RESEARCH PROJECT

PROJECT OVERVIEW

1. Study title

Non-alveolar vestibular bone sites for temporary anchorage devices in orthodontics: a quantitative and qualitative analysis

2. Study investigators

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3. Introduction

The study titled "Non-alveolar vestibular bone sites for temporary anchorage devices in orthodontics: a quantitative and qualitative analysis" aims to evaluate the suitability of infrazygomatic crest (IZC) and mandibular buccal shelf (MBS) regions for the placement of temporary anchorage devices (TADs) in orthodontic treatment. Conducted at the University of L'Aquila, this research leverages cone beam computed tomography (CBCT) scans that were previously acquired for orthodontic or dental treatment at the practice of Dr. Daniele Parrello. Over the past two decades, TADs have revolutionized orthodontic treatment by offering enhanced patient compliance, anchorage preservation, and simplification of complex procedures. While the interradicular area has traditionally been used for TAD placement, new sites like the IZC and MBS are being explored to avoid root damage and enhance biomechanical advantages.

This study will use CBCT scans to assess bone quantity and quality in these regions across individuals with varying ages, sexes, and craniofacial patterns. By analyzing cortical bone thickness and cancellous bone density, the research aims to determine the adequacy of these sites for TAD placement, hypothesizing that cortical bone thicknesses exceeding 1 mm and higher cancellous bone density contribute to primary stability of TADs.

The expected outcomes include detailed measurements of cortical and total bone thickness, along with an assessment of bone density. These findings will provide critical insights into the feasibility of using IZC and MBS regions for TADs, potentially enhancing clinical practice in orthodontics by identifying optimal anchorage sites.

4. Protocol registration

This study has been registered with the ISRCTN registry under the title 'Evaluation of cortical bone thickness and spongy bone density in non-dental maxillary and mandibular regions for orthodontic mini-implant placement' (Trial ID: ISRCTN12531499). The registration date is 08/05/2024. For more information, please visit the following link: https://www.isrctn.com/ISRCTN12531499

5. Background

In the past two decades, the use of temporary anchorage devices (TADs) has revolutionized orthodontic treatment.[1] TADs have led to a paradigm shift in terms of patient compliance, preservation of anchorage, and the simplification of treatment for various difficult malocclusions. TADs can be inserted into different areas based on therapeutic procedures and bone supply available, such as sections of the edentulous maxilla, the interradicular septum of the dentulous alveolar process, the infra-apical and supra-apical areas, the palate (median or paramedian), the retromolar area, the zygomatic bone, and the basal mandibular body.[2,3]

While the interradicular area is an excellent site for TADs insertion, it limits the versatility of these devices, for example, when the alveolar ridge represents the pathway in which the tooth moves.[4] Additionally, there is a risk of damaging tooth roots when placing TADs in the interradicular area.[5,6] The introduction of palatal TADs has significantly revolutionized the concept of skeletal anchorage in orthodontics. Palatal TADs provide a larger area of cortical bone, allowing for the development of more versatile devices and reducing the risk of root damage compared to interradicular TADs.[7–9] However, their cost is often considerably higher due to patented device planning techniques offered by various companies in the industry.

The constant search for new areas in which to apply TADs has led to the adoption of the infrazygomatic crest (IZC) and mandibular buccal shelf (MBS) as new sites of choice.[10] Biomechanical advantages related to the simplification of techniques normally used to perform complex movements are the main reasons for this adoption.[11] TADs on the IZC are advantageous in hyperdivergent subjects where intrusion of the upper posterior teeth is desired,[12] as well as in cases of class II distalization, which are often associated with molar mesiorotation.[13] The force vector that can be expressed by using TADs on the IZC is more favourable for these types of correction. Further advantages can be provided by TADs applied in the mandibular buccal shelf region, for example, in cases of molar uprighting movements or distalization of the entire lower arch for class III correction.[14]

Recent literature has investigated the bone characteristics of IZC and MBS in relation to sex, age, and different skeletal patterns with the aim of defining the quantity of bone at these sites.[15,16] According to Laursen et al., we know that these characteristics are crucial to obtain the primary stability of skeletal anchorage.[17] The authors have reported that the cortical bone should have a thickness of more than 1 mm in order to achieve a stable TAD placement. On the other hand, at the time of writing, only one author has delved into the qualitative aspects of these sites by investigating bone density.[18] This is surprising, especially considering that, as in implant surgery, in the context of skeletal anchorage in orthodontics, the prior assessment of bone quality at the chosen site, along with other treatment planning parameters, constitutes a crucial factor in achieving good primary stability.[19–21]

6. Aims

This study aims to utilize cone beam computed tomography (CBCT) scanning to assess both bone quantity and quality in the IZC and MBS regions across individuals with varying age, sex, and craniofacial patterns.

By analyzing the regions typically used for the application of TADs, our research question posits the hypothesis that cortical bone thicknesses in IZC and MBS are sufficient to ensure primary stability, specifically above a cut-off value of 1 mm.

Simultaneously, the authors intend to evaluate the cancellous bone density in the same IZC and MBS regions, recognizing that higher density contributes to the enhanced primary stability of these devices.

During the execution of linear measurements, we also aim to record overall bone widths (cortical and cancellous) as typical paths for the insertion of TADs. This data will help determine the average lengths of TADs suitable for different sites.

7. Objectives

- To assess cortical bone thickness in the IZC and MBS regions using CBCT scanning.
- To evaluate cancellous bone density in the IZC and MBS regions through CBCT scanning.
- To measure total bone thickness, including cortical and cancellous components, in the IZC and MBS regions.
- To investigate the association between cortical bone thickness and craniofacial pattern, considering different age and sex demographics as covariates.
- To determine if cortical bone thickness in the IZC and MBS regions meets the threshold for ensuring primary stability of TADs, with a specific cutoff value set at 1 mm.
- To examine the potential influence of age, sex, and craniofacial pattern on cortical bone thickness and overall bone structure in the selected regions.
- To provide comprehensive data on bone quantity and quality in the IZC and MBS regions, contributing to a better understanding of the suitability of these sites for TAD placement in orthodontic treatment.

8. Hypotesis

Primary hypotesis

Null Hypothesis (H0): Cortical bone thicknesses in IZC and MBS are not sufficient to ensure primary stability, specifically above a cut-off value of 1 mm.

Alternative Hypothesis (HA): Cortical bone thicknesses in IZC and MBS are sufficient to ensure primary stability, specifically above a cut-off value of 1 mm.

Secondary hypotheses

1. <u>Cancellous bone density</u>
   H0: Cancellous bone density in IZC and MBS is not high enough to contribute to enhanced primary stability of TADs.
   HA: Cancellous bone density in IZC and MBS is high enough to contribute to enhanced primary stability of TADs.
2. <u>Total bone thickness</u>
   H0: Total bone thickness in IZC and MBS is not wide enough to host most of the TADs that are offered in the market for these specific locations.
   HA: Total bone thickness in IZC and MBS is wide enough to host most of the TADs that are offered in the market for these specific locations.

9. Study setting

Single-centre study conducted at:

Dental Clinic - University of L'Aquila
Via G. Petrini, Building "Rita Levi Montalcini"
67100 L'Aquila Italy

10. Study design

This research will adopt a **retrospective cross-sectional study design**. A retrospective approach allows for the collection and analysis of data from a predefined period, enabling a comprehensive assessment of cortical and cancellous bone characteristics in the IZC and MBS regions.

The cross-sectional nature of the study involves the examination of a single point in time, providing a snapshot of bone quantity and quality across individuals with varying age, sex, and craniofacial patterns. This design facilitates the simultaneous assessment of cortical bone thickness, cancellous bone density, and overall bone structure in the selected regions.

The choice of a retrospective cross-sectional design is justified by its efficiency in exploring associations between cortical bone thickness and craniofacial pattern while accounting for demographic factors such as age and sex. Additionally, this design allows for the investigation of bone characteristics without the need for longitudinal follow-up, making it suitable for the objectives of this study.

By employing a retrospective cross-sectional approach, this research aims to provide valuable insights into the suitability of the IZC and MBS regions for temporary anchorage device placement in orthodontic treatment, contributing to the advancement of clinical practice in the field.

11. Study timeline

The study began with the protocol development phase, which started in December 2022 and is currently ongoing. Once the protocol receives approval from the regional ethics committee (application in progress), the measurement phase will commence. This phase will involve detailed assessments of the radiographic records and is expected to start shortly after ethical approval, continuing until December 2024. Following the measurement phase, data analysis will be conducted, with the study scheduled for completion by February 2025.

Key milestones include:

- **Protocol Development:** December 2022 - Present
- **Ethics Committee Approval:** Application in progress

- **Measurement Phase:** Start date contingent on ethical approval, expected completion by December 2024
- **Data Analysis:** January 2025 - February 2025
- **Study Completion:** February 2025

12. Study population

The study population consists of patients who were referred to a private practice in the city of Legnano, Italy. Patients underwent CBCT scans performed for orthodontic purposes, prosthetic planning, or orthognathic surgery. The total population size comprises 84 subjects, representing the number of CBCT records available for individual patients. There is no subdivision of patients into subgroups.

13. Eligibility criteria

Inclusion criteria

- Radiographic examinations (CBCT) performed for orthodontic purposes, prosthetic planning, or orthognathic surgery.
- Patients in good health.

Exclusion criteria

- Radiographic examinations (CBCT) of patients with congenital craniofacial abnormalities (e.g. micrognathia, cleft lip or palate, etc) or syndromes.
- CBCT images with motion artifacts in the region of interest due to patient movement during the scanning process.
- CBCT images with metal artifacts in the region of interest due to the presence of dental implants, amalgam filling, etc.
- Patients previously undergoing oral rehabilitation with zygomatic implants.
- Patients with a history of fractures in the region of interest.
- Patients with systemic or local pathologies that have an effect on bone metabolism.
- Patients under bisphosphonate therapy.

14. Study outcomes

Primary outcome

- **Cortical Bone Thickness:** The primary outcome of this study is the measurement of cortical bone thickness in the IZC and MBS regions using CBCT scanning. Cortical bone thickness will be assessed to determine its adequacy for ensuring primary stability of TADs, with a specific cutoff value set at 1 mm.

Secondary outcomes

- **Cancellous Bone Density:** Secondary outcome measures include the assessment of cancellous bone density in the IZC and MBS regions using CBCT scanning. A higher cancellous bone density is known to contribute to enhanced primary stability of TADs.
- **Total Bone Thickness:** Another secondary outcome is the measurement of total bone thickness in the IZC and MBS regions. This includes the combined measurement of cortical and cancellous bone thickness to provide comprehensive information on bone structure.

Association factors

- **Craniofacial Pattern:** The association between cortical bone thickness and craniofacial pattern will be explored as a factor potentially influencing primary stability of TADs. Different craniofacial patterns may impact the thickness of cortical bone in the IZC and MBS regions.
- **Covariates:** Patient age and sex will be considered as covariates in the analysis to account for potential demographic influences on cortical bone thickness and overall bone structure.

15. Study procedures

Data sources

This study utilizes pre-existing radiographic records. The CBCT scans were acquired using an i-CAT scanner (Imaging Sciences International, Hatfield, PA, USA) with the following acquisition parameters:

- **Voltage:** 120 kVp
- **Current:** 37 mA
- **Slice Thickness:** 1 mm
- **Acquisition Time:** 40 seconds
- **Voxel Size:** 0.3 mm
- **Field of View:** 23x17 cm

Craniofacial pattern analysis

All CBCT records will be processed and analyzed using a freely available open-source medical image processing platform (3D Slicer v 5.4.0; link: https://slicer.org).[22] Since Kumar et al.[23] demonstrated no significant differences between linear distances and angles assessed with reconstructed lateral cephalogram (RLC) obtained from a CBCT scan and lateral skull teleradiography, we have chosen to obtain 2D images from each CBCT scan through lateral radiographic projection of the entire volume. The RLCs will be analyzed using a web-based digital cephalometric analysis program (WebCeph version 1.5.0, AssembleCircle Corp., Pangyoyeok-ro, Bundang-gu, Seongnam-si, Gyeonggi-do, Republic of Korea) to define their craniofacial pattern.

The Facial Height Ratio (FHR) or Jarabak's quotient will be considered and measured for this purpose.[24] This approach determines the facial type by establishing a correlation between the posterior facial height, which is divided by the anterior facial height and then multiplied by 100. The results are presented as percentages with values below 59% classified as hyperdivergence, values between 59% and 63% falling within the neutral range (normodivergence), and values above 63% indicating hypodivergence.

Additionally, the authors intend to employ the vertical skeletal relationships used by the European Orthodontic Society (EOS) in the context of cephalometric morphological evaluation of clinical cases to be presented at the European Board of Orthodontics (EBO) examination, as per the society's guidelines.[25] These relationships are represented by the following parameters: Maxillary Inclination (S-N/ANS-PNS) within the normal range of 8° ± 3°; Mandibular Inclination (S-N/Go-Gn) within the normal range of 33° ± 2.5°; Vertical Jaw Relation (ANS-PNS/Go-Gn) within the normal range of 25° ± 6°. While the method based on the Facial Height Ratio provides a nonspecific value of subject verticality, not defining which skeletal component is responsible for any alteration, the EBO's vertical skeletal relationships allow for defining which segment is responsible for such alteration.

Standardisation of head position

Due to non-standardised head positions during the acquisition of 3D scans, we may end up with images that are suboptimally oriented or off-axis. This can lead to variations in measurements, making them less reliable compared to reality.[26] To address this issue, it is advisable to subject the DICOM data set to a re-slicing process to visualize sections that are more consistent with the anatomical symmetry of both sides. For this purpose, we will first use the Transforms module, available within the 3D Slicer platform.

In the frontal view, the coronal plane will be rotated around the sagittal axis until the most internal points of the right and left frontozygomatic sutures at the margin of the orbital rim (anatomical reference points ZR and ZL used in posteroanterior cephalometry) are simultaneously positioned on the axial plane. In the lateral view, the sagittal plane will be rotated around the transverse axis until the Frankfurt plane (defined by anatomical reference points Porion and Orbitale on the right side) is parallel to the axial plane. In the axial view, the transverse plane will be rotated around the vertical axis until the mid-sagittal line (the line passing through the center of the foramen magnum and the crista galli) is perpendicular to the coronal plane.

At the end of the reorientation process of the sections, the volume will then be resampled using the Resample Scalar Volume module, which is also part of the 3D Slicer platform. Subsequently, the Crop module will be applied to realign the region of interest (ROI) box with the new spatial coordinates of the resampled volume.

Standardisation of measurements

To standardise measurements on CBCT images and minimise errors, all images will be oriented before the measurement step. For this purpose, the Transforms Reformat Widget Module, available within the 3D Slicer platform, will be used.

For the MBS region, spatial reference planes will be positioned following a specific procedure to ensure repeatability for all subjects and thus make all subsequent measurements reliable. The axial plane (transverse) will initially be positioned so that it is tangent to the furcations of the first and second mandibular molars. The sagittal plane (anteroposterior) will be placed at the center of the dentoalveolar process, passing through the axes of the mesial root of the first mandibular molar and the distal root of the second mandibular molar. At this point, by establishing an origin point along the intersection between the two planes described above and halfway between the two furcations, the axial plane will be rotated along the sagittal axis until it is perpendicular to the sagittal plane. Finally, the coronal plane (vertical) will be oriented so that it is perpendicular to the two above planes and can be slid along the intersection direction to bring it, in the subsequent measurement phase, to the level of the respective long axes of the mesial and distal root of the second mandibular molar.

For the IZC region, spatial reference planes will be arranged using another specific procedure, again in order to ensure consistency across all subjects, thereby enhancing the reliability of subsequent measurements. The axial plane (transverse) will be positioned tangentially at the furcation of the first mandibular molar. The sagittal plane (anteroposterior) will be placed to pass through the furcations of the first and second mandibular molars. These two planes will already be perpendicular to each other. Subsequently, the coronal plane (vertical) will be oriented to be perpendicular to the aforementioned planes. It can then be slid along the intersection direction to align it, in the subsequent measurement phase, with the respective long axes of the mesiobuccal and distobuccal roots of the first and second maxillary molars.

MBS region measurements

The measurement phase of bone thicknesses will then be conducted using the Markups module available within the 3D Slicer platform.

As defined by the studies of Chang and subsequently confirmed by Ghosh, the preferred site for the application of TADs in the MBS is the buccal region adjacent to the roots of the second mandibular molar.[27–29] Measurements in the MBS region will then be taken using four coronal slices. Two of these slices (LMR7 and LDR7) will pass through the centers of the mesial and distal roots of the second molar. The additional two slices (LMPR7 and LDPR7) will be tangential to the proximal surfaces, both mesial and distal, of the same second molar, thus also providing assessment of the proximal regions. In Figure 1, coronal sections as described above are illustrated on the sagittal view of the MBS.

Several studies have measured the apicocoronal depth (bone height) of the MBS by tracing vertical lines at distances of 4, 5, and 6 mm buccally from the cementoenamel junction (CEJ), intersecting the outer cortical bone of the mandible at two points.[30–33] This type of measurement seems appropriate, considering that Ghosh defines the initial insertion point of

TADs in the MBS to be located 2 mm below the mucogingival junction in the alveolar mucosa.[29] Additionally, Chang states that MBS bone screws should be positioned with an axial inclination as parallel as possible to the roots of the first and second mandibular molars.[34] Some studies have also focused on the position of the Inferior Alveolar Nerve Canal (IANC) or its distance from the site of TADs insertion.[32,35] This also appears to be a valid observation, given that the variability of the nerve pathway in the MBS area poses a tangible risk of injury during the insertion of these devices.

Measurements on each coronal radiographic slice will therefore be conducted, simulating the trajectory of TADs in the MBS according to the aforementioned characteristics. The measurement steps will be as follows:

- The sagittal plane and the axial plane will first be taken as the reference lines, and for this purpose, they will both be positioned at the CEJ of the second molar. At this point, on each radiographic slice, starting from where the axial plane and the sagittal plane intersect, three vertical lines will be drawn buccally respectively at 4, 5, and 6 mm from the intersection. These lines, thanks to the previous image orientation phase, will be parallel to the roots of the first and second mandibular molars;
- The thickness of the coronal cortical bone will be measured on these lines, and it will be classified according to the new jawbone classification system recently introduced by Wang et al.[36] into three levels: A, B, and C, with classification intervals of A: > 1.1 mm, B: 0.7-1.1 mm, and C: < 0.7 mm;
- Finally, the bone height (apicocoronal depth) will be measured within the segments between the points of intersection of the vertical lines with the outer cortical bone of the MBS. In the context of this last step, the position of the IANC relative to the measured segment will be observed: a minimum distance of 2 mm will be the limit parameter considered to exclude the risk of nerve damage. In cases of IANC interference, for distances less than 2 mm, the bone height will be measured from the coronal point of intersection with the outer cortical bone, along the vertical line, up to the point of first interference with the IANC. This measurement will then be indicated with a distinctive mark.

Since bone thickness is typically symmetrical on both sides of the mandible in patients with a symmetrical structure, the above measurements will only be carried out on the right side.[37]

In Figure 2, vertical lines and IANC marking as described above are illustrated on the coronal view of the MBS.

IZC region measurements

The preferred site for placing TADs in the IZC has been defined by Liou as the buccal area adjacent to the roots of the first maxillary molar; subsequently, Lin advocated for a more posterior position, buccal to the roots of the second maxillary molar.[38–40] Measurements in the IZC region will then be taken using five coronal slices. Four slices (UMBR6 and UDBR6, as well as UMBR7 and UDBR7) will pass through the centers of the mesiobuccal and distobuccal roots of the first and second molars. The last slice (UPR67) will be tangential to the point of proximal contact between the first and second molars, thus also providing assessment of the proximal region. In Figure 3, coronal sections as described above are illustrated on the sagittal view of the IZC.

Vargas et al. conducted a study on the bone thickness of the IZC, using as the upper limit for their measurements the highest apex between the distobuccal root of the first maxillary molar and the mesiobuccal root of the second maxillary molar.[30] This precaution seems to be appropriate since, according to the indications of Liou and Lin, the initial insertion of TADs in this area should be performed perpendicular to the buccal plate or the tooth axis.[38,39] Establishing this upper limit therefore helps reduce the risk of root damage during TADs placement. It should also be added that Liou and Lin themselves agree that, once the screw tip has penetrated the cortical bone, the TADs must be gradually angled 55-70° relative to the occlusal plane to reach their final position.

Based on the aforementioned characteristics, simulating the trajectory of TADs in the IZC, the measurement phase will be carried out on each individual coronal radiographic slice. This will involve the following steps:

- The axial plane will first be taken as the reference line, and for this purpose, it will be positioned at the highest apex between the mesiobuccal and distobuccal roots of the first and second molars. At this point, on each radiographic slice, starting from where the axial plane intersects the buccal cortical bone of the maxilla, four lines will be drawn at 55, 60, 65, and 70 degrees of angulation relative to the axial plane line;
- The thickness of the outer cortical bone will be measured on these lines, and it will be classified according to the new jawbone classification system recently introduced by Wang et al.[36] into three levels: A, B, and C, with classification intervals of A: > 1.1 mm, B: 0.7-1.1 mm, and C: < 0.7 mm;
- Finally, the total bone thickness will be measured within the segments between the points of intersection of the lines previously drawn with the outer surfaces (buccal and sinus) of the cortical bones of the IZC.

In this case as well, we will consider symmetric bone thickness on both sides of the maxilla, with our assessment focused on the right side.

In Figure 4, lines as described above are illustrated on the coronal view of the IZC.

Cancellous bone density assessment

Finally, within the 3D Slicer platform, the analysis phase of cancellous bone density in the areas previously subjected to linear measurements will be conducted.

The literature on maxillary bone quality has long described and distinguished various types, also defining their predominance in terms of distribution in the four maxillary regions: anterior and posterior maxilla, and anterior and posterior mandible.[41,42] Therefore, we believe that the density of cancellous bone is likely to be the same throughout the MBS or IZC region. For this reason, the density assessment will be performed only once within the MBS and IZC of each patient.

On the selected radiographic slice for assessing cancellous bone density, proceeding along the direction of one of the linear measurements described above, an area 2 mm wide will be delimited using the Segment Editor module, with a length sufficient to ensure that no portions

of cortical bone are included. The 2 mm width was chosen based on the diameter of TADs typically used for the MBS and IZC regions.[29,34] In Figure 5 and 6, areas as described above are illustrated on the coronal views of the MBS and IZC.
Finally, the Segment Statistics module will determine the level of cancellous bone density as a mean value in grayscale (GV). The cancellous bone density will then be classified according to the new mandibular bone classification system recently introduced by Wang et al.[36] into three levels: 1, 2, and 3, with a classification range of 1: > 600 GV (=420g/cm^3), 2: 300-600 GV (=160 g/cm^3-420 g/cm^3), and 3: < 300 GV (=160 g/cm^3).

16. Statistical considerations and data analysis

Sample size and statistical power
The sample size calculation was based on the requirement to identify significant correlations in our dataset. Given that our study involves 84 subjects, we aimed to ensure sufficient statistical power to detect meaningful relationships. The calculation was performed using the mean and standard deviation values for IZC bone thickness from the study by Murugesan et al..[43]
To achieve an 80% power with a type I error rate of 0.05, a sample size of at least 26 subjects is necessary to identify a correlation coefficient of 0.5 or higher. For detecting a smaller correlation coefficient of 0.3, a sample size of 82 subjects is required. Our study population of 84 subjects therefore meets the criteria to robustly analyze correlations between cortical bone thickness, cancellous bone density, and various demographic and craniofacial characteristics (see Figure 7).
This approach ensures that our analyses will have adequate power to detect significant associations, providing a comprehensive understanding of the bone characteristics in the IZC and MBS regions relevant for temporary anchorage device placement in orthodontics.

Statistical methods
The statistical analysis will be conducted using IBM SPSS Statistics for Windows, Version 29.0.2.0 (IBM Corp., Armonk, NY, USA). To analyze the repeated measurements of cortical bone thickness, cancellous bone density, and total bone thickness at different sites (IZC and MBS) within subjects, we will employ repeated measures ANOVA. This method is chosen to account for the within-subject correlation and reduce error variance associated with individual differences, thereby increasing the statistical power of our analysis.
In addition to repeated measures ANOVA, we will perform correlation analysis to examine the relationships between bone characteristics and demographic factors such as age, sex, and craniofacial patterns. This will help identify significant associations and enhance our understanding of the factors influencing bone characteristics.
Furthermore, multivariate linear regression will be used to model the relationships between the primary and secondary outcomes and the various demographic and craniofacial variables.

This comprehensive approach will allow us to assess the impact of multiple predictors simultaneously and control for potential confounding variables.
By utilizing these statistical methods, we aim to provide a thorough analysis of the bone characteristics in the IZC and MBS regions, offering insights relevant to the placement of temporary anchorage devices in orthodontics.

17. Ethical considerations

The study will be conducted in full compliance with the principles of the Declaration of Helsinki, Good Clinical Practice (GCP), and the laws and regulations of Italy. The research will be conducted at the University of L'Aquila, using CBCT scans that were previously acquired for orthodontic purposes, prosthetic planning or orthognathic surgery at the practice of Dr. Daniele Parrello.

Quality assurance of technical aspects
The quality of technical aspects has been ensured through rigorous procedures in the acquisition and analysis of CBCT scans. All scans were performed using standardized protocols to ensure high-resolution images, which are essential for accurate measurements and reliable data.

Potential risks and proposed benefits
The primary potential risk involves the use of previously acquired CBCT scans. However, as the scans are retrospective and anonymized, there are no direct risks to participants. The proposed benefits of the study include enhanced understanding of cortical bone thickness and cancellous bone density in the mandibular and maxillary regions. This knowledge could significantly improve the effectiveness of temporary anchorage devices in orthodontics.

Participants' interests
The interests of the participants are prioritized above those of science and society. The anonymization process ensures that personal data is protected, and all data shared for research purposes will be anonymized to maintain confidentiality.

Liability and informed consent
Responsibility for liability in the event of any injury during the study is covered under the ethical standards of the study, though the nature of the study minimizes any physical risk. Participants have provided informed consent, acknowledging their understanding of the study's purpose and their voluntary participation. This consent was obtained through a detailed information sheet and consent form provided by Dr. Daniele Parrello's practice, ensuring that participants had the opportunity to ask questions and understand the study fully before consenting.

Informed consent process

The informed consent form, signed by all participants, details the study's objectives, procedures, potential risks, and benefits. Participants were informed that their data would be anonymized and used exclusively for research purposes at the University of L'Aquila. The process ensures participants can make an informed decision about their voluntary participation.

Data anonymization and management

Data will be anonymized by assigning unique codes to each participant's information, ensuring privacy and confidentiality. This anonymized data will be securely stored, with paper copies kept in locked files and electronic files protected by encryption and password access. Data extraction methods will ensure non-identifiable information is used, maintaining the highest standards of data security and privacy.

Ethical approval

The ethical approval process is underway with the competent territorial ethics committee for the Abruzzo region (C.Et.R.A.) in Italy. This approval is in line with Italian laws governing clinical research, ensuring the study meets all legal and ethical requirements.

18. References

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19. Appendices

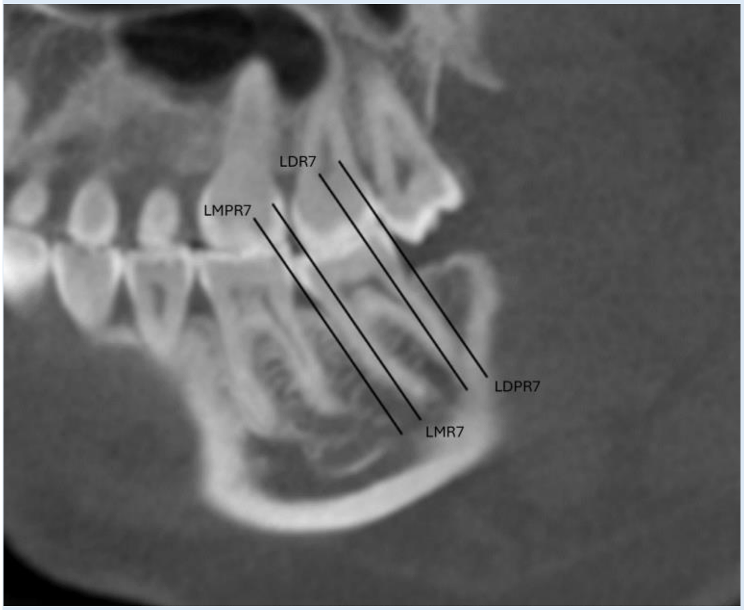

Figure 1: Coronal Sections of the Mandibular Buccal Shelf

Coronal sections (LMR7, LDR7, LMPR7, and LDPR7) illustrating the reference grid within the MBS region. Slices LMR7 and LDR7 pass through the centers of the mesial and distal roots of the second molar. Slices LMPR7 and LDPR7 are tangential to the proximal surfaces of the second molar.

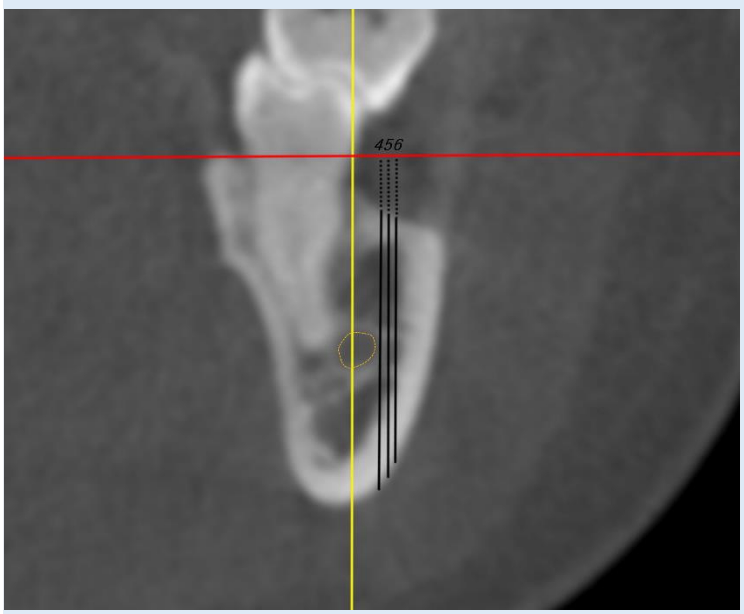

Figure 2: Linear measurements of the Mandibular Buccal Shelf

Three vertical lines will be drawn at distances of 4, 5, and 6 mm from the cementoenamel junction, the point of intersection of the sagittal and axial planes. These lines will be used to perform linear measurements of cortical bone thickness in the coronal region and apicocoronal depth. For the latter measurement, potential interference from the inferior alveolar nerve canal will also be considered.

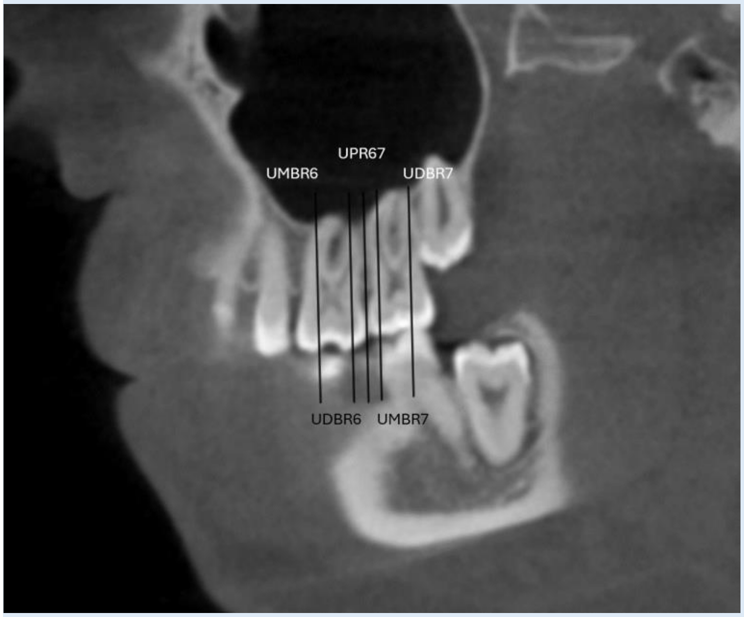

Figure 3: Coronal Sections of the Infrazygomatic Crest

Coronal sections (UMBR6, UDBR6, UPR67, UMBR7, and UDBR7) illustrating the reference grid within the IZC region. Slices UMBR6, UDBR6, UMBR7, and UDBR7 pass through the centers of the mesiobuccal and distobuccal roots of the first and second molars, respectively. Slice UPR67 is tangential to the point of proximal contact between the first and second molars.

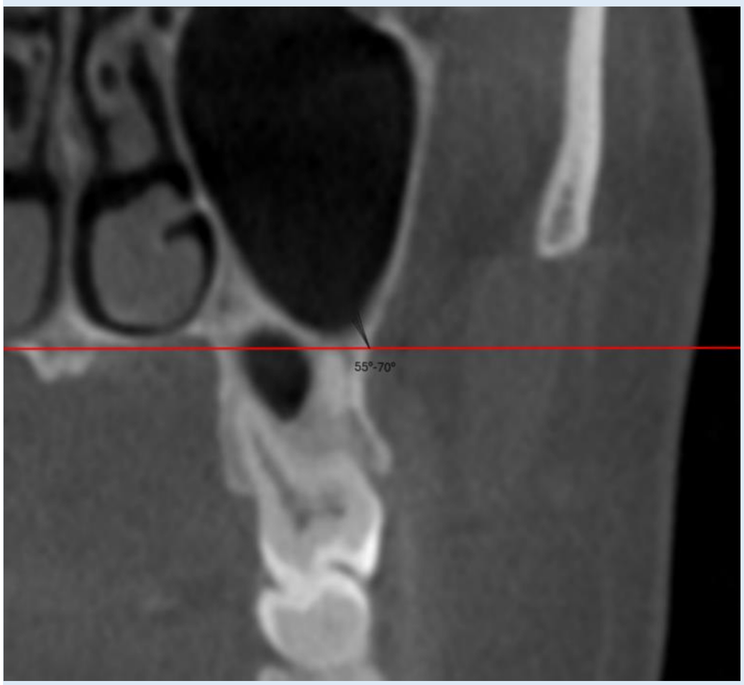

Figure 4: Linear measurements of the Infrazygomatic Crest

The axial plane will be positioned at the highest apex between those of the mesiobuccal and distobuccal roots of the first and second molars. Starting from the intersection point of the axial plane with the vestibular cortical bone, four lines will be drawn respectively at 55, 60, 65, and 70 degrees of angulation relative to the plane itself. These lines will be used to perform linear measurements of the outer cortical bone thickness and the total bone thickness.

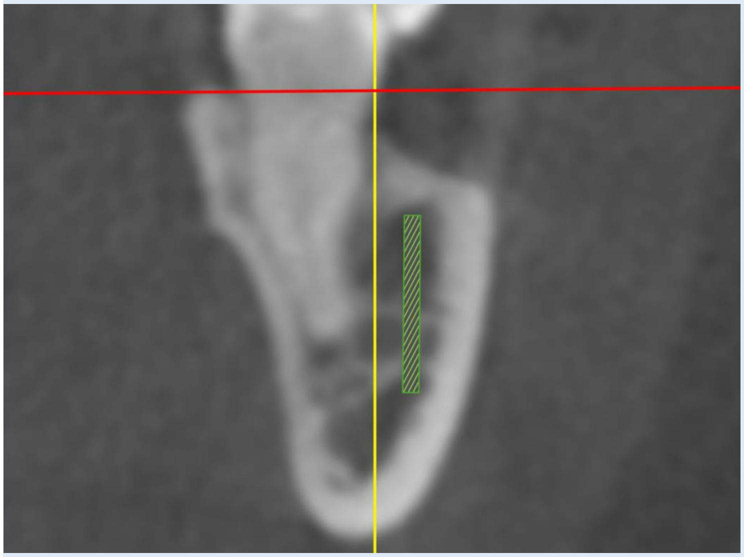

Figure 5: Density measurement of the Mandibular Buccal Shelf

On one of the coronal radiographic slices, an area 2 mm wide will be outlined along the trajectory of one of the previously linear measurements. The length will be adjusted to exclude cortical bone portions.

Figure 6: Density measurement of the Infrazygomatic Crest

On one of the coronal radiographic slices, an area 2 mm wide will be outlined along the trajectory of one of the previously linear measurements. The length will be adjusted to exclude cortical bone portions.

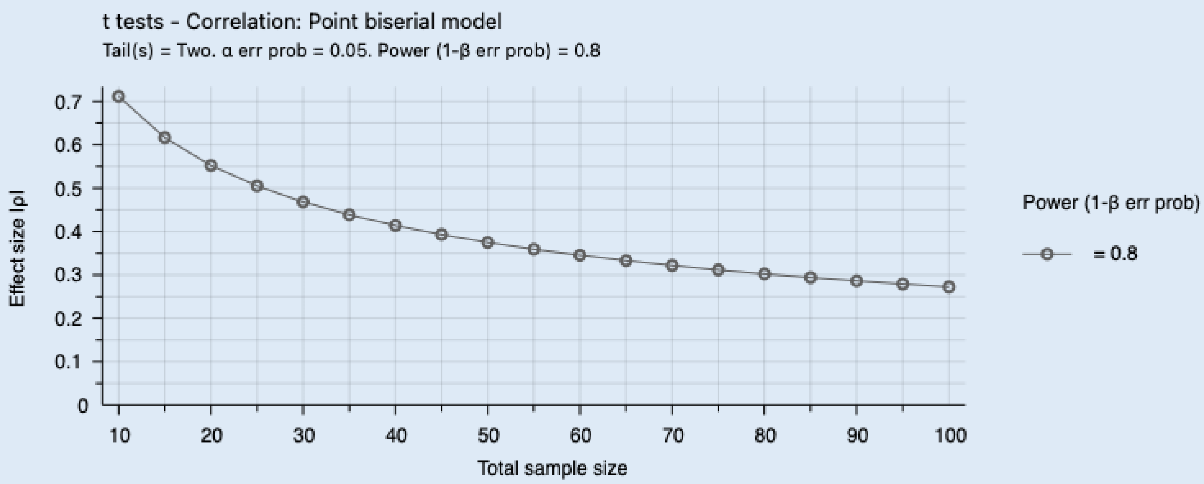

Figure 7: T-test correlation analysis for sample size determination

This graph illustrates the relationship between sample size and the power to detect various correlation coefficients (0.3 and 0.5) with an 80% power and a type I error rate of 0.05.