of our study are consistent with previous studies indicating that pre-operative amoxicillin, with or without clavulanic acid, does not significantly impact post-operative clinical outcomes such as patient convenience, mouth opening, and pain (Mendes et al., 2023; Sologova et al., 2022). However, another study found that prophylactic antibiotics reduced the need for painkillers (Yanine et al., 2021), which was not evaluated in our study. While other articles have shown that pre-operative antibiotics can reduce the risk of surgical site infections (Arteagoitia, Barbier, et al., 2016; Cao et al., 2023; Marcussen et al., 2016; Mariscal-Cazalla et al., 2021), our study observed only one infection, and the rate of surgery site infection was not significantly different between the two groups. In contrast to our findings, Torof et al.'s meta-analysis revealed a 69% reduction in infection risk in groups treated with antibiotics before or after surgery. However, similar to our study, their results indicated that different antibiotic regimens did not affect intraoral inflammation, wound dehiscence, lymphadenopathy, post-operative facial edema, trismus, or post-operative pain (Torof et al., 2023).

By ensuring adequate accessibility, visibility, and the use of appropriate surgical procedures and instruments, assisted by high-quality radiographs and a precise assessment of the surgical difficulty, dentists play a critical role in reducing the risk of infection following third molar surgeries (Arteagoitia, Barbier, et al., 2016; Dym & Weiss, 2012; Ku et al., 2020). Furthermore, lowering the oral bacterial load before to surgery with chlorhexidine mouthwash or gels is a valid strategy to lessen difficulties following surgery (Coello-Gómez et al., 2018; Torres-Lagares et

al., 2006). For instance, Chugh et al.'s published research results demonstrated that a chlorhexidine irrigation local delivery system with a curved plastic needle on a syringe can be just as effective as taking oral amoxicillin with clavulanic acid before surgery (500 mg of amoxicillin plus 125 mg of clavulanic acid one hour prior to surgery) and three days following the operation (same antibiotic three times per day) in order to reduce discomfort, edema, and trismus by helping patients irrigating and cleaning the surgical site and tooth socket properly (Chugh et al., 2024). This discovery is crucial because it offers a simple and alternative method of replacing antibiotic treatments in regular third molar extractions.

Furthermore, studies have highlighted that the length of the procedure, the surgeon's skill, and the degree of tissue damage sustained during extraction are important variables affecting the problems that follow surgery (Herrera-Barraza et al., 2022; Torof et al., 2023). All surgeries in our research were performed by a highly skilled oral surgeon in order to minimize these variables.

Despite the significant difference founded in the Difficulty Index between the two groups in our investigation, the surgery duration was comparable and indifferent. However, similar to the findings of Mobilio et al., we observed that the duration of surgery is correlated with acute postoperative symptoms such as facial swelling and changes in maximum mouth opening (Mobilio et al., 2017). Additionally, performing an osteotomy can lead to more long-term pain and short-term facial swelling and MMO changes, which have been identified as significant factors in postoperative complications in previous studies (Lupi et al., 2021; K. B. Marcussen et al., 2016).

Dry socket or alveolar osteitis, which is a non-life-threatening post-surgery complication, with an incidence rate of 0.5% to 5%, is treated by antibiotics, while scientists have not found the main pathogenesis and aetiology of this problem (Aguilar-Durán et al., 2019; Camps-Font et al., 2024). In addition, the incidence of infection after third molar surgery ranges from 1% to 16% (Arora et al., 2014). As we mentioned before, research has indicated that even in cases where antibiotic therapy successfully prevents infections or dry sockets after third molar surgeries, a significant number of patients must be treated in order to reap this benefit (Camps-Font et al., 2024).

Given the importance of preventing antibiotic resistance (*Global antimicrobial resistance and use surveillance system (GLASS) report 2022. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO.*) and the low incidence of infections after third molar surgeries in healthy individuals (Menon, Kar yan, et al., 2019), it is essential to consider patients' systemic and local conditions when prescribing antibiotics before or after surgery (Cervino et al., 2019).

Other factors that significantly influence the rate of post-operative complications, such as infection and alveolitis, include patient characteristics and medical comorbidities (Arteagoitia, Barbier, et al., 2016). While antibiotic prophylaxis is effective in high-risk patients, like those with cardiac diseases or in risk of osteonecrosis (Isiordia-Espinoza et al., 2015; Straub et al., 2024), or in cases of active local infections (Camps-Font et al., 2024; Knezevic et al., 2018; Martin et al., 2005), the overuse and misuse of antibiotics in dentistry, along with patients' non-adherence to prescriptions (Contaldo et al., 2023), have led researchers to explore the use of local antibiotics. For instance, using platelet-rich fibrin (PRF) with local antibiotics has been found to be as effective as systemic antibiotics in reducing postoperative complications, without the associated risks of systemic antibiotic use (Donmezer & Bilginaylar, 2021). Furthermore, in 2021, Verma et al. discovered that antibiotic microbeads impregnated containing 500 mg of amoxicillin and 100 mg of clavulanic acid, when embedded in the surgical site and extraction socket, can be just as beneficial in lowering pain, swelling, and infection rate as oral amoxicillin 500mg with clavulanic acid 125 mg three times a day for three days post-surgery (Verma et al., 2022).

Antibiotic prophylaxis has been shown to be beneficial in high-risk patients (Arteagoitia, Barbier, et al., 2016), as was indicated in the previous paragraph, and it is essential to prevent infective endocarditis in patients having dental extraction procedures (Thornhill et al., 2022). Antibiotics are also necessary for the healing of severely infected teeth. We chose healthy individuals with sound third molars who were at no risk of developing infectious endocarditis in order to secure the health and safety of these patient groups and due to ethical considerations. By doing this, we were able to confirm that the participants who took the placebo capsules did not require them due to any local or systemic conditions.

6.2. LABORATORY PART

Only the clinical features of inflammatory problems after third molar procedures have been the subject of the majority of published investigations (Mendes et al., 2023; Rohit & Reddy, 2014; Stošić, Šarčev, et al., 2022). Our findings are remarkable because, as far as we are able to keep track, no prior research has investigated the effect of prophylactic antibiotic medication on salivary PGE2 levels.

According to Cei et al., the surgical removal of third molars causes a mild systemic inflammatory response that lasts for one week, peaks at 24 hours, and then fades away after two months (Cei et al., 2012). Urine and saliva are two other bodily fluids that exhibit this inflammatory reaction. Mohn et al. reported that the highest salivary PGE2 levels occurred 24 hours post-extraction, significantly higher than at 48 and 72 hours after the surgery (Mohn et al., 2015). In light of these results, we made the decision to assess salivary PGE2 levels at follow-up visits 24 hours and seven days after surgery.

Studies have shown that the removal of impacted or semi-impacted teeth is associated with elevated inflammatory responses comparing with simple extraction (Graziani et al., 2017). Factors such as impaction level, periodontal diseases, and pre-existing infections contribute to increased inflammatory complications following third molar removal (Chuang et al., 2008). To minimize these effects, besides the ethical issues mentioned before, we selected sound impacted third molars with healthy periodontal status to be included in this study.

Our results indicated that the difficulty index of the surgery did not correlate with PGE2 concentration. Interestingly, longer surgery duration was associated with lower PGE2 concentrations in saliva seven days post-surgery, suggesting a quicker return to normal levels. This finding aligns with previous studies, which also found no correlation between the difficulty index of the surgery and levels of salivary amylase or blood inflammatory factors (Jumaah & Al-Adili, 2022; B. Sainz de Baranda, F. J. Silvestre, & J. Silvestre-Rangil, 2019). However, we discovered a correlation between decreased PGE2 values 24 hours after surgery and performing tooth sectioning during surgery. This could be because less pressure was used to remove the tooth fragments from the socket, which might result in less inflammatory reactions.

However, there appears to be a problem with the difficulty index that was used in both our study and earlier research. This technique for calculating the difficulty index assesses the third molar's depth of impaction, angulation, and relationship to the mandibular ramus' mesial wall. Even though these factors obviously influence how difficult it is to extract the mandibular third molar, other characteristics, such as the density of bone and the shape and curvature of the roots, as well as how they relate to one another, can make the procedure more or less challenging. In addition, mandibular alveolar nerve place and its relationship to the third molar roots are another crucial element during tooth removal. It is advised to carefully examine the panoramic radiograph or CBCT in order to identify abnormal root shapes, such as those depicted in Figure 36, and to evaluate other surgical aspects as well.

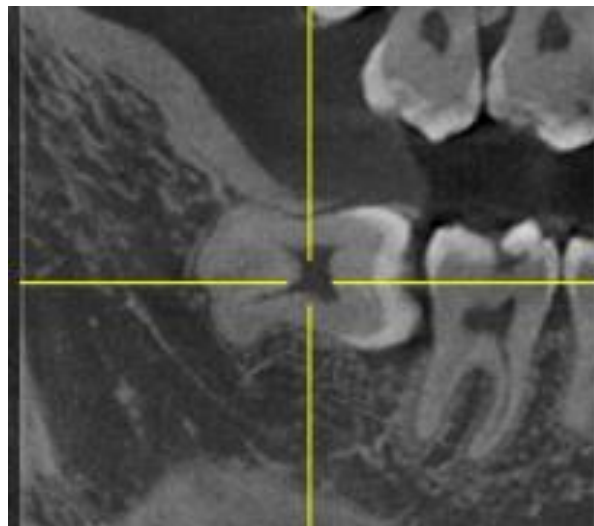


Figure 36. Abnormal root shapes in the third mandibular molar, which can affect the surgical difficulty. Author's elaboration

Salivary prostaglandins are essential for oral wound healing, with PGE₂ playing a significant role in bone fracture healing (Mohn et al., 2015; Mohn et al., 2018). According to Wu-Wang et al., the development of recurrent aphthous stomatitis is associated with decreased levels of PGE₂ in saliva (Wu-Wang et al., 1995). Additionally, PGE₂ serves as a measurable post-traumatic marker for evaluating pain, fever, and inflammation. Although it is challenging to measure PGE₂ accurately in the blood, the scientific community recommends saliva as a well-accepted sample for its measurement (Mehra et al., 2013). Previous studies

have shown that salivary PGE2 levels increase in smokers and individuals with periodontal diseases (Almeslet et al., 2024; Sánchez et al., 2013). Zetner K. et al. reported decreased levels of PGE2 in dogs after 14 days of antibiotic treatment for periodontitis (Zetner et al., 2002). However, our double-blinded, randomized clinical trial showed that antibiotic therapy administered one hour before mandibular third molar extraction surgery does not alter the inflammatory parameter, PGE2, in saliva.

As previously mentioned, PGE2 is a valuable marker for post-traumatic pain (Mehra et al., 2013). Our results confirmed this, showing that PGE2 concentrations correlated with reported pain from 48 hours to 5 days after third molar surgery. Patients with higher baseline concentrations of PGE2 experienced more pain. However, Chandini R. et al. found no correlation between another salivary inflammatory factor, α -amylase, and reported pain after surgery. They measured α -amylase in salivary samples immediately after surgery, which is too soon to assess the inflammatory factors that cause pain (Chandini et al., 2022).

Additionally, one study showed that PGE2 levels are higher in females than in males (Maan & Patil, 2019). However, in our study, baseline PGE2 concentrations were similar between the two groups and genders.

It is important to note one drawback of salivary samples: despite their simplicity, non-invasive collection method, and other positive aspects mentioned in the introduction, the concentration of salivary markers is more variable than serum factors, and there is no definitive normal range for them (Thomas et al., 2009). However, as previously stated, salivary samples were a preferable option for our investigation because PGE2 cannot be accurately detected in blood samples (Mehra et al., 2013).

Our clinical trial results supported the null hypothesis, demonstrating no significant effects of antibiotics on salivary and clinical inflammation following third mandibular molar surgery. Consequently, it is crucial to emphasize reducing antibiotic prescriptions for healthy individuals without infection risks in such cases.

VII – CONCLUSIONS

VII - CONCLUSIONS

This thesis has evaluated the effect of antibiotic prophylaxis on the inflammatory factor (PGE2) in salivary samples following mandibular third molar surgery. The primary objectives were to compare postoperative concentrations of PGE2 and subjective pain assessments between the experimental and placebo groups.

According to this study's findings and limitations, taking two grams of amoxicillin two hours prior to surgery did not significantly alter postoperative salivary PGE2 concentrations or experienced pain in healthy patients with sound gingiva and mandibular third molars. Furthermore, there were no significant differences in the groups' maximum mouth opening or facial swelling, suggesting that giving an antibiotic prior to the extraction of the third molar cannot lessen the patient's postoperative surgical consequences. These results are noteworthy because they imply that prophylactic antibiotics might not have the anticipated effects on lowering pain or inflammation following third molar surgery.

The study also investigated several minor objectives, including estimating the difficulty index of the surgery, measuring baseline and postoperative concentrations of PGE2, and assessing correlations between clinical outcomes and various variables. The results revealed that while the difficulty index of the surgeries in the placebo group was more than experimental group, surgery duration was similar in both groups and no significant difference was found between the PGE2 concentrations in different times in two groups.

Correlation assessments showed that age, gender, number of sutures, and performing osteotomy were associated with reported pain at different time points. Surgery duration and performing osteotomy correlated with 24-hour facial swelling, while surgery duration and difficulty index were related to short-term difficulties in mouth opening. Performing osteotomy was also correlated with seven-day MMO changes. Additionally, surgery duration and performing tooth section were correlated with PGE2 levels after seven days and 24 hours, respectively. A correlation between pain and PGE2 was detected, although PGE2 concentration did not correlate with facial swelling or MMO changes.

This study is the first to evaluate the salivary inflammatory factor to assess the effect of antibiotics on postoperative inflammatory reactions, and it utilized a reliable and repeatable measure for evaluating the postoperative swelling instead of visual analogue scales. Furthermore, daily pain measurements allowed for a thorough evaluation of postoperative suffering.

All things considered, the results of this study advance our knowledge of the function of preventative antibiotics in third molar surgery and imply that their use could not have a major effect on postoperative pain or inflammation. Future research with larger sample sizes and diverse populations is needed to further explore these relationships and validate the findings.

VIII – LIMITATIONS AND FUTURE WORK

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8.1. SAMPLE COLLECTION AND SIZE

In our study, we implemented strict inclusion and exclusion criteria to achieve controlled conditions for evaluating PGE2 in saliva and to limit differences between the two groups to antibiotic use before third molar extraction. Participants had to be healthy individuals with sound gingiva and teeth, with no history of inflammatory diseases in the four months preceding surgery. Additionally, those who took anti-inflammatories, antibiotics, or contraceptives during the month before surgery were excluded. These stringent criteria resulted in a small pool of eligible participants.

Moreover, participants had to agree not to take any antibiotics or anti-inflammatory medications post-surgery, relying solely on analgesics for pain management. This requirement proved challenging, as eighteen potential participants were unwilling to forgo these medications, believing that painkillers alone would be insufficient for managing their pain and inflammation. This highlights the need for more extensive community education about the adverse effects of long-term and unreasonable use of antibiotics and anti-inflammatories.

Small sample size derived from the formula which made the study feasible. Given small sample size, the low power of the study in both clinical (12.18%) and laboratory (20.43%) parts, and the medium effect size observed in PGE2 concentrations 24 hours post-surgery, it is likely that a significant difference between the two groups could be detected with a larger sample size. Therefore, it is recommend repeating the study with a larger sample to validate these findings.

8.2. OTHER LIMITATIONS

Another limitation was the timing of the surgeries each day. Due to the schedule of the single surgeon performing all surgeries to control for the effect of surgical experience on postoperative complications and the availability of the participants, surgeries were conducted at different times. It is recommended to perform surgeries at a consistent time each day to better control PGE2

concentration levels in different patients. However, since the baseline concentrations of PGE2 were similar in both groups, this issue did not affect our results.

Human error, such as mistakes in reading the ruler or recording data, and random error, which includes unpredictable variations due to slight hand movements, are possible when one investigator performs the assessments. While limiting evaluations to one person helps control the method and consistency, it is advisable to repeat the measurements by one or two persons in facial dimensions analysis and difficulty index assessment on the CBCT to minimize human and random error. This feature was missed in our study, and we strongly recommend implementing it in future studies

In studies with human subjects, it is impossible to control some patient-related conditions. For example, in our investigation, we asked participants not to take analgesics and to follow the post-surgery instructions provided in the methodology section. However, we could not control their situation at home or work. Some participants had to work after the surgery, which led to less post-surgery self-care and possibly more facial swelling or pain, while others went home and rested, which could affect facial swelling. This limitation is almost impossible to control in clinical studies unless conducted in a hospital setting with hospitalized patients.

Pain is a subjective variable, and people have different thresholds for expressing and enduring it. Although VAS and a 11-point scale have been agreed to be useful tools for assessing pain levels (E. K. Breivik et al., 2000), recording the number and type of analgesics taken by patients and the intervals between taking painkiller pills are additional helpful methods for assessing pain experienced by the patient. This aspect was missed in our investigation and is recommended for implementation in future studies.

In this study, the length of the surgery was taken into consideration without the sutures being placed since we allowed the patient to close their mouth after extracting the final piece of the tooth while preparing the sutures. However, the results were unaffected by this issue, because the number of sutures was considered as a variable. By the way, in other studies, the length of surgery was measured until the last suture (Stošić, Šarčev, Mirković, Bajkin, et al., 2022). This

may be taken into account in future research since it may be significant because it causes patients to keep their mouth open for a longer period of time.

IX – BIBLIOGRAPHICAL REFERENCES

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X – APPENDIXES

X - APPENDIXES

APPENDIX 1. Ethical committee approval



UCAM ETHICS COMMITTEE

PROJECT DATA

Title:	"Evaluation of the effect of Antibiotic prophylaxis on inflammatory factor in salivary samples after third molars surgery"	
Principle Researcher	Name	Email
PhD.	José Eduardo Maté Sánchez de Val	jemate@ucam.edu

COMITTEE REPORT

Date	30/03/2023	Code	CE032315
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Type of Experimentation

Experimental clinical research involving human subjects	X
Non-clinical experimental research with humans	
Using human tissues from patients, healthy people, embryonic or fetal tissue	
Using human tissues, embryonic or fetal tissue from banks or tissue samples	
Use of personal data, genetic information, etc.	X
Animal studies	
Use of biological agents of risk to human health, animal or plant	
Use of genetically modified organisms (GMOs)	

Comments regarding the type of experimentation
No comments

Comments regarding the methodology of experimentation
No comments



APPENDIX 2. Ethical committee approval



UCAM ETHICS COMMITTEE

Suggestions for the researcher

In view of the application of the attached report by the Researcher and the above mentioned recommendations, the opinion of the Committee is to:

Issue a favorable report	☒
Issue an unfavorable report	☐
Issue a favorable report with subject to correction	☐

MOTIVATION

It will increase knowledge in this area

Approved by the President,

Sig.: José Alberto Cánovas Sánchez



Approved by the Secretary,

Sig.: José Alarcón Teruel

APPENDIX 2. Information sheet for participants



ANEXO V

DOCUMENTO DE INFORMACIÓN PARA SUJETOS SOMETIDOS A ESTUDIO
(HOJA INFORMATIVA)

1. EN QUÉ CONSISTE Y PARA QUÉ SIRVE:

Este proyecto mide los factores inflamatorios en la saliva tras una cirugía de terceros molares. Los investigadores quieren evaluar el efecto de los antibióticos en los parámetros inflamatorios de la saliva tras una cirugía dental.

2. COMO SE REALIZA:

Los participantes se dividirán en grupos aleatoriamente. Según su grupo, recibirán antibióticos (Amoxicilina, 2 g, 1 hora antes de la cirugía) o un placebo (materiales no eficaces con forma de cápsula, 1 hora antes de la cirugía). Los investigadores necesitan algunos datos sobre su estado general de salud. Además, te piden que participes en sesiones de seguimiento para cuidarte. A partir de ahí, registran datos sobre la inflamación, el dolor y las probables infecciones en el sitio de la cirugía. Se necesitan tres muestras de saliva: 1. Antes de la intervención, 2. 24 horas después de la intervención y 3. Siete días después de la intervención. Buscamos la concentración de PGE2 (un factor inflamatorio) en las muestras de saliva.

3. QUÉ EFECTOS LE PRODUCIRÁ:

Todo el proceso no tiene ningún efecto adicional en los pacientes. La amoxicilina es un antibiótico común que se utiliza desde hace mucho tiempo. La extracción del tercer molar es una intervención sencilla que realiza un dentista profesional bajo anestesia local. Las complicaciones más frecuentes son dolor en las 72 horas siguientes a la intervención, hinchazón e inflamación. Durante el estudio, se le permite tomar el medicamento paliativo (paracetamol 500) para reducir el dolor.

4. EN QUÉ LE BENEFICIARÁ:

Al participar en este proyecto, un cirujano experimentado le operará de los terceros molares. Se beneficiará de los cuidados de los investigadores tras la cirugía durante siete días para anotar su dolor, edema y otras complicaciones.

5. QUÉ RIESGOS TIENE:

5.1 LOS MÁS FRECUENTES: dolor, hemorragia, trismo, edema, infección, osteítis alveolar, retraso en la cicatrización y alteraciones sensoriales

5.2 LOS MÁS GRAVES:

Reacciones alérgicas a los fármacos (informe a los investigadores si tiene antecedentes de reacciones alérgicas), Fracturas óseas durante la intervención (poco frecuentes), Lesiones nerviosas sensoriales temporales o permanentes

APPENDIX 2. Information sheet for participants**6. SITUACIONES ESPECIALES QUE DEBEN SER TENIDAS EN CUENTA:**

Por favor, informe a los investigadores si ha tenido algún problema médico/dental o ha consumido algún fármaco/medicamento durante los últimos cuatro meses. Llame a los investigadores si tiene algún problema durante el proyecto.

Tiene derecho a abandonar el proyecto en cualquier momento.

7. OTRAS INFORMACIONES DE INTERÉS (a considerar por el/la profesional)

Para obtener resultados precisos y fiables, le pedimos que nos facilite con confianza todos los datos necesarios. Por favor, anote completamente en los formularios todos los datos que le pidan los investigadores.

8. OTRAS CUESTIONES PARA LAS QUE LE PEDIMOS SU CONSENTIMIENTO

Utilizaremos los datos de esta investigación para analizarlos y publicar un artículo. Sin embargo, todos los datos se utilizarán sin ninguna información identificativa. Esto significa que no publicaremos su nombre, apellidos ni ningún dato privado.

APPENDIX 3. Informed consent

ANEXO IICONSENTIMIENTO INFORMADO

Yo, ..., con DNI:.....

DECLARO:

Haber sido informado/a del estudio y procedimientos de la investigación del Proyecto titulado: **Evaluación del efecto de la profilaxis antibiótica sobre el factor inflamatorio en muestras salivales tras cirugía de terceros molares**

Los investigadores que van a acceder a mis datos personales y a los resultados de las pruebas son: **Prof. Dr. José Eduardo Maté Sánchez de Val, Prof. Dr. Carlos Pérez-Albacete Martínez, Fatemeh Soleymani**

Asimismo, he podido hacer preguntas del estudio, comprendiendo que me presto de forma voluntaria al mismo y que en cualquier momento puedo abandonarlo sin que me suponga perjuicio de ningún tipo.

CONSIENTO:

1.-) Someterme a las siguientes pruebas: **Cirugía del tercer molar, Tres muestras de saliva**

2.-) El uso de los datos obtenidos según lo indicado en el párrafo siguiente:

En cumplimiento del Reglamento (UE) 2016/679 del Parlamento Europeo y del Consejo, de 27 de abril de 2016 y Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y Garantía de los Derechos Digitales, le comunicamos que la información que ha facilitado y la obtenida como consecuencia de las pruebas a las que se va a someter pasará a formar parte del fichero automatizado INVESALUD, cuyo titular es la FUNDACIÓN UNIVERSITARIA SAN ANTONIO, con la finalidad de INVESTIGACIÓN Y DOCENCIA EN LAS ÁREAS DE CONOCIMIENTO CIENCIAS EXPERIMENTALES Y CIENCIAS DE LA SALUD. Tiene derecho a acceder a esta información y cancelarla o rectificarla, dirigiéndose al domicilio de la entidad, en Avda. de los Jerónimos de Guadalupe 30107 (Murcia). Esta entidad le garantiza la adopción de las medidas oportunas para asegurar el tratamiento confidencial de dichos datos.

En Guadalupe (Murcia) a.....de.....de 202

El / La participante,

El / La investigador/a,

Fdo:.....

Fdo:.....

APPENDIX 4. www.clinicaltrials.gov registration

This registration is available at:

<https://clinicaltrials.gov/study/NCT06613776?locStr=Murcia,%20Spain&country=Spain&state=Region%20of%20Murcia&city=Murcia&rank=4>

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Completed ⓘ

Evaluation of the Effect of Antibiotic Prophylaxis (2g Amoxicillin One Hour Before Surgery) on an Inflammatory Factor (PGE2) in Salivary Samples After Third Molars Surgery

ClinicalTrials.gov ID ⓘ NCT06613776

Sponsor ⓘ Universidad Católica San Antonio de Murcia

Information provided by ⓘ Carlos Pérez-Albacete Martínez, Universidad Católica San Antonio de Murcia (Responsible Party)

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