

Special Issue Reprint

Advances in the Diagnosis of Oral and Maxillofacial Disease

2nd Edition

Edited by
Luís Eduardo Almeida

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Advances in the Diagnosis of Oral and Maxillofacial Disease: 2nd Edition

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Guest Editor

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Basel • Beijing • Wuhan • Barcelona • Belgrade • Novi Sad • Cluj • Manchester

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About the Editor

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Article

Bilateral Condylar Hyperplasia: Importance of Its Diagnosis in the Treatment and Long-Term Stability of Skeletal Class III Correction

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Abstract: Background: Condylar hyperplasia (CH) leads to mandibular overgrowth with anatomical, aesthetic, and functional consequences, particularly affecting facial harmony. It is characterized by severe mandibular prognathism (MP) in bilateral cases. This study aims to propose a therapeutic algorithm for diagnosing and treating bilateral condylar hyperplasia (BCH) based on demographic, clinical, craniofacial growth, and clivus ratio uptake conditions. **Methods:** Ten patients with severe skeletal Class III by MP, whose alteration was clinically associated with BCH, were consecutively evaluated in a specialized dentofacial deformity center between the period of 2019 and 2024. A detailed protocol was followed to gather clinical history, assess anatomical features, evaluate malocclusion, and identify potential BCH. When suspicion arose, a nuclear medicine test measured condylar scintigraphy uptake. If the result was positive, patients underwent bilateral condylectomy, following one of three treatment protocols. **Results:** Severe PM, pronounced Class III with excessive negative overjet, elongated condyles of normal anatomy, absence of family history, and accelerated growth since preadolescence and adolescence were common characteristics in these patients. Regarding the treatment protocol chosen according to the characteristics of the patients, five cases followed treatment protocol A: condylectomy and surgical correction of the alteration in two surgical stages. Two cases followed protocol B: bilateral condylectomy and orthognathic surgery in the same surgical time, and three cases followed protocol C: condylectomy and later post-surgical orthopedics and/or orthodontics without a second surgical intervention. Histopathological results confirmed bilateral hyperplastic growth and stability in mandibular size, and occlusion was observed during follow-up. **Conclusions:** Specialists need to recognize the clinical signs of BCH and use scintigraphy tests to measure condylar metabolic activity when suspected. Early detection of BCH is crucial, as it influences treatment decisions and helps prevent relapses in orthodontic or surgical interventions aimed solely at correcting or compensating for Class III malocclusion caused by MP.

Keywords: hyperplasia; mandibular condyle; radioisotope scanning; diagnostic imaging; mandibular prognathism

1. Introduction

Condylar hyperplasia (CH) affects facial growth due to overgrowth of the mandibular condyle, histologically characterized by an increase in the number of cells and the thickness of the layers that compose the soft condyle [1,2]. Although it is a self-limiting entity, it can be deforming because condylar overgrowth can be present during the whole stage of growth and development and even continue well beyond the residual growth [3], affecting, according to its time of evolution, type of hyperplasia, and severity, structures not only related to the temporomandibular joint (TMJ) (condyle, disc, fossa) but also to the mandibular ramus and producing in the upper jaw, secondary vertical compensations and malocclusions with sagittal, transverse, and vertical compromises [4]. Its etiology may be due to genetic, hormonal, functional, neoplastic, molecular, or traumatic factors [5].

All of the above not only leads to possible functional alterations of the TMJ [6] but also progressive facial asymmetries in the case of unilateral conditions [5,7] and severe mandibular prognathism in the case of bilateral conditions [8].

Although the diagnosis of CH and its different variations depend on a thorough and detailed correlation between extraoral and intraoral clinical features with radiographic and/or tomographic findings [4], the metabolic information of the active state of CH, which represents the subchondral bone growth activity in the mandibular condyle, can only be obtained through nuclear medicine tests, which have evolved from planar bone scans to single photon emission computed tomography (SPECT) [9] and, currently, to SPECT/CT tests, which seek to obtain combined information of the anatomical alteration together with the metabolic activity [10,11].

Regarding bilateral condylar hyperplasia (BCH), its diagnosis and, therefore, its treatment has been a challenge for clinicians because, on the one hand, there is no clarity in the anatomical characteristics of the alteration, the time of onset of the pathology, or the parameters of differentiation with skeletal Class III due to mandibular prognathism (MP), only what was proposed by Wolford et al. in 2014 [8] and the hypotheses of Goulart et al., 2015 [12] and López et al., 2024 [13]. On the other hand, few studies provide reference values for condylar metabolic activity in healthy populations that take the clivus as a reference structure for comparison with the condyle [14–16]. Almost all studies have focused on the percentage comparison of uptake between condyles, and this is useful only in cases of suspected unilateral CH [9].

In turn, the MP, which represents the exaggerated sagittal projection of the mandible, leads to a Class III skeletal and occlusal relationship that affects the facial profile (concave) with esthetic and functional compromise [8]. Although its etiologic factor is associated with the underdevelopment of the upper jaw, overdevelopment of the lower jaw, or a combination of both, in some cases, the excess longitudinal growth of the condyle may be the etiologic factor triggering the alteration [8].

Therefore, the aim of this article is to present a series of cases in which a clinical, anatomical, radiographic, and gammagraphic diagnostic methodology was followed for the identification of BCH according to the available evidence. A therapeutic algorithm for the clinical and metabolic diagnosis of the disorder is also presented, and three treatment protocols for BCH are proposed according to the individual characteristics of each patient.

2. Materials and Methods

All skeletal Class III patients for MP whose prognathism was clinically associated with BCH were retrospectively evaluated from 2019 to 2024. These patients were sent to nuclear medicine to obtain information on the bone metabolic activity of each condyle with respect to the clivus, and, if the result was positive for abnormally active condylar growth, the patients underwent bilateral condylectomy with an extraoral approach in a

center specialized in the treatment of dentofacial deformities. The Declaration of Helsinki guidelines on medical protocol and ethics were followed at all stages of treatment. The conduct of this study did not alter the ethically approved protocol for the diagnosis and treatment of CH and Class III skeletal discrepancy with MP at the specialized center; therefore, it was exempt from the requirement for additional ethical approval.

Patients with any type of syndrome affecting the craniofacial configuration, as well as the mandibular fossa and the development of the middle and lower third, were excluded from this study. Also, patients with a history of craniofacial trauma or previous orthognathic surgeries. To be included, in addition to a complete clinical history, it was indispensable to have a positive SPECT test result and post-surgery histopathological result.

2.1. Diagnostic Evaluation and Treatment Planning

For detecting suspected bilateral condylar hyperplasia (BCH), the specialized center for dentofacial deformities follows this protocol (Figure 1):

1. Clinical History: A detailed history is taken from the patient and family members, focusing on any family history of mandibular prognathism (MP), the onset of disproportionate growth, previous related treatments, TMJ issues, and psycho-functional concerns.
2. Clinical Evaluation: The sagittal relationship of the bony bases, severity of Class III malocclusion, facial profile, growth type, and vertical compromise are assessed.
3. Imaging: Two-dimensional and three-dimensional imaging are used to determine the anatomical characteristics, measure condylar length, mandibular length, and maxillary sagittal size, and compare these with population-specific reference values.
4. SPECT/CT Scan: A SPECT/CT scan of the TMJ is performed, with radiopharmaceutical absorption ratios calculated for each condyle relative to the clivus. The formula used is as follows:

$$\frac{\text{Condylar counts} - \text{Background counts}}{\text{Clivus counts} - \text{Background}}$$

Five transaxial tomographic slices define a fixed region of interest (ROI).

5. Surgical Planning: Surgical planning according to the information collected and the characteristics of the alteration, following three treatment schemes.
 - Protocol A: Bilateral condylectomy via an extraoral approach with a modified endaural incision, followed by bracket placement and orthodontic alignment. After condylectomy, orthodontic decompensation is performed, with a second surgical phase planned using virtual surgical planning. This approach is for patients with no growth potential, severe skeletal discrepancies, and aberrant malocclusions. Post-condylectomy, clinical improvements are noted (Figures 2–4).
 - Protocol B: Bilateral condylectomy and orthognathic surgery performed in one session, followed by post-surgical orthodontics. The surgery order is first the high Le Fort I osteotomy in bilateral step, then the bilateral condylectomies, and then the sagittal osteotomies of the bilateral mandibular ramus, ending with advancement mentoplasty when necessary. This approach is suitable for patients with no growth potential, severe skeletal issues, aberrant malocclusions, and significant psychoemotional distress due to facial appearance. It is indicated for those needing rapid correction to improve their quality of life (Figures 5 and 6).
 - Protocol C: Bilateral high condylectomy followed by maxillary orthopedics (if growth potential exists) or compensatory orthodontics. This approach is for patients with growth potential or mild conditions, where early detection allows for correction using only condylectomy and proper orthodontics without additional surgery (Figures 7–10).

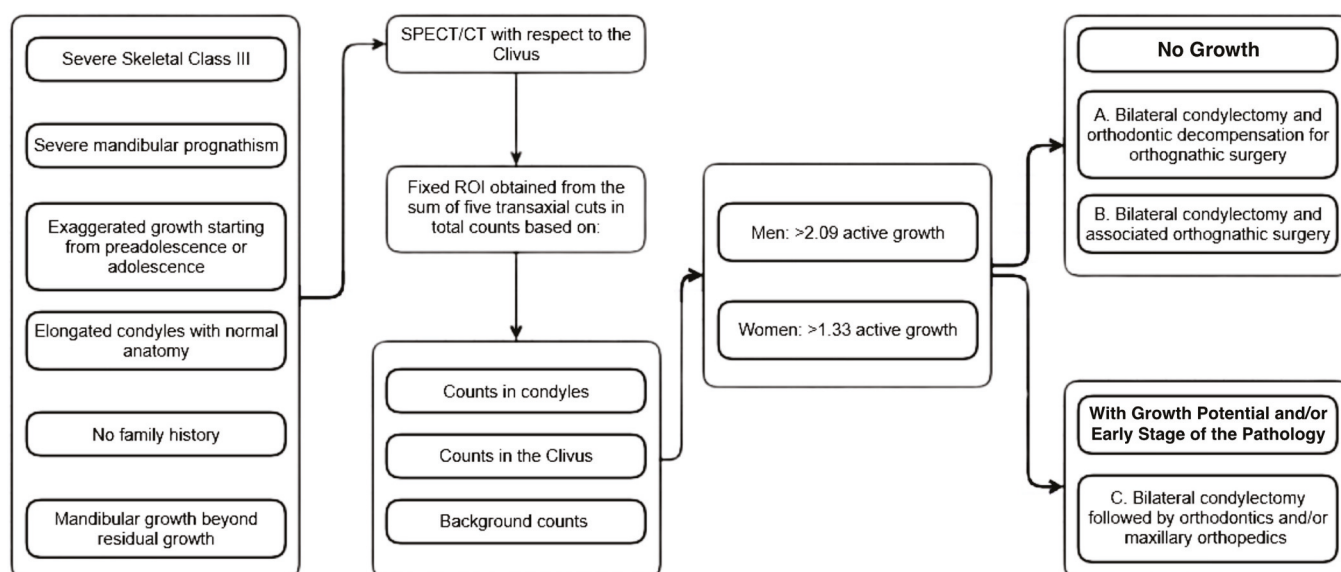


Figure 1. Therapeutic algorithm for identifying and treating bilateral condylar hyperplasia, considering the rate of radiopharmaceutical uptake by each condyle concerning clivus. Values > 2.09 in men and 1.33 in women are suggested to confirm active status, and three treatment protocols are presented.

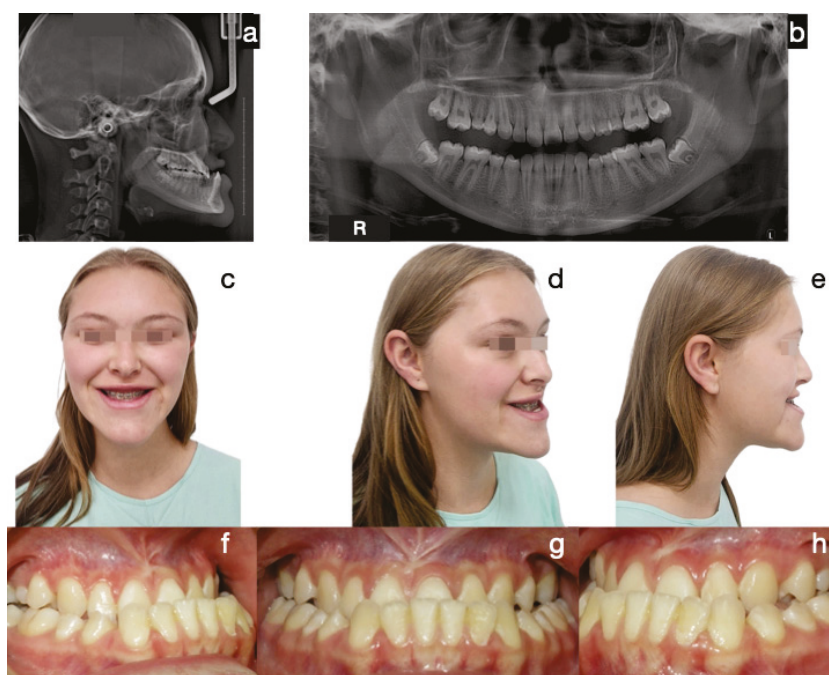


Figure 2. Case 1 corresponds to patient 1, described in Table 1. Protocol A (bilateral condylectomy and orthognathic surgery in two surgical stages): initial photos. The severity of the Class III skeletal and dental discrepancy with negative overjet of -12 mm and concave profile is evidenced. (a) Lateral skull radiograph, (b) panoramic radiograph, (c) frontal extraoral photograph, (d) $3/4$ extraoral photograph, (e) lateral extraoral photograph, (f) right lateral intraoral photograph, (g) frontal intraoral photograph, (h) left lateral intraoral photograph.

Table 1. Demographic characteristics, clinical findings, ratio result, surgical procedure, histopathological results, treatment protocol, and post-surgical follow-up.

Patient	Age	Gender	Clinical Highlights	Ratio Results	Magnitude of the Condilectomy	Histopathological Findings	Treatment Type	Follow Up
1	14	F	Severe mandibular prognathism. Elongated condyles.			Soft condyle thickness.	Type A. Bilateral condylectomy, orthodontic decompensation and 12 months later bimaxillary orthognathic surgery.	12 months
			Accelerated mandibular growth after menarche (13 years). Negative overjet of 12 mm. No family history of Class III.	Right condyle: 1.4 Left condyle: 1.4	Right condyle: 6 mm Left condyle: 6 mm	Right 0.6 mm. Left 0.7 mm. Positive findings for BCH.		
2	12	F	Severe mandibular prognathism. Elongated condyles.			Soft condyle thickness. Right 0.6 mm. Left 0.6 mm.	Type C. Bilateral condylectomy, followed by corrective orthodontics.	36 months
			Accelerated mandibular growth after menarche (11 years). Negative overjet of 8 mm No family history of Class III	Right condyle: 1.38 Left condyle: 1.38	Right condyle: 6 mm Left condyle: 6 mm	Positive findings for BCH.		
3	25	M	Severe mandibular prognathism. Elongated condyles.			Increased thickness of the layers of the soft condyle, without giving thickness value.	Type A. Bilateral condylectomy, orthodontic decompensation and 12 months later bimaxillary orthognathic surgery with mentoplasty.	60 monts
			Accelerated mandibular growth after 15 years of age. Negative overjet of 14 mm. Patient with bicigotic twin presenting unilateral condylar hyperplasia.	Positive report for uptake ratio above normal values in condyles, with respect to clivus. Did not provide value	Right condyle: 5 mm Left condyle: 7 mm	Increased proliferation of hypertrophic chondrocytes on medullary bone. Positive findings for BCH.		

Table 1.
 Cont.

Patient	Age	Gender	Clinical Highlights	Ratio Results	Magnitude of the Condilectomy	Histopathological Findings	Treatment Type	Follow Up
4	30	M	Severe mandibular prognathism. Elongated condyles. Accelerated mandibular growth from the age of 15 years and beyond the age of 20 years. Negative overjet of 12 mm. No family history of Class III. Failed orthodontic treatment.	Positive report for uptake ratio above normal values in condyles, with respect to clivus. Did not provide value	Right condyle: 6 mm Left condyle: 6 mm	Increased thickness of the layers of the soft condyle, without giving thickness value. Increased proliferation of hypertrophic chondrocytes on medullary bone. Positive findings for BCH.	Type B. Bilateral condylectomy, bimaxillary orthognathic surgery and mentoplasty all in the same surgical time.	60 months
5	17	M	Severe mandibular prognathism. Elongated condyles. Accelerated mandibular growth since the age of 14 years. Negative overjet of 16 mm. No family history of Class III. Failed orthopedic treatments. Depressive mood episodes.	Positive report for uptake ratio above normal values in condyles, with respect to clivus. Did not provide value	Right condyle: 6 mm Left condyle: 6 mm	Increased thickness of the layers of the soft condyle, without giving thickness value. Increased proliferation of hypertrophic chondrocytes on medullary bone. Positive findings for BCH.	Type B. Bilateral condylectomy, bimaxillary orthognathic surgery and mentoplasty all in the same surgical time.	60 months

Table 1.
 Cont.

Patient	Age	Gender	Clinical Highlights	Ratio Results	Magnitude of the Condilectomy	Histopathological Findings	Treatment Type	Follow Up
6	18	F	Severe mandibular prognathism. Elongated condyles.	Right condyle: 1.36 Left condyle: 1.36	Right condyle: 6 mm Left condyle: 6 mm	Increased thickness of the layers of the soft condyle, without giving thickness value.	Type A. Bilateral condylectomy, orthodontic decompensation for future bimaxillary orthognathic surgery.	12 months
			Accelerated mandibular growth since the age of 11 years. Hypoplasia of the middle third of the face. Negative overjet of 6 mm. No family history of Class III. Failed orthopedic and orthodontic compensation treatment.			Increased proliferation of hypertrophic chondrocytes on medullary bone. Positive findings for BCH.		
7	16	M	Severe mandibular prognathism. Elongated condyles and posterior divergence.	Positive report for uptake ratio above normal values in condyles, with respect to clivus. Did not provide value	Right condyle: 7 mm Left condyle: 5 mm	Increased thickness of the layers of the soft condyle, without giving thickness value.	Type A. Bilateral condylectomy, orthodontic decompensation for future bimaxillary orthognathic surgery.	24 months
			Accelerated mandibular growth since the age of 14 years. Hypoplasia of the middle third of the face. Negative overjet of 8 mm. No family history of Class III. Low self-perception of facial appearance.			Increased proliferation of hypertrophic chondrocytes on medullary bone. Positive findings for BCH.		

Table 1.
 Cont.

Patient	Age	Gender	Clinical Highlights	Ratio Results	Magnitude of the Condylectomy	Histopathological Findings	Treatment Type	Follow Up
8	17	M	Severe mandibular prognathism.	Right condyle: 2.25 Left condyle: 2.30	Right condyle: 9 mm Left condyle: 10 mm	Increased thickness of the layers of the soft condyle, without giving thickness value. Increased proliferation of hypertrophic chondrocytes on medullary bone. Positive findings for BCH.	Type C. Bilateral condylectomy, followed by corrective orthodontics of malocclusion.	8 months
			Elongated condyles and posterior divergence. Accelerated mandibular growth since the age of 12 years. Hypoplasia of the middle third of the face. Negative overjet of 8 mm. No family history of Class III. Low self-perception of facial appearance.					
9	11	F	Mandibular prognathism.	Right condyle: 1.45 Left condyle: 1.38	Right condyle: 5 mm Left condyle: 5 mm	Soft condyle thickness. Right 0.6 mm. Left 0.6 mm. Positive findings for BCH.	Type C. Bilateral condylectomy, followed by skeletally anchored orthopedics, use of elastics with orthopedic forces and corrective orthodontics of the malocclusion.	48 months
			Elongated and posterior divergent condyles. Accelerated mandibular growth after menarche (11 years). Edge-to-edge bite. Incisive compensation. No family history of Class III.					

Table 1.
 Cont.

Patient	Age	Gender	Clinical Highlights	Ratio Results	Magnitude of the Condilectomy	Histopathological Findings	Treatment Type	Follow Up
10	16	M	Class III since infancy. Severe mandibular prognathism. Elongated condyles. Accelerated growth from the age of 14 years. Negative overjet of 5 mm. Severely retroinclined lower incisors.	Right condyle: 2.1 Left condyle: 2.2	Right condyle: 9 mm Left condyle: 12 mm	Increased thickness of the layers of the soft condyle, without giving thickness value. Increased proliferation of hypertrophic chondrocytes on medullary bone. Positive findings for BCH.	Type A. Bilateral condylectomy, orthodontic decompensation and 9 months later bimaxillary orthognathic surgery.	42 months

F, female; M, male.

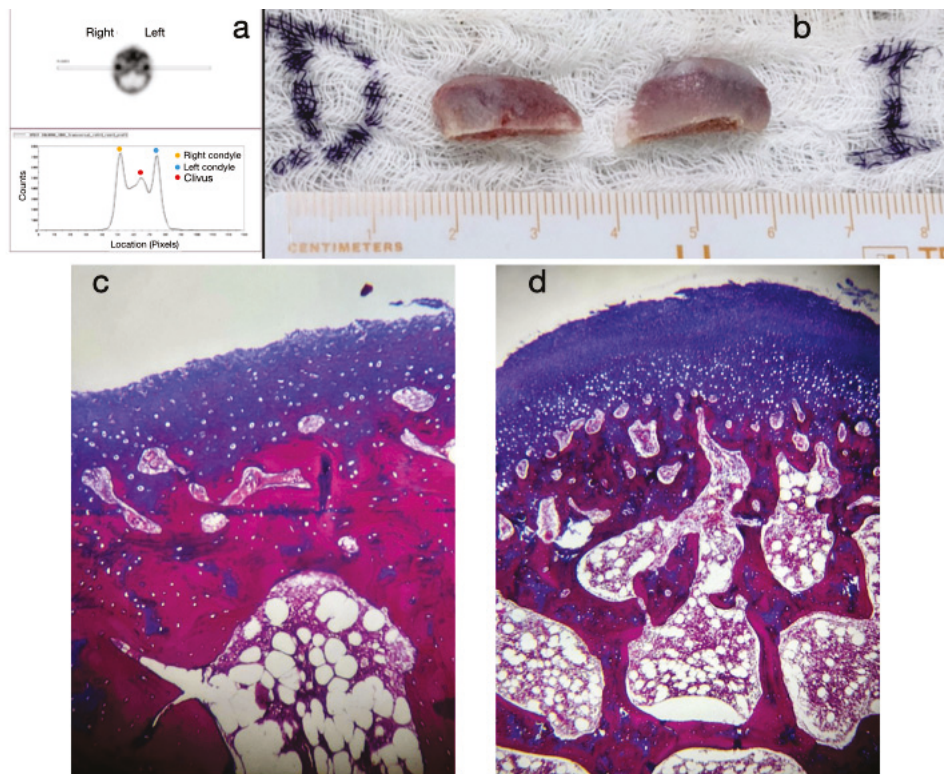


Figure 3. (a) Histogram of the SPECT test with uptake ratio for both condyles to clivus of 1.4; (b) bilateral high postcondylectomy specimens; (c) image of the right histological slice with 0.6 mm thickness in the soft condyle; (d) image of the left histological slice with 0.7 mm thickness in the soft condyle.

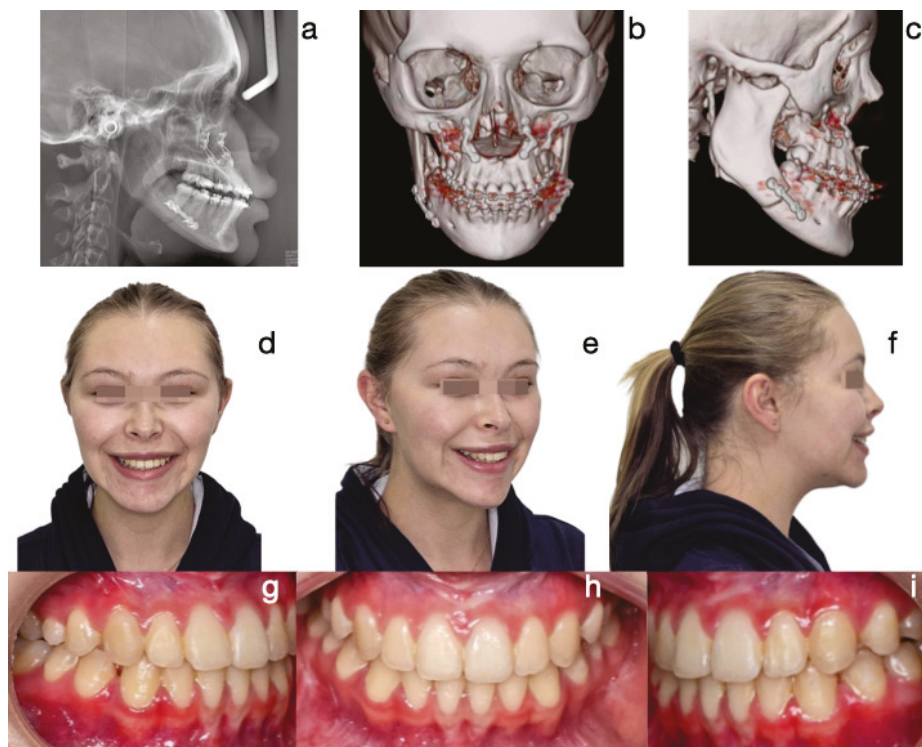


Figure 4. Post-surgery photographs: (a) lateral skull X-ray, (b) frontal CBCT, (c) lateral CBCT. Final photographs. Correction of Class III skeletal and dental discrepancy. Straight facial profile, positive overjet, and stable occlusion. (d) Frontal extraoral photograph, (e) ¾ extraoral photograph, (f) lateral extraoral photograph, (g) lateral right intraoral photograph, (h) frontal intraoral photograph, (i) lateral left intraoral photograph.

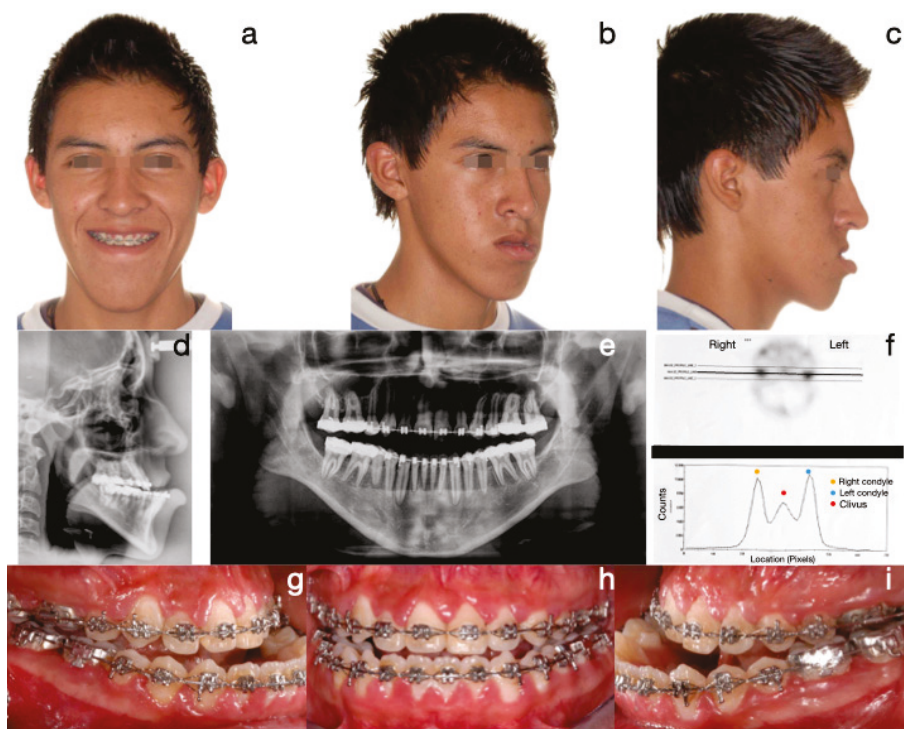


Figure 5. Case 2 corresponds to patient 5, described in Table 1. Protocol B (condylectomy and orthognathic surgery simultaneously): pre-surgical photos. The severity of the Class III skeletal and dental discrepancy with negative overjet of -16 mm and concave profile is evidenced. (a) Frontal extraoral photograph, (b) $3/4$ extraoral photograph, (c) lateral extraoral photograph, (d) lateral skull radiograph, (e) panoramic radiograph, (f) histogram of the SPECT test with uptake ratio above normal values to clivus. Pre-surgical photos: (g) intraoral right lateral photograph, (h) intraoral frontal photograph, (i) intraoral left lateral photograph.

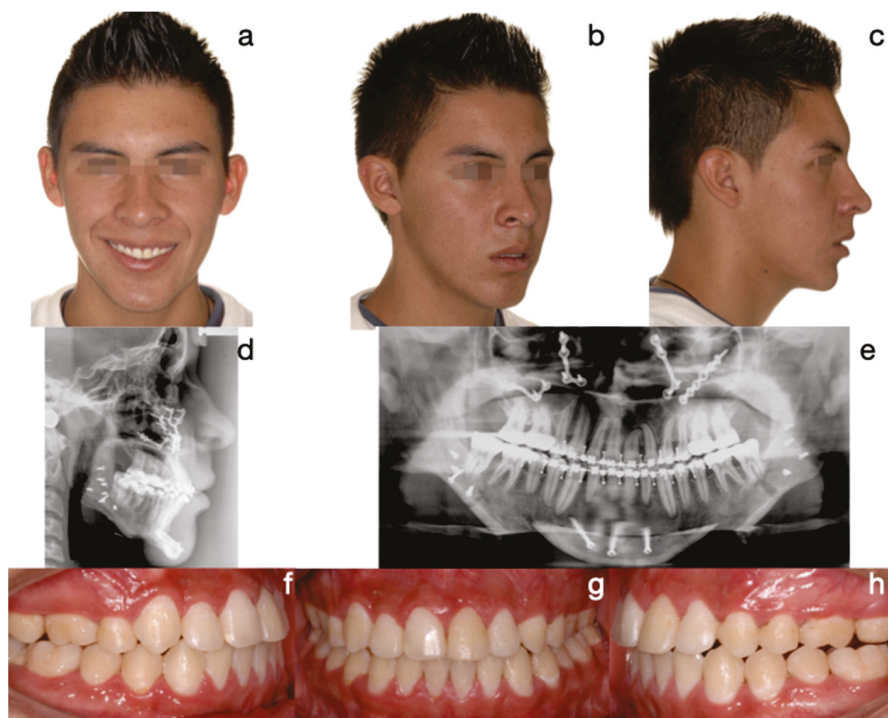


Figure 6. Final photos. Correction of skeletal discrepancy, positive overjet, improvement of profile, and malocclusion. (a) Extraoral frontal photograph, (b) $3/4$ extraoral photograph, (c) extraoral lateral photograph, (d) lateral skull radiograph, (e) panoramic radiograph, (f) intraoral right lateral photograph, (g) intraoral frontal photograph, (h) intraoral left lateral photograph.

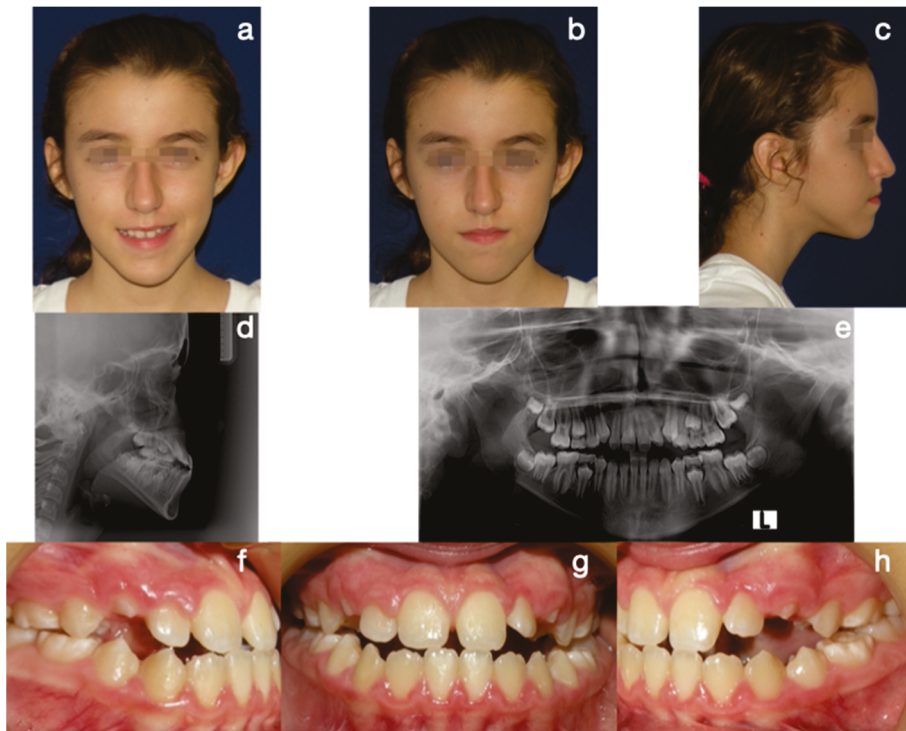


Figure 7. Case 3 corresponds to patient 9 described in Table 1. Protocol C (bilateral condylectomy followed by orthopedics and/or corrective orthodontics without second surgical intervention). Initial photos: (a) frontal extraoral photograph, (b) frontal smile photograph, (c) lateral extraoral photograph, (d) lateral skull radiograph, (e) panoramic radiograph, (f) lateral right intraoral photograph, (g) frontal intraoral photograph, (h) lateral left intraoral photograph.

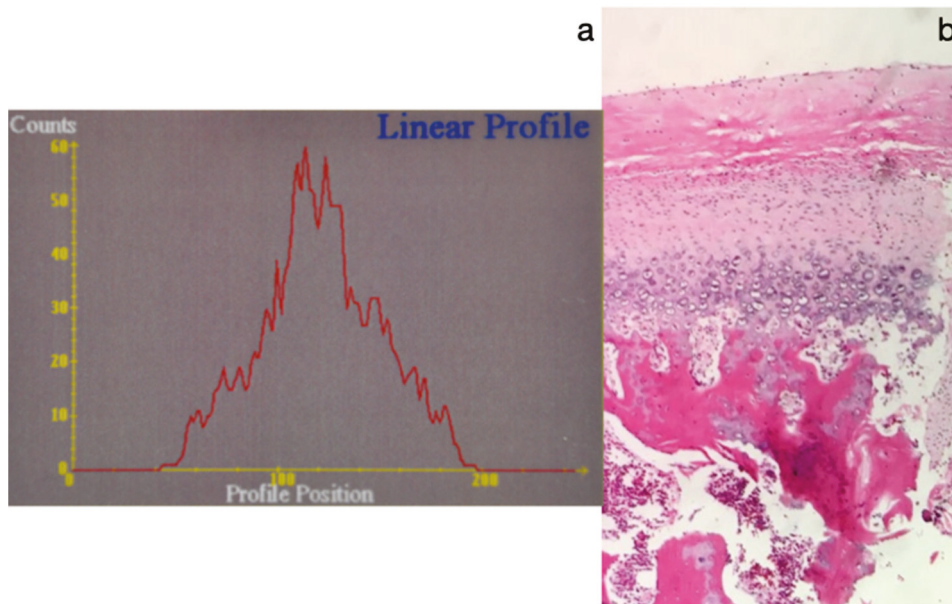


Figure 8. (a) Histogram of the SPECT test, with an uptake ratio in the right condyle of 1.45 and left condyle of 1.38. (b) Histological slice image with bilateral 0.6 mm thickness in the soft condyle.

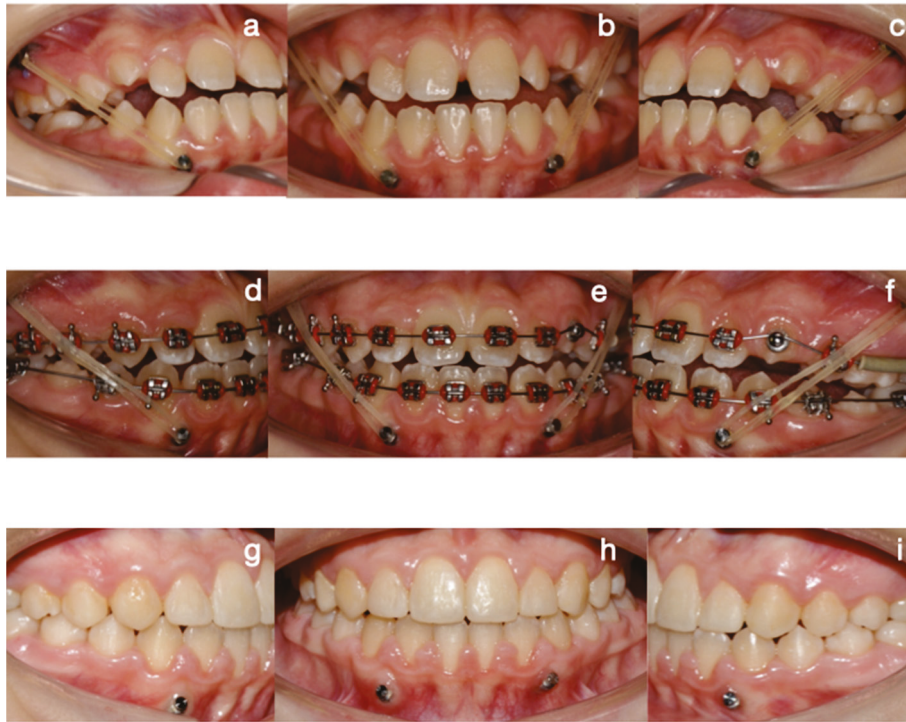


Figure 9. Intraoral treatment sequence: (a) right lateral post-surgical photograph, (b) frontal post-surgical photograph with skeletal anchorage and elastic mechanics, (c) left lateral post-surgical photograph with Class III elastic vector and 250 g force, (d) right lateral corrective orthodontic photograph with Class III elastic vector and 250 g force, (e) frontal photograph, (f) left lateral photograph, (g) right lateral final photograph, (h) frontal final photograph, (i) left lateral final photograph.

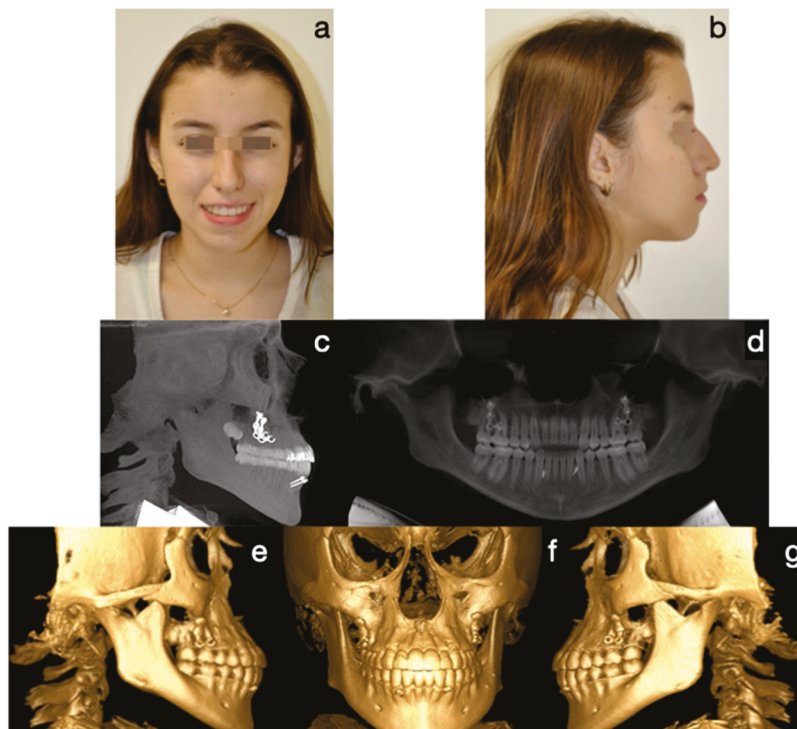


Figure 10. Final photos. Correction of the skeletal discrepancy, positive overjet, and adequate incisor emergence profile. Zygomatic plates and symphyseal screws were kept for the retention stage. (a) Frontal extraoral photograph, (b) lateral extraoral photograph, (c) lateral skull radiograph, (d) panoramic radiograph, (e) lateral CBCT, (f) frontal CBCT, (g) left lateral CBCT.

2.2. Surgical Technique

The procedure is performed under general anesthesia with nasotracheal intubation. A cotton swab, impregnated with 2 mg/g Furacin ointment, is placed in the ear canal. The incision design for the modified endaural approach is marked [17], starting at the anterior third of the helix and extending to its root, passing endaurally through the internal surface of the tragus to the earlobe junction. The preauricular region is infiltrated with 1% xylocaine with epinephrine (1:200,000), 5 cm³, at the helix and tragus to achieve hydrodissection and vasoconstriction.

Using a #15 scalpel, the incision is parallel to the auricular cartilage, separating the skin, subcutaneous tissue, and superficial temporal fascia while protecting the facial nerve branches (frontal, zygomatic, superior buccal). The dissection proceeds with Dean scissors until the deep temporal fascia and articular capsule are reached. Lidocaine with epinephrine is used for hydrodissection, separating the capsule from the condyle. The articular disc is carefully separated from the secondary condylar cartilage with a Molt 9 dissector. TMJ separators are placed backward and outward, and the planned measurements from the tomography are marked with a caliper. Condylectomy is performed with the Mectron[®] piezoelectric from the lateral to the medial pole.

It is decided to perform a high condylectomy when the characteristics of the alteration are not severe, generally diagnosed at an early age and in its initial stages [18]. A low or proportional condylectomy is used for severe cases where reducing the effective mandibular length is necessary [19]. The osteotomized segments are sent for pathology, and osteoplasty of the condylar stump is performed. Meniscopexy is performed from the disc to the capsule. Suturing is performed in layers using Vicryl 4/0, and the skin is closed with Prolene 6/0. A dressing with Bactigras and steri-strips is applied.

Postoperatively, the patient is prescribed cephalexin 500 mg every 8 h for 7 days and etoricoxib 120 mg once daily for 5 days. Stitches are removed seven days later, and Class III elastic vector orthodontic therapy is initiated one week postoperatively. In the third case, 250 g of orthopedic force is applied via skeletal temporary anchorage systems.

3. Results

From August 2019 to November 2024, 10 patients (6 males and 4 females) aged from 11 to 30 years underwent bilateral condylectomy at the study center due to progressive, exaggerated mandibular growth, resulting in severe Class III malocclusion and negative overjet. Table 1 summarizes the patients' demographic characteristics, clinical findings, scintigraphy results, condyle removal volumes, histopathological reports, treatment scheme followed, and follow-up durations.

All patients exhibited elongated but otherwise normal condylar anatomy. The onset of disproportionate mandibular growth typically coincided with the prepubertal growth spurt in males (ages 14–16) and menarche in females. Except for case 3, who reported having a dizygotic twin with unilateral condylar hyperplasia, no family history of Class III or MP was noted. Cases 3, 4, and 6, all over 18, reported continued mandibular growth beyond the cessation of overall body growth.

The scintigraphy results revealed that, for female patients (cases 1, 2, 6, and 9), the condylar uptake ratio concerning the clivus was higher than 1.33, while male patients (cases 8 and 10) had uptake ratios exceeding 2.09. In the other cases, the nuclear medicine center report showed increased condylar uptake concerning clivus above normal age-adjusted values but did not provide quantitative data.

High condylectomy (5–6 mm condylar cut) was the predominant procedure, performed on cases 1, 2, 4, 5, 6, and 9, and for the right condyle of case 3 and the left condyle of case 7. Low condylectomy (beyond 6 mm) was performed on the left condyle of case 3

and the right condyle of case 7, and bilaterally for cases 8 and 10. The authors reported that, as more of the condylar portion was removed, the effective mandibular length decreased and, therefore, the sagittal mandibular projection diminished.

Histopathological analysis revealed that cases 1, 2, and 9 had soft condyle thicknesses exceeding 0.6 mm. The remaining patients exhibited increased proliferation of hypertrophic chondrocytes in the medullary bone, consistent with BCH.

The most common treatment approach was Protocol A (cases 1, 3, 6, 7, and 10), characterized by bilateral condylectomy, to stop the aggressive growth, then orthodontic therapy to prepare the patient for a second surgical intervention to correct the sequel according to the patient's alterations. One of the patients who followed this protocol is the one presented in Figures 2–4, which shows a 14-year-old female patient (Case 1, Table 1), with severe mandibular prognathism, that was accentuated after menarche with no family history of Class III. Anatomically, as evidenced by the radiographic images, the condyles are elongated but have normal anatomy. During the diagnostic process, a nuclear medicine test was conducted to obtain the radiopharmaceutical uptake in the condyles concerning the clivus, and the result was 1.4 for each condyle, confirming the active state of CH. With this diagnosis, it was decided to proceed with a first surgical intervention consisting of a 6 mm bilateral high condylectomy. The results of the histopathological test reported a soft condyle thickness of 0.6 mm for the right condyle and 0.7 mm for the left condyle, with positive findings for BCH. After surgery, orthodontic decompensation was performed for 12 months, and a second surgical intervention was scheduled, this time, bimaxillary to correct the skeletal alteration. Post-surgical orthodontics were then carried out for 8 months to achieve adequate occlusal stability.

Protocol C followed in frequency (cases 2, 8, and 9), a single-surgical approach, involved bilateral condylectomy followed by orthodontics and/or orthopedics to correct sagittal discrepancies, avoiding a second surgery. One of the patients who followed this protocol is the one presented in Figures 7–10, which show a 12-year-old female patient (Case 9, Table 1) with a severe mandibular prognathism that was accentuated after menarche. Anatomically, as evidenced by radiographic images, the condyles are elongated with posterior divergence but have normal anatomy. In addition, she has a lingual offset of the lower incisors. The result of the nuclear medicine test was 1.45 for the right condyle and 1.38 for the left condyle, confirming the active state of the CH. The surgical intervention consisted of a 5 mm high bilateral condylectomy. Histopathologic examination reported a soft condyle thickness of 0.6 mm for both condyles, with positive findings for CHB. In this case, in which the sequelae in the three planes of space were not severe and, additionally, there was growth potential, it was decided to correct the sagittal relationship of the jaws with skeletally anchored orthopedics and then with corrective orthodontics to achieve an ideal occlusion. In the active phase, the treatment lasted 24 months, and, in the follow-up phase, it lasted 48 months with adequate stability.

The third scheme in frequency was Protocol B (cases 4 and 5), which involved condylectomy and orthognathic surgery in one session, followed by post-surgical orthodontics. One of the patients who followed this protocol is the one presented in Figures 5 and 6, which show a 17-year-old male patient (Case 5, Table 1) with severe mandibular prognathism, accentuated since a pubertal growth spurt, and with no family history of Class III. Anatomically, as evidenced by radiographic images, the condyles were elongated with posterior divergence but presented normal anatomy. In addition, the clinical history evidenced episodes of depression due to her facial appearance and also failed orthodontic treatments to correct the skeletal alteration. The nuclear medicine center sent a positive report for bilateral hypercaptation in condyles concerning age-adjusted clivus, without providing quantitative data. In this case, because of her psychoemotional compromise, everything

was performed in the same surgical time. The order of the procedure was an 8 mm high Lefort I osteotomy followed by 6 mm high bilateral condylectomies and, finally, a sagittal osteotomy of the mandibular ramus for setback, accompanied by an 8 mm advancement mentoplasty and 5 mm ascension. The histopathological findings were confirmatory of BCH and post-surgical orthodontics was performed for 24 months. The follow-up has been for 60 months, observing adequate stability in mandibular size and position, as well as in the occlusal relationship.

Follow-up durations ranged from 8 months for the most recent case to 60 months for the longest. No secondary growth was observed, and mandibular position and size remained stable post-condylectomy, as did occlusion after the completion of treatment.

4. Discussion

Class III skeletal malocclusion can be classified into clusters and subclusters [20]. It is characterized by the fact that about 75% of Class III patients have a skeletal component. This sagittal discrepancy of the jaws can be a consequence of maxillary underdevelopment (prevalence: 19.5%), mandibular overdevelopment (prevalence: 47.4%), or a combination of both (incidence: 8.7%) [21]. Southeast Asian populations have the highest incidence (18.5%), followed by the Middle East (10.18%) and Latin America (9.1%) [22]. The etiology is multifactorial, involving both environmental and genetic factors, and it is considered a polygenic condition influenced by ethnicity [23]. Prognosis and treatment are largely influenced by the age of onset and the severity of the condition.

BCH may be one of the etiological factors of severe MP where condylar growth, on the one hand, goes far beyond the residual mandibular growth and, on the other hand, to an exaggerated growth rate with severe esthetic, functional, and psychoemotional consequences. This continuous condylar growth could even be one of the causes of the recurrence of Class III surgical or orthodontic corrections when the diagnosis of BCH is overlooked.

Concerning BCH, there is limited literature that specifies its characteristics in detail and gives clarity to the clinician with respect to the development of the disease. For now, there are only postulations and hypotheses derived from results in morphological and metabolic studies.

Wolford et al. (2014) [8] described BCH as accelerated, disproportionate condylar growth extending into the second decade of life, resulting in Class III malocclusion with MP and anatomically elongated but normal-shaped condyles. For its treatment, they recommended bilateral condylectomy with disc repositioning and orthognathic surgery according to the patient's age.

Regarding these anatomical changes, Mohsen et al. (2023) [24] conducted a morphological and morphometric evaluation of the mandibular condyles using CBCT, linking them to skeletal malocclusions. They found that the length of mandibular condyles in Class III malocclusions was significantly greater than in Class I or II. Similar findings were reported by Krisjane et al. (2009) [25], Saccucci et al. (2012) [26], and Noh et al. (2021) [27], all concluding that larger condylar size is a characteristic of skeletal Class III malocclusions.

Goulart et al. (2015) [12] studied a sample of 30 patients—15 with unilateral condylar hyperplasia (CH) and 15 with Class III malocclusion due to mandibular prognathism (MP). Using CBCT, they compared condylar length and the distances of the mediolateral and anteroposterior poles. Their findings showed no significant differences in condylar size between the two groups, suggesting that some cases diagnosed as Class III malocclusion could actually be cases of BCH.

Lopez et al. (2024) [13] conducted a study on volumetric differences in the temporomandibular joint (TMJ) structures—condyle, fossa, and disc—using 3D tomographic

segmentation in a sample of 138 patients with facial asymmetry. Of these, 64 had hemimandibular elongation, 12 had hemimandibular hyperplasia, 20 had hybrid forms within the condylar hyperplasia (CH) group, and 25 presented with asymmetric MP. The study found that condylar volume was increased in both CH and MP cases compared to healthy controls and other conditions causing facial asymmetry. However, there were no significant volumetric differences between CH and MP, suggesting that condylar overdevelopment may be an etiological factor of MP in Class III malocclusions, as seen in BCH. This implies that many cases historically diagnosed as MP could be BCH.

Gammagraphic studies provide valuable information on bone metabolism, as radio-tracers used in these studies are absorbed in areas of increased blood flow and active bone mineralization [10]. Historically, these studies have focused on comparing condylar uptake to assess active states in unilateral CH cases with facial asymmetry. Recently, efforts have been made to establish reference values for radiopharmaceutical uptake based on age and gender, using the clivus as a reference structure to compare condylar uptake [10]. This approach allows for a more individualized assessment when suspecting BCH.

In this regard, Anzola et al. conducted two studies [14,16]. In the first, involving 72 healthy patients, they found radiopharmaceutical uptake ratios concerning the clivus up to 1.28. In the second study, which included 48 healthy patients, the maximum uptake was 0.84. Lopez et al. [15], studying 80 healthy patients, found sexual dimorphism in uptake, indicating the need to differentiate values by gender. The ranges observed were from 0.46 to 1.33 for females and from 0.61 to 2.09 for males, suggesting that uptake above these ranges indicates active hyperplasia. To calculate the ratio, both condylar and clivus uptake and background radiation (near the study area) were considered.

The differences in the results, despite being studies conducted in similar populations, could be attributed to variations in methodology, such as the selection of the region of interest (ROI) for uptake extraction or the formula used. This generates confusion for clinicians because decisions regarding this pathology are related to surgical procedures and, if the best decision is not made, conditions that are deforming from the beginning could be aggravated.

To address this, the literature has proposed standardizing methodologies to ensure the test is reliable and reproducible. Therefore, our study used a fixed ROI derived from the sum of five transaxial tomographic slices based on total counts. This technique offers greater reproducibility between operators and improved intraoperator repeatability, with minimal variability and better diagnostic agreement [28]. Additionally, we applied the following formula to calculate the ratio [15]:

$$\frac{\text{Condylecounts} - \text{Backgroundcounts}}{\text{Clivuscounts} - \text{Backgroundcounts}}$$

Histopathologically, CH is characterized by an increase in the thickness of the articular layer of fibrous tissue, an increase in the proliferative layer of undifferentiated mesenchymal cells, and, also, an increase in the hypertrophic cartilage layer, which together represent the soft condyle [2]. In the patients reported in this article, this thickness was increased with regard to the reference values, with some having thicknesses above 0.6 mm and also increased proliferation of hypertrophic chondrocytes on the medullary bone with a histopathological report confirming BCH.

The clinical characteristics and common background observed in these patients, as well as those treated by the dental–skeletal disorders team to which the authors belong, include the following: (1) exaggerated mandibular growth starting in preadolescence or adolescence, surpassing the typical growth rates in the population [29]; (2) progressive development of severe mandibular prognathism (MP) with increasing age; (3) severely

progressive skeletal Class III malocclusion with negative overjet; (4) elongated condylar anatomy with a normal shape; (5) no family history of Class III malocclusion or prognathism; (6) mandibular growth extending beyond the typical cessation of facial growth.

The treatment approach for CH is heavily influenced by factors such as the patient's growth potential, the timing of diagnosis, the severity of the skeletal alteration, and the presence of any psycho-functional concerns. When growth potential remains, or when the severity of the alteration is not yet fully manifested, a combination of bilateral post-condylectomy, skeletally-anchored maxillary orthopedics, and/or corrective orthodontics may be used to control the sagittal relationship of the jaws [30]. In the absence of growth potential, bilateral condylectomy and orthognathic surgery are necessary, performed in one or two stages to correct the skeletal discrepancies. The choice of treatment is often influenced by the patient's healthcare system and insurance coverage, as well as potential mood disturbances that can affect the patient's quality of life due to aesthetic concerns [31]. In these cases, a comprehensive approach involving condylar and maxillary surgery is typically preferred for a quicker correction of the deformity. It is important to note that, the more condyle is removed during the condylectomy, the greater the reduction in effective mandibular length and, consequently, the reduction in mandibular projection.

Although the literature has not provided data on the prevalence of BCH, nor on the natural history of the disease that characterizes this entity, nor has it standardized the diagnostic processes in it, and, although we understand that this is a novel proposal that we hope will create clinical awareness for its correct diagnosis and treatment, we understand that one of the limitations of this study is the size of the sample since it still represents a series of cases. It is necessary to carry out longitudinal studies with a larger sample size in which a comparison is made with a population of patients with severe MP not associated with BCH.

5. Conclusions

One important implication for clinical practice is that specialists must be aware of the existence of BCH and the clinical conditions that may indicate its presence. Scintigraphy tests can assess condylar metabolic activity when there is suspicion of this condition. BCH not only alters mandibular growth but does so disproportionately and progressively, affecting the aesthetics, function, and self-esteem of patients. Therefore, early detection is crucial, as it directly impacts treatment decisions and helps prevent relapses in orthodontic or surgical treatments solely focused on correcting or compensating for Class III malocclusion caused by mandibular prognathism. The choice of treatment protocol varies according to the patient's growth potential, the severity of the condition, the time at which the excessive overgrowth is detected, the psycho-emotional compromise, and the magnitude of the sequelae.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in this study, both for surgical procedures performed corresponding to the pathology and for taking records. Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement: Data are available upon request due to restrictions, such as privacy or ethics.

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Abbreviations

The following abbreviations are used in this manuscript:

CH	Condylar hyperplasia
MP	Mandibular Prognathism
BCH	bilateral condylar hyperplasia
TMJ	temporomandibular joint
SPECT	single photon emission computed tomography

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Article

Three-Dimensional Displacement of Upper Cervical Vertebrae in Severe Mandibular Deviation Caused by Condylar Hyperplasia: A Tomographic Segmentation Study

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Abstract

Objective: To evaluate the three-dimensional (3D) angular displacement (Roll, Yaw, and Pitch) of the upper cervical vertebrae (C1, C2, and C3) in patients with severe mandibular deviation (MD) due to condylar hyperplasia (CH), utilizing a computed tomography (CT)-based segmentation approach. **Methods:** This retrospective cross-sectional study included 50 patients with MD ≥ 6 mm caused by hemimandibular elongation (HE) or a hybrid form (HF) of CH. The skull, mandible, and cervical vertebrae (C1–C3) were segmented using 3D Slicer software. Angular deviations (Pitch, Yaw, Roll) were measured relative to the Frankfurt plane. Patients were categorized by the side of CH (right or left), and intergroup comparisons were performed using Kruskal–Wallis and Mann–Whitney U tests. Spearman’s correlation analyses assessed associations between MD magnitude and cervical angles. **Results:** CH was significantly more prevalent in females (58%; $p = 0.021$). C2 and C3 exhibited significantly increased lateral Roll inclination toward the side of deviation ($p = 0.006$ and $p = 0.045$, respectively). C2 Pitch negatively correlated with MD severity bilaterally ($r \approx -0.51$, $p = 0.02$ right; $r \approx -0.50$, $p = 0.02$ left). Strong intra-vertebral correlations between Pitch and Yaw were observed in C1 and C2, indicating synchronized vertical and rotational motion. No significant intergroup differences were found in Yaw angles ($p > 0.05$). **Conclusions:** Patients with CH and severe MD exhibit consistent patterns of 3D cervical displacement, particularly in lateral inclination and vertical movement, suggesting compensatory postural adaptations in the upper cervical spine.

Keywords: cervical vertebrae; condylar hyperplasia; mandibular deviation; x-ray computerized tomography; imaging three-dimensional

1. Introduction

Condylar hyperplasia (CH) is characterized by overgrowth of the mandibular condyle, which disrupts normal facial development. Histologically, this condition presents as an increase in cell number and thickness within the soft condylar layers [1,2]. This overgrowth can lead to severe deformities because it can occur not only during growth and development but also in adulthood [3]. Depending on its duration, type, and severity, CH can affect the temporomandibular joint (TMJ) structures (condyle, disc, fossa) and the mandibular ramus. This can result in secondary vertical compensations in the maxilla and malocclusions

with sagittal, transverse, and vertical implications [4]. The origin of CH may be linked to hormonal, traumatic, neoplastic, genetic, molecular, or functional factors [5].

Condylar hyperplasia (CH) presents with varying vectors of alteration, leading to distinct clinical manifestations and associated complications. Three primary forms are recognized [4,6], as detailed below.

1.1. Hemimandibular Elongation (HE)

This form is characterized by a horizontal vector of alteration, which shifts the mandible away from the sagittal midline. This results in consistent occlusal changes among patients. Its main etiologic factors are trauma and hormonal influences, often leading to onset during pre- and adolescence. HE also shows a higher female prevalence and is the most common form of hyperplasia.

1.2. Hemimandibular Hyperplasia

This type involves a vertical vector of alteration, producing three-dimensional growth on one side of the face without displacing the mandible from the mid-sagittal plane. Consequently, its occlusal characteristics differ significantly from HE. Its main etiologic factor is neoplastic growths, such as osteochondroma, and it is the least common of the hyperplasia.

1.3. Hybrid Form (HF)

The HF combines both horizontal and vertical vectors of alteration. This makes it a particularly severe and aggressive expression of the pathology.

Although human anatomy is not entirely symmetrical, anthropometric standards derived from population-based studies provide parametric thresholds to identify when a craniofacial asymmetry falls outside the normal range [7]. When a patient's craniofacial anthropometric assessment (i.e., quantitative anatomical measurements) deviates from these parameters, facial asymmetry (FA) is evident, typically associated with mandibular deviation (MD) [4].

In such cases, the midpoint of the mandibular symphysis extends beyond the sagittal midplane of the cranial structures. This deviation is linked to multiple etiological factors, including genetic [8], neurological [9], neoplastic [10], molecular [11], developmental [12], environmental and traumatic [13], functional laterognathism [14], glenoid cavity asymmetry [15] and condylar resorption [16]. Such FA not only impacts bone and soft tissue structures but can also lead to alterations in extracranial structures, such as the cervical vertebrae.

The mandible and cervical spine are anatomically and functionally interconnected. This complex relationship is facilitated by shared muscle chains, including the sternocleidomastoid, suprahyoid, infrahyoid, and suboccipital muscles, as well as the hyoid bone. Furthermore, neurological connections between the trigeminal nerve and brainstem nuclei, which also regulate cervical functions, contribute to this interrelationship [17]. The C1 (Atlas) and C2 (Axis) vertebrae form the craniovertebral junction, acting as the crucial transition between the brain and the rest of the cervical spine. This area is vital as it houses neural and vascular structures while allowing for significant spinal mobility. Most vertebral rotation, flexion, and extension occur between C1 and C2, while the intervertebral discs are located from the C2-C3 level downwards [18].

Craniofacial alterations linked to FA have been thoroughly investigated, but research into extracranial alterations remains in its early stages. A crucial question in a patient's comprehensive evaluation is how MD affects neck posture and the position of the cervical vertebrae. This is especially relevant given that some scientific literature has established an anatomical-functional and pathophysiological connection between craniomandibular and craniocervical dysfunction, integrating them into a single tonic-postural system [19].

In line with this, Guan et al. [20] and Nakashima et al. [21] have postulated an inherent correlation between MD and cervical posture, affecting it three-dimensionally. Additionally, Cardinal et al. [22] noted a positive correlation between posterior crossbite, a common feature of MD, and deviation of the first cervical vertebrae.

However, the direct correlation between specific conditions that cause FA, such as CH, and associated vertebral changes remains unexplored. Furthermore, previous studies have neither differentiated cohorts based on the severity of MD nor segmented individual vertebrae to assess their behavior comprehensively.

Given that computed tomography (CT) has proven helpful in the morphological evaluation of anatomical structures, providing detailed views of their size, shape, and position from various angles and slices [23], this study aims to evaluate the three-dimensional behavior of the upper cervical vertebrae to CH with severe MD, as observed in HE and HF.

2. Materials and Methods

2.1. Study Design and Patient Selection

We conducted a retrospective cross-sectional study, approved by the Institutional Ethics Committees of Clínica Imbanaco (CEI-856) and Universidad del Valle Faculty of Health (E 008-024). We retrieved CT datasets in DICOM format from the institutional repository, encompassing patients with a clinical diagnosis of FA who received care at Clínica Imbanaco between August 2015 and December 2023.

Our selection criteria for FA were based on the classification by López et al. [4]. We specifically chose datasets from patients with severe FA, defined as mandibular symphysis deviation of 6 mm or more from the sagittal midplane, caused by CH with a horizontal vector of alteration, such as hemimandibular elongation HE and hybrid form HF (Figure 1A).

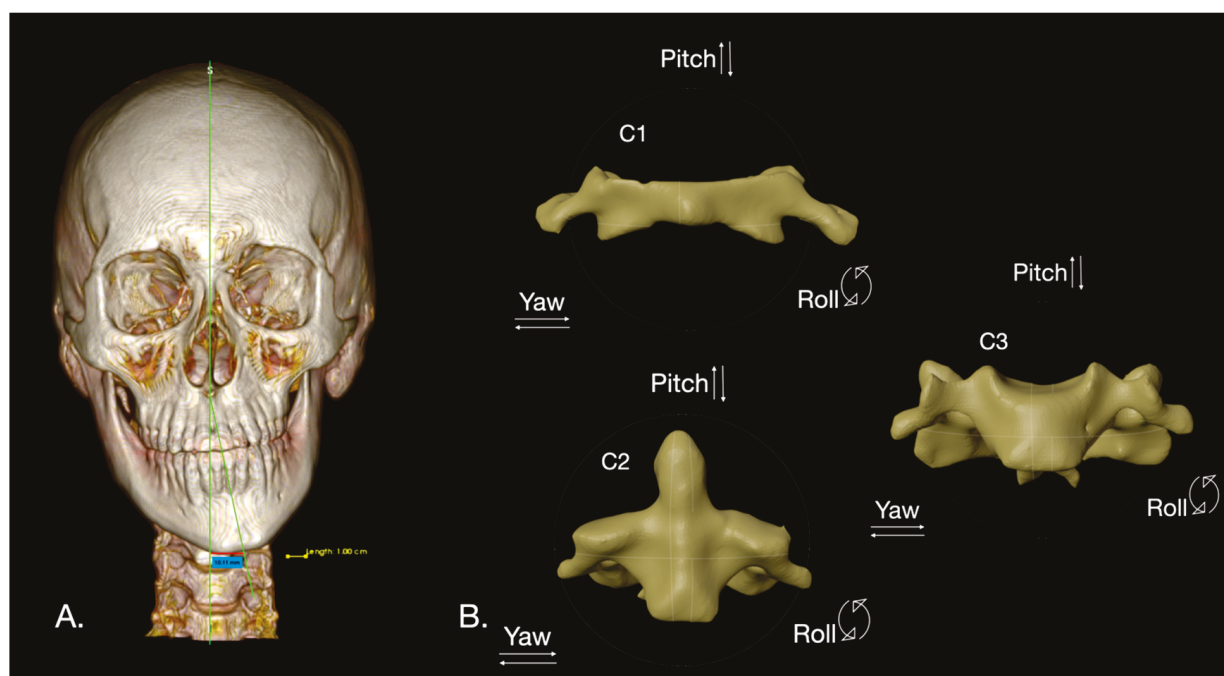


Figure 1. Front view. (A) Severe mandibular deviation (10.11 mm) to the left in a subject with condylar hyperplasia of the hybrid form. (B) C1, C2 and C3 vertebrae with Pitch, Roll and Yaw planes.

2.2. CT Acquisition and Reconstruction

All CT datasets were acquired using a Biograph mCT20 PET/CT system (Siemens, Erlangen, Germany) at Clínica Imbanaco. The imaging parameters included:

- No contrast medium.
- Volume of interest: From the vertex to the sternal notch.
- Slice thickness: 0.75 mm.
- Pitch: 1.0.
- Cubic matrix: 512×512 with an isotropic voxel size of 0.58 mm.

CT images were reconstructed using a homogeneous low-dose B26F filter for precise anatomical localization.

2.3. Exclusion Criteria and Side Definition

We excluded incomplete CT datasets and those from patients with a history of:

- TMJ disease or surgery.
- Orthognathic surgery.
- Craniofacial trauma.
- Dentofacial syndrome.
- Pathologies like arthritis or other spinal diseases.

For consistency, the affected side was defined as the side with condylar overgrowth, and MD side was considered the contralateral side.

2.4. CT Dataset Segmentation

For each dataset, the skull, mandible, and cervical vertebrae (C1–C3) were segmented. A trained researcher manually selected hard tissue structures using the Threshold tool within the Segment Editor of the open-source software 3D Slicer (v. 5.8.1 r33241/11eaf62, USA) [24]. This selection was based on Hounsfield Units (HU) ranging from 140 HU to 5734 HU. Subsequently, a semi-automatic segmentation generated Standard Tessellation Language (.stl) files for the merged skull and mandible, as well as for each cervical vertebra (Figure 1B).

2.5. Cephalometric Landmark Selection

The Frankfort horizontal plane was used as the reference plane because it is defined by stable cranial landmarks that are minimally influenced by mandibular growth, asymmetry, or positional alterations. Since this study evaluates three-dimensional displacement of the upper cervical vertebrae associated with severe mandibular deviation secondary to condylar hyperplasia, a cranial-based reference system prevents mandibular displacement from being incorporated into the reference itself and allows cervical positional changes to be assessed relative to the cranial base.

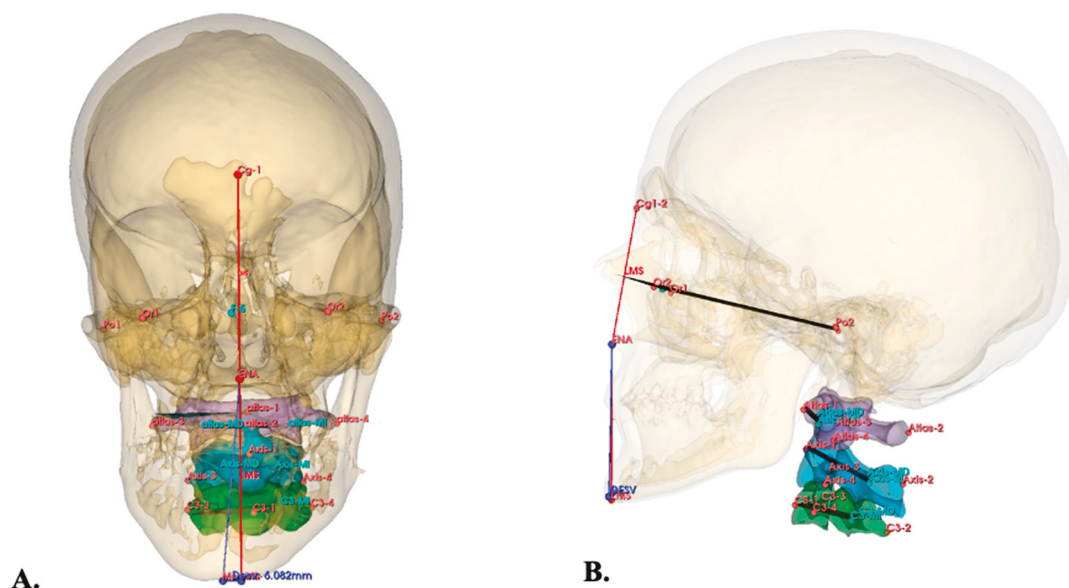
The Frankfort plane, serving as the reference, was established by placing four primary cephalometric landmarks on the skull using the Markups module in 3D Slicer (Table 1). Following this, primary cephalometric landmarks were similarly placed on the C1 (Atlas), C2 (Axis), and C3 vertebrae to define their respective planes of interest (Table 1). To determine the three-dimensional (3D) position of each vertebra based on Pitch, Yaw, and Roll angle deviations relative to the Frankfurt plane, the Angle Planes module and the Middle Point tool in 3D Slicer were utilized. Derivative landmarks were automatically generated by the software (Table 1, Figure 2).

2.6. Outcomes of Interest

MD was quantified in millimeters (mm) using the method described by López et al. [4] (Figure 1). For the C1–C3 vertebrae, their 3D positions relative to the Frankfurt plane were recorded as Pitch, Yaw, and Roll angles in degrees (°) (Figure 2).

Table 1. Description of the cephalometric landmarks used to determine the reference plane on the skull and the planes of interest in the cervical vertebrae.

STRUCTURE	PRIMARY	DESCRIPTION	ABBREVIATION
SKULL	Porion (Po)	Most superior point of the margin of the external acoustic meatus.	Po1: Right Porion Po2: Left Porion
	Orbitale (Or)	Most inferior point on the margin of the orbit.	Or1: Right Orbitale Or2: Left Orbitale
	DERIVATE	DESCRIPTION	ABBREVIATION
	F5	Middle Point between Or1 and Or2.	F5
CERVICAL VERTEBRAE	PRIMARY	DESCRIPTION	ABBREVIATION
	Point 1	The most prominent part of the anterior tubercle.	C1-1; C2-1; C3-1
	Point 2	The most prominent part of the posterior tubercle.	C1-2; C2-2; C3-2
	Point 3	The most lateral edge of the right transverse process.	C1-3; C2-3; C3-3
	Point 4	The most lateral edge of the left transverse process.	C1-4; C2-4; C3-4
	DERIVATE	DESCRIPTION	ABBREVIATION
	Medium Right	The midpoint between Point 2 (posterior tubercle) and Point 3 (right transverse process)	MR
	Medium Left	The midpoint between Point 2 (posterior tubercle) and Point 4 (left transverse process).	ML

**Figure 2.** (A) Frontal view. Severe subject diagnosed with CH of the HF type, anatomical points located Cg1-2 (Crista Galli), ENA (Anterior Nasal Spine), LMS (Mid Sagittal Line), Me (Menton), in

yellow the segmented skull is illustrated, in purple the C1 vertebra, in blue the C2 vertebra, in green the C3 vertebra, all this to evaluate the magnitude of MD and the Roll and Yaw of the cervical vertebrae. **(B)** Sagittal view. Subject with severe MD, diagnosed with CH. Anatomical points located Cg1-2 (Crista Galli), ENA (Anterior Nasal Spine), LMS (Mid Sagittal plane), Me (Menton), Or1 (Right Orbit), Or2 (Left Orbit), Po1 (Right Porion), Po2 (Left Porion), Atlas 1-2-3-4-MD-MI, Axis 1-2-3-4-MD-MI, C3 1-2-3-4-MD-MI, in yellow the segmented skull is illustrated, in purple the C1 vertebra, in blue the C2 vertebra, in green the C3 vertebra, all this to evaluate the up–down movement (pitch) of the first three cervical vertebrae.

2.7. Statistical Analysis

A single researcher (CMR) obtained all measurements. To assess intra-examiner agreement, 10 randomly selected CT datasets were re-measured after two weeks, with agreement evaluated using the Wilcoxon signed-rank test.

Data are reported as Mean $\pm$ SD. The Shapiro–Wilk test was used to assess data normality. Samples were divided into two groups based on the side of CH (right and left). The Kruskal–Wallis test, followed by Dunn’s multiple comparisons test, was used to compare outcomes of interest between these groups. Spearman’s correlation coefficient was used to calculate the correlation between MD and the 3D position of the vertebrae.

All analyses were performed using GraphPad Prism 10 for macOS (Version 10.4.2/534). A p -value < 0.05 was considered statistically significant.

3. Results

From an initial 247 revised CT datasets, we included 50 from patients with CH. The mean age was 22 ± 7.83 years, with similar mean ages for both right-sided CH (22.5 ± 9.37 years; $n = 28$) and left-sided CH (21.6 ± 6.52 years; $n = 22$). Females predominated in the left CH group (77%), while males were more frequent in the right CH group (57%). High-frequency (HF) was more common in both groups (59% left, 61% right) compared to low-frequency (HE) (41% left, 39% right).

When comparing right versus left CH, no significant differences were found for age ($p = 0.842$), CH type ($p > 0.999$), or amount of MD ($p = 0.729$). However, there was a significant difference in patient distribution based on sex ($p = 0.021$) (Table 2).

Table 2. Sample distribution according to age, sex, condylar hyperplasia type, and affected side.

	Affected Side	
	Right (28)	Left (22)
Age		
Median $\pm$ SD (95% CI)	21.6 ± 6.52 (19.1–24.1)	22.5 ± 9.37 (18.3–26.6)
Sex		
Female	12 (43%)	17 (77%)
Male	16 (57%)	5 (23%)
Diagnosis		
HE	11 (39%)	9 (41%)
HF	17 (61%)	13 (59%)
MD in mm		
Median $\pm$ SD (95% CI)	7.43 ± 1.37 (6.90–7.96)	7.39 ± 1.75 (6.60–8.15)

To compare the groups based on the age and the amount of MD, the Mann–Whitney test was used, whereas, to compare the groups based on the distribution of the sex and the CH types, the Chi-square test (Fisher’s exact test) was used. Abbreviations: MD, mandibular deviation; HF, hybrid form; HE, hemimandibular elongation.

We evaluated the lateral inclination (roll) and axial rotation (yaw) of cervical vertebrae C1, C2, and C3, classifying movements as rightward, leftward, or no movement, relative to the CH side. Although a consistent lateral Roll orientation of C2 and C3 toward the MD side was observed, it is important to distinguish between directional distribution and angular magnitude. Table 3 reports the frequency of Roll orientation toward each side and therefore reflects a significant directional pattern. In contrast, the comparison of mean Roll angles evaluates the magnitude of inclination, which did not show statistically significant differences between sides. Thus, the significant findings indicate a preferential direction of Roll rather than a greater degree of angular displacement.

Table 3. Distribution of the direction of the Roll and Yaw movements of the cervical vertebrae (C1–C3) according to the side of the CH.

Variable	Affected Side		<i>p</i> -Value
	Right (28)	Left (22)	
Inclination Roll C1 Atlas (right/left)			0.12
Right	5 (18%)	10 (45%)	
Left	15 (54%)	8 (36%)	
No inclination	8 (29%)	4 (18%)	
Inclination Roll C2 Axis (right/left)			0.006
Right	10 (36%)	17 (77%)	
Left	14 (50%)	5 (23%)	
No inclination	4 (14%)	0 (0%)	
Inclination Roll C3 (right/left)			0.045
Right	9 (32%)	14 (64%)	
Left	18 (64%)	8 (36%)	
No inclination	1 (4%)	0 (0%)	
Rotation Yaw C1 Atlas (right/left)			0.7
Right	11 (39%)	11 (50%)	
Left	8 (29%)	6 (27%)	
No rotation	9 (32%)	5 (23%)	
Rotation Yaw C2 Axis (right/left)			0.4
Right	17 (61%)	9 (41%)	
Left	9 (32%)	10 (45%)	
No rotation	2 (7%)	3 (14%)	
Rotation Yaw C3 (right/left)			0.2
Right	17 (61%)	10 (45%)	
Left	9 (32%)	12 (55%)	
No rotation	2 (7%)	0 (0%)	

The bold format of *p*-value indicates that $p < 0.05$.

For C1, the lateral roll inclination pattern showed no statistically significant difference between groups ($p = 0.12$). However, leftward inclination was more prevalent in the right CH group (54%), while rightward inclination predominated in the left CH group (45%).

At C2, we identified statistically significant differences in lateral roll inclination ($p = 0.006$). Most patients with left CH presented C2 inclination to the right (77%), align-

ing with the side of MD. Conversely, leftward inclination predominated in the right CH group (50%).

Significant differences were also noted for C3's lateral roll inclination pattern ($p = 0.045$). In both the right and left CH groups, 64% of patients exhibited lateral inclination toward the side of their MD.

Regarding axial rotation (yaw) movement, no statistically significant differences were observed in any of the three evaluated vertebrae (C1: $p = 0.7$; C2: $p = 0.4$; C3: $p = 0.2$). However, there was considerable dispersion in rotation directions, with similar proportions across both groups. For example, 50% of the left CH group's C1 vertebrae rotated to the right, compared to 39% in the right CH group.

Movement Angles (Supplementary Table S1)

Pitch (Flexion/Extension)

Mean pitch angles were similar between right and left CH groups across all three evaluated vertebrae:

- **C1:** $11.6^\circ \pm 7.26^\circ$ (95% CI: 8.79–14.4°) in right CH versus $12.1^\circ \pm 5.40^\circ$ (95% CI: 9.74–14.5°) in left CH.
- **C2:** Higher in left CH ($18.6^\circ \pm 5.53^\circ$) than right CH ($16.0^\circ \pm 6.67^\circ$), though not statistically significant ($p > 0.05$).
- **C3:** Similar between groups (5.43° in right CH vs. 4.73° in left CH; $p > 0.05$).

Yaw (Axial Rotation)

Axial rotation angles (yaw) displayed marked interindividual variability, with no significant association with the side of CH:

- **C1:** $44.0^\circ \pm 54.7^\circ$ (right CH) versus $46.6^\circ \pm 57.6^\circ$ (left CH).
- **C2:** $45.5^\circ \pm 54.2^\circ$ (right CH) versus $59.4^\circ \pm 63.8^\circ$ (left CH).
- **C3:** $45.3^\circ \pm 54.4^\circ$ (right CH) versus $19.5^\circ \pm 23.3^\circ$ (left CH).

Roll (Lateral Inclination)

Roll angles were low and similar between groups, with no significant differences:

- **C1:** $2.25^\circ \pm 2.49^\circ$ (right CH) versus $2.41^\circ \pm 2.61^\circ$ (left CH).
- **C2:** 2.18° (right CH) versus 2.32° (left CH).
- **C3:** Slightly higher in right CH ($2.68^\circ \pm 1.74^\circ$) than left CH ($1.91^\circ \pm 1.66^\circ$), without statistical significance.

A Spearman's correlation analysis investigated the relationship between MD magnitude (mm) and the 3D motion angles (Pitch, Yaw, and Roll) of cervical vertebrae C1, C2, and C3. The results were analyzed independently for each side of MD (Figure 3). Because several correlations were explored, the statistical analysis was designed as exploratory and hypothesis-generating. Accordingly, no adjustment for multiple comparisons was applied, and findings are intended to inform future confirmatory studies.

For patients with rightward MD, there was a significant negative correlation between MD and C2 Pitch ($r = -0.50$; $p = 0.02$). A non-significant negative correlation was also noted with C1 Pitch ($r = -0.38$; $p = 0.08$). These findings suggest that greater leftward MD correlates with lower pitch angles in both C1 and C2.

In patients with leftward MD, a significant positive correlation was observed between MD magnitude and C1 Pitch angle ($r = 0.41$; $p = 0.03$). Conversely, a significant negative correlation was found with C2 Pitch ($r = -0.51$; $p = 0.01$). This indicates that greater rightward MD is associated with increased C1 pitch and decreased C2 pitch.

Relevant Intravertebral Correlations (Figure 3)

Analysis of correlations between movement planes within each vertebra revealed strong and statistically significant associations, especially in the Atlas (C1).

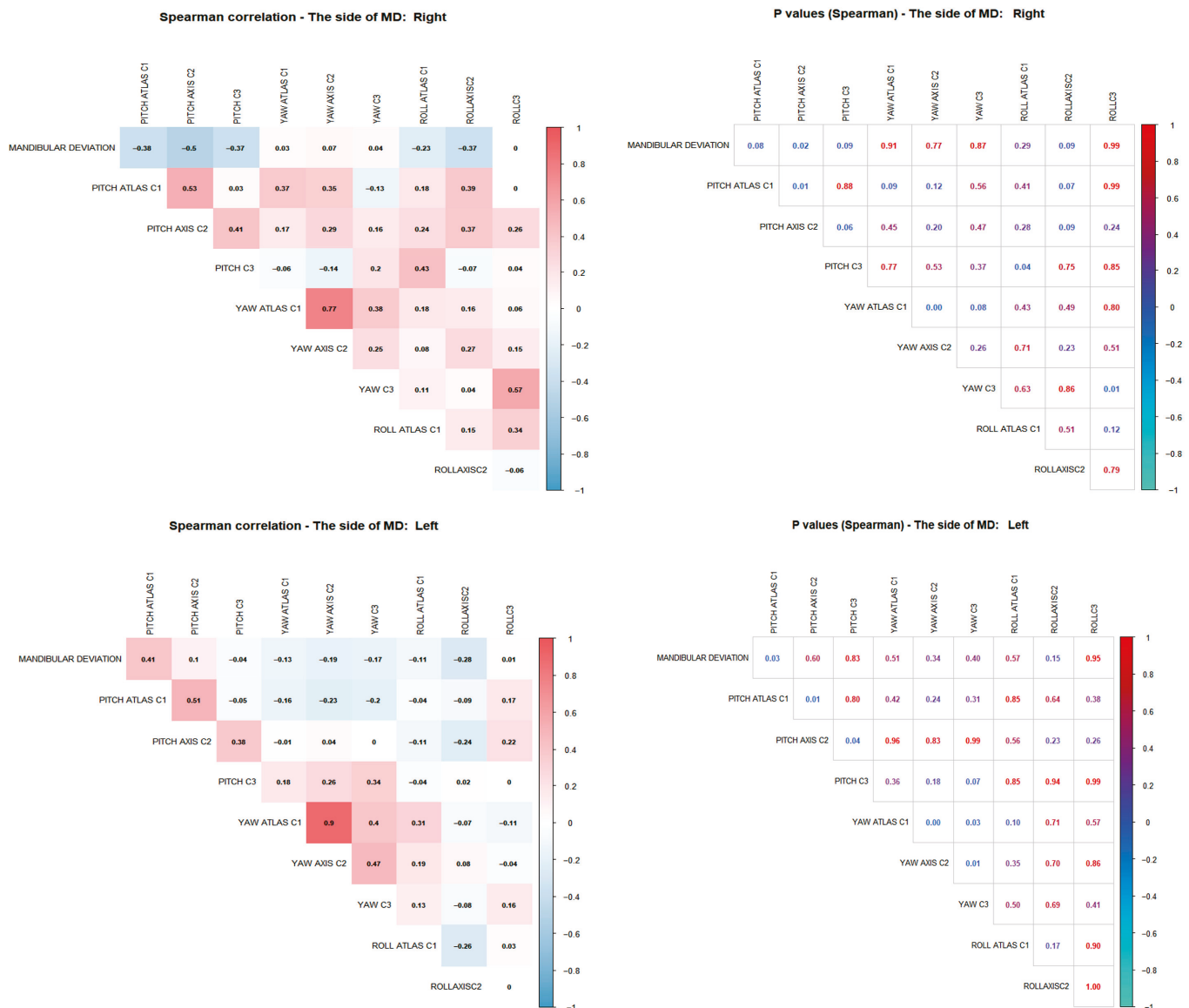


Figure 3. Spearman's correlation matrix between mandibular deviation and the three-dimensional movements of the cervical vertebrae (Pitch, Yaw, and Roll), differentiated by side of the deviation. statistically significant (Spearman's correlation coefficient). Abbreviations: MD, mandibular deviation; r, correlation coefficient.

In both right and left MD patient groups, we observed a strong positive correlation between the Pitch and Yaw angles of C1 ($r = 0.77$, $p < 0.001$ in the right group; $r = 0.90$, $p < 0.001$ in the left group). This indicates a high degree of synchrony between vertical and rotational movements at this level.

Similarly, in patients with left MD, a significant positive correlation was found between the Pitch and Yaw angles of the Axis (C2) ($r = 0.47$, $p = 0.01$), suggesting that, as in C1, the Axis may also exhibit coordinated movement as part of a superior cervical adaptive pattern in response to MD (Figure 3). Additionally, in the group with right deviation, a significant correlation was identified between Yaw and Roll in the C3 vertebra ($r = 0.57$, $p = 0.01$). (Figure 3).

4. Discussion

This study utilized computed tomography to analyze craniofacial structure and MD in patients with CH. Through tomographic segmentation, we individualized the cervical

vertebrae to assess their three-dimensional behavior relative to the hyperplasia. Our results indicate changes in vertebral inclinations, predominantly towards the contralateral side of the hyperplasia—the side of mandibular deviation. These findings provide a comprehensive understanding of how craniofacial alterations can manifest in extracranial structures, such as the cervical vertebrae, potentially as adaptive responses or due to altered mechanical loads.

Our research sample consisted of patients diagnosed with unilateral CH. This condition involves disproportionate growth of one condyle compared to the other, leading to not only FA but also significant articular and occlusal changes that affect the entire maxillomandibular system [6]. While unilateral CH presents in three forms, this study focused exclusively on HE and HF. These are the most common CH entities and share a horizontal component in their alteration, effectively displacing the mandible away from the sagittal midplane. We included only severe cases, defined as those with MD of 6 mm or more. This threshold signifies deviations that typically exceed personal acceptance and social perception, as reported by Wang et al. [7], and commonly necessitate orthodontic or surgical correction.

In our sample, 58% of severe unilateral CH cases occurred in women. This aligns with numerous studies and systematic reviews, including that of Raijmakers et al. [25], which suggest a higher female predisposition, potentially considering female gender a risk factor for unilateral CH. This predisposition, along with other TMJ disorders, may be linked to hormonal differences, particularly estrogen, which regulates bone growth and is evidenced in the TMJ [26]. This study also revealed a predominance of right condyle alteration (56%), leading to left-sided MD (mandibular levognathism), an incidence also reported by Lopez et al. [27].

Regarding the relationship between CH, MD, and vertebral inclinations, our study demonstrated that the lateral roll inclination for both C2 and C3 vertebrae was significantly ($p < 0.05$) more pronounced towards the side contralateral to the CH, which corresponds to the side of MD.

Similar findings were reported by Cardinal et al. [22], who observed lateral roll inclination of C2 and C3 on the side of MD and unilateral posterior crossbite in a 26-patient sample analyzed via CBCT. Expanding on these observations, Guan et al. [20] conducted a CBCT study on 30 subjects with MD, comparing them to individuals without FA to analyze 3D cervical vertebral changes. They concluded that a correlation exists between MD and vertebral deflection, which influences the 3D position of the cervical vertebrae. Despite methodological differences—Guan et al. [20] included asymmetric cases from 2 mm of MD, while our study focused solely on severe cases from 6 mm—the results align. Therefore, as stated by Lippold et al. [28], these studies collectively provide evidence of a relationship between jaw position and upper spinal body posture.

It is important to note that MD not only affects facial aesthetics and produces differential joint loading but also leads to asymmetrical occlusal changes, often resulting in crossbite on the deviating side [3,5]. In this regard, Matsaberidze et al. [19] theorized that inadequate occlusion can cause misalignment of the upper cervical vertebrae, leading to skull tilting and deviation from a symmetrical and balanced position above the shoulders. This, in turn, can sometimes result in postural collapse that affects, strains, and compresses the brainstem, thereby suggesting a relationship between occlusion and posture.

This theory aligns with various research findings. For instance, Meibodi et al. [29], Sonnesen et al. [30], and Aranitasi et al. [31] found a significant correlation between cervical vertebrae anomalies and deviations with skeletal malocclusions, such as Class III. Furthermore, studies like Korbmacher et al. [32] have demonstrated an association between unilateral crossbite and cervical orthopedic disorders.

The above findings suggest a possible association between MD and 3D characteristics of the cervical vertebrae; however, the clinical relevance of these changes for craniocervical anatomy remains uncertain. Previous studies have explored potential links between mandibular position and postural behavior. For example, Wakano et al. [33] reported that horizontal deviations in jaw position may be associated with alterations in postural stability, while Ben-Bassat et al. [34] described a higher prevalence of asymmetric malocclusion features in patients with idiopathic scoliosis. Likewise, Ozturk et al. [35] reported relationships between the cranio-cervico-mandibular system and temporomandibular disorders. These reports provide contextual background but do not establish direct causal pathways between malocclusion and global postural alterations.

In the present study, no statistically significant differences were observed in vertebral anthropometric measurements between groups based on the CH side or MD side across any of the evaluated planes (Pitch, Yaw, or Roll). However, the high dispersion of Yaw values, particularly in C1 and C2, is notable. This might reflect greater individual variability in the axial rotation of these vertebrae in patients with severe MD, possibly due to the wide range of flexion, extension, and rotational movements inherent to these cervical segments [36].

Regarding Pitch (up–down) motion, we observed a significant decrease in the C2 angle for both sides of MD ($p < 0.05$). This means greater MD correlates with a more pronounced downward motion at C2.

Our correlation analysis of motion planes within each vertebra revealed strong, statistically significant associations, especially in the Atlas (C1). This indicates high synchrony between C1's vertical (Pitch) and rotational (Yaw) movements, suggesting an integrated functional response of C1 to postural changes linked to MD.

Similarly, in the left CH and right MD group, a significant positive correlation between Pitch and Yaw was found in the Axis (C2) vertebra ($r = 0.47, p = 0.01$). Like C1, C2 appears to participate coordinately in cervical adaptation to MD. This finding may reflect a functional coupling in the upper cervical spine, possibly associated with compensatory mechanisms or stabilization of the postural axis in relation to MD. This is plausible given that the craniovertebral junction—the complex transition between the brain and cervical spine involving the atlas and axis joined by the odontoid apophysis—houses vital neural and vascular structures while allowing for the greatest range of spinal movements [17,18].

The 3D positional deviations observed at C1, C2, and C3 in the present study may reflect a neuromuscular compensatory response to MD associated with CH. However, due to the cross-sectional design of this investigation, the absence of a non-asymmetrical control group, and the limited body of literature addressing this specific interaction, a definitive cause-and-effect relationship between MD and cervical spine positioning cannot be established.

Although existing theoretical frameworks suggest that the stomatognathic system may influence cervical alignment, its overall contribution to global postural control remains insufficiently understood. Moreover, without comparison to individuals presenting mandibular asymmetry of non-pathological origin, it remains uncertain whether the observed alterations in cervical vertebral angulation exceed normal anatomical variability or represent adaptive postural responses.

Accordingly, these findings should be interpreted as exploratory and hypothesis-generating, as it remains uncertain whether the observed changes in vertebral angulation fall within normal anatomical variability or represent postural alterations with potential clinical relevance. In addition, the synchronized Pitch–Yaw behavior observed at C1 and C2 may reflect different, non-exclusive mechanisms, including biomechanical adaptation to maintain cranio-cervical balance, neuromuscular compensation aimed at preserving head orientation, or simply a coincidental anatomical association in the absence of a control group. Future

prospective and longitudinal studies, incorporating both symmetrical healthy controls and appropriately matched asymmetrical groups, are required to elucidate the temporal and causal relationships between mandibular deviation, occlusal characteristics, cervical spine alignment, and overall body posture, as well as their potential implications for orthodontic diagnosis, orthognathic surgical planning, and postural assessment.

Study Limitations: This study has several limitations. First, its retrospective design involves an inherent risk of selection bias, as cases were included based on the availability of imaging records rather than predefined sampling criteria. Second, the absence of a control group of subjects with facial symmetry limits the ability to establish causal relationships between condylar hyperplasia, mandibular deviation, and upper cervical vertebral displacement. Therefore, the present results should be interpreted as descriptive and associative rather than causal.

In addition, although three-dimensional spatial changes were quantified, potential confounding variables were not controlled. Future investigations should adopt prospective designs including age- and sex-matched control groups to better isolate the effect of condylar hyperplasia on upper cervical morphology and orientation and to improve the generalizability of the findings.

5. Conclusions

The key findings from this study are:

- **Vertebral Lateral Inclination:** Patients with CH exhibiting a horizontal alteration vector (HE and HF) show a greater proportion of lateral Roll inclination in their C1, C2, and C3 vertebrae. This inclination is consistently directed towards the contralateral side of the hyperplasia, which is also the side of MD.
- **Mandibular Deviation and C2 Pitch:** As the magnitude of MD increases (for both right and left deviation), the Pitch of the C2 vertebra becomes more negative. This indicates a downward, or inferior, movement of the C2 vertebra.
- **Synchronized Upper Cervical Movement:** There is a high degree of synchrony between Pitch and Yaw movements in both the C1 and C2 vertebrae. This suggests that these vertebrae exhibit coordinated motion, likely as part of an adaptive pattern in response to MD.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/diagnostics16040579/s1>, Table S1: Mean values of the three-dimensional angles (Pitch, Yaw and Roll) of the cervical vertebrae according to the CH side.

Author Contributions: Conceptualization, D.F.L. and C.M.R.; methodology, C.M.R. and D.F.L.; software, R.C.-P.; validation, C.M.R., L.E.A. and R.C.-P.; formal analysis, D.F.L. and C.M.R.; investigation, C.M.R. and D.F.L.; resources, L.E.A. and R.C.-P.; data curation, R.C.-P. and L.E.A.; writing—original draft preparation, D.F.L.; writing—review and editing, D.F.L.; visualization, C.M.R. and D.F.L.; supervision, L.E.A.; project administration, D.F.L. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Ethics Committees of Clínica Imbanaco (code CEI-856; date 18 March 2024) and Universidad del Valle Faculty of Health (code E 008-024; date 28 February 2024).

Informed Consent Statement: We conducted a retrospective cross-sectional study where tomographic images were evaluated. All patients signed the institutional informed consent form for these procedures before the scans were performed.

Data Availability Statement: Data are available upon request due to restrictions, such as privacy or ethics.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

MD	Mandibular deviation
CH	Condylar hyperplasia
CT	Computed Tomography
HE	Hemimandibular elongation
HF	Hybrid form
TMJ	Temporomandibular joint
FA	Facial asymmetry
C1	Atlas
C2	Axis

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Article

New Classification of Midpalatine Suture Maturation Using Cone Beam Computed Tomographic Study

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Abstract

Background/Objectives: Assessment of the midpalatine suture is vital for making clinical decisions regarding the correction of transverse growth discrepancies of the maxilla. Several studies have used Cone Beam Computed Tomography (CBCT) to evaluate skeletal maturity by midpalatine suture staging (MPS) in various populations. A few patterns of staging did not fit the standard classification. Hence, the rationale of this research was to explore potential new subcategories of maturation staging using CBCT. The study aimed to develop a new comprehensive classification subcategorization system for midpalatine suture maturation staging based on CBCT scans. **Methods:** The study involved the retrospective analysis of 168 CBCT scans. The standard reference for MPS staging was taken from a previous published classification in 2013 using CBCT. Each classification stage of the standard classification was subcategorized into Pattern A and Pattern B. **Results:** Both classifications (standard reference and new) can rely on age to predict the possibility of maturation of the MPS compared to non-maturation. Age is a predictable variable of suture opening in both classifications. **Conclusions:** The new classification demonstrated increased sensitivity in detecting midpalatine suture maturity and also increased the likelihood of utilizing non-surgical maxillary expansion compared to the previous classification. Evaluating suture staging in individual cases using CBCT is recommended for personalized diagnosis and optimal treatment planning for maxillary expansion. This advancement allows clinicians to use the new classification as a reliable tool to confidently predict non-surgical expansion success for more mature patients, thereby broadening the scope of orthodontic treatment without compromising patient outcomes.

Keywords: palatal expansion; midpalatine suture; cone beam computed tomography; cranial sutures; palatine bone; rapid maxillary expansion

1. Introduction

During growth, a series of joints grows at the boundaries of the adjacent bones of the cranium, mainly belonging to a type of syndesmosis defined as ‘suture’. Sutures permit

stress dispersal and bone remodeling through growth, which occurs over the period of bone formation during sutural distraction [1]. The midpalatine suture is known as the junction of two bones that meet at the same end and undergo changes in their morphology as they grow. Its shape is described as a Y shape in the frontal part [2].

The assessment of the development of the mid-palatine suture is extremely vital before a clinical decision on correction of a transverse growth discrepancy of the maxilla, termed as slow maxillary expansion (SME), rapid maxillary expansion (RME) with a conventional palate expander in cases with opened suture (children and adolescents) or surgically using MARPE (mini screw-assisted rapid palatal expander) and SARPE (surgical assisted rapid palatal expander) in closed suture cases such as adult patients [3]. Cone Beam Computed Tomography (CBCT), being an advanced imaging modality in dentistry, can be used to study the growth patterns of the midpalatine region.

Mid-Palatine Suture (MPS) development was studied using CBCT in different populations to evaluate skeletal maturity [2,4–6]. These studies aimed to classify the different stages of midpalatine suture fusion, which is crucial in determining the optimal timing for treatments involving maxillary bone expansion, particularly in adolescents and young adults [5]. Notably, previous studies by Haghanifar et al. (2017) and Soman et al. (2025) found new observed patterns of MPS staging, other than those mentioned by the previous classification [2,4,7].

Interestingly, age is usually correlated with suture maturation. It is expected that the suture will be closed entirely at a certain age. However, variations in suture closure can exist within the same bone, mainly when the bone is formed from more than one component, like the palatine bone that has the maxillary part (premaxilla) and the palatine part. There are no reported studies that have explored the possibilities of new subcategories beyond the standard classification by the previous classification [4]. The current lack of detailed sub-staging may contribute to inconsistent diagnostic interpretations and sub-optimal treatment planning in cases that require maxillary expansion fine ambiguous radiographic presentation. Hence, the rationale of this study was to explore the possibilities of identifying varying patterns in the maturation staging of MPS, aiming to provide a more detailed and clinically relevant assessment tool.

The aim of the study was thus to develop a comprehensive subcategorization system for midpalatine suture maturation staging based on Cone Beam Computed Tomography. The null hypothesis of the study was that there is no significant difference between mid-palatine suture maturation staging using CBCT and the previous reference classification by and staging using the proposed new classification.

2. Materials and Methods

Study design: Retrospective cross-sectional study on CBCT scans.

Study Setting: The study was conducted in a private dental university affiliated with the investigators. Retrospective CBCT scans were collected (specification-Sirona Galileos, Germany, Bensheim city at 85 kV, 57 mA, 14s) between January 2018 and January 2024. The study was conducted in accordance with the Declaration of Helsinki 2013, and approved by the Institutional Review Board (or Ethics Committee) of Riyadh Elm University (IRB no. FUGRP/2024/368/1162/1045 on 8 October 2024) and a waiver of informed consent for the analysis of retrospective data was approved.

Participants/Samples: CBCT scans were screened following the eligibility criteria below.

Inclusion Criteria:

- Both genders.
- CBCT scans with clear images of the region of interest.

Exclusion Criteria:

- Impacted teeth in the region of interest.
- History of or ongoing orthodontic treatment or use of orthodontic appliances.
- History of maxillofacial trauma or surgery in the maxilla.
- Presence of systemic diseases affecting the bone or bone diseases.
- Presence of pathologies, developmental abnormalities, or syndromic conditions affecting the maxilla.
- CBCT scans with noise or blurred images.

Sampling: Samples were segregated during data analysis using a stratified random sampling technique, where age served as the basis for stratification. The stratified sampling method was used to ensure that all biological maturation stages, from early adolescence to young adulthood, were included in the study sample. This approach enabled the new classification to be tested across various age groups where the MPS varies, and the growth stage can be harnessed for maxillary expansion, allowing for increased precision in this process. Randomization was subsequently carried out within each stratified group to recruit samples into the study, ensuring unbiased representation of the study sample and facilitating statistical validity. The stratified samples included four groups: 10–13 years (early stage of adolescence), 14–17 years (mid-adolescent stage), 18–21 years (late adolescent stage), and 22–25 years (young adulthood). After segregation, random sampling was conducted from these groups.

Sample size: The sample size calculation was derived from a previous study by Jimenez-Valdivia et al. (2019) [5]. The sample size parameters of Jimenez-Valdivia et al.'s 2019 study provided the statistical rationale for validating the new classification in the quest to detect an open MPS (one proportion) in each category or stratum of the sample. This calculation increased the likelihood of managing maxillary expansion non-surgically. Thus, by using this sample size calculation, it was ensured that this study had adequate samples to measure this one proportion reliably with a precision of 5%, confidence interval 95%, 80% power, and an anticipated proportion of 10% (which guaranteed to detect even a few samples in a group with clinical significance); the minimum required sample size was 163 CBCTs. The current study gathered 168 CBCT scans, applied the new classification, and compared it to the previous standard classification regime [2].

Variables:

- *Independent Variables:* Age, Gender, stages of maturation of the MPS.
- *Dependent Variables:* Open or Fused MPS (open sutures are non-surgically expandable and fused being surgically expandable), prediction of possibility of open MPS according to the new classification.
- *Covariates:* Age and Gender.

Data sources/measurement: The analysis of the CBCT images was done using Galileos viewer (Sidexis XG), version 1.6. The sample CBCT images were viewed using multiplanar reformatted windows, allowing for detailed analysis of the axial, coronal, and sagittal planes. The skeletal stages of maturation of the midpalatal suture were visualized and classified using axial sections following the reference staging below and further scrutinized for a newly proposed subcategorization of maturation of the MPS. All of the patient identifiers were removed to anonymize the data after the data collection was completed.

Reference Staging for the Maturation of Midpalatine Suture [2]

Stage A: Nearly straight high-density sutural line with no or minimal interdigitation in the maxilla and palate.

Stage B: One or two irregular, high-density, scalloped sutural lines in the maxilla and palate. When two lines are present, they may be parallel with a few small low-density radiolucent spaces in between the lines.

Stage C: Straight or irregular, two well-defined high-density lines in the maxilla and palate, scalloped with small low-density intra-sutural spaces.

Stage D: Fusion of the palatal suture with bone, while maxillary suture is still two high-density lines with radiolucent spaces in between.

Stage E: Fusion of mid-palatine suture in the maxilla and palate where no visible suture is identifiable.

Proposed new classification [Figures 1 and 2]

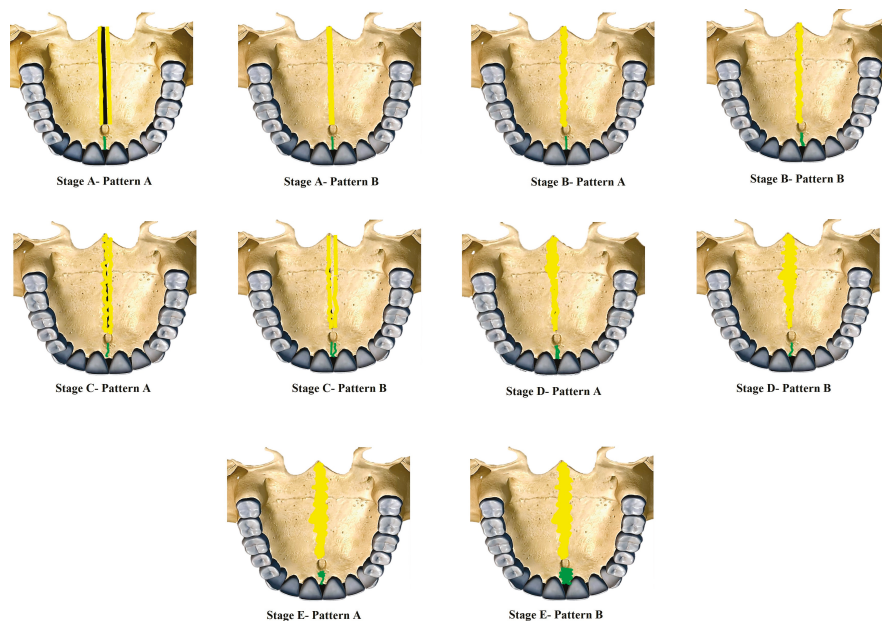


Figure 1. New classification of Midpalatine Suture Staging with five stages and two patterns per stage represented in skull based pictorial representation. Yellow lines represent the palatine part and green line represents the maxillary/premaxillary part of Midpalatine suture.

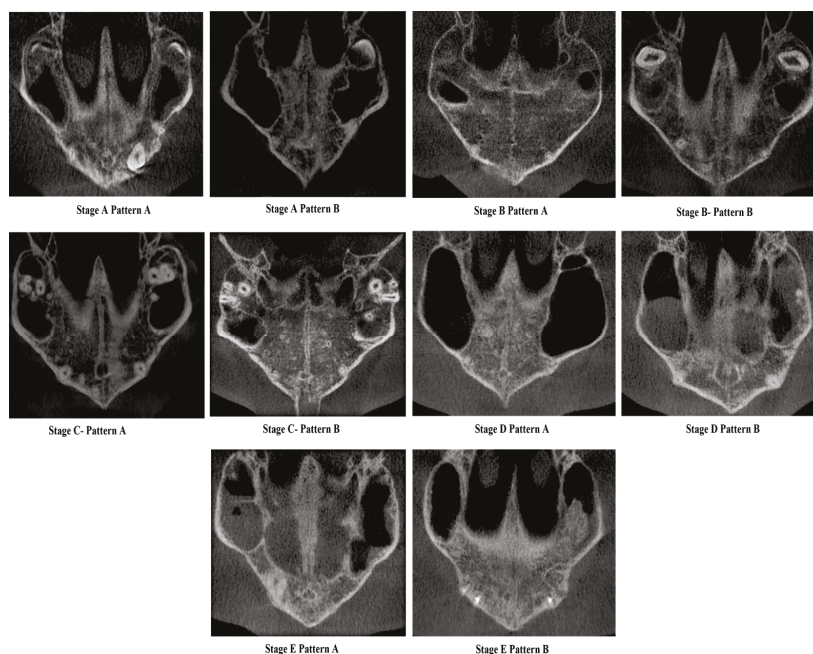


Figure 2. Cone Beam Computed Tomographic images of the New classification of Midpalatine Suture Staging with five stages and two patterns per stage.

Stage A—Pattern A—Single, wide, continuous radiolucent linear space bounded by well-defined radiopaque lines extending from posterior palate anteriorly.

Stage A—Pattern B—Same as the previous standard classification Stage A—Single high-density relatively straight radiopaque (RO) line [2].

Stage B—Pattern A—Single straight high-density sutural line in the maxillary region, comparable to Stage A. The suture is single scalloped toward the palatal bone region.

Stage B—Pattern B—Equivalent to the previous standard classification Stage B—Single scalloped line of high-density radiopacity.

Stage C—Pattern A—Single scalloped line in the region of the premaxilla and toward the palate with two parallel RO lines with small radiolucent (RL) areas.

Stage C—Pattern B—Similar to the previous standard classification Stage C—Continuous or irregular-2 RO, non-scalloped or scalloped, parallel lines separated by low-density (RL) areas in the palatal and premaxillary region.

Stage D—Pattern A—Palatine suture (not completely fused)—50% or less than 50% fused in the posterior maxilla and continues anteriorly as two parallel radiopaque straight or irregular lines with radiolucent spaces, maxillary suture seen as two continuous or irregular RO lines or a single radiopaque line.

Stage D—Pattern B—Palatine suture—more than 50–100% fusion of posterior palatine region and continues anteriorly as straight or irregular lines with radiolucent spaces in between, maxillary suture seen as two continuous or irregular RO lines or a single radiopaque line.

Stage E—Pattern A—Palatine suture completely fused, but maxillary suture 50% or less than 50% fused and seen as two RO lines toward maxilla.

Stage E—Pattern B—Palatine suture completely fused and maxillary suture more than 50–100% or near complete fusion.

2.1. Validity and Reliability of New Classification

A validation study of the novel midpalatal suture maturational stages classification was carried out by three experienced investigators (C.S., N.A., F.A.): an oral radiologist, an orthodontist, and an oral pathologist. Each had more than five years of experience interpreting CBCT scans for research and diagnostic purposes. Prior to the study, the investigators were shown the definitions and illustrations (Figures 1 and 2) of the maturational stages via a PowerPoint presentation against a black background. Calibration took place using ten images, where all investigators independently classified the sutures, followed by a discussion of the different stages. For the validation phase, 30 images—randomly chosen to represent all maturational stages—were classified blindly by the three investigators in a controlled, dimly lit environment, using the same high-definition monitor. The same images were randomly reordered and reclassified by the investigators two weeks later to assess intra-rater reliability.

2.2. Statistical Analysis

The collected data were analyzed using SPSS version 25.0. Descriptive statistics were used for categorical and ordinal variables as frequencies and percentages. A normality check was not required, as the data being compared were categorical. The inter- and intra-examiner reliability and reproducibility was conducted to establish the validity and reliability of the new proposed MPS staging classification. Sensitivity and specificity tests were applied to detect MPS between the new proposed and standard classifications. Furthermore, the association between the possibility of an open MPS in the staging of MPS maturation using the new classification and gender across different age groups was examined using the chi-square test. Comparing the presence of an open MPS during MPS staging between

males and females across four age groups, a binary logistic regression model was used to identify predictors of MPS maturation. A value of $p < 0.05$ was considered significant.

3. Results

The results derived from the study provides the following key information with regard to the maturation of MPS staging using the new classification and its predictability. A total of 168 CBCT images, spanning from 2018 to 2024, were investigated in this study.

The assessment of both intra-examiner (within the same reviewer) and inter-examiner (between different reviewers) reliability revealed outstanding agreement. This was quantified by weighted kappa coefficients ranging from 0.94 (with a 95% confidence interval [CI] of 0.89 to 0.99) down to 0.84 (95% CI, 0.78 to 0.89) (Table 1).

Table 1. Sensitivity and specificity midpalatine suture staging of the new classification versus previous classification.

Sensitivity and Specificity MPS Stages					
New classification	Maturity	Count	Previous Classification		Total
			Maturity	No Maturity	
		% within previous classification	100.0%	21.2%	69.0%
	No maturity	Count	0	52	52
		% within previous classification	0.0%	78.8%	31.0%
	Total	Count	102	66	168
		% within previous classification	100.0%	100.0%	100.0%

Table 2 presents the distribution of the study variables (age group, gender, current and proposed classification systems). The majority of CBCTs belonged to young adults aged 22–25 years (34.5%), followed by late adolescents aged 18–21 years (31.0%). Mid-adolescents (14–17 years) and early adolescents (10–13 years) accounted for 23.2% and 11.3%, respectively.

Table 2. Distribution of study variables, their frequency, and percentage.

Study Variables ($n = 168$)		n	%
Age	10–13 (Early adolescence)	19	11.3%
	14–17 (Mid-adolescence)	39	23.2%
	18–21 (Late adolescence)	52	31.0%
	22–25 (Young adults)	58	34.5%
Gender	Male	73	43.5%
	Female	95	56.5%
Current classification of maturation of MPS	Stage A	11	6.5%
	Stage B	34	20.2%
	Stage C	57	33.9%
	Stage D	39	23.2%
	Stage E	27	16.1%

Table 2. Cont.

Study Variables (<i>n</i> = 168)		<i>n</i>	%
Proposed classification of maturation of MPS	Stage A—Pattern A	5	3.0%
	Stage A—Pattern B	6	3.6%
	Stage B—pattern A	19	11.3%
	Stage B—pattern B	14	8.3%
	Stage C—pattern A	21	12.5%
	Stage C—pattern B	37	22.0%
	Stage D—pattern A	14	8.3%
	Stage D—pattern B	25	14.9%
	Stage E—pattern A	7	4.2%
	Stage E—pattern B	20	11.9%

The study included 73 males (43.5%) and 95 females (56.5%), indicating a higher proportion of female participants. Based on the stages, the participants were classified into five stages (A–E), with the highest proportion in Stage C (33.9%) and the lowest in Stage A (6.5%). Within each stage, a further classification was made into patterns A and B. The most common group was Stage C—Pattern B (22.0%), followed by Stage D—Pattern B (14.9%) and Stage B—Pattern A (11.3%). The least common group was Stage A—Pattern A (3.0%).

The distribution of the maturation of the MPS by age group and gender is shown in Table 3. The maturation of the MPS progressed with age, with both males and females exhibiting a shift from early stages (A, B, C) in younger age groups to later stages (D, E) as age increased. In the maturation of the MPS, gender differences indicate that females tend to reach higher stages earlier than males, particularly evident in the 18–21 and 22–25 age groups. Early adolescence showed maturation of MPS stages of A, B, and C. Mid-to-late adolescents predominantly showed Stages B and C, with a gradual emergence of Stage D observed. However, in young adulthood, there was an increase in representation in Stages D and E (Figure 3).

Table 3. Maturation of midpalatine suture staging based on previous classification (2013).

Maturation of Midpalatine Suture Staging												
Age	Gender	Stage A		Stage B		Stage C		Stage D		Stage E		Total
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	
10–13	Male	4	50.0	3	37.5	1	12.5	0	0.0	0	0.0	8
	Female	3	27.3	4	36.4	3	27.3	0	0.0	1	9.1	11
14–17	Male	0	0.0	7	41.2	8	47.1	2	11.8	0	0.0	17
	Female	2	9.1	8	36.4	10	45.5	2	9.1	0	0.0	22
18–21	Male	0	0.0	4	15.4	8	30.8	7	26.9	7	26.9	26
	Female	0	0.0	3	11.5	14	53.8	8	30.8	1	3.8	26
22–25	Male	2	9.1	2	9.1	4	18.2	7	31.8	7	31.8	22
	Female	0	0.0	3	8.3	9	25.0	13	36.1	11	30.6	36

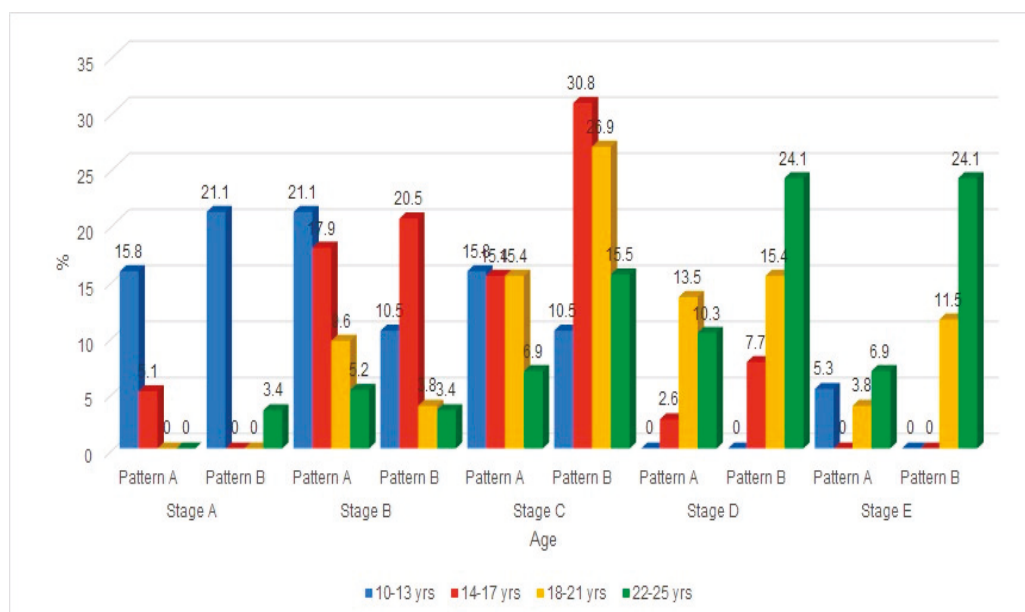


Figure 3. Cone Beam Computed Tomographic images of the New classification of Midpalatine Suture Staging with five stages and two patterns per stage.

The maturation of the midpalatal suture (MPS) was analyzed across males and females within four age groups (10–13, 14–17, 18–21, and 22–25 years). The analysis reveals a clear trend in which the possibility of maturation of the MPS decreases with increasing age across both genders. In the youngest age groups (10–13 and 14–17 years), a high proportion of both males and females were identified as having the possibility of maturation of the MPS, with percentages exceeding 88% and minimal differences between genders. In the 18–21 and 22–25-year groups, the proportion of individuals with the possibility of maturation of the MPS declined in both males and females, with the lowest rates observed in the oldest age group (22–25 years). Statistical comparisons within each age group demonstrated no significant differences between males and females regarding the possibility of maturation of the MPS, as indicated by p -values greater than 0.05. These findings suggest that while age is associated with a reduced likelihood of maturation of the MPS, gender does not significantly influence this association within any specific age group ($p > 0.05$).

The classification across five developmental stages (Stage A to Stage E) is further divided into two patterns (Pattern A and Pattern B), as proposed. The data was organized by age group (10–13, 14–17, 18–21, 22–25 years) and gender (male, female). In younger age groups (10–13, 14–17), more subjects are found in the earlier stages (A, B, C), with a larger spread across both patterns. As age increases (18–21, 22–25), a noticeable shift occurs, with more individuals progressing to later stages (D, E), particularly in Pattern B. The distribution between Pattern A and Pattern B within each stage shows some gender and age-related variation.

The maturation of the midpalatal suture (MPS) was analyzed across males and females within four age groups (10–13, 14–17, 18–21, and 22–25 years). The results showed that all males and 90.9% of females in the 10–13 age group displayed evidence of MPS maturation. Similarly, 88.2% of males and 95.5% of females aged 14–17 years showed a possibility of maturation of the MPS. Over half of the males (53.8%) and 84.6% of females in the age range of 18–21 revealed possibilities of maturation of the MPS. In contrast, less than half of the males (45.5%) and females (44.4%) showed the possibility of maturation of the MPS within the age range of 22–25 years. Hence, it is noted that individuals aged 10–17 exhibited high maturation rates in both males and females. The age range of 18–25 years showed a decline in maturation rates, especially among males. The gap between males and females is most

pronounced in the 18–21 age group, although it is not statistically significant. However, maturation rates dropped to similarly low levels in both genders. All age groups showed a $p > 0.05$, indicating no statistically significant association between gender and maturation status in any age group.

A comparison of the proportion of individuals classified as having “Maturity” or “No maturity” using the current and proposed maturation of MPS classifications is detailed in Table 4. The current classification showed that 60.7% (102 out of 168) are classified as mature, while the proposed classification showed that 69.0% (116 out of 168) are classified as mature. The $p < 0.001$ indicates a statistically significant difference between the current and proposed classification systems in identifying “Maturity” versus “No maturity” of maturation of the MPS. This suggests that the proposed classification is more likely to classify individuals as mature compared to the current classification.

Table 4. Comparison of maturation of Midpalatine Suture (MPS) between current and proposed classification.

Maturation of MPS—Current and Proposed Classification				
Current Classification		Proposed Classification		<i>p</i>
Maturity	No maturity	Maturity	No maturity	
102 (60.7%)	66 (39.3%)	116 (69%)	52 (31%)	<0.001

The predictors of maturation of the MPS are shown in Table 5. Both classifications indicate that age is a strong, statistically significant predictor of maturation of the MPS ($p < 0.001$). For the current classification, each unit increase in age increases the odds of maturation of the MPS by 3.57 times (95% CI: 2.28–5.58). For the proposed classification, a one-unit increase in age increases the odds of maturation of the MPS by 3.42 times (95% CI: 2.11–5.53), confirming its statistical significance. Hence, it can be noted that older age is consistently and strongly associated with a higher likelihood of maturity, regardless of classification type. Contrarily, gender was not found to be a significant predictor of maturation of the MPS in either the current classification ($p = 0.419$, $\text{Exp(B)} = 0.74$, 95% CI: 0.36–1.53) or the proposed classification ($p = 0.114$, $\text{Exp(B)} = 0.55$, 95% CI: 0.26–1.16). This suggests that gender has no significant effect on the maturation of the MPS.

Table 5. Predictors of maturation of Midpalatine Suture (MPS).

Predictors of Maturation of MPS									
		B	S.E.	Wald	df	Sig.	Exp(B)	95% CI for Exp(B)	
								Lower	Upper
Previous classification	Age	1.272	0.228	31.221	1	0.000	3.569	2.284	5.577
	Gender	−0.297	0.367	0.654	1	0.419	0.743	0.362	1.526
	Constant	−3.849	0.890	18.720	1	0.000	0.021		
Proposed classification	Age	1.228	0.246	25.001	1	0.000	3.416	2.110	5.529
	Gender	−0.601	0.380	2.501	1	0.114	0.548	0.260	1.155
	Constant	−3.705	0.937	15.645	1	0.000	0.025		

Variable(s) entered on step 1: Age, Gender.

A logistic regression analysis to predict a new classification based on age and gender indicates a good fitness of the new classification model. According to the observed and analyzed data, both classifications (standard reference and new) can rely on age ($p < 0.001$

and odds ratio 3.416) to predict the likelihood of maturation of the MPS compared to non-maturation, indicating that the new classification is a good fit. The new classification offers a higher possibility of detecting a midpalatine suture opening compared to the standard classification.

4. Discussion

The present study was conducted for applicability of the new classification to detect an open MPS and thus expandable sutures that can be managed without surgery. The highest prevalence of the samples (60.6%) was in the potentially expandable, non-surgical expansion categories (Stage A: 11, B: 34, C: 57). The potentially non-expandable stage that would require surgical intervention accounted for up to 39.3% of the study samples (Stage D and E). Stage C, with 33.9%, was the single most common stage in both classifications that were partially open/partially fused and possibly had high resistance to expansion, with a definite possibility of expansion. The new classification subcategorizes each stage into Pattern A and Pattern B for greater diagnostic accuracy in detecting stages with patterns that utilize non-surgical expansion. The possibility of detection of an open MPS was highest in the younger age groups (100%) (10–13-year old males) and 90% in 14–17-year old females. The likelihood of open MPS sharply decreased with increasing age, as shown in the results, with 33% in females and 36.4% in males in the 22–25-year-old group. The above results reinforced the notion that age is a determining factor in MPS maturation. Gender was not a determining factor in MPS maturation, as the Chi-squared test revealed no significance between gender and the likelihood of detecting an open MPS. The few deviations from the above are likely due to chance. The standard classification and the new classification thus relied on age as a predictable variable, with the new classification displaying increased sensitivity in detecting open MPS.

The midpalatine suture region is one of the key areas where the biological process of suture maturation can be harnessed for the prevention and treatment of malocclusion due to underdevelopment of the maxilla, resulting in size/shape discrepancies in the maxillary arch (transverse maxillary deficiency). Maxillary transverse deficiency (MTD) can cause various dentofacial abnormalities, including tooth crowding, anterior tooth protrusion, posterior tooth crossbite, mouth breathing, and obstructive sleep apnea. It is also associated with various abnormalities such as cleft lip/palate and craniosynostosis [8]. MTD cannot be resolved over time during the individual's development. Therefore, an early diagnosis and intervention are imperative. Treatment of such conditions involves maxillary expansion. One of the key factors in the treatment plan is the stage of maturation of MPS and the patient's age.

The present study evaluated the maturation of the midpalatine suture by exploring new subcategories of classification and comparing them with the staging of the previous MPS classification by. In the present study, the majority of the sample consisted of young adults or late adolescents, which can be attributed to the most common age group seeking dental care within the specified age range selected for this study. Additionally, the female gender was more prevalent than the male gender in the study population, possibly due to the greater proportion of females seeking dental care, which may be attributed to higher oral health literacy and oral care [9].

The midpalatal sutural fusion was thought to be complete by the age of 15 to 19 years. Unsurprisingly, the possibility of RME was previously considered only until 16 years of age, as with older age, the midpalatal suture and adjacent articulations start to fuse and become more rigid, leading to a higher resistance to expansion forces [10]. During the period of late adolescence and adulthood, extra force is indispensable to open the MPS due to its increased degree of interdigitation. Consequently, after the age of 16, MARPE

or SARPE was frequently used to overcome these limitations by surgically releasing the interdigitated suture prior to maxillary expansion [11]. Some studies suggest that RME should be performed before puberty, based on the above observation [12,13]. However, contrasting results of fusion of the MPS were found in studies across various age groups, with variations observed up to the age of 71 years, indicating that age may not be a completely reliable factor for MPS maturation [10,14,15]. These findings were noted in the present study, where there were cases in which age and the maturation of the MPS were inconsistent. In this study, it was also noted that although younger individuals are more likely to represent immature stages of the MPS, a notable share of young adults and late adolescents may be in stages that are amenable to non-surgical expansion. Although the majority of the scan age groups could predict the MPS's maturation and staging, it was interesting to find that in some cases, the MPS staging differed from that predicted by age. The stages of fusion of the midpalatal suture were found to vary significantly with age. These findings were similar to those of Angelieri et al. (2013) and Haghanifar et al. (2017) [2,4]. However, further analysis showed that age was a significant predictor of MPS maturation in this study, while gender did not play a significant role in maturation of the MPS. Gender may not be a significant factor, as the closure of palatine sutures is influenced by various factors, including age, vascularity of the region, the genetic composition of the individual, hormonal and locoregional conditions, and other factors [16].

CBCT is the most widely used imaging technology in dentistry for assessing the maturation of the MPS. In the previous classification of maturation of the MPS using CBCT, Stage C was found to be the most prevalent [2]. This finding is noteworthy, as at this stage, maxillary expansion is still achievable without surgical intervention. Although this classification can explain the maturation stages, variations were observed that did not fit into specific stages of maturation [4,7]. Additionally, the results from a cohort study evaluating 63 adolescent patients in the pre- and post-phases of adolescence, using pre- and post-expansion with RME, assessed the applicability of the classification. The study concluded that this classification requires further research and validation and did not support the efficacy of this classification in predicting changes in maturation of the MPS [17].

The proposed classification provided more details for maturation staging, with the most prevalent being Stage C—Pattern B (22.0%), followed by Stage D—Pattern B (14.9%) and Stage B—Pattern A (11.3%). The distinguishing ability of these patterns within the same stage enables diagnostic precision. Subtle variations in maturation within the same stage, represented as Pattern A and Pattern B, can influence the extent of resistance to maxillary expansion in the MPS. The prognosis of RME is determined by the maturation of the MPS and the bone strength at this level, along with the multitude of sutures in the facial skeleton [18]. The choice of RME, such as conventional RME, MARPE, or SARPE, and the differences in design of these RME appliances, their confirmation, and the techniques directed to achieve specific treatment outcomes rely on the maturation of the MPS [19]. Hence, for RME, maturation of the MPS should be evaluated as part of the treatment plan rather than relying solely on age.

The new classification of MPS maturation using CBCT also demonstrated a higher detection rate of MPS maturity compared to the previous classification. The higher detection rate of MPS maturation in identifying an open MPS indicates that the new system of classification showed improved sensitivity in identifying maturity stages and thus has greater diagnostic accuracy. The applied aspect of the new classification emphasizes that individual or personalized suture assessment is crucial in treatment planning for maxillary expansion, rather than relying on standard chronological age-based treatment planning.

Thus, in stages and patterns of MPS maturation according to the new classification, where the MPS is open or partially ossified from Stage A to Stage D, Pattern A, non-surgical maxillary expansion techniques can be considered including RME, SME, and MARPE [3,4,20–22]. In the previous classification, this possibility was limited to Stage C [2]. Additionally, Stage D—Pattern B and Stage E—Pattern A in the new classification indicated the possibility of maxillary expansion results being feasible, confined to the maxillary segment alone of the hard palate. For Stage E—Pattern B, where ossification is complete, surgical techniques such as SARPE or Maxillary Le Fort I osteotomy can be considered [23,24].

The most compelling evidence that the new classification is superior to the previous classification in terms of clinical applicability is the extension of stages where non-surgical maxillary expansion is considered achievable, and the identification of a larger sample of mature patients who can benefit from the aforementioned less invasive maxillary expansion techniques. The new classification also guides maxillary expansion, even in stages such as D and E, where limited maxillary expansion is still attainable, confined to the maxillary segment of the hard palate. The new classification allows clinicians to achieve non-surgical, targeted results in maxillary expansion. The new classification thus offers more detailed predictions an open MPS compared to the previous classification, which treats cases deemed mature, making it a superior clinical tool compared to the previous method of assessing MPS maturation.

Limitations of the study included that the research was conducted as a single center study; interobserver bias, the investigators required calibration twice to achieve good inter-examiner agreement and reliability, as well as to standardize the detection of staging patterns and absence of clinical validation. The study did not compare the MPS maturation with the facial growth patterns of the face.

Prospective studies are necessary to determine whether the new patterns identified in the staging of the MPS in the new classification translate into measurable outcomes in treatment planning, treatment outcomes, and stability, thereby evaluating the efficacy and validity of the proposed new classification. A larger sample size will definitely increase the validity and applicability of the new classification. Further, studies integrating artificial intelligence training models to detect the maturation stages of the MPS will ease the detection of MPS and be beneficial. Recently, an artificial intelligence model has been tried for maturation of the MPS using 2D convolutional models, as proposed by the previous classification) [2,25]. The study also states that if future studies challenge the classification, the validity of the aforementioned study will be affected. Thus, the present study and the new classification should be explored as a new scaffold for AI training and the detection of open sutures.

5. Conclusions

The study observed a clear progression of maturation, as indicated by midpalatine suture staging, and this finding was valid in both classifications. Age was a strong predictor of the maturation staging in both classifications. In the new classification, each stage was divided into Pattern A and Pattern B, with the most common patterns being Stage C—Pattern B and Stage D—Pattern A, which provides a crucial diagnostic differentiation, suggesting a new classification of superior choice. The new classification demonstrated increased sensitivity in detecting midpalatine suture maturity. This improved accuracy enables more reliable staging and assessment, thereby enhancing the potential to guide palatal expansion decisions where previous classifications have shown limitations. The present study highlights the role of CBCT-based suture staging in personalized interven-

tions, establishing a new classification system that improves clinical decision-making. The results are preliminary and require multicenter and prospective validation.

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Article

Volumetric Analysis of Maxillary Sinus and Nasal Conchae According to Skeletal Classes and Cranio-Maxillary Relation

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Abstract

Background: This study aimed to evaluate the volumetric characteristics of the inferior and middle nasal conchae and maxillary sinuses in individuals with different skeletal malocclusion classes and cranio-maxillary relationships using cone beam computed tomography (CBCT). **Methods:** A total of 150 adult patients were retrospectively analyzed. CBCT scans were used to obtain volumetric measurements of the right and left inferior nasal conchae (INC), middle nasal conchae (MNC), and maxillary sinuses (MS). Patients were categorized into skeletal Classes I, II, or III based on ANB angles, and into retrognathic, normal, or prognathic groups according to SNA angles. Gender- and age-related differences were also analyzed. Statistical comparisons were performed using appropriate parametric and non-parametric tests. **Results:** Class II individuals exhibited significantly lower conchal volumes compared to Class I and III groups, while MS volumes were highest in Class II, although statistical significance was reached only on the left side. Gender differences were evident, with males presenting greater volumes than females in both the right and left INC and MS; however, significant differences were observed only for the left INC and left MS. A significant age-related decrease in left INC volume was found between the 21–30 and 61+ age groups. No statistically significant correlation was detected between conchal and sinus volumes. **Conclusions:** Skeletal malocclusion patterns, gender, and age significantly influence concha and sinus volumes. These findings emphasize the utility of CBCT-based three-dimensional assessments in enhancing diagnostic accuracy and informing interdisciplinary treatment planning in orthodontics and craniofacial care.

Keywords: nasal concha; maxillary sinus; volumetric assessment; skeletal malocclusion; CBCT

1. Introduction

The viscerocranium, which encompasses key anatomical components such as the nasal conchae and nasal cavity, plays an integral role in the development and structural configuration of the craniofacial complex. These components develop in a coordinated

and interconnected manner, beginning early in fetal life and continuing through postnatal growth, particularly in conjunction with the paranasal sinuses and maxillofacial bones [1].

Among these, the nasal conchae—bony projections forming the lateral walls of the nasal cavity—are of particular anatomical and functional significance [2,3]. They participate in regulating nasal airflow, humidification, and filtration, and their development is closely linked with adjacent structures, including the maxillary sinuses [1]. The growth of these structures is strongly influenced by respiratory patterns [4].

Alterations in normal breathing, especially chronic mouth breathing induced by conditions such as adenoid hypertrophy, allergic rhinitis, or nasal concha enlargement, may disrupt the balance of orofacial forces. In children, persistent mouth breathing may result in maladaptive tongue posture, reduced muscle tone in the lips and cheeks, and a lowered resting position of the tongue [5]. These conditions may contribute to the development of malocclusions and craniofacial growth disturbances [6].

In addition to their impact on dental occlusion and facial symmetry, altered breathing patterns can influence the pneumatization and volumetric growth of the paranasal sinuses [7]. Adequate nasopharyngeal airflow is essential for the proper aeration and development of these air-filled spaces. It has been reported that individuals with prolonged oral breathing patterns tend to exhibit significantly reduced maxillary sinus volumes when compared to nasal breathers [8].

Skeletal malocclusions, which are typically classified based on the sagittal relationship between the maxilla and mandible, may also correlate with anatomical variations in the nasal cavity and paranasal sinuses [9]. Understanding the volumetric characteristics of nasal conchae and maxillary sinuses has important clinical implications. Differences in these anatomical structures may influence nasal airflow, orthodontic treatment outcomes, and surgical planning in patients with skeletal malocclusion. In particular, volumetric variations can provide additional diagnostic information for orthodontists and maxillofacial surgeons when planning interventions such as rapid maxillary expansion, orthognathic surgery, or the management of airway-related problems. However, no study has been found in the literature regarding the volumetric data of nasal conchae and sinuses across different skeletal malocclusion types.

Therefore, this study aimed to investigate the volumetric characteristics of the maxillary sinuses and nasal conchae—specifically the inferior and middle conchae—in individuals with different skeletal malocclusion classes, utilizing cone beam computed tomography (CBCT). Understanding these anatomical relationships could provide insights into the etiopathogenesis of malocclusion and offer guidance for clinical planning in orthodontics and maxillofacial interventions.

2. Materials and Methods

This study adhered to the Declaration of Helsinki guidelines, with ethical approval granted by the Near East University Scientific Research Evaluation Ethics Committee (approval number: YDU 2019/67-785). Written informed consent was obtained from all participants prior to undergoing CBCT imaging.

In this study, the effect size, calculated as Cohen's f , was found to be 0.258. This effect size was utilized to determine the sample size required to achieve sufficient statistical power. The study was designed with a 95% confidence level and an 80% theoretical power. To meet these criteria, the study was conducted with a total of 150 participants, with at least 50 subjects allocated to each skeletal malocclusion group. A retrospective analysis was conducted of 150 full-head CBCT scans from patients treated at the Near East University, Faculty of Dentistry Department of Dentomaxillofacial Radiology between 2009 and 2017. The study consisted of 86 female and 64 male patients with a mean age

of 38.67 ± 16.53 years old. These scans were initially performed for various diagnostic purposes including pre-osteotomy planning, paranasal sinus evaluation, implant planning, impacted teeth surgery and temporomandibular joint disease assessment.

The study included adult participants aged 18 years and older. Exclusion criteria encompassed patients with known hereditary or systemic diseases that could potentially influence the study's outcomes, as well as those with tumoral lesions in the regions of interest or prior surgical interventions affecting these areas. Additionally, CBCT scans that exhibited artifacts such as scatter caused by metallic dental fillings, or any other conditions that compromised the clarity or diagnostic quality of the images, were also excluded from the study.

The Newtom 3G (Quantitative Radiology s.r.l., Verona, Italy) was used for CBCT imaging. Patients were positioned horizontally with their teeth in natural occlusion, and their head and jaw stabilized; the Frankfurt horizontal plane was maintained perpendicular to the ground. Imaging parameters included isotropic voxels with an axial slice thickness of 0.3 mm, operating at 120 kVp and 3–5 mA within a 12-inch field. All evaluations were carried out on a 21.3-inch flat panel color active matrix TFT medical [(Nio Color 3MP, Barco, Belgium), a resolution of 2048×1536 at 76 Hz] screen.

Axial images, exported in DICOM format, were imported into the InVivoDental 5.1.2[®] software (Anatomage Inc., San Jose, CA, USA) for analysis. For volume measurements of the nasal conchae (Figure 1) and maxillary sinuses (Figure 2), we adjusted the “Soft Tissue 1” visualization setting in the “Volume Render” tab. This involved fine-tuning the brightness and opacity to optimize the visibility of the soft tissue structures within the nasal cavity. Each nasal concha and sinus was clearly delineated using the software's segmentation/cutting tool, adhering to anatomical boundaries to isolate the areas of interest. The “Volume Measurement” tool in the software was utilized to measure the volumetric data. Volume measurements of the maxillary sinus, inferior and middle nasal conchae, and 3D cephalometric analysis were conducted using the InVivoDental program.

Angular measurements were conducted based on cephalometric landmarks identified in this study, which helped assess the spatial orientation of the maxilla and mandible. Specifically, the SNA angle was used to determine the position of the maxilla, the SNB angle to assess the position of the mandible, and the ANB angle to evaluate the maxillomandibular relationship within the midsagittal plane. Subsequently, skeletal classifications were categorized based on the ANB angle values: Class I ($0-4^\circ$), Class II ($>4^\circ$), and Class III ($<0^\circ$). Also to assess the effect of sagittal maxillary position on the measured volumes, patients were classified into three categories based on their SNA angle: retrognathic ($\text{SNA} < 80^\circ$), normal ($\text{SNA } 80-84^\circ$), and prognathic ($\text{SNA} > 84^\circ$).

To assess intra-observer reliability, the same observer re-evaluated a random subset of the images after a 2-week interval. The intra-observer reliability was calculated using the Intraclass Correlation Coefficient (ICC).

For the evaluation of age-related differences, participants were divided into six categories (18–20, 21–30, 31–40, 41–50, 51–60, and 61+ years). These intervals were selected to ensure a balanced distribution across decades and to reflect distinct physiological stages of adulthood. This categorization allowed the detection of potential volumetric changes not only between younger and older adults but also across intermediate decades.

Statistical analyses were performed using SPSS 23 (IBM Corp., Armonk, NY, USA). Data normality was assessed with the Shapiro–Wilk test, guiding the selection of appropriate statistical tests. Depending on the normality of the data, the Independent sample *t*-test and ANOVA were used for normally distributed variables, complemented by the Bonferroni correction. For non-normally distributed variables, the Kruskal–Wallis H test

was applied. A significance level of 0.05 was maintained throughout, where p -values below 0.05 were considered statistically significant.

The intra-observer reliability analysis yielded an ICC of 0.92, confirming excellent measurement consistency.

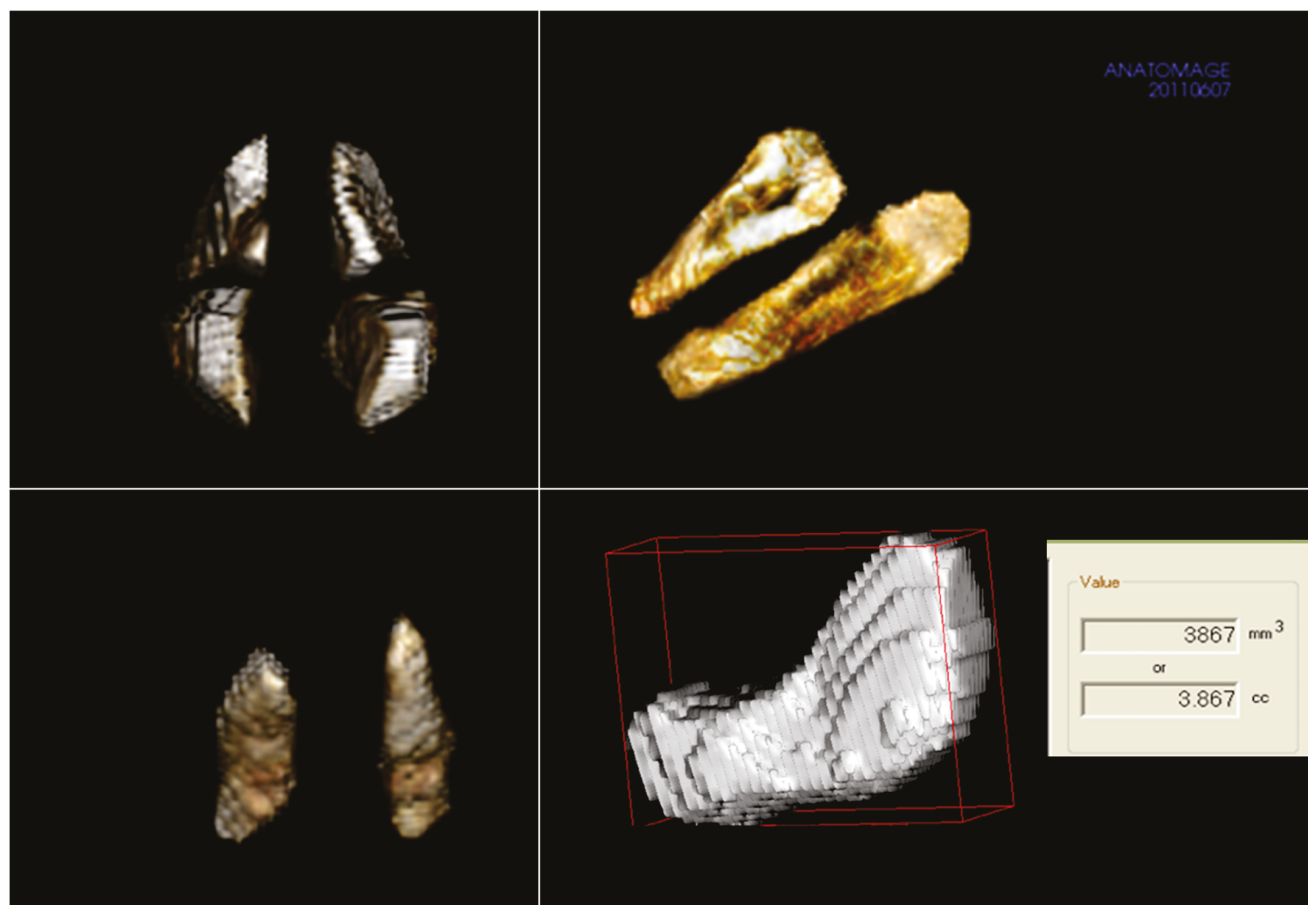


Figure 1. Three-dimensional volume renderings and segmentation of the INC, and MNC obtained from CBCT images. The segmented structures represent the measured volumes of the INC, as part of the volumetric assessment process.

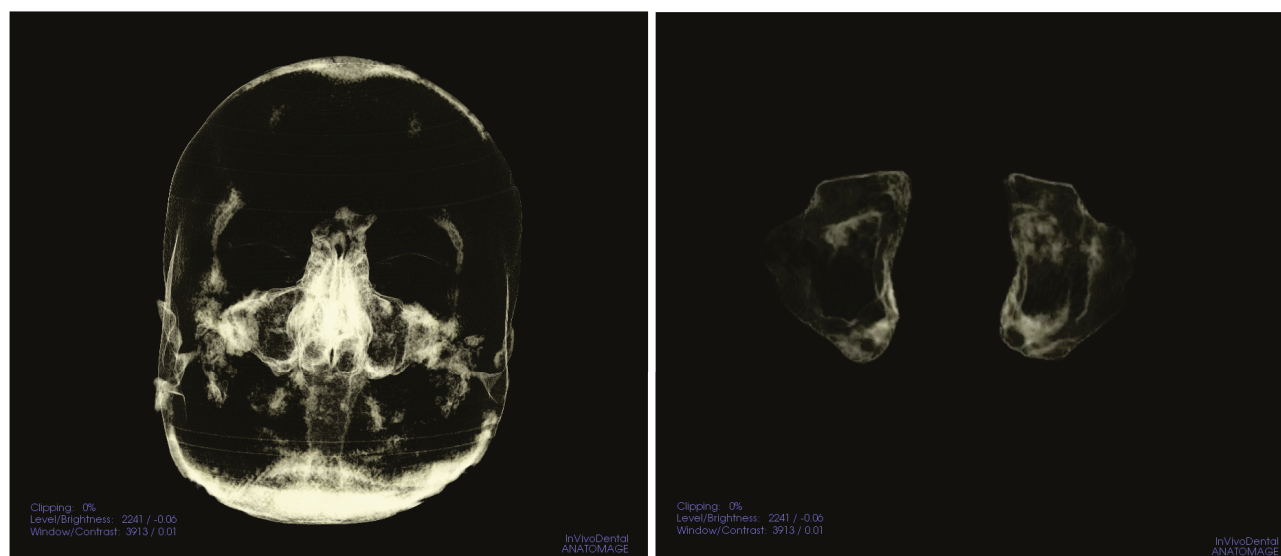


Figure 2. Cont.

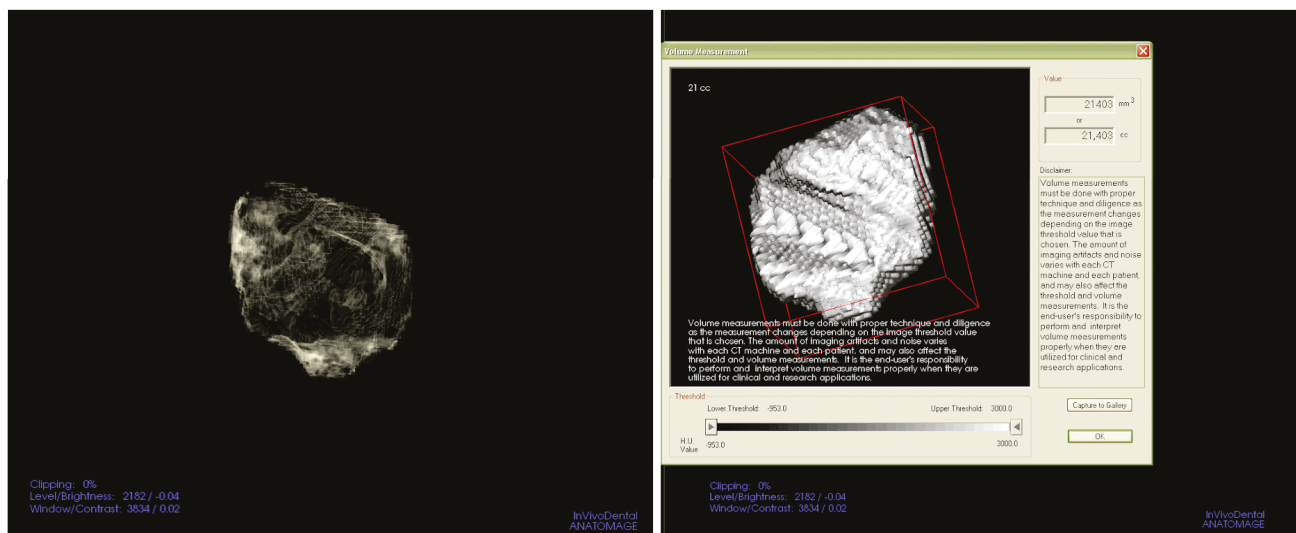


Figure 2. Three-dimensional CBCT image volume renderings and segmentations of the MS. The figure illustrates bilateral maxillary sinus boundaries delineated for volumetric analysis.

3. Results

The overall average volumes for both genders were as follows: The average volume for the right inferior nasal conchae was 5.64 cm³, and for the left inferior nasal conchae, it was 5.75 cm³. The right middle nasal conchae had an average volume of 2.61 cm³, while the left middle nasal conchae averaged 2.58 cm³. Regarding the maxillary sinuses, the average volume of the right maxillary sinus was 14.30 cm³, and the left maxillary sinus was 14.38 cm³.

Gender-based differences were observed in the volumetric analysis of nasal conchae and maxillary sinuses. The average volumes for the right and left inferior nasal conchae in males were 5.82 cm³ and 6.27 cm³ respectively, compared to 5.50 cm³ and 5.36 cm³ in females. This difference was statistically significant for the left inferior nasal conchae ($p = 0.001$). Also, while the average volumes for the right and left maxillary sinuses in males were 15.51 cm³ and 15.82 cm³ respectively, females exhibited lower volumes of 13.41 cm³ and 13.31 cm³ respectively, with the difference in the left maxillary sinus being significant ($p = 0.007$). These findings suggest that there are discernible gender-based differences in the volumetric dimensions of these anatomical regions (Table 1).

Table 1. Comparative analysis of nasal conchae and maxillary sinus volumes by gender.

Anatomical Structure		Gender	Average Volume $\pm$ SD (cm ³)	p Value
Inferior nasal conchae (INC)	Right	Female	5.50 $\pm$ 1.52	0.201
		Male	5.82 $\pm$ 1.51	
	Left	Female	5.36 $\pm$ 1.43	0.001 *
		Male	6.27 $\pm$ 1.96	
Middle nasal conchae (MNC)	Right	Female	2.57 $\pm$ 1.04	0.540
		Male	2.67 $\pm$ 1.02	
	Left	Female	2.46 $\pm$ 1.09	0.156
		Male	2.75 $\pm$ 1.27	
Maxillary sinus (MS)	Right	Female	13.41 $\pm$ 5.28	0.055
		Male	15.51 $\pm$ 7.37	
	Left	Female	13.31 $\pm$ 5.35	0.007 *
		Male	15.82 $\pm$ 5.94	

* Statistical significance at $p < 0.05$.

Similarly, age-group differences were observed in the volumetric analysis of nasal conchae and maxillary sinuses. Patients were categorized into the six age groups and the average volumes of these structures and their standard deviations were calculated for each group. The findings indicate significant volumetric differences only in the left inferior conchae volume, notably between the 21–30 and 61+ age groups ($p = 0.013$). No significant difference was observed for the other structures between the age groups (Table 2).

Table 2. Comparison of nasal conchae and maxillary sinus volumes in different age groups.

Anatomical Structure	Age Groups	<i>n</i>	Average Volume $\pm$ SD (cm ³)	<i>p</i> Value
INC	Right	18–20	6.00 $\pm$ 1.36	0.100
		21–30	5.78 $\pm$ 1.36	
		31–40	5.48 $\pm$ 1.61	
		41–50	5.87 $\pm$ 1.49	
		51–60	5.78 $\pm$ 1.50	
		61+	4.75 $\pm$ 1.78	
	Left	18–20	5.90 $\pm$ 1.21	0.037 * 21–29 vs. 61+ $p = 0.013$ *
		21–30	6.13 $\pm$ 1.74	
		31–40	5.61 $\pm$ 1.40	
		41–50	5.81 $\pm$ 1.58	
		51–60	5.80 $\pm$ 1.73	
		61+	4.75 $\pm$ 2.28	
MNC	Right	18–20	2.60 $\pm$ 0.93	0.838
		21–30	2.69 $\pm$ 1.23	
		31–40	2.66 $\pm$ 0.90	
		41–50	2.45 $\pm$ 0.83	
		51–60	2.73 $\pm$ 0.98	
		61+	2.38 $\pm$ 1.01	
	Left	18–20	2.74 $\pm$ 1.26	0.693
		21–30	2.55 $\pm$ 1.15	
		31–40	2.78 $\pm$ 1.14	
		41–50	2.45 $\pm$ 1.01	
		51–60	2.72 $\pm$ 1.41	
		61+	2.25 $\pm$ 1.09	
MS	Right	18–20	16.54 $\pm$ 7.35	0.184
		21–30	15.49 $\pm$ 5.70	
		31–40	13.41 $\pm$ 7.12	
		41–50	14.50 $\pm$ 7.03	
		51–60	12.68 $\pm$ 6.39	
		61+	12.48 $\pm$ 4.21	
	Left	18–20	15.62 $\pm$ 6.11	0.156
		21–30	15.58 $\pm$ 5.47	
		31–40	12.67 $\pm$ 6.17	
		41–50	15.29 $\pm$ 6.35	
		51–60	13.08 $\pm$ 5.24	
		61+	13.18 $\pm$ 4.95	

* Statistical significance at $p < 0.05$.

The analysis of nasal conchae and maxillary sinus volumes across skeletal malocclusion classes revealed significant differences, especially in the nasal conchae. The inferior and middle nasal conchae displayed notable variations between the malocclusion classes, with Class II generally exhibiting lower volumes both compared to Class I and Class III patients. These differences underscore the impact of skeletal malocclusion on nasal conchae

volume. In contrast, differences in maxillary sinus volumes among the classes were less and, in most cases, did not reach statistical significance, suggesting that malocclusion has a more variable impact on these structures (Table 3).

Table 3. Volumetric differences in nasal conchae and maxillary sinuses across different skeletal malocclusion classes.

Anatomical Structure	Malocclusion Class	Average Volume $\pm$ SD (cm ³)	<i>p</i> Value	Pairwise Comparison
INC	Right	Class I	<0.0005 *	CI vs. CII <i>p</i> = 0.003 *
		Class II		CI vs. CIII <i>p</i> = 0.899
		Class III		CII vs. CIII <i>p</i> = 0.001 *
	Left	Class I	<0.0005 *	CI vs. CII <i>p</i> < 0.0005 *
		Class II		CI vs. CIII <i>p</i> = 0.370
		Class III		CII vs. CIII <i>p</i> < 0.0005 *
MNC	Right	Class I	<0.0005 *	CI vs. CII <i>p</i> < 0.0005 *
		Class II		CI vs. CIII <i>p</i> = 0.226
		Class III		CII vs. CIII <i>p</i> < 0.0005 *
	Left	Class I	<0.0005 *	CI vs. CII <i>p</i> < 0.0005 *
		Class II		CI vs. CIII <i>p</i> = 0.705
		Class III		CII vs. CIII <i>p</i> < 0.0005 *
MS	Right	Class I	0.662	-
		Class II		-
		Class III		-
	Left	Class I	0.011 *	CI vs. CII <i>p</i> = 0.458
		Class II		CI vs. CIII <i>p</i> = 0.165
		Class III		CII vs. CIII <i>p</i> = 0.008 *

* Statistical significance at *p* < 0.05.

The average volumes of the inferior nasal conchae (INC), middle nasal conchae (MNC), and maxillary sinuses (MS) were compared across SNA groups. While mean values for each structure varied among the different groups, none of the differences reached statistical significance. The right maxillary sinus volume showed the most notable variation, with prognathic individuals demonstrating the highest average volume (15.27 ± 6.78 cm³) and the normal group the lowest (12.89 ± 5.98 cm³), though this difference was not statistically significant (*p* = 0.119). Similar nonsignificant trends were observed for the inferior and middle nasal conchae (Table 4).

Table 5 presents a comprehensive correlation analysis among various nasal and maxillary sinus structures. The Pearson correlation coefficients and their significance levels revealed notable relationships: A significant correlation exists between the right and left inferior nasal conchae, suggesting a symmetrical growth or reduction pattern in nasal structures across the population. The middle nasal conchae (right and left) also show a strong positive correlation with each, which further supports the bilateral symmetry in nasal anatomy. Additionally, the right maxillary sinus volume is strongly correlated with the left maxillary sinus volume, indicating consistent volumetric changes between the two sides (Figure 3). Age showed negative correlations with inferior nasal conchae and maxillary sinus in both sides, suggesting a decrease in volume with increasing age. Middle nasal conchae did not correlate with age. Interestingly, no significant correlations were observed between inferior and middle nasal conchae and the maxillary sinuses.

Table 4. Volumetric differences in nasal conchae and maxillary sinuses among SNA groups.

Anatomical Structure		SNA Based Classification	Average Volume $\pm$ SD (cm ³)	<i>p</i> Value
INC	Right	Retrognathic	5.78 $\pm$ 1.59	0.611
		Normal	5.52 $\pm$ 1.59	
		Prognathic	5.53 $\pm$ 1.27	
	Left	Retrognathic	6.01 $\pm$ 1.78	0.260
		Normal	5.61 $\pm$ 1.85	
		Prognathic	5.46 $\pm$ 1.36	
MNC	Right	Retrognathic	2.77 $\pm$ 1.09	0.224
		Normal	2.44 $\pm$ 0.88	
		Prognathic	2.58 $\pm$ 1.10	
	Left	Retrognathic	2.72 $\pm$ 1.13	0.435
		Normal	2.53 $\pm$ 1.20	
		Prognathic	2.41 $\pm$ 1.20	
MS	Right	Retrognathic	15.00 $\pm$ 6.22	0.119
		Normal	12.89 $\pm$ 5.98	
		Prognathic	15.27 $\pm$ 6.78	
	Left	Retrognathic	14.93 $\pm$ 5.36	0.243
		Normal	13.33 $\pm$ 5.30	
		Prognathic	15.04 $\pm$ 6.82	

Table 5. The correlation between nasal conchae and maxillary sinus volume with age.

		Right INC	Left INC	Right MNC	Left MNC	Right MS	Left MS	Age
Right INC	Pearson correlation	1	0.573 *	0.443 *	0.271 *	0.148	0.060	−0.182 *
	Sig. (2-tailed)		0.000 *	0.000 *	0.001 *	0.070	0.466	0.026 *
Left INC	Pearson correlation	0.573 *	1	0.389 *	0.448 *	0.132	0.045	−0.199 *
	Sig. (2-tailed)	0.000 *		0.000 *	0.000 *	0.107	0.587	0.015 *
Right MNC	Pearson correlation	0.443 *	0.389 *	1	0.630 *	0.135	0.053	−0.068
	Sig. (2-tailed)	0.000 *	0.000 *		0.000 *	0.099	0.520	0.406
Left MNC	Pearson correlation	0.271 *	0.448 *	0.630 *	1	−0.009	−0.020	−0.058
	Sig. (2-tailed)	0.001 *	0.000 *	0.000 *		0.911	0.807	0.480
Right MS	Pearson correlation	0.148	0.132	0.135	−0.009	1	0.620 *	−0.222 *
	Sig. (2-tailed)	0.070	0.107	0.099	0.911		0.000 *	0.006 *
Left MS	Pearson correlation	0.060	0.045	0.053	−0.020	0.620 *	1	−0.179 *
	Sig. (2-tailed)	0.466	0.587	0.520	0.807	0.000 *		0.029 *

* Statistical significance at $p < 0.05$.

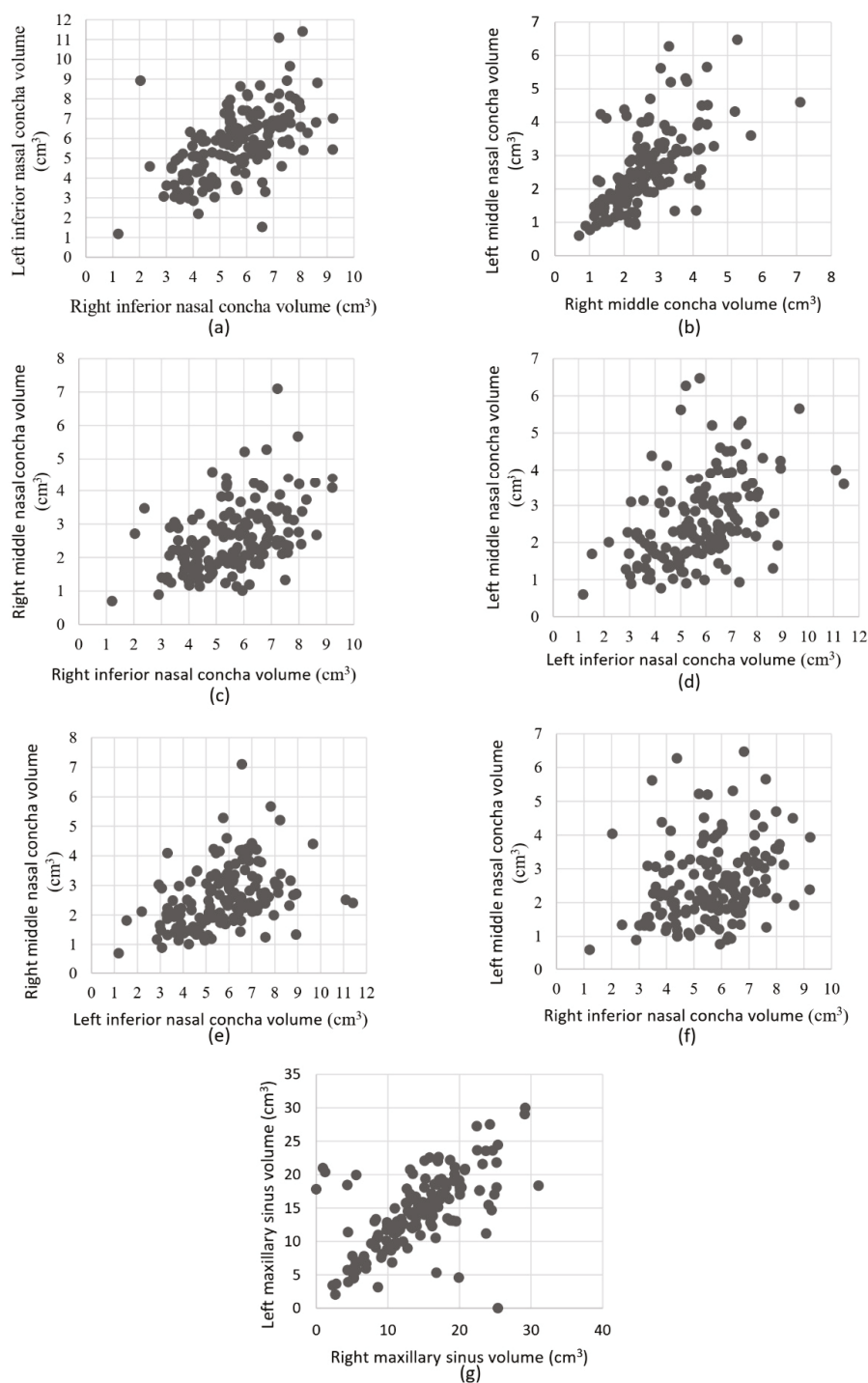


Figure 3. Scatter plots illustrating the correlations between bilateral and inter-structural volumetric measurements of nasal conchae and maxillary sinuses. (a) Left vs. right INC volumes (b) Left vs. right MNC volumes (c) Right INC vs. right MNC volumes (d) Left INC vs. left MNC volumes (e) Left INC vs. right MNC volumes (f) Right INC vs. left MNC volumes (g) Left vs. right maxillary sinus (MS) volumes.

4. Discussion

This study assessed the three-dimensional volumes of the inferior and middle nasal conchae and maxillary sinuses in individuals with different skeletal malocclusion classes using CBCT imaging. The results revealed statistically significant differences in nasal conchal volumes among skeletal classifications, with Class II individuals consistently exhibiting lower nasal conchae volumes. Interestingly, these individuals also demonstrated the high-

est maxillary sinus volumes, despite the absence of a statistically significant correlation between conchal and sinus volumes.

4.1. Gender Differences

Numerous anatomical studies have assessed nasal conchae and maxillary sinus volumes to better understand their structural variability and potential clinical implications. In the present study, although male participants exhibited higher mean volumes in all measured regions, statistically significant gender differences were only observed in the left inferior nasal concha and the left maxillary sinus. A borderline difference was also noted in the right maxillary sinus volume ($p = 0.055$).

These findings are partially consistent with previous literature. Turhan et al. [10] reported significantly larger conchal volumes in male subjects, while Emirzeoğlu et al. [11] observed gender-related differences only in the MNC but not in the INC. Similarly, Shetty et al. [12] found no statistically significant gender-based differences in total INC volume. Pediatric research by Ertekin et al. [13] demonstrated an increase in conchal volumes in both sexes during growth, with gender differences becoming significant only after the completion of craniofacial development. Since our study comprised only adults, age-dependent developmental differences may not have been captured.

Additionally, a study evaluating inferior nasal concha volumes in patients with obstructive sleep apnea (OSA) found significantly higher INC volumes in OSA patients compared to healthy controls (right INC: $5.57 \pm 2.14 \text{ cm}^3$ vs. $4.34 \pm 1.77 \text{ cm}^3$; left INC: $5.12 \pm 2.04 \text{ cm}^3$ vs. $3.90 \pm 1.07 \text{ cm}^3$) [14]. Interestingly, the conchal volumes observed in the present study closely resemble those of the OSA group, despite the exclusion of individuals with diagnosed respiratory disorders. This similarity may suggest underlying anatomical predispositions or subclinical variability. Moreover, the higher volumetric values compared to earlier stereological studies may be attributed to differences in imaging modalities (CBCT vs. CT), segmentation protocols, or population-based anatomical variation.

Gender-related differences in maxillary sinus volumes have also been widely studied, with most findings indicating larger MS volumes in males [9,15]. In line with these reports, male participants in the present study showed significantly greater left MS volumes, and a borderline significant difference on the right side. Aktuna Belgin et al., Dinç and İçöz, and Oruç et al. similarly reported significantly larger MS volumes in males [16–18]. These consistent findings suggest a robust biological basis for sexual dimorphism in maxillary sinus dimensions, possibly linked to overall differences in craniofacial size and morphology.

4.2. Age-Related Differences

Several studies have highlighted the influence of age on the volumetric development of nasal and paranasal structures. Prior research has demonstrated that nasal cavity and paranasal sinus volumes tend to increase during childhood and adolescence, reaching a peak in early adulthood, followed by a gradual volumetric decrease with advancing age. For instance, Velasco-Torres et al. and Ariji et al. reported that maxillary sinus volumes peak around the third decade of life, after which sinus pneumatization appears to regress slightly [19,20].

In a stereological study by Ertekin et al., nasal conchae volumes were shown to increase progressively until age 15, after which significant gender-based differences became apparent [13]. Similarly, Turhan et al. compared nasal structure volumes in adults and children using CBCT and found statistically significant differences in all anatomical regions [10]. Specifically, adults had consistently higher volumes than children across both the inferior and middle nasal conchae as well as the nasal septum. Since our study exclusively included adult participants, the age-related volumetric increases reported in pediatric populations

may not be directly observable in our cohort. Nevertheless, the subtle volume reduction in older age groups supports previous evidence of anatomical remodeling and possible mucosal involution in the nasal cavity over time.

Collectively, these observations align with known craniofacial growth trajectories and emphasize the relevance of age when interpreting volumetric data of the nasal and paranasal structures.

4.3. Skeletal Classification Differences

This study uniquely contributes to the literature by evaluating the volumes of nasal conchae and maxillary sinuses across different skeletal malocclusion classes and cranio-maxillary relationships. In our results, Class II individuals exhibited significantly reduced nasal concha volumes alongside increased maxillary sinus volumes. This dual alteration may point to complex compensatory mechanisms in craniofacial development.

Nearly consistent with our findings, Dinç and İçöz observed that skeletal pattern did not influence MSV in terms of ANB or SNA-based classifications [17]. The present study only found significant differences in the Class II group, showing higher MS volume on the left side. Similarly, Oruç et al. found no significant difference in MSV among malocclusion classes [18]. In contrast, Shrestha et al. reported that individuals with skeletal Class II malocclusion had significantly larger MSV than those with Class III malocclusion [21]. Abate et al., in their comprehensive 3D analysis, found that although MSV and surface area did not differ significantly between ANB groups, patients with retrusive ($SNA < 80^\circ$) or protrusive ($SNA > 84^\circ$) maxillae exhibited greater MSV and linear dimensions compared to those with a normal SNA [22]. Although our findings were similar to those of previous studies, and the normal group exhibited lower MSV than the others, this result did not reach statistical significance.

In light of these comparisons, present study offers a broader anatomical perspective by incorporating the nasal conchae into the analysis. The inclusion of volumetric nasal concha assessment provides novel insights into how different skeletal patterns may influence not only sinus development but also adjacent nasal structures. Given the lack of prior studies exploring this relationship, the present study results highlight the need to broaden volumetric evaluations beyond the maxillary sinuses when investigating craniofacial variations in orthodontic populations.

Although maxillary sinus volumes have been extensively studied in relation to skeletal malocclusions, the inferior and middle nasal conchae have not been evaluated in this context. Their inclusion provides clinically relevant insights, as smaller bony conchae may indicate relatively wider nasal passages, whereas larger ones may suggest narrower spaces. Such distinctions can influence orthodontic treatment planning, rapid maxillary expansion, and orthognathic surgery.

The potential correlation between nasal conchae and maxillary sinus volumes has been a subject of ongoing investigation. However, in the present study, no statistically significant correlation was observed between the volumetric measurements of the inferior or middle nasal conchae and the maxillary sinuses. This finding is consistent with the results of multiple studies. For instance, Tassoker et al. reported no significant correlation between MSV and anatomical variations such as nasal septal deviation (NSD) and concha bullosa (CB) [23]. In contrast, Al-Rawi et al. found that while CB was associated with increased MSV, NSD showed no significant impact on sinus volume, suggesting that the presence of certain anatomical variations may not consistently influence sinus pneumatization [24]. Kapusuz Gencer et al. further examined the influence of NSD and found that contralateral maxillary sinus volumes were significantly larger in cases of severe deviation [25]. However, no correlation was found between the degree of deviation and overall MSV in milder cases,

indicating that only severe anatomical alterations may induce volumetric adaptations in adjacent structures.

Taken together, these findings support the view that although nasal and paranasal structures are anatomically and functionally interconnected, their volumetric development may follow independent pathways or be influenced by additional factors such as local airflow dynamics, chronic mucosal conditions, or genetic predispositions. Further studies incorporating functional assessments and standardized segmentation protocols are needed to elucidate the interplay between nasal cavity morphology and paranasal sinus development.

Although volumetric evaluation of nasal conchae and maxillary sinuses is not currently a standard component of diagnostic protocols, our findings indicate that such analyses can provide valuable supplementary information in selected clinical scenarios. Since our study assessed the osseous structure of the conchae, smaller bony volumes may actually reflect relatively wider airway passages, whereas larger conchal volumes may correspond to narrower spaces. This nuance is important when interpreting the clinical relevance of volumetric measurements. In orthodontic patients with skeletal Class III malocclusion, in cases with suspected airway obstruction such as obstructive sleep apnea, or in preoperative planning for orthognathic and sinus surgery, these assessments may contribute to a more comprehensive understanding of craniofacial and airway morphology.

These results have important clinical implications, particularly in interdisciplinary planning involving airway management, orthodontic expansion, and orthognathic surgery. Furthermore, volumetric assessment via CBCT offers a practical, reliable method for non-invasive evaluation of airway-related structures.

Our findings further confirm that nasal conchae volumes are influenced by gender and age, independent of skeletal classification. The consistent trend of higher volumes in male individuals and volume reduction in older age groups aligns with previous anatomical studies and enhances our understanding of upper airway morphology in orthodontic populations.

However, this study has certain limitations. First, the retrospective design restricts control over confounding factors such as breathing patterns, nasal pathologies, or previous treatments that might influence volumetric outcomes. Second, the study population consisted largely of patients seeking dental or orthodontic care, which may introduce sampling bias toward individuals with craniofacial anomalies. Additionally, functional assessments of airway resistance or airflow, such as rhinomanometry or acoustic rhinometry, were not included, which limits the correlation between anatomical volume and functional outcomes.

Future research should incorporate longitudinal follow-up, a broader population base, and integrate functional assessments to explore how anatomical variation translates into clinical symptoms or treatment outcomes. Combining volumetric analysis with airflow modeling or respiratory function testing could further improve interdisciplinary care in orthodontics, otolaryngology, and sleep medicine.

5. Conclusions

This study highlights significant volumetric differences in nasal conchae and maxillary sinuses across skeletal malocclusion types. Class II individuals had smaller conchae and larger sinus volumes. Gender and age were influential, with males showing higher volumes and older individuals showing reductions. These findings underline the potential clinical relevance of CBCT-based volumetric assessment. Although not part of standard diagnostic protocols, such measurements may provide supplementary information in selected scenarios, such as evaluation of suspected airway obstruction, or preopera-

tive assessment before orthognathic and sinus surgery. Incorporating these volumetric data into clinical decision-making could therefore enhance both diagnostic accuracy and treatment outcomes.

Author Contributions: All authors contributed to the study conception and design. Conceptualization, Project administration, Supervision were performed by İ.T. and K.O. Methodology was performed by all authors. Investigation was performed by B.Ç.Ö. Data curation was performed by S.A. Statistical analysis was performed by N.İ.A. The first draft of the manuscript was written by B.Ç.Ö. and all authors commented on previous versions of the manuscript. Review and editing of the manuscript were performed by S.A. and K.O. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Near East University Scientific Research Evaluation Ethics Committee (approval number: YDU 2019/67-785, approved on 28 March 2019).

Informed Consent Statement: Written informed consent was obtained from all participants prior to undergoing CBCT imaging.

Data Availability Statement: The data presented in this study are available on request from the corresponding author due to ethical reasons.

Conflicts of Interest: The authors declare no conflicts of interest.

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Article

Salivary Cortisol as a Biomarker for Assessing Fear and Anxiety in Patients with Molar–Incisor Hypomineralization

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Abstract: Background/Objectives: Molar–incisor hypomineralization (MIH) is a prevalent dental condition characterized by hypomineralized enamel affecting the first permanent molars and incisors. It leads to visible enamel opacities, with varying severity. Children with MIH often experience dental hypersensitivity, which can result in increased dental fear and anxiety, complicating dental treatment. Salivary cortisol, a well-established biomarker of stress, has been used to assess stress levels in various pediatric conditions but has not been extensively studied in MIH. This study aimed to assess salivary cortisol levels as a stress biomarker in children with MIH and compare them to those in children without MIH. **Methods:** Sixty children aged 5–9 years were divided into two groups: 31 with MIH and 29 healthy controls. Salivary cortisol levels were measured using ELISA, and statistical analysis was performed using IBM SPSS software, version 23. The Mann–Whitney test was used for group comparison, and the Kruskal–Wallis test evaluated the correlation between MIH severity and cortisol levels. **Results:** Children with MIH showed significantly higher mean cortisol levels (2.63 ng/mL) compared to controls (0.96 ng/mL), with a *p*-value of 0.0001. A progressive increase in cortisol levels was observed with the severity of MIH, with the highest levels recorded in grade 3 (4.38 ng/mL), in contrast to grade 0 (0.95 ng/mL), with a *p*-value of 0.001. **Conclusions:** Salivary cortisol levels are significantly higher in children with MIH, suggesting that MIH-related stress may contribute to dental anxiety and hypersensitivity. These findings highlight the importance of stress management in pediatric dental care.

Keywords: molar hypomineralization; cortisol; saliva; stress; psychological; dental anxiety

1. Introduction

Molar–incisor hypomineralization (MIH) is a prevalent dental condition in children, primarily affecting the first permanent molars and incisors with hypomineralized enamel. This results in visible opacities on the enamel that vary in color from white to yellow brown. The global prevalence of MIH varies widely, with reports of the lowest prevalence in China in 2008 (2.8%), a mean prevalence of 9.4% in Europe, and of 14.3% in Romania [1,2].

Despite its recognition as a significant global public health issue, the exact cause of MIH remains unclear. The condition is categorized into four severity grades that help guide treatment decisions: mild, moderate, severe, and atypical restoration. Grade 3, or PEB (post-eruptive enamel breakdown), refers to severe cases characterized by demarcated opacities and post-eruptive loss of dental hard tissue [3,4]. Beyond the cosmetic effects, MIH is often linked to dental hypersensitivity and a heightened susceptibility to caries [5].

In 2022, the European Academy for Pediatric Dentistry (EAPD) proposed an updated guide for practitioners that serves as a valuable tool for evaluating, documenting, and communicating the severity of MIH. This guide aids in treatment planning, monitoring progress, and supporting research. The EAPD guide emphasizes that affected teeth often display sensitivity, varying from a mild reaction to external stimuli to spontaneous hypersensitivity, and MIH-affected molars can be challenging to anesthetize, complicating dental treatment [6]. However, a more commonly used system classifies MIH-related hypersensitivity as follows:

- Grade 0: Absence of hypersensitivity—The tooth shows no sensitivity to stimuli.
- Grade 1: Mild hypersensitivity—The tooth shows reduced sensitivity without significantly affecting daily activities.
- Grade 2: Moderate hypersensitivity—Tooth sensitivity is moderate, causing occasional discomfort during certain activities.
- Grade 3: Severe hypersensitivity—Sensitivity is intense, causing frequent or continuous pain that significantly affects the patient's daily activities and quality of life [7].

It is well known that children diagnosed with severe MIH exhibit significantly higher levels of dental fear and anxiety compared to children who do not have this enamel defect [8]. The fear is mainly related to the hypersensitivity and pain associated with the affected teeth, which are aggravated during dental procedures [9].

Due to increased fear and anxiety, children with MIH and active caries grafted on enamel defects have avoidant behaviors and reduced cooperation during dental treatments. This presents a challenge for dental practitioners, who may need to apply advanced behavior management techniques.

Possible causes of anxiety may be pain experienced during daily activities such as eating or brushing, previous traumatic experiences during dental procedures caused by MIH-specific hypersensitivity, or anticipation of pain during treatments involving the affected teeth [10].

Studies regarding dental hypersensitivity and behavioral management difficulties in children with MIH are conflicting, with some research indicating that these children exhibit behavioral problems associated with dental anxiety and hypersensitivity, while other studies claim that there are insufficient objective tools to demonstrate that patients with MIH are more difficult to manage in the dental office compared to those without MIH [11,12].

Dental fear and anxiety (DFA) represent intense emotional responses related to dental treatment, often influenced by childhood experiences. Dental fear occurs as a reaction to specific stimuli in the dental office, whereas dental anxiety involves a generalized state of anxiety about possible discomfort. Distinguishing between the two can be difficult, but both can affect dental treatments [13].

Acute stress is defined as the body's immediate and short-lived physiological response to a situation perceived as threatening or demanding. This type of stress also occurs in dental office anxiety as an emotional and behavioral reaction immediately following the action of a specific, unexpected, or high-intensity factor, such as danger, conflict, or unusual challenge [14]. DFA in response to an acute stressor is characterized by the activation of the

sympathetic nervous system and the release of stress hormones, such as adrenaline and cortisol, which prepare the body for the fight-or-flight response [13].

Regarding salivary cortisol as a biomarker, it can be used to objectively assess children's stress levels as a marker of anxiety and can be a valuable diagnostic tool for oral health. It can serve in the identification, prevention, and optimal management of dental anxiety, involving behavioral management techniques, as well as creating a friendly environment for children and giving them the necessary emotional support [15].

Research on salivary cortisol levels in children during dental treatments has provided important insights into how these procedures impact physiological stress responses. Salivary cortisol is a noninvasive biomarker widely used to assess stress, and its levels can reflect the anxiety experienced by children in dental settings. In 1998, Klingberg et al. found that cortisol levels were higher in children who reported greater dental fear, suggesting a close relationship between subjective anxiety and physiological stress markers [16].

A 2013 study demonstrated that children undergoing dental extractions exhibited significant increases in salivary cortisol levels, indicating elevated stress. Moreover, interventions aimed at reducing dental anxiety, such as cognitive-behavioral techniques or the use of audiovisual distractions, have been shown to effectively lower cortisol levels, thus improving the dental experience for young patients. These findings underscore the importance of managing stress in pediatric dental care to promote better health outcomes and patient cooperation [17,18].

The aim of this study was to determine salivary cortisol levels in children with MIH and to compare these levels with those in children without this condition, based on the hypothesis that salivary cortisol levels, as a biomarker of stress, are significantly higher in children with MIH compared to children without this condition.

2. Materials and Methods

This case-control study was carried out between July and September 2024, with the approval of the Ethics Commission (Decision of the Scientific Research Ethics Commission No. 3307 of 12 July 2024), assessing salivary cortisol levels in children with mixed dentition who were patients in the Natural Smile by Dr Pop Dental Clinic in the Transylvania region. The study included children, regular patients of the clinic, aged between 5 and 9 years. The initial group consisted of 70 participants, of which 10 were excluded based on the exclusion criteria outlined below. A total of 60 participants remained in the study, divided into two groups: the study group, which included 32 children diagnosed with MIH of varying degrees of severity, and the control group, which included 28 children without MIH. The protocol for determining the varying degrees of severity of MIH was developed based on the classification of K. Mathu-Muju and J.T. Wright. Mild (Grade 1): Limited demarcated opacities located in non-stress-bearing areas, with mild involvement of the incisors, but no hypersensitivity. Moderate (Grade 2): Enamel defects limited to one or two surfaces without cuspal involvement, and the presence of hypersensitivity. Severe (Grade 3): Post-eruptive enamel breakdown, crown destruction, caries associated with affected enamel, and a history of dental hypersensitivity [19].

The evaluation of salivary cortisol levels was performed using the quantitative competitive ELISA (enzyme-linked immunosorbent assay) method using salivary cortisol ELISA tests by DRG International, Inc., Springfield, NJ, USA. Kit sensitivity: the detection limit for cortisol is 0.082 ng/mL. Kit precision: the intra-assay precision has a coefficient of variation below 8.3%, and the inter-assay precision has a coefficient of variation below 13.6%.

Patient inclusion criteria specified children with mixed dentition, patients with regular dental visits, with known medical history, absence of general chronic or acute pathologies,

absence of any medication or dietary supplements in the last month, absence of untreated carious lesions, and with permanent incisors and molars present on the arch.

Exclusion criteria ruled out patients with recent acute illnesses (infections, viruses, trauma), patients with any type of recent medication (including anti-inflammatory or anesthetic), and those with inflammation or any type of intra-oral lesion.

After informing parents and children about the conduct of the study and obtaining consent for the voluntary participation of patients, verbal and written informed consent was obtained from participants and their legal guardians, who were given a detailed explanation of the procedure and its purpose.

Patient preparation:

1. Collection of unstimulated saliva was carried out in the first part of the day, between 9 and 11 a.m., in 1.5 mL Eppendorf containers
2. The patients were scheduled at least 60 min after waking up, after consuming any type of food or drink.
3. Teeth brushing or mouthwash use was avoided for at least 1 h before.
4. A state of physical and emotional rest before harvesting was ensured.
5. The 1.5 mL samples were stored at -80 degrees Celsius in hermetically sealed Eppendorf containers, then slowly thawed to prevent biomarker degradation.

Sample processing included:

1. Centrifugation—to remove cell debris or precipitates, the unstimulated saliva samples were centrifuged for 5 min at 5400 rpm using the MiniSpin Eppendorf (Eppendorf SE, Eppendorf SE, Germany, 2021) (Figure 1a–c).
2. According to the standardized protocol, controls, samples, and the enzymatic conjugate (cortisol labeled with horseradish peroxidase) were added by pipetting into wells coated with a monoclonal antibody specific to cortisol.
3. Incubation for one hour at room temperature (25 degrees). During incubation, the cortisol present in the standards, controls, or samples competed with the enzymatic conjugate for binding to the monoclonal antibody immobilized at the bottom of the well.
4. After 5 washing cycles, the complete elimination of unbound reagents was ensured (Figure 2).
5. The chromogenic substrate was added, generating a color change proportional to the cortisol concentration in the sample (Figure 3a,b).
6. Final incubation: the plate was incubated again for 30 min at room temperature.
7. Stop reaction: stopping solution was added, which turned the color to a deep yellow (Figure 3c,d).
8. Plate reading: the absorbance of each well was measured using a DYNEX DSX Automated ELISA System spectrophotometer, Dynex Technologies, Chantilly, VA, USA at the specified wavelength (450 nm). The cortisol concentration in the sample was determined by measuring the absorbance of each well and interpolating the values on the calibration curve, with cortisol concentration being inversely proportional to the color intensity in the well.
9. Analysis and interpretation: the standard curve was constructed from the absorbance values of the standards (Figure 4). Cortisol concentration in salivary samples was determined by interpolation using spectrophotometer software, Version 6.25.

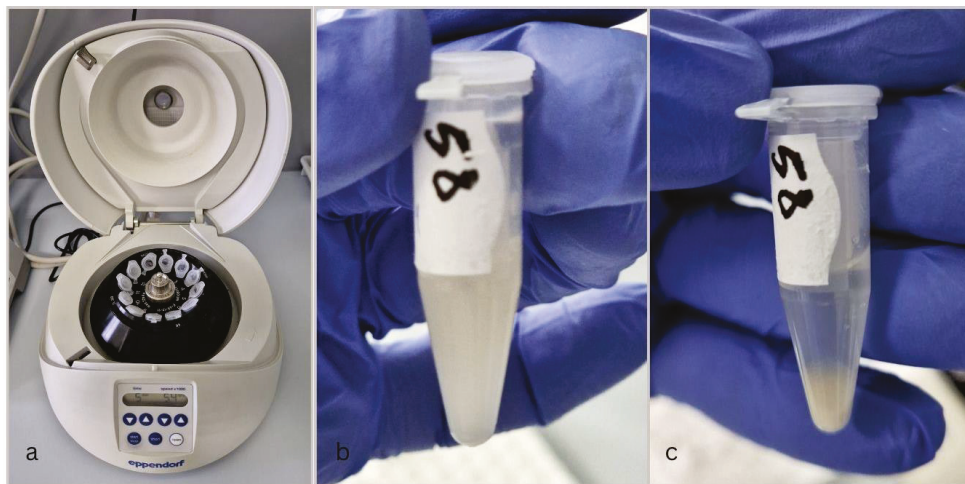


Figure 1. (a) MiniSpin Eppendorf centrifuge; (b) saliva sample before centrifugation; (c) saliva sample after centrifugation.



Figure 2. The washing process.

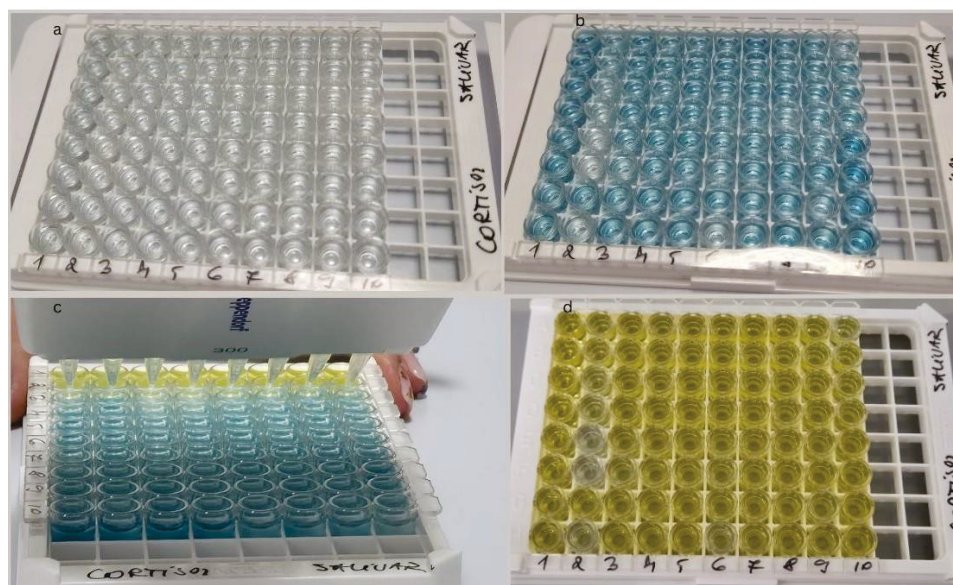


Figure 3. (a) The plate before adding the chromogenic substrate; (b) the plate after adding the chromogenic substrate; (c) the addition of the stopping solution; (d) chromogenic reaction turning deep yellow.

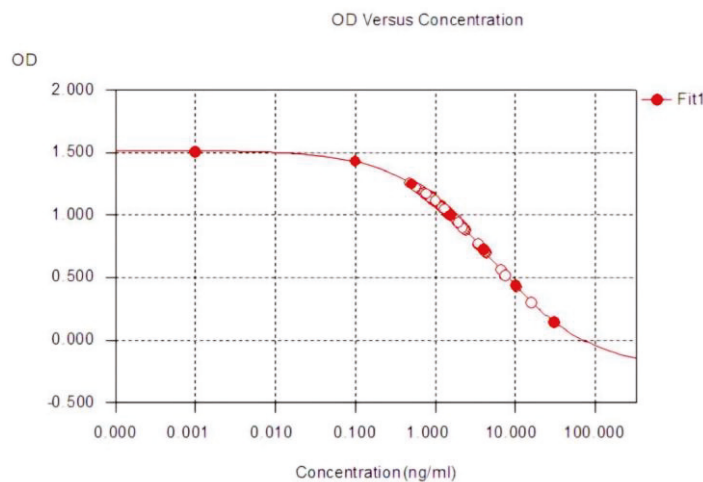


Figure 4. The standard curve.

All data were entered into a database, and statistical analysis was performed using IBM SPSS software, version 23 (Armonk, NY, USA: IBM Corp), with a confidence interval (CI) set at 95% and a significance level set at 5% (0.05) for the statistical analysis (p -value < 0.05). The nonparametric Mann–Whitney test was applied for the comparative analysis of cortisol levels between the two groups, and the nonparametric Kruskal–Wallis test was applied to establish a correlation between the severity of MIH and salivary cortisol levels.

3. Results

The study group included 60 patients, comprising 35 girls and 25 boys, aged between 5 and 9 years, with a mean age of 7.2 (standard deviation of 1.09), with no significant differences between the two subgroups. Of the total 31 MIH cases, 14 (45.16%) had a severity grade of 2, 11 (35.48%) had a severity grade of 3, and 7 (22.58%) had a severity grade of 1. In the case group, the mean value of salivary cortisol level was considerably higher compared to the mean value of the control group, the values having no Gaussian distribution, suggesting that the stress level experienced by patients with MIH was higher than that experienced by patients in the control group ($2.63306 > 0.95954$ ng/mL). Comparative evaluation of salivary cortisol values in the two groups showed statistically significant differences, with a p -value of 0.0001 ($p < 0.005$) following the application of the nonparametric Mann–Whitney test.

Mean cortisol values and standard deviation in the two groups are presented in Table 1: (Table 1)

Table 1. Comparison of cortisol values (ng/mL) between the case and control groups.

MIH	Mean Value	Standard Deviation	Minimum Value	Maximum Value
YES	2.63306	2.065567	1.133	10.129
NO	0.95954	0.330475	0.474	1.922

In terms of sex distribution, the mean value was 1.89606 ng/mL in girls and 1.76104 ng/mL in boys, with p -value 0.576 ($p > 0.05$), with no statistical significance, although the highest recorded value in the study was among girls (10,129 ng/mL) (Table 2).

Table 2. Comparison of cortisol levels (ng/mL) between boys and girls.

Sex	Mean Value	Standard Deviation	Minimum Value	Maximum Value
Female	1.89606	1.932252	0.568	10.129
Male	1.83885	1.424165	0.474	7.498

To correlate the degree of severity with the salivary cortisol level, the nonparametric Kruskal–Wallis test was applied, which showed statistically significant differences, indicating a progressive increase in cortisol level from grade 0 (MIH absent) to grade 3, with a statistically significant ($p < 0.05$) p -value of 0.001 (Table 3). The highest salivary cortisol level (10.129 ng/mL) recorded in this study corresponds to severity grade 3.

Table 3. Salivary cortisol levels (ng/mL) by MIH severity grade.

MIH Severity Grade	Mean Value	Standard Deviation	Minimum Value	Maximum Value
0	0.94678	0.329669	0.474	1.922
1	1.43757	0.418561	1.133	2.095
2	1.76693	0.611382	1.199	3.358
3	4.37536	1.722815	1.809	10.129

The values in the table shows a progressive increase in the mean cortisol level, correlated with the severity of MIH (0–3), according to the Kruskal–Wallis test (p -value = 0.001, $p < 0.05$), suggesting that the anxiety experienced by a child patient is proportional to the severity of the enamel defects and objectified by the salivary cortisol level.

4. Discussion

MIH is a qualitative enamel developmental defect, of systemic etiology, affecting one or more first permanent molars and sometimes incisors of the same dentition. Clinically, it is manifested by visible opacities, with shades ranging from opaque white to yellow brown, their severity being asymmetrically distributed, the affected teeth being prone to post-eruptive fractures [20,21].

Therapies for this pathology are varied and correlated with the degree of severity, ranging from noninvasive remineralization or sealing therapies, to minimally invasive restorative options with glass ionomer cements or composite resins, to indirect restorations with pedodontic zirconia crowns or even extraction [22,23].

Regardless of the therapeutic option chosen, one of the critical elements of the clinical picture is represented by the specific hypersensitivity of MIH, which may be accompanied by the failure of anesthetic techniques. Thus, by suboptimal pain control during restorative procedures or even during routine check-ups, child patients experience acute episodes of dental fear and anxiety (DFA), requiring holistic therapeutic approaches, including psychological support and personalized interventions, behavioral modulation, optimal pain control, and appropriate restorative therapy [24,25].

In pediatric dentistry, behavior management techniques such as tell–show–do, distraction, and positive reinforcement are often employed to help children feel more comfortable and relaxed during dental procedures [12,13]. These techniques, being indispensable, were rigorously applied in the present study, the patients benefiting from the psycho-emotional comfort necessary for further collaboration [26].

The results of the present study suggest that dentists may incorrectly interpret the noncooperative behavior of pediatric patients with MIH [27] when, in fact, patients who seemingly have difficulty accepting or refuse dental procedures may actually be experiencing psychological stress, objectively reflected in elevated cortisol levels.

In order to develop an appropriate treatment plan that integrates behavioral management, effective pain control, and long-lasting restorative methods, it is essential for practitioners to accurately assess the actual level of dental fear and anxiety as well as the degree of hypersensitivity of children. Currently, the lack of a universal standardized assessment tool is a challenge in the complex management of patients with MIH.

Behavioral analysis tools such as the Wong–Baker Faces Pain Rating Scale and FLACC (face, legs, activity, cry, consolability) index are useful for assessing pain and discomfort, especially in children, but they are not sufficient, nor are they specific or objective; they do not quantify dental hypersensitivity, do not distinguish between dental pain and dental anxiety, and do not provide relevant information for specific treatments for this condition [28,29].

The Wong–Baker Faces Pain Rating Scale is a visual assessment tool commonly used to measure pain intensity in children. It consists of a series of faces displaying different expressions, from a smiling face representing “no pain” to a crying face representing “the most pain”.

This scale is a useful but not sufficient or objective tool for assessing children’s anxiety, being more suitable for illustrating the discomfort perceived by children through the six expressive faces corresponding to different degrees of pain. For a more accurate and objective assessment of stress, it is necessary to complement this with the analysis of a biological parameter such as salivary cortisol levels, which can provide a concrete indicator of the physiological response to stress during office visits [30]. The FLACC index is an essential tool used by dentists and pediatric specialists to objectively assess and communicate the level of hypersensitivity or pain experienced by the patient, especially in children who cannot verbally express discomfort. This score allows a continuous monitoring of the patient’s physiological and behavioral reactions and is crucial for adapting therapeutic strategies during each treatment session [31]. By carefully evaluating each component of the FLACC index, the clinician can adjust the dental approach according to the intensity of pain and discomfort experienced, thus optimizing behavioral management and patient comfort. The use of this tool also helps to reduce the risk of stress and anxiety among patients, ensuring a more efficient and less traumatizing treatment experience.

Stress can be defined as the result of physical and mental reactions generated by our inability to differentiate between real risks and personal expectations. It produces anxiety and can negatively influence the nervous, endocrine, and immune systems. Anxiety is an organic response, manifested both psychologically and physiologically, characterized by somatic, emotional, cognitive, and behavioral conditions [32].

A recent longitudinal study highlights that even a simple professional dental prophylaxis procedure increases salivary cortisol levels in children with behavioral management problems. This finding is supported by our study, which shows that children with MIH, of varying degrees of severity, frequently exhibit behavioral issues, reflected in elevated cortisol levels [33].

Salivary cortisol can also be used as an evaluation tool for other pain-associated conditions in the oro-maxillofacial region, such as temporomandibular disorders (TMD). It has been demonstrated that salivary cortisol levels are reliable for assessing self-reported pain perception in adolescents with and without TMD. The findings showed that adolescents with TMD had higher salivary cortisol levels compared to those without TMD. Additionally, a direct relationship was observed between self-reported pain in the temporomandibular joint area and salivary cortisol levels [34].

Regarding sex distribution, similar results were reported in the study on salivary cortisol levels and early childhood caries, where no significant differences were observed between the examined groups in terms of gender ($p = 0.943$) and age ($p = 0.060$). Similarly, in our study, the mean salivary cortisol level was 1.89606 ng/mL in girls and 1.76104 ng/mL in boys, with a p -value of 0.576 ($p > 0.05$), indicating no statistically significant difference. This supports the idea that DFA does not specifically affect a particular age group or sex [35].

Other studies have highlighted the role of salivary cortisol in monitoring stress and anxiety, factors that can influence oral health. For instance, a 2010 study investigated

the salivary cortisol response to psychological stress in children with early childhood caries, revealing a significant correlation between these two [36]. More recent research has indicated that salivary cortisol levels are correlated with various oral conditions, such as recurrent aphthous stomatitis, highlighting the importance of monitoring this marker in dental practice [37]. In 2012, the significance of evaluating salivary cortisol in the diagnosis of Cushing's syndrome was highlighted, particularly in children and individuals for whom stress factors may influence cortisol secretion at the adrenal level [38]. A 2024 study further underscores the utility of salivary cortisol as a noninvasive tool for assessing stress in children during medical procedures, providing valuable insights into how children respond to surgical stress without the need for invasive methods [39]. All these findings are consistent with the results of the present study, which indicate a strong correlation between cortisol levels and the severity of the MIH condition.

Cortisol belongs to the group of glucocorticoid hormones synthesized from cholesterol and secreted by the adrenal cortex, the majority of cortisol in the blood circulating in the form bound by corticosteroid-binding globulin (called transcortin) and albumin, while 5–10% of cortisol remains in free and biologically active form. Although determination of blood cortisol levels is widely used, it can lead to false-positive results due to the additional stress associated with blood collection [40]. Measurement of salivary cortisol is a simpler, safer, and less invasive method of determining active cortisol levels, with a strong correlation with free cortisol in the blood. The salivary cortisol level represents about 70% of the free cortisol in the bloodstream, and saliva is easier to collect than blood, not requiring special equipment or additional patient preparation. One of the main advantages of salivary cortisol is that it can be obtained in the participant's natural environment, avoiding additional stress. This makes it an innovative approach in acute stress biomarker research, with its simple collection method and wide potential applications [41].

The mean physiologic levels of salivary cortisol in children aged 5–9 years in the morning are in the range 5.7–7.2–32.4–36.9 nmol/L, corresponding to values of 0.2052–0.2592–1.175–1.338 ng/mL. These values are compared to the mean recorded in the control group of the study (0.95954 ng/mL) and to the significantly higher values identified in the cohort of MIH patients (2.63306 ng/mL) [42,43]. These differences underline that dental visits may act as triggers of acute cortisol fluctuations, with a more significant impact in children with MIH compared to healthy children, indicating a deeper level of anxiety among MIH patients and thus imposing the need for a more rigorous behavioral management strategy. This notion is further corroborated by other studies that have demonstrated increased cortisol levels in different pediatric populations undergoing various dental therapeutic interventions [44].

The correlation between severity of MIH and salivary cortisol levels (absent MIH—0.94678 ng/mL, Grade 1—1.43757 ng/mL, Grade 2—1.76693 ng/mL, and Grade 3—4.37536 ng/mL) clearly underlines the link between the severity of the condition and the body's intensified physiological response, reflected by increased cortisol concentrations. These results suggest that MIH-specific hypersensitivity poses a significant challenge in the therapeutic approach to this abnormality, negatively influencing treatment efficacy and increasing the complexity of behavioral management of patients. Practitioners have reported considerable difficulties in the behavioral management of children with MIH, their anxiety being both justified and objectified by the significant elevation of this biomarker [45,46].

The main causes and mechanisms involved in the development of hypersensitivity, which underlie the behavioral problems among children with MIH, include dentin exposure due to reduced enamel mineralization, increased porosity, and changes in the thermal and mechanical properties of enamel, such as decreased hardness and elastic modulus, increased carbon and carbonate concentrations, and higher protein content [40]. These

changes lead to premature breakdown of enamel and exposure of subjacent dentin, favoring increased sensitivity, which contributes to deterioration of oral hygiene and a higher susceptibility to caries.

Detailed and exhaustive knowledge of the local and general clinical picture of patients with MIH is essential for the development of tailored practice guidelines that integrate effective strategies for the management of dentin hypersensitivity and behavioral difficulties associated with DFA specific to this category of patients. In this regard, the use of hydroxyapatite-based desensitizing pastes represents a promising approach to ameliorate dentin sensitivity by rebuilding the enamel microstructure and restoring its structural integrity. In parallel, the promotion of an effective anesthetic protocol, with an emphasis on preemptive administration of analgesics, may contribute to optimal pain control and prevention of acute discomfort associated with dental procedures [47,48].

Furthermore, techniques to reduce anxiety, including the use of effective means of communication and different behavioral therapy techniques adapted to the age and needs of each patient, are crucial to prevent intense emotional reactions and to facilitate cooperation during treatment [49]. These techniques should be correlated with the patient's psycho-emotional pattern, identifying individual traits, such as fear of dental treatments or heightened emotional sensitivity, in order to implement personalized behavioral management. Thus, therapeutic options can be calibrated according to the particularities of each case, taking into account not only the pathophysiological features of MIH, but also the patient's behavioral response, to ensure effective treatment and a less traumatic dental experience. This holistic approach will help improve patients' quality of life and minimize the risks of long-term complications, such as further enamel damage and the development of other associated dental conditions. It facilitates patient-centered care, reducing the need for invasive interventions, such as general anesthesia, thereby helping to optimize costs and improve the accessibility of dental treatments.

The fact that salivary cortisol levels are higher in children with MIH but not in those with other diseases like caries, suggests that the anxiety and stress experienced by MIH patients may not be driven only by pain, as clinically, pain is present in both conditions. Instead, the stress response in MIH children could be linked to a combination of factors, including chronic hypersensitivity, prior negative dental experiences, and even psychological distress associated with esthetic concerns. Moreover, it is worth considering whether cortisol might play a dual role in MIH—both as a contributing factor and as a consequence. Since elevated cortisol levels during early childhood development have been identified in enamel hypomineralization, chronic stress exposure in critical periods could be a potential etiological factor for MIH. This bidirectional relationship may suggest that cortisol is not just a response to MIH-related challenges but may also be an underlying factor in its development, creating a cycle where stress contributes to MIH and MIH exacerbates stress [50,51].

A recent study suggests a potential link between elevated cortisol levels during early childhood and the development of MIH, revealing a significant correlation between stress and MIH, indicating that children with higher stress scores experience a greater occurrence of this enamel defect. The study concluded that stress is potentially one of the main causal factors contributing to MIH development [52].

Moreover, glucocorticoid receptors have been detected on ameloblasts, suggesting that elevated cortisol levels could directly influence the enamel developmental process. This finding implies that stress-induced cortisol secretion during critical periods of tooth development may disrupt normal enamel mineralization, leading to conditions like MIH [53].

The hypothesis that children with MIH have significantly higher levels of salivary cortisol and thus a higher degree of DFA than children without MIH is confirmed by the data

provided by the study. These results suggest that MIH is not only pathophysiological but also behaviorally challenging and requires a tailored and integrated therapeutic approach to ensure adequate management of pain, anxiety, and dentin hypersensitivity.

5. Conclusions

This study confirms that children with MIH exhibit significantly higher salivary cortisol levels compared to those without MIH, reflecting an increased level of dental fear and anxiety. These findings underscore the critical need for personalized therapeutic strategies aimed at behavioral management and effective pain control to enhance the dental care experience for these patients.

The successful management of hypersensitivity and anxiety in children with MIH requires a multidisciplinary and proactive approach. This includes the implementation of desensitization techniques, preemptive analgesia for optimal pain control, and targeted anxiety reduction methods. By integrating these strategies, clinicians can minimize treatment-related stress, reduce trauma, and create a more comfortable and accessible dental environment for affected children.

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Article

External Validation of Periodontal Screening Using Self-Reports in Dental Settings

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Abstract

Background/Objectives: This study aimed to externally validate the screening model for severe PD proposed by Verhulst in a Portuguese population, assessing its discriminative performance and clinical applicability. **Methods:** A cross-sectional study was conducted with 310 adults recruited from a university dental clinic in Portugal. The participants completed a validated self-reported oral health questionnaire (screening model) and underwent a full-mouth periodontal examination using the CPITN index, with severe PD defined as a score of 4. Model performance was evaluated for discrimination, calibration, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). **Results:** Of the 310 participants, 51% were diagnosed with severe PD. The Verhulst model showed an area under the curve (AUC) of 0.72 (95% CI: 0.67–0.77), with sensitivity and specificity of 0.709 (95% CI: 0.639–0.779) and 0.724 (95% CI: 0.651–0.796) and PPV and NPV of 0.727 (95% CI: 0.673–0.784) and 0.704 (95% CI: 0.651–0.764), respectively. Calibration was reasonable, with an observed-to-expected ratio of 1.10 (95% CI: 0.939–1.291). **Conclusions:** The model demonstrated acceptable external validity and could serve as a feasible screening tool for severe PD in Portuguese dental settings. Its integration may enhance early diagnosis and interdisciplinary care. Future studies should consider broader diagnostic criteria to refine accuracy.

Keywords: periodontitis; screening model; self-reported questionnaire; prediction model; CPITN; oral health; model validation

1. Introduction

Periodontitis (PD) is a chronic inflammatory disease of the periodontium that is estimated to affect over 60% of the dentate population worldwide [1]. This disease is characterised by inflammation of the tissues around the tooth, and its screening is a key step to identify people at risk of having the disease undiagnosed [2].

According to the World Health Organization, there is urgency to strengthen the integration of oral health into noncommunicable disease and universal health coverage strategies [3]. This implies that non-dental health professionals should have tools and knowledge to screen periodontitis at primary health care and hospital settings in order to promote an integrated health response [4]. To reach this goal, the Division of Oral Health at the National Centers for Disease Control and Prevention (CDC), in collaboration with

the AAP, proposed a self-reported questionnaire [5,6]. This tool has already been validated in other countries worldwide [7–14].

Patients' self-report is recognized as valuable and reliable information in periodontal medicine with public health benefits [15]. This preventive approach is particularly relevant where an accurate screening process might promote early diagnosis, allowing better therapeutic outcomes and less oral complications [5,6]. Implementing such a strategy in an integrated universal oral health coverage has potential as the restricted human and material resources necessary require approaches that are precise and less time- and labour-intensive [16,17]. Recently, Verhulst et al. [14] validated a screening model for severe PD to be implemented in the medical care setting, with potential for validation in other populations but with the need to adjust individual predicted probabilities [14].

With this in mind, this study aims to validate Verhulst et al.'s screening model for severe PD in a Portuguese population and to examine whether its original and newly computed predicted probability shows adequate performance to be implemented in a medical setting [14].

2. Materials and Methods

2.1. Source of Data

The present cross-sectional study is reported following the 'Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis' (TRIPOD) checklist [18] for prediction model validation. The present study was carried out following the Declaration of Helsinki, updated in 2024, approved by the Egas Moniz Ethical Committee (research number PT-348/23 on 27 February 2024).

In this study, adult subjects (≥ 18 years) were recruited following consecutive sampling between February 2024 and April 2025 and signed an informed consent. This independent Portuguese cohort was used to externally validate and test the prediction performance of the model originally developed by Verhulst et al. [14], which had been trained and validated in a Dutch population.

2.2. Participants

Consecutive enrolment occurred, as adult participants were asked to take part in the initial screening visit at a Portuguese university's dental clinic (located at the Egas Moniz School of Health & Science). This study's protocol and objectives were explained to potential participants, who provided written consent prior to involvement. To be eligible, individuals had to be between 18 and 80 years old and possess at least one natural tooth. This criterion was adopted to avoid potential bias arising from the exclusion of individuals who had lost most of their teeth due to severe periodontitis, ensuring that the model accounted for participants across the full spectrum of periodontal health. Including such cases prevented underestimation of disease prevalence and maintained the representativeness of the population, while the CPITN scoring system remained applicable to the existing sextants. Edentulous patients and those who were mentally incapacitated were excluded. Individuals requiring antibiotic prophylaxis before periodontal probing or were unable to communicate in Portuguese were also excluded from participation. Severe periodontitis was defined as a CPITN score of 4, corresponding to the presence of periodontal pockets ≥ 6 mm in at least one sextant. For each participant, the highest sextant score was used as the diagnostic classification, following the CPITN guidelines. To ensure accurate assessment in individuals with reduced dentition, CPITN scoring was applied to all remaining sextants with at least two functional teeth, and the distribution of remaining teeth within this group was recorded. This allowed for consistent and reliable evaluation of periodontal status, even in participants with substantial tooth loss.

2.3. Outcome

One calibrated member (J.V.) performed a full-mouth periodontal examination and was blinded for all patient data. Intra-rater reliability was assessed by the intra-class correlation coefficient (ICC) in ten patients not involved in this study, one week apart between each observation. Obtained ICC intra-examiner values were 0.92 and 0.89, for CAL and PD, respectively. Each clinical examination was performed under proper lighting in a dental chair.

All fully erupted teeth, excluding third molars, implants, and retained roots, were examined with manual periodontal measurements (CP-12, Hu-Friedy; Chicago, IL, USA). We recorded the number of missing teeth. Gingival recession (REC), periodontal pocket depth (PPD), and bleeding on probing (BoP) were circumferentially recorded at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual). PPD, REC, and CAL were assessed as previously detailed [19].

The periodontal chart with full-mouth data was then transformed to community periodontal index of treatment needs (CPITNs) [20]. ‘Severe’ PD was categorized following Verhulst et al. (2019) as a CPITN score of 4 [14].

2.4. Predictors

The SROH questionnaire was administered as previously validated [10]. The questions included were those used in the original model developed by Verhulst et al., which identified the most relevant self-reported oral health indicators for predicting severe periodontitis. No additional items were added or excluded. The questions present in this questionnaire had been previously translated and culturally adapted for the Portuguese population following standard cross-cultural validation guidelines to ensure conceptual and linguistic equivalence [10]. It was administered to the participants by one trained researcher (J.V.) using an electronic device linked to a Google Form. The selected questions were as follows: “Q3: Have you ever had treatment for gum disease such as scaling and root planing, sometimes called ‘deep cleaning’?”; “Q4: Have you ever had any teeth become loose on their own, without an injury?”; “Q6: During the past 3 months, have you noticed a tooth that doesn’t look right?”; and “Q8: Aside from brushing your teeth with a toothbrush, in the last 7 days, how many times did you use mouthwash or other dental rinse product that you use to treat dental disease or dental problems?”. Additionally, demographic information (age and sex) was extracted from the comprehensive health and oral hygiene questionnaire completed during the screening process. Demographic and behavioural data were extracted from the screening questionnaire and included age, sex, education level, employment status, and smoking habits. Smoking status was categorized as never smoker, former smoker (individuals who had quit smoking for at least 12 months prior to examination), and current smoker. Employment status was recorded according to self-reported occupation at the time of data collection. Oral hygiene behaviours (e.g., frequency of toothbrushing and mouthwash use) were also documented to complement the self-reported oral health questionnaire.

2.5. Sample Size

To estimate the required sample size for validating the model’s performance, we used the reported AUC of 0.75 with a 95% confidence interval of 0.66 to 0.84; the margin of error was calculated as 90%. All analyses were conducted in R version 3.1 (www.r-project.org) using the ‘pROC’ package, we used the Hanley & McNeil (1982) [21] approximation for the standard error (SE) of the AUC, which allows estimation of the sample size required to achieve a specified confidence interval width around an AUC. We assumed a two-sided 95% confidence level ($z = 1.96$) and solved iteratively for the minimum sample size. Using this

approach, we determined that a minimum of 83 cases and 83 controls (total 166 participants) was required to estimate the AUC with the desired precision, at a 1:1 ratio.

2.6. Statistical Analysis

After confirming the non-normal distribution of our sample, we performed a Mann–Whitney test for continuous variables and a chi-square test for the categorical variables (p -value = 0.00067).

To assess the discriminative performance of the Verhulst model when it is applied in the Portuguese patients, a Receiver Operating Characteristic (ROC) analysis was conducted. The predicted individual risks of severe periodontitis based on the model were compared against the outcome (severe PD) to generate the ROC curve and compute the area under the curve (AUC). An $AUC \geq 0.80$ indicates a good to excellent discriminative ability of the model, while an $AUC \geq 0.70$ indicates an acceptable discriminative ability. Youden's Index was calculated, which is the point on the ROC curve closest to the top-left corner, depicting the best performance trade-off (sensitivity and specificity).

To assess the calibration of the model in the Portuguese patients, a calibration plot and the overall observed-to-expected ratios (O:E ratios) were generated [1]. The calibration plot was made by plotting the predicted individual outcomes against the observed actual outcomes [2]. For this, the patients in the dataset were grouped into deciles based on their predicted probabilities for the outcomes. The prevalence of the outcome events within each decile represents the observed probability. The mean of the individual predicted probabilities within each decile represents the predicted probability. In the calibration plot, the observed and predicted probabilities were compared across the range of predicted risk. The O:E ratio was also calculated for the assessment of overall calibration of the models. The O:E ratio was obtained by dividing the prevalence of the outcomes (observed) by the mean of individual predicted probabilities of the outcomes (expected) within the cohort [3]. An O:E ratio < 1 indicates an overestimation of the models, while an O:E ratio > 1 indicates an underestimation of the models [2]. An O:E ratio between 0.8 and 1.2 indicates that the calibration of the model is acceptable [1,3].

In addition, we computed sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the models. Sensitivity is defined as the proportion of the patients who had severe PD based on the model among those who had severe PD in reality. Specificity is defined as the proportion of the patients who had no severe PD based on the model among those who had no severe PD in reality. The negative predictive value (NPV) is defined as the proportion of patients without severe PD in reality among those predicted by the model as not having severe PD, while the positive predictive value (PPV) is defined as the proportion of patients with severe PD in reality among those predicted by the model as having severe PD.

All analyses were conducted in R software (version 3.1) (www.r-project.org) and SPSS software 29.0 (IBM, New York, NY, USA). Statistical significance was set at $p < 0.05$.

Schematic diagrams and graphical elements were created with assistance from a generative artificial intelligence tool (ChatGPT (GPT-5.1), OpenAI, San Francisco, CA, USA). No text, data analysis, or interpretation of results was generated using AI tools.

3. Results

3.1. Participants

From a total of 356 invited participants, 46 were excluded, giving a final sample of 310 patients (Figure 1). The patients had an average age of 52.8 years old (± 16.3), with a predominance of 40-year-olds. Among the included patients, 48.0% were males. When asked about their smoking habits, 51.0% of the participants said they were not

smokers. Regarding to the employment status and the education level, the majority was employed (59.7%) and had a middle education level (43.9%) (Table 1).

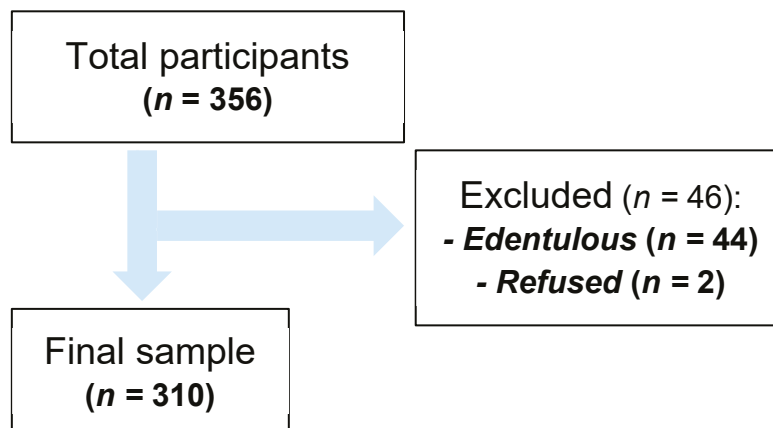


Figure 1. Flow diagram summarising the screening and selection processes.

Table 1. Participant characteristics according to the periodontal status via CPITN ($n = 310$). Values are presented as mean (standard deviation) for continuous variables and percentage (n of participants) for categorical variables.

Variables	Total ($n = 310$)	Severe PD ($n = 158$)	Non-Severe PD ($n = 152$)	<i>p</i> -Value
Age, mean (SD) (years)	52.8 (16.3)	58.5 (12.0)	46.9 (18.0)	<0.0001
Sex, % (n)				
Female	52.3 (162)	44.9 (71)	59.9 (91)	0.0118
Male	47.7 (148)	55.1 (87)	40.1 (61)	
Smoking, % (n)				
Never	51.0 (158)	44.3 (70)	57.9 (88)	0.0552
Former	21.3 (66)	24.7 (39)	17.8 (27)	
Active	27.7 (86)	31.0 (49)	24.3 (37)	
Employment status, % (n)				
Student	12.6% (39)	4.4 (7)	21.1 (32)	<0.0001
Employed	59.7% (185)	61.4 (97)	57.9 (88)	
Unemployed	7.4% (23)	8.9 (14)	5.9 (9)	
Retired	20.3% (63)	25.3 (40)	15.1 (23)	
Education, % (n)				
Elementary	24.8% (77)	31.6 (50)	17.8 (27)	0.0066
Middle	43.9% (136)	43.7 (69)	44.1 (67)	
Higher	31.3% (97)	24.7 (39)	38.2 (58)	
Number of remaining teeth	20.4 (5.7)	20.5 (5.6)	22.4 (5.6)	0.0007

Out of the 310 patients evaluated, 158 (51%) were classified with a CPITN score of 4, while 152 (49%) had scores below 4. The mean age of patients with a CPITN of 4 was 58.5 years (± 12.0), whereas the mean age of those with scores below 4 was 46.9 years (± 18.0).

3.2. Model Performance

The ROC analysis demonstrated an AUC of approximately 0.72 (95% CI: 0.67–0.77) for screening of severe PD (Figure 2). This indicates an acceptable discrimination of the model in Portuguese dental patients. The optimal predicted probability cutoff value was 0.420 based on the ROC curve. This indicates that if the predicted probability of a patient is >0.420 , this patient is very likely to have a severe form of PD. If the predicted probability of a patient is <0.420 , this patient is unlikely to have severe PD.

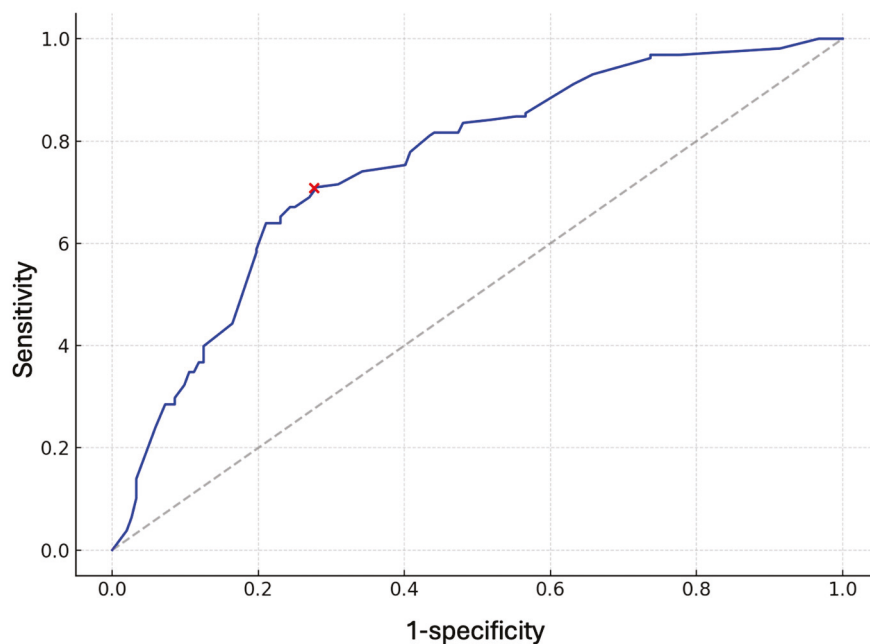


Figure 2. ROC curves with 95% confidence intervals for the screening model for severe periodontitis (the red cross in the curve indicates the optimal cutoff for the predicted risk, which is 0.420).

The observed-to-expected (O:E) ratio was estimated at 1.10 (95% CI: 0.939–1.291), which indicates that the model had a slight underestimation. That is, the predicted risk was slightly lower than the actual risk of severe PD overall. However, the O:E ratio was between 0.8 and 1.2, which indicates that the calibration of the model was acceptable.

This calibration plot illustrates the agreement between predicted and observed risks (Figure 3). Each point represents a decile of predicted risk, with the x -axis indicating the average predicted probability, and the y -axis representing the corresponding observed event rate. The 45-degree reference line denotes perfect calibration. In this plot, most points lie close to the diagonal, suggesting acceptable calibration across risk groups. However, slight deviations in some areas may indicate underestimation or overestimation of risk by the model in specific ranges.

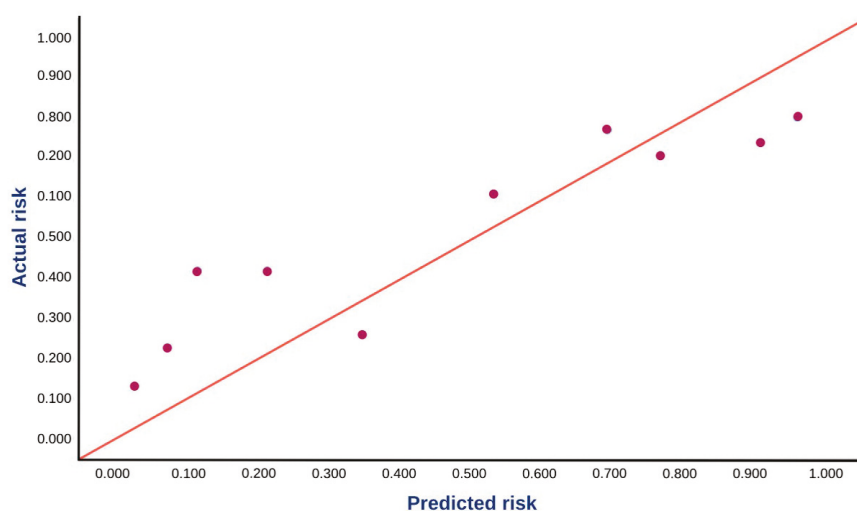


Figure 3. Calibration plot comparing predicted risk with observed risk. The 45-degree line represents perfect calibration.

Sensitivity was 0.709 (95% CI: 0.639–0.779) and specificity 0.724 (95% CI: 0.651–0.796), with a PPV of 0.727 (95% CI: 0.673–0.784) and an NPV of 0.704 (95% CI: 0.651–0.764).

4. Discussion

This prediction validation study aimed to explore the external validity of a model for screening severe periodontitis, proposed by Verhulst et al., in a Portuguese cohort of participants. Overall, the performance of the model was clinically acceptable when it was applied in Portuguese patients in dental settings, with an acceptable discrimination and calibration.

Before screening models can be extensively used in different populations, the performance of the models must be robust across diverse populations to ensure their generalisability and clinical usefulness [22,23]. Therefore, the present study externally validated Verhulst's prediction model to test this external generalizability and application. In 2019, Verhulst et al. [14] developed three different screening models for severe periodontitis using self-reported oral health, demographics, and/or salivary biomarkers, based on 156 patients from Academic Center for Dentistry Amsterdam (ACTA), The Netherlands. The model showed a good performance for the screening of severe periodontitis, with an AUC of 0.78–0.89. In 2021, Nijland et al. [24] externally validated one of the screening models developed by Martijn, including SROH questions, age, and sex only, based on 159 patients from an outpatient medical setting in The Netherlands. The performance of the model for severe periodontitis in the medical setting was shown to be acceptable, with an AUC of 0.72. In 2025, Nijland et al. [24] further attempted to modify the screening model using five different update methods. However, the updating methods did not further improve the performance of the original screening model for severe PD. Therefore, in the present study, we still used the original model developed by Verhulst et al. for the external validation. Our study was the first one which externally validated the model in the Portuguese population. Despite the expected population-specific factors of this cohort sample (e.g., socioeconomic conditions, access to dental care, and oral hygiene habits), our results provide concrete evidence on the model's transferability to new settings. Thus, it has the potential to be integrated into routine dental assessments within the Portuguese healthcare context.

The ability of individuals to self-assess their periodontal status reveals two fundamental dimensions [5]. On the one hand, it points to the development of intrapersonal knowledge, reflected in awareness of one's own oral health [25]. On the other hand, it shows a basic understanding of periodontal disease and its implications, which is essential for encouraging a patient's active involvement in managing their own health [26]. These assumptions support the importance of health education and individual empowerment, promoting a more autonomous and preventive approach to oral health care [25,27].

The results obtained reinforce the relevance of this approach. The prevalence of severe periodontal disease was significant, with 51% of individuals having a CPITN index of 4 [27–29]. This is in line with previous studies, reporting a high prevalence of moderate to severe periodontitis in adult and elderly populations [30,31]. This phenomenon could be explained by the cumulative effect of exposure to risk factors and indicators such as poor oral hygiene, smoking, and systemic diseases, combined with less frequent preventive care in older age groups [32,33]. However, the problem of poor performance of CPITN reported in the literature raises concerns regarding its sensitivity in accurately reflecting the extent and severity of periodontal destruction [10,24,27]. Several studies have highlighted its limitations, including underestimation of disease prevalence, inability to detect clinical attachment loss, and its reduced diagnostic validity in longitudinal monitoring [8,32,33]. Therefore, while the CPITN offers a practical screening tool, caution is warranted in interpreting its findings as representative of true periodontal status [1,8,34–36].

In the present study, the discrimination of the model was shown to be clinically acceptable, with an AUC of 0.72. This indicates that if there are 100 pairs of patients, each pair consisting of one patient with the outcome and one without, the model would

correctly distinguish 72 of those pairs. The calibration of the model was also clinically acceptable, only with a very slight underestimation. Additionally, the sensitivity (0.709) and specificity (0.724) values reveal a balanced performance of the model in correctly identifying both positive and negative cases. This indicated that the model can be used for both ruling in the severe PD and ruling out the non-severe PD cases. In addition, the positive predictive value (PPV) and the negative predictive value (NPV) are both measures of a model's performance in clinical decision-making. The PPV represents the proportion of individuals predicted as positive who truly have the condition, while the NPV indicates the proportion of individuals predicted as negative who are indeed free of the condition. Each metric offers complementary insights: the PPV is clinically valuable, as it reflects the likelihood that a positive test result corresponds to a true case, guiding treatment decisions. The NPV, in turn, is essential for ruling out disease, as it provides confidence that a negative test result reliably excludes the condition. In our model, the PPV was 0.727 (95% CI: 0.673–0.784), while the NPV was slightly lower, at 0.704 (95% CI: 0.651–0.764). This also indicated that the model can be reliably used to screen in or out the severe PD, with minimal false positives and false negatives. This shows that the model can be well used in Portuguese dental patients for the screening of severe PD.

Beyond statistical performance, the clinical and societal added value of this screening model should also be emphasised. For clinicians, it provides a simple and efficient tool to identify patients at high risk of severe periodontitis without the need for complex or time-consuming examinations, thereby supporting earlier intervention and personalised preventive strategies. For patients, the model promotes timely diagnosis and treatment, reducing the risk of disease progression, tooth loss, and the associated negative impacts on quality of life. At a societal level, implementing such models may contribute to lowering healthcare costs by reducing the burden of advanced periodontal treatments, decreasing productivity losses linked to oral disease, and ultimately supporting public health strategies aimed at improving population-wide oral health outcomes.

Strengths and Limitations

This study presents several strengths, including adherence to the TRIPOD checklist, the use of CPITN based on a full-mouth periodontal examination, the employment of a calibrated examiner with high intra-examiner reliability, and a sample size determined through prior calculation, ensuring adequate statistical power. Nevertheless, the use of CPITN as the primary diagnostic criterion represents a limitation, as it may underestimate disease severity and compromise diagnostic accuracy [36]. Future research should consider more comprehensive diagnostic criteria to improve validity.

An important limitation of this study concerns the target population. That is, the patients included in the present study were dental patients. Therefore, the findings are strictly generalisable only to individuals in dental settings, rather than the patients from medical settings. It is essential to acknowledge that medical patients may differ significantly from dental patients in various aspects, such as the prevalence of severe periodontitis, age distribution, and gender proportions. These differences may influence the model's performance and limit its applicability in broader clinical settings. Moreover, although the model may have considerable potential in medical contexts, where clinical periodontal examinations are more challenging to perform, its performance in such settings was not assessed in the present study. Future research should aim to validate the model in medical populations to ensure its effectiveness and generalisability beyond dental care environments.

5. Conclusions

This study validated the screening model by Verhulst et al. in the studied population. The model demonstrated to have an acceptable screening performance, but future research is needed to enhance its clinical utility.

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Institutional Review Board Statement: This study was conducted in accordance with the Ethics Committee of Egas Moniz (IDs: number PT-348/23, 27 February 2024), adhering to the principles of the Declaration of Helsinki of 1975, as revised in 2024.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in the study are openly available in 10.5281/zenodo.17711524.

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Article

A Simplified CAMBRA-Based Diagnostic Caries Risk Assessment Tool for Young Adults: Development and Clinical Validation

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Abstract

Background/Objectives: Young adulthood is a transitional period associated with changes in lifestyle and preventive dental attendance, which may influence caries risk. In routine practice, the use of comprehensive caries risk assessment systems is often limited by time and diagnostic requirements, highlighting the need for simplified diagnostic screening tools. This study aimed to develop and clinically validate a simplified, questionnaire-based caries risk assessment tool derived from the CAMBRA framework for use in young adults. **Methods:** An observational cross-sectional study was conducted among 246 Romanian young adults aged 18–25 years. The instrument was designed to enable rapid caries risk stratification based exclusively on questionnaire data, without radiographic or laboratory investigations. Internal consistency, test–retest reliability, and construct validity were evaluated by comparison with clinically recorded indicators, including DMFT values, early enamel changes, visible dental plaque, and active carious lesions. **Results:** The questionnaire showed acceptable internal consistency (Cronbach’s alpha = 0.71) and good temporal stability (ICC = 0.82). Higher caries risk categories were consistently associated with unfavorable clinical findings, including increased DMFT values, a higher prevalence of early enamel changes, greater plaque accumulation, and more frequent active caries ($p < 0.01$). **Conclusions:** The simplified CAMBRA-based questionnaire demonstrated satisfactory reliability and clinical relevance in young adults. It may serve as a practical diagnostic screening and decision-support tool for risk-based caries prevention in routine and community dental settings.

Keywords: caries risk assessment; CAMBRA; young adults; preventive dentistry

1. Introduction

In routine dental practice, caries remains a common finding across the lifespan. Despite the widespread availability of preventive information, preventive measures are not consistently applied in all settings, and clinical examination frequently reveals new carious lesions, early enamel changes, or signs of ongoing disease activity. Epidemiological data consistently show that dental caries remains highly prevalent across age groups, with a

substantial proportion of lesions remaining untreated despite the availability of preventive strategies [1–5].

Against this background, caries risk assessment has assumed a central role in everyday caries management. The recognition that caries is a dynamic, multifactorial disease, with risk profiles varying substantially between individuals, has supported a broader shift from lesion-centered treatment toward risk-based prevention and individualized care planning [6]. Within this framework, the Caries Management by Risk Assessment (CAMBRA) approach integrates biological, behavioral, and protective factors into a structured assessment process and is widely used in clinical practice [7,8]. Nevertheless, the routine implementation of comprehensive CAMBRA-based protocols may be challenging in some settings due to time constraints and the need for clinical, radiographic, or laboratory-based information.

Within this broader risk-based framework, young adulthood represents a transitional stage in terms of caries risk and preventive needs. During this period, changes in lifestyle, dietary habits, oral hygiene routines, and dental attendance are common and may increase susceptibility to caries. At the same time, regular dental visits often become less frequent after adolescence, and preventive interventions may be inconsistently applied [9,10]. In this context, early identification of individuals at increased caries risk becomes essential to allow timely preventive measures and to limit progression toward cavitated lesions requiring restorative or endodontic treatment.

In everyday preventive dental practice, particularly in community or population-based contexts, clinicians often require simplified and rapid tools that allow initial caries risk stratification without extensive diagnostic procedures. As highlighted by Featherstone et al., existing caries risk assessment systems differ substantially in terms of structure, complexity, and data requirements, which may limit their routine application in real-world clinical settings [11]. Questionnaire-based instruments represent a pragmatic alternative for this purpose, as they rely on self-reported information related to established caries risk indicators, including dietary habits, oral hygiene behaviors, fluoride exposure, and relevant medical factors. When appropriately designed and applied, such tools may facilitate large-scale screening, support prioritization of preventive resources, and assist clinical decision-making, particularly in settings where comprehensive clinical assessment is not immediately feasible [12,13].

Several caries risk assessment systems have been described in the literature, including comprehensive models based on the CAMBRA framework and other multifactorial risk assessment approaches [7,11]. However, many of these approaches rely on detailed clinical, radiographic, or biological information, which may limit their use as rapid screening tools in routine preventive settings. Although questionnaire-based approaches have been explored in epidemiological research [12,13], simplified instruments specifically designed for rapid caries risk screening in young adult populations remain relatively limited. Within this context, the development of practical tools that allow initial risk stratification using easily obtainable information may be clinically useful.

Simplified questionnaire-based instruments may serve as practical diagnostic screening tools, enabling early identification of individuals at increased caries risk and supporting risk-based preventive decision-making in routine dental practice.

The clinical usefulness of simplified caries risk questionnaires, however, depends on their psychometric robustness and their ability to reflect actual disease patterns. Validation against clinical indicators of caries experience and activity is therefore essential to ensure that questionnaire-derived risk scores correspond to meaningful differences in oral health status [14–16]. Demonstrating coherent associations with parameters such as DMFT values,

early enamel changes, visible dental plaque, and active cavitated lesions provides evidence that a questionnaire captures clinically relevant aspects of caries risk.

The aim of the present study was to develop and clinically validate a simplified CAMBRA-based caries risk assessment instrument for young adults. The proposed tool was designed to enable rapid risk stratification based exclusively on questionnaire data, without the need for radiographic or laboratory investigations. Its validity was evaluated by assessing internal consistency, temporal stability, and associations between questionnaire-derived risk scores and established clinical indicators of caries experience and activity, with the objective of supporting efficient, risk-based caries prevention in routine preventive dental practice.

2. Materials and Methods

Study design: Our study was designed as an observational, cross-sectional investigation aimed at developing and validating a simplified caries risk assessment instrument derived from the CAMBRA conceptual framework. The study comprised two consecutive components: (1) the development of a simplified CAMBRA-based questionnaire and (2) its initial psychometric and clinical validation. In addition, a subsample of participants was reassessed after 14 days to evaluate the temporal stability of the instrument.

The study protocol was conducted in accordance with the principles of the Declaration of Helsinki and received approval from the Ethics Committee of Denta Aur Private Medical Center, Tîrgu Mureş, Romania (protocol no. 11/3 September 2024). All participants provided written informed consent prior to enrolment.

Study population and recruitment: Participants were recruited from the general population of Romanian young adults aged 18–25 years, using both academic and non-academic settings. Recruitment sites included university environments as well as community-based locations, allowing the inclusion of individuals both enrolled and not enrolled in formal education at the time of the study. This approach was chosen to reflect the heterogeneity of young adults encountered in routine preventive dental practice and to enhance the external applicability of the proposed instrument.

Participants were invited through direct approach and informational announcements in university-associated and community-based settings. Young adults who presented for the clinical visit completed the questionnaire under supervision immediately prior to the oral examination. Following clinical assessment, three participants were excluded due to the identification of exclusion criteria not declared at recruitment, resulting in a final sample of 246 participants. A formal a priori sample size calculation was not performed; however, the final sample size ($n = 246$) exceeds commonly recommended thresholds for exploratory psychometric validation studies and was therefore considered adequate for the analyses performed. **Eligibility criteria.** Individuals were included if they met the following criteria: age between 18 and 25 years, presence of natural dentition with at least 20 permanent teeth, ability to complete a self-administered questionnaire in Romanian, and provision of informed consent.

Participants were excluded if they were undergoing active fixed orthodontic treatment, presented systemic conditions with a known major impact on oral health, were under chronic medication with significant xerogenic effects, or were unable to reliably complete the questionnaire. This category referred to systemic conditions known to significantly affect salivary function or oral health status, such as Sjögren's syndrome, uncontrolled diabetes, previous head and neck radiotherapy, or conditions requiring long-term xerogenic medication. Common systemic conditions without a substantial direct impact on oral health were not considered exclusion criteria. Participants with incomplete clinical or questionnaire data were also excluded from the final analysis.

2.1. Development of Simplified CAMBRA-Based Instrument

The caries risk assessment instrument evaluated in this study was newly developed by adapting and simplifying key domains from the CAMBRA framework. Item selection was guided by clinical relevance, feasibility in non-specialized settings, and applicability to young adult populations. Domains requiring radiographic, salivary, or microbiological investigations were intentionally excluded to retain parameters that can be reliably captured through questionnaire-based assessment and applied in routine preventive settings. Item selection was performed by four clinicians with experience in preventive dentistry and caries risk assessment, based on parameters commonly evaluated during preventive dental consultations, with the contribution of an external expert involved in the development of the instrument.

The resulting questionnaire included a reduced number of items addressing biological risk factors, protective factors, and behavioral aspects commonly assessed during preventive dental consultations. The instrument was intentionally designed to be completed within a few minutes and without the need for laboratory tests, salivary analyses, or radiographic investigations, making it suitable for large-scale screening and routine preventive use.

Prior to data collection, the questionnaire underwent a pilot testing phase to ensure clarity, comprehensibility, and feasibility of completion in a clinical setting. Minor wording adjustments were made to improve clarity, but no structural modifications to the questionnaire domains were introduced. Items corresponding to protective factors were coded as present (1) or absent (0) at the time of data collection. During score computation, the cumulative protection score was subtracted from the sum of disease indicators and risk factors, in accordance with the CAMBRA conceptual framework.

The questionnaire used in this study is provided in Supplementary File S1, and the detailed scoring algorithm and risk categorization procedure are provided in Supplementary File S2.

2.2. Clinical Oral Examination

All participants underwent a standardized clinical oral examination performed by three calibrated clinicians. Prior to data collection, the examiners underwent a calibration session to standardize the clinical assessment criteria. During this phase, clinical findings were independently evaluated and subsequently discussed to ensure consistent interpretation of DMFT scoring and plaque assessment criteria. Inter-examiner agreement for DMFT and plaque assessment was evaluated during the calibration phase, and a high level of agreement between examiners was achieved before the start of the study. Clinical examinations were performed under standardized lighting conditions, using dental mirrors and air-drying when necessary. The examination focused on the identification of visual clinical indicators relevant to caries risk assessment, including visible dental plaque, oral hygiene status, initial enamel changes, cavitated carious lesions, and selected structural features associated with increased caries susceptibility.

The DMFT index was assessed exclusively through visual clinical examination, without radiographic evaluation. Only clinically cavitated carious lesions were recorded as decayed teeth. Initial non-cavitated enamel lesions, including white spot lesions, were not included in the DMFT score and were analyzed separately as indicators of early disease activity. Missing teeth were recorded as part of the DMFT index only when tooth loss was attributable to dental caries, based on clinical judgment and participant-reported history. Teeth lost for orthodontic, traumatic, or congenital reasons were not considered. Filled teeth were defined as teeth presenting permanent restorations, including crowns, in the absence of clinical signs of secondary caries. Preventive sealants and temporary restorations were not recorded as filled teeth.

This conservative scoring approach was adopted to ensure that caries risk assessment reflects current disease activity rather than historical disease experience alone.

2.3. Data Collection Procedure

The questionnaire was completed by participants using a secure online platform in a clinical setting, under investigator supervision, immediately prior to the oral examination. The clinical assessment was subsequently performed independently by calibrated examiners who were blinded to questionnaire responses, to minimize observational bias. For the assessment of test–retest reliability, the questionnaire was re-administered and completed after a 14-day interval by a subsample of 73 participants.

2.4. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA). Internal consistency of the questionnaire was evaluated using Cronbach’s alpha coefficient. Test–retest reliability was assessed using the intraclass correlation coefficient (ICC). Construct validity was explored by examining the relationships between questionnaire-derived risk scores and clinically recorded indicators obtained during oral examination, including DMFT values and the presence of early enamel changes. Differences in risk scores across predefined clinical categories were analyzed using the Kruskal–Wallis test due to the non-normal distribution of the data. This test evaluated overall differences across the predefined caries risk categories, and the reported *p*-values correspond to these global comparisons between groups. Associations between caries risk categories and clinical indicators were examined using non-parametric comparisons across predefined groups. Statistical significance was set at a two-tailed *p* value < 0.05. Exploratory analyses were conducted to assess potential differences in caries risk scores according to participants’ enrolment in formal education at the time of the study, using non-parametric comparative tests as appropriate.

2.5. Methodological Considerations

The study was conceived from a pragmatic clinical perspective, aiming to balance methodological rigor with applicability in everyday preventive dentistry. The proposed instrument was not intended to replace comprehensive caries management protocols, but rather to provide a rapid and clinically meaningful tool for caries risk stratification in young adult populations.

3. Results

3.1. Characteristics of the Study Population

A total of 246 young adults aged between 18 and 25 years were included in the final analysis. The mean age of the participants was 21.4 years (SD 2.1). Females were slightly more represented than males. Most participants were enrolled in formal education at the time of recruitment, while a smaller proportion were not enrolled in formal education (Table 1).

Table 1. Characteristics of the study population.

Variable	Value
Number of participants, <i>n</i>	246
Age, mean ± SD (years)	21.4 ± 2.1
Female sex, <i>n</i> (%)	142 (57.7)
Male sex, <i>n</i> (%)	104 (42.3)
Enrolled in formal education, <i>n</i> (%)	162 (65.9)
Not enrolled in formal education, <i>n</i> (%)	84 (34.1)

3.2. Distribution of Caries Risk Scores and Categories

The simplified CAMBRA-based instrument generated a broad distribution of caries risk scores within the study population. Based on the predefined cut-off values, participants were classified into four categories (Table 2). Low caries risk was identified in a minority of participants, whereas the moderate-risk category accounted for the largest proportion of the sample. A substantial number of participants were classified as high risk, while a smaller but clinically relevant group fell into the very high-risk category. Overall, the distribution of risk categories suggested that the instrument was able to differentiate between distinct levels of caries risk in this young adult population.

Table 2. Distribution of caries risk categories.

Caries Risk Category	n (%)
Low risk	48 (19.5)
Moderate risk	96 (39.0)
High risk	72 (29.3)
Very high risk	30 (12.2)

3.3. Reliability of the Instrument

The internal consistency of the questionnaire was acceptable. The Cronbach's alpha coefficient for the total score was 0.71, indicating adequate coherence between items addressing disease indicators, risk factors, and protective behaviors.

Test–retest reliability was assessed in a subsample of 73 participants who completed the questionnaire twice, with a 14-day interval between administrations. The intraclass correlation coefficient (ICC) for the total risk score was 0.82 (95% CI: 0.74–0.88), indicating good temporal stability of the instrument (Table 3).

Table 3. Reliability analysis of the simplified CAMBRA-based instrument.

Parameter	Value
Cronbach's alpha	0.71
Test–retest sample (n)	73
ICC (95% CI)	0.82 (0.74–0.88)

3.4. Construct Validity

Higher caries risk categories were associated with less favorable clinical findings (Table 4). Median DMFT values increased progressively across caries risk categories, with lower values observed in participants classified as low risk and higher values in those classified as very high risk. Differences between groups were statistically significant.

Initial enamel changes (white spot lesions) were more frequently observed in participants classified as high and very high risk compared to those in the low- and moderate-risk categories. Similarly, visible dental plaque and poor oral hygiene scores were more commonly recorded in the higher-risk groups.

Table 4. Clinical indicators across caries risk categories.

Indicator	Low Risk	Moderate Risk	High Risk	Very high Risk	p-Value
DMFT, median (IQR)	3 (2–4)	5 (4–6)	7 (6–9)	9 (8–12)	<0.001
White spot lesions, n (%)	6 (12.5)	20 (20.8)	28 (38.9)	16 (53.3)	<0.01
Visible plaque (score = 2), n (%)	4 (8.3)	18 (18.8)	30 (41.7)	18 (60.0)	<0.001
Active caries (D > 0), n (%)	2 (4.2)	12 (12.5)	26 (36.1)	18 (60.0)	<0.001

Active cavitated lesions ($D > 0$) were predominantly identified among participants classified as high or very high risk, supporting the clinical relevance of the proposed risk stratification.

3.5. Exploratory Analysis According to Enrolment in Formal Education

Exploratory analyses showed modest differences in caries risk distribution between participants enrolled in formal education and those not enrolled at the time of the study. Participants not enrolled in formal education tended to be more frequently classified in the higher-risk categories. However, the overall pattern of associations between caries risk categories and clinical indicators remained consistent across educational groups.

4. Discussion

The present study reports the clinical performance of a simplified CAMBRA-based caries risk assessment instrument when applied to a young adult population in a real preventive care setting. Within this context, the proposed instrument may also function as a practical diagnostic screening tool, supporting early identification of individuals at increased caries risk and facilitating risk-based preventive decision-making in routine clinical practice. The questionnaire showed acceptable internal consistency and good temporal stability, together with consistent associations with clinically recorded indicators of caries experience and activity. These findings support the clinical relevance of questionnaire-based approaches for initial caries risk stratification in routine preventive dental practice, in line with contemporary risk-oriented caries management concepts [7,11,17–20].

In the present sample, higher questionnaire-derived risk categories corresponded to progressively less favorable clinical findings. Participants classified as high or very high risk exhibited higher DMFT values, a greater prevalence of initial enamel changes, increased levels of visible dental plaque, and a higher frequency of active cavitated lesions compared with those classified as low or moderate risk. The observed gradients across risk categories indicate that the instrument can distinguish between clinically distinct risk profiles, capturing both accumulated caries experience and signs of ongoing disease activity. Similar associations between risk assessment outcomes and clinical indicators have been reported in previous validation studies of CAMBRA-based tools and other caries risk assessment models, supporting the construct validity of the present approach [21–23].

The pragmatic design of the proposed tool addresses several practical limitations associated with comprehensive caries risk assessment systems. While full CAMBRA protocols and multifactorial models such as the Cariogram integrate a wide range of clinical, behavioral, and biological parameters, including radiographic findings and salivary or microbiological assessments, their routine application may be limited in busy clinical environments or community-based preventive programs [13,24,25]. Unlike predictive models such as the Cariogram, which aim to estimate future caries incidence or risk, the present cross-sectional study evaluated associations between questionnaire-derived risk categories and current clinical indicators. Longitudinal studies will be required to assess the predictive performance of the proposed instrument. By relying exclusively on questionnaire-based information, the proposed instrument allows rapid initial risk stratification without the need for radiographic assessment, salivary testing, or additional chairside procedures, which may facilitate its integration into routine preventive care. Importantly, the present findings indicate that this simplified approach retains clinical relevance when appropriately validated against objective oral health indicators [11,26,27]. Although biological parameters such as salivary flow, buffering capacity, or microbial load were not included in the simplified instrument, the observed associations between

questionnaire-derived risk categories and clinical indicators of caries experience and activity suggest that the instrument is able to distinguish between clinically relevant risk profiles.

Several methodological considerations should be acknowledged when interpreting these results. The cross-sectional design does not permit conclusions regarding the predictive performance of the instrument over time, and longitudinal studies are required to assess its ability to predict caries incidence or progression, as highlighted in previous evaluations of caries risk assessment tools [10,13,28]. In addition, DMFT assessment was based on visual clinical examination without radiographic evaluation, which may have resulted in underdiagnosis of certain interproximal lesions. According to recent expert consensus, caries management in clinical practice should integrate caries risk assessment with lesion activity and be supported by individualized treatment planning and periodic reassessment over time [29]. In addition, although the questionnaire was administered under supervision to reduce missing data and misinterpretation, the use of self-reported information may still introduce recall or reporting bias. This may also include the influence of social desirability bias, whereby participants could overestimate adherence to recommended oral hygiene behaviors or underreport cariogenic dietary habits, particularly when completing the questionnaire in a supervised clinical environment. The distribution of educational status within the sample may also have influenced the observed risk profile; however, the associations between risk categories and clinical indicators remained consistent across groups. In addition, socioeconomic variables such as income, occupation, or parental education level were not systematically collected and may represent potential confounding factors. Nevertheless, questionnaire-based assessments are widely used in preventive and epidemiological research, and their utility is supported when consistent associations with objective clinical indicators are demonstrated [12,30–32]. The coherence observed between questionnaire-derived risk categories and multiple clinical parameters in the present study supports the validity of the approach within the investigated population.

Taken together, these findings support the use of the simplified CAMBRA-based questionnaire as an initial screening and decision-support aid in young adults, intended to complement, rather than substitute, comprehensive clinical caries assessment. Its application may be particularly valuable in preventive and community-based settings where rapid risk stratification is required to support efficient, risk-oriented preventive decision-making and allocation of preventive resources [13,17,33,34]. From a practical perspective, the simplified structure of the instrument may also facilitate future digital implementation. Further studies could explore the application of the questionnaire in broader population groups and support the development of web-based platforms that would allow automated scoring and easy access for dental practitioners, potentially facilitating large-scale preventive screening.

5. Conclusions

The simplified CAMBRA-based caries risk assessment instrument demonstrated acceptable reliability and clinically meaningful associations with established indicators of caries experience and activity in young adults, supporting its potential use as a diagnostic screening tool in routine preventive dental practice. By enabling rapid risk stratification based exclusively on questionnaire data, the proposed tool may support efficient, risk-based preventive decision-making in routine dental practice. Its use may be particularly valuable in community and population-based settings where comprehensive clinical assessment is not immediately feasible. Further longitudinal studies are warranted to evaluate its predictive performance over time.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/diagnostics16060859/s1>, File S1: Simplified CAMBRA-Based Caries Risk Assessment Questionnaire; File S2: Scoring Algorithm and Risk Categorization Procedure.

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Data Availability Statement: The data presented in this study are not publicly available due to ethical and privacy restrictions involving human participants. Anonymized data may be made available from the corresponding author upon reasonable request, subject to institutional and ethical approval.

Conflicts of Interest: The authors declare no conflicts of interest.

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Article

The Inflammatory–Dysplastic Spectrum in Oral Lichen Planus: A Study on Six Immunohistochemical Markers

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Abstract

Background/Objective: Oral lichen planus (OLP) is a chronic inflammatory, immune-mediated mucosal condition classified as a potentially malignant disorder due to its risk of progression to oral squamous cell carcinoma (OSCC). The molecular events linking chronic inflammation in OLP to epithelial dysplasia remain poorly defined. To evaluate the expression of six immunohistochemical markers: IL-17, Maspin, β -Catenin, TIMP-1, MMP-14 and Syndecan-4 in OLP specimens and to explore their association with clinico-pathological features and early dysplastic changes. **Methods:** We conducted a retrospective, cross-sectional study including 63 cases of OLP and 20 healthy controls. Formalin-fixed, paraffin-embedded sections underwent immunohistochemical staining for the six markers. Semi-quantitative scoring of staining intensity and percentage of positive cells was performed independently by two blinded pathologists. **Results:** IL-17 was markedly up-regulated in 82.5% of OLP lesions versus absence in controls, correlating strongly with inflammatory infiltrate intensity. β -Catenin exhibited cytoplasmic and nuclear accumulation in 88.9% of OLP samples, with nuclear localization significantly associated with moderate dysplasia. Syndecan-4 membrane expression was reduced in dysplastic lesions, while Maspin and TIMP-1 co-expression were more prevalent in non-dysplastic OLP. MMP-14 was weakly positive in 87.3% of OLP cases and correlated with neovascularization. **Conclusions:** Elevated IL-17 expression and nuclear localization of β -Catenin may contribute to the progression of OLP toward dysplastic transformation, with this pattern being most evident in the erosive subtype. These findings suggest that a combined immunohistochemical panel may support risk stratification in OLP, although validation in larger, prospective cohorts is warranted.

Keywords: oral lichen planus; immunohistochemistry; IL-17; maspin; β -Catenin; TIMP-1; MMP-14; Syndecan-4; inflammation; dysplasia

1. Introduction

Oral lichen planus (OLP) is a chronic, immune-mediated inflammatory disorder of the oral mucosa that affects approximately 1–2% of the general population, with a strong female predilection in middle age [1]. The World Health Organization recognizes OLP as an oral potentially malignant disorder (OPMD) because longitudinal studies report a pooled malignant transformation rate of about 1.1% (range 0.4–1.8%) over follow-up periods averaging six years [2–4]. Clinically, OLP presents in several distinct variants—reticular (marked by asymptomatic Wickham’s striae), atrophic/erosive (painful, ulcerated), plaque-like (mimicking leukoplakia), and rarely bullous—with atrophic and erosive forms conferring the highest risk of progression to OSCC [3,5,6].

The pathogenesis of OLP centers on an antigen-specific, CD8⁺ T-cell attack against basal keratinocytes, resulting in vacuolar degeneration, apoptotic cell death, and disruption of epithelial integrity, accompanied by a dense, band-like lymphocytic infiltrate in the superficial lamina propria [7,8]. Persistent inflammation in this microenvironment generates pro-inflammatory cytokines (notably IL-17), reactive oxygen species, and growth factors that can induce DNA damage, aberrant repair, and epithelial dysplasia [9,10]. However, histopathologic distinction between reactive atypia and true dysplasia in OLP is notoriously challenging due to overlapping morphological features and significant inter-observer variability [11,12], underscoring the need for objective molecular markers to improve diagnostic reproducibility and risk stratification.

Recent advances in molecular pathology have highlighted several immunohistochemical (IHC) markers involved in inflammation, epithelial adhesion, and extracellular matrix remodeling, which may clarify the inflammatory–dysplastic continuum in OLP. Interleukin-17 (IL-17), secreted by Th17 cells, sustains chronic mucosal inflammation and promotes angiogenesis and keratinocyte proliferation [13–15]. Syndecan-4, a transmembrane heparan-sulfate proteoglycan, regulates cell–matrix adhesion and cytoskeletal dynamics; its downregulation correlates with loss of epithelial polarity and increased invasiveness in premalignant lesions [16–18]. Maspin, a serpin family protein with tumor-suppressor activity, exhibits nuclear localization in differentiated epithelia and its loss associates with poor prognosis in head and neck cancers [19,20]. β -Catenin, a central mediator of Wnt signaling and cell–cell adhesion, accumulates in the cytoplasm and nucleus during early dysplasia and OSCC, driving transcription of oncogenic targets [21–23]. Matrix metalloproteinase-14 (MMP-14) degrades basement membrane components to facilitate invasion, while its inhibitor TIMP-1 maintains extracellular matrix homeostasis; disruption of their balance underpins early stromal remodeling and epithelial–mesenchymal transition (EMT) [24–26].

The primary objective of this study was to investigate the immunohistochemical expression of six biomarkers—IL-17, Maspin, β -Catenin, TIMP-1, MMP-14, and Syndecan-4—in oral lichen planus (OLP) compared with healthy oral mucosa. Specifically, we aimed to assess their association with the presence and severity of epithelial dysplasia and to determine whether particular expression patterns may serve as predictors of malignant risk.

2. Materials and Methods

2.1. Study Design and Specimen Collection

This retrospective observational study was based on biopsy specimens archived between January 2015 and January 2023 at the Departments of Anatomical Pathology of the “Sfântul Spiridon” Emergency Clinical Hospital. A total of 63 cases with histopathologically confirmed oral lichen planus (OLP) were included. All cases strictly met the modified WHO clinical and histological diagnostic criteria for OLP. Lesions attributable to lupus erythematosus were excluded using a combination of clinical and histopathological criteria: (a) absence of systemic or mucocutaneous features suggestive of lupus, (b) no prior history

of autoimmune disease, and (c) lack of histopathological hallmarks typical of lupus erythematosus, such as basement membrane thickening, perivascular inflammatory infiltrates, and deep connective tissue involvement.

Patients with systemic inflammatory diseases were excluded on the basis of medical history, clinical records, and routine laboratory screening. At the time of biopsy, all participants underwent a standard laboratory panel, including complete blood count, C-reactive protein (CRP), and liver and renal function tests. Individuals with autoimmune conditions (e.g., lupus erythematosus, rheumatoid arthritis) or chronic infections were also excluded.

For the control group, perilesional specimens of clinically normal, non-inflamed oral mucosa were obtained during minor surgical procedures. Inclusion criteria comprised adults over 18 years of age with no chronic illnesses and a confirmed clinical and histopathological diagnosis of either OLP or normal mucosa. Exclusion criteria included current smoking or alcohol abuse, prior radiotherapy to the head and neck, immunosuppressive therapy, systemic inflammatory disease, or absence of histopathological confirmation of the clinical diagnosis (Table 1).

Table 1. Characteristics of the study group (OLP) and control group.

Characteristic	OLP Group (<i>n</i> = 63)	Control Group (<i>n</i> = 20)
Timeframe	January 2015–January 2023	January 2015–January 2023
Source	Archived biopsy specimens from the Department of Anatomical Pathology at “Sfântul Spiridon” Emergency Clinical Hospital	Normal oral mucosa obtained during minor surgical procedures (e.g., third molar extraction)
Inclusion Criteria	Age > 18 years; no other chronic illness; histopathologically confirmed OLP (modified WHO criteria)	Age > 18 years; no other chronic illness; clinically and histologically normal mucosa (no inflammation or dysplasia)
Exclusion Criteria	Current smokers or alcohol abuse; prior head and neck radiotherapy; immunosuppressive therapy; systemic inflammatory diseases; lack of histopathological confirmation;	Current smokers or alcohol abuse; prior head and neck radiotherapy; immunosuppressive therapy; systemic inflammatory diseases; lack of histopathological confirmation;

The study was approved by the Ethics Committee of the Grigore T. Popa University (approval no. 61/23 March 2021), and written informed consent was obtained from all patients.

2.2. Reagents and Antibodies

The primary antibodies used for immunohistochemical analysis are listed in Table 2.

Table 2. Reagents and Antibodies.

Marker	Clone	Host	Dilution	Supplier	Catalog No.
IL-17	Polyclonal	Rabbit	1:100	Biorbyt, Cambridge, UK	ORB13500
β-Catenin	SAB4500543	Rabbit	1:50	Sigma-Aldrich, St. Louis, MO, USA	SAB4500543-100UG
Maspin	Clone C-8	Mouse	1:100	Santa Cruz Biotechnology, Dallas, TX, USA	SC-271694
MMP-14	SAB4501901	Rabbit	1:100	Sigma-Aldrich, St. Louis, MO, USA	SAB4501901-100UG
Syndecan-4	SAB4502721	Rabbit	1:100	Sigma-Aldrich, St. Louis, MO, USA	SAB4502721-100UG
TIMP-1	HPA053417	Rabbit	1:100	Atlas Antibodies, Bromma, Sweden	HPA053417-100UL

2.3. Tissue Processing and Staining Protocols

For immunohistochemical analysis, 4- μ m sections were first deparaffinized through three consecutive xylene baths (10 min each) and then rehydrated in descending ethanol series (100%, 95%, 70%; 10 min each). Antigen retrieval was carried out in citrate buffer (pH 6.0) at 97–99 °C for 15 min. Endogenous peroxidase activity was quenched and nonspecific protein binding blocked by incubating the slides for 20 min each in commercial Peroxidase Block and Protein Block solutions. Sections were then incubated overnight at 4 °C with primary antibodies directed against IL-17, Syndecan-4, Maspin, β -Catenin, MMP-14, and TIMP-1 at their optimized dilutions. After PBS washes, slides were treated for 10 min with a biotinylated secondary antibody, followed by a 60-min incubation with streptavidin–peroxidase. Chromogenic detection was achieved by applying DAB substrate (30 μ L DAB chromogen in 1.5 mL buffer) for 1 min, producing a brown reaction product at antigen sites. Finally, sections were counterstained in Mayer’s hematoxylin (1 min), dehydrated through graded ethanols (10 min each), cleared in xylene (9 min), coverslipped, and examined on an Olympus BX40 (Olympus Corporation, Tokyo, Japan) microscope. Positive controls included breast and colon carcinoma tissues. Negative controls were obtained by omitting the primary antibody.

2.4. Evaluation of Immunohistochemical Staining

Immunoreactivity for all six markers (IL-17, Maspin, β -Catenin, TIMP-1, MMP-14, and Syndecan-4) was semi-quantitatively scored against resident tissue lymphocytes, using a four-tier system: Negative (–); Weak (+, 0–10% of positive cells); Moderate (++); and Strong (+++, intensity equivalent to lymphocytes). Two independent, board-certified pathologists—both co-authors of this study—performed blinded evaluations of at least 1000 epithelial cells per case across ten high-power fields (40 $\times$). Oral lesions were further classified by anatomical location and histological grade of dysplasia: 0 = none, 1 = mild, 2 = moderate, 3 = severe. Cellular localization was documented for each antibody: IL-17 and Syndecan-4 (cytoplasmic/membranous), Maspin (nuclear and/or cytoplasmic), β -Catenin (membranous, cytoplasmic, nuclear), and MMP-14 and TIMP-1 (cytoplasmic and membranous), with notation of focal versus diffuse staining. Normal mucosa served as the baseline control. Any inter-observer discrepancies exceeding 10% were resolved by joint review and consensus. No automated or digital image-analysis software was applied.

2.5. Statistical Analysis

Data were entered into Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and analyzed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Statistical significance was defined as $p < 0.05$. Group means were compared using Student’s *t*-test (according to degrees of freedom), while frequency distributions were assessed with the Chi-squared (χ^2) test, applying Yates’ correction when appropriate. Odds Ratios (OR) and Relative Risks (RR) were also calculated to evaluate the likelihood of disease occurrence in exposed versus unexposed groups.

3. Results

3.1. Clinicopathological Characteristics

Given that 95% of the cohort represented erosive OLP, the findings of this study primarily reflect this clinical subtype. The buccal mucosa was the most commonly involved site, followed by the lateral tongue border and gingiva, with many patients presenting lesions at more than one oral location (Table 3). The control group comprised 20 specimens of perilesional normal mucosa, obtained from non-inflamed tissue adjacent to the lesion during biopsy.

Table 3. Distribution of Affected Sites by OLP.

Affected Site	Number of Cases (<i>n</i> = 63)	Percentage (%)
Buccal mucosa	56	89.2
Lateral border of the tongue	37	58.6
Gingiva	11	16.7
Labial mucosa (lips)	11	16.7

3.2. Histological and Dysplasia Grades

All OLP cases met the World Health Organization histological diagnostic criteria, exhibiting basal cell degeneration, saw-tooth rete ridges, and a band-like lymphocytic infiltrate. Epithelial dysplasia was present in a subset of lesions: 24 cases (38.1%) showed mild dysplasia, and 8 cases (12.7%) displayed moderate dysplasia. No dysplastic changes were observed in control tissues (Table 4).

Table 4. Comparison of Histopathological Features Between OLP and Control Groups.

Histopathological Feature	OLP Group (<i>n</i> = 63)	Control Group (<i>n</i> = 20)
Basal cell degeneration	Present in 63 (100%)	Absent
Saw-tooth rete ridges	Present in 63 (100%)	Absent
Band-like lymphocytic infiltrate	Present in 63 (100%)	Absent
Epithelial dysplasia—none	31 (49.2%)	20 (100%)
Epithelial dysplasia—mild	24 (38.1%)	0 (0%)
Epithelial dysplasia—moderate	8 (12.7%)	0 (0%)

3.3. Immunohistochemical Expression

3.3.1. Interleukin-17 (IL-17)

In the OLP cohort, 52 cases (82.5%) exhibited strong (+++) cytoplasmic and membranous IL-17 staining in basal and suprabasal layers, with no nuclear signal. These also showed intense (+++) inflammatory infiltrates and neovessel formation (+) in the lamina propria. The remaining 11 cases (17.5%) demonstrated moderate (++) IL-17 expression, moderate infiltrates (++), and presence of neovessels (+). All 20 control specimens were considered negative (−), as only occasional faint cytoplasmic staining in scattered basal keratinocytes was below the threshold (<10% cells at 1+). (Table 5) (Figure 1a,d).

Table 5. Immunohistochemical Expression of IL-17, Syndecan-4, Maspin, β -Catenin, MMP-14, and TIMP-1 in OLP versus control.

Marker	Localization	OLP Group				Control Group			
		(0)	(+)	(++)	(+++)	(0)	(+)	(++)	(+++)
IL-17	Cytoplasmic/Membranous	0 (0.0%)	11 (17.5%)	0 (0.0%)	52 (82.5%)	20 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Maspin	Cytoplasmic/Nuclear	0 (0.0%)	0 (0.0%)	5 (7.9%)	58 (92.1%)	19 (95.0%)	1 (5.0%)	0 (0.0%)	0 (0.0%)
β -Catenin	Cytoplasmic/Nuclear	0 (0.0%)	0 (0.0%)	7 (11.1%)	56 (88.9%)	20 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
TIMP-1	Membranous/Cytoplasmic	0 (0.0%)	0 (0.0%)	14 (22.2%)	49 (77.8%)	0 (0.0%)	20 (100.0%)	0 (0.0%)	0 (0.0%)
MMP-14	Cytoplasmic/Membranous	0 (0.0%)	63 (100%)	0 (0.0%)	0 (0.0%)	20 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Syndecan-4	Membranous	0 (0.0%)	0 (0.0%)	12 (19.0%)	51 (81.0%)	0 (0.0%)	20 (100.0%)	0 (0.0%)	0 (0.0%)

Immunoreactivity was scored semi-quantitatively as + (weak), ++ (moderate), and +++ (strong, intensity equivalent to lymphocytes).

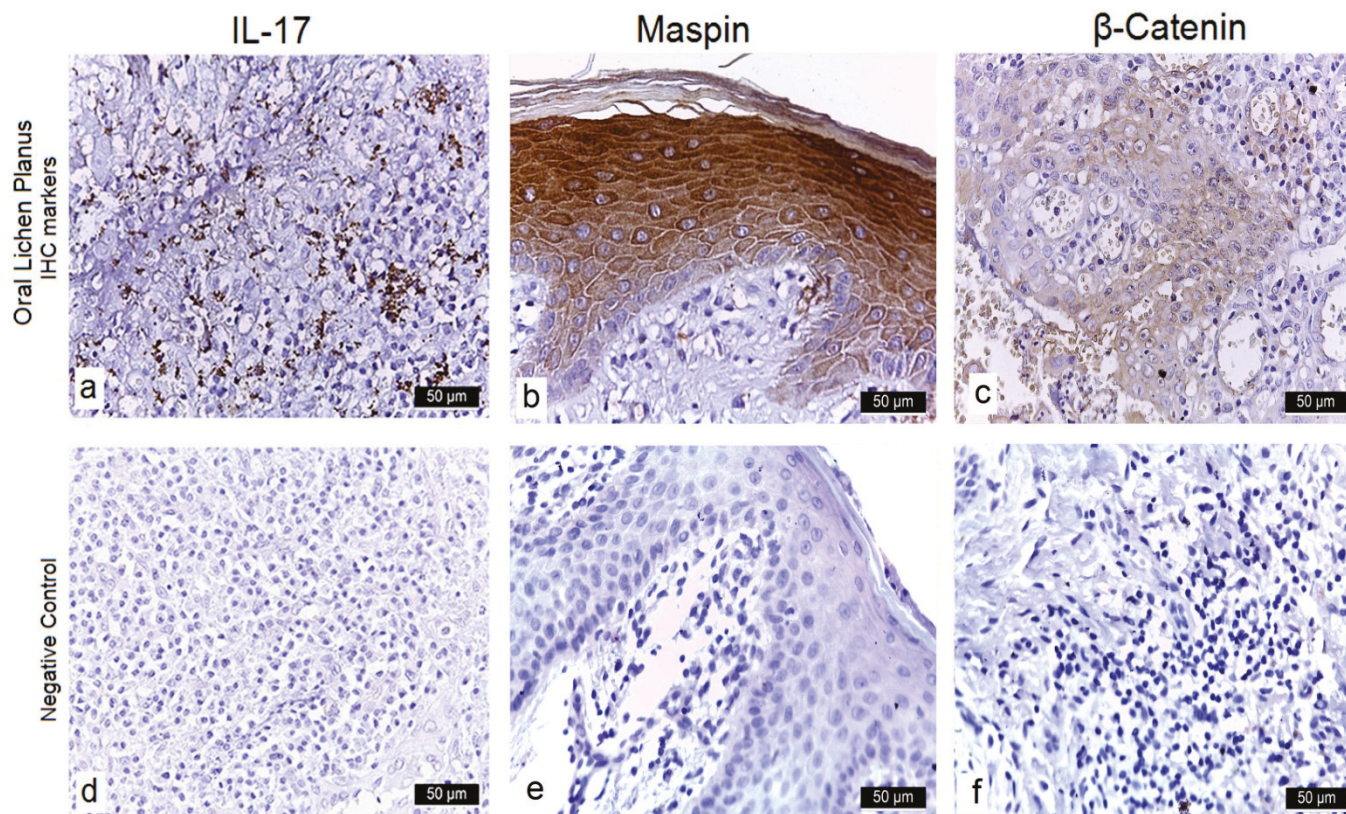


Figure 1. Immunohistochemical analysis. (a) Lichen planus—buccal mucosa, $\times 20$ (IL-17): IL-17 immunostaining reveals an inflammatory lymphocytic infiltrate at the junction between the stratified squamous epithelium and underlying connective tissue, with degenerated basal keratinocytes. Strong IL-17 expression is present in lymphocytic aggregates and in keratinocytes of the basal layer. (b) Lichen planus—jugal mucosa, $\times 40$ (maspin): Maspin immunostaining of a fragment of stratified squamous epithelium with hyperkeratosis over loose connective tissue shows collagen fibers, granulocytic inflammatory cells, isolated lymphocytes, and newly formed vessels with open lumens. Intense cytoplasmic positivity with occasional nuclear staining is observed in the epithelial cells. (c) Lichen planus—buccal mucosa, $\times 10$ (β -catenin): β -Catenin immunostaining highlights superficial micro-erosions, hyperkeratosis, and loss of cellular cohesion in the deeper and granular layers of the stratified squamous epithelium, with an accompanying inflammatory infiltrate at the epithelium–lamina propria interface and within the superficial and deep lamina propria. Intense cytoplasmic staining and focal nuclear positivity are noted in the granular and clear layers, as well as superficially. (d) Normal buccal mucosa, $\times 20$ (IL-17): IL-17 immunostaining of non-lesional stratified squamous epithelium overlying unremarkable lamina propria reveals occasional faint cytoplasmic staining in basal keratinocytes was observed, but below the threshold for positivity ($<10\%$ at $1+$) and thus classified as negative. (e) Normal buccal mucosa, $\times 40$ (maspin): Maspin immunostaining demonstrates weak, diffuse cytoplasmic positivity restricted to basal and parabasal epithelial layers, with no nuclear signal and absence of staining in the underlying connective tissue or vascular endothelium. (f) Normal buccal mucosa, $\times 10$ (β -catenin): β -Catenin immunostaining highlights crisp membranous localization throughout all epithelial layers, preserving intercellular adhesion complexes and showing no cytoplasmic or nuclear accumulation in keratinocytes or stromal cells.

3.3.2. Maspin

Strong (+++) cytoplasmic and weak (+) nuclear Maspin labeling occurred in 58 OLP samples (92%), accompanied by moderate (++) inflammatory infiltrates and neovessels (+). The other 5 cases (8%) showed moderate (++) cytoplasmic and weak (+) nuclear staining, with moderate (++) infiltrates and no neovascularization (−). Among controls, 19/20 were negative (−) in both compartments; one showed weak (+) cytoplasmic positivity without stromal inflammation (Table 5) (Figure 1b,e).

3.3.3. β -Catenin

Fifty-six OLP specimens (88.9%) displayed intense (+++) cytoplasmic and moderate (++) nuclear β -catenin staining, with intense (+++) infiltrates and no neovessels (–). The remaining 7 cases (11.1%) exhibited moderate (++) cytoplasmic and nuclear reactivity and moderate (++) infiltrates. In controls, β -Catenin remained strictly membranous in basal layers, with no cytoplasmic or nuclear signal (Table 5) (Figure 1c,f).

3.3.4. TIMP-1

In 49 OLP cases (77.8%), TIMP-1 showed strong (+++) membranous and moderate (++) cytoplasmic expression, coupled with weak (+) infiltrates and absence of neovascularization (–). Fourteen cases (22.2%) had moderate (++) membranous and weak (+) cytoplasmic staining with similar stromal features. Control tissues exhibited only faint (+) diffuse cytoplasmic positivity in scattered basal keratinocytes, without stromal or inflammatory cell labeling. (Table 5) (Figure 2a,d). When stratified by subtype, erosive OLP cases ($n = 60$) exhibited the same overall patterns of IL-17 and β -Catenin upregulation as the full cohort, while the three non-erosive cases showed weaker expression levels across markers. Due to the small number of non-erosive cases, no reliable statistical comparison could be performed.

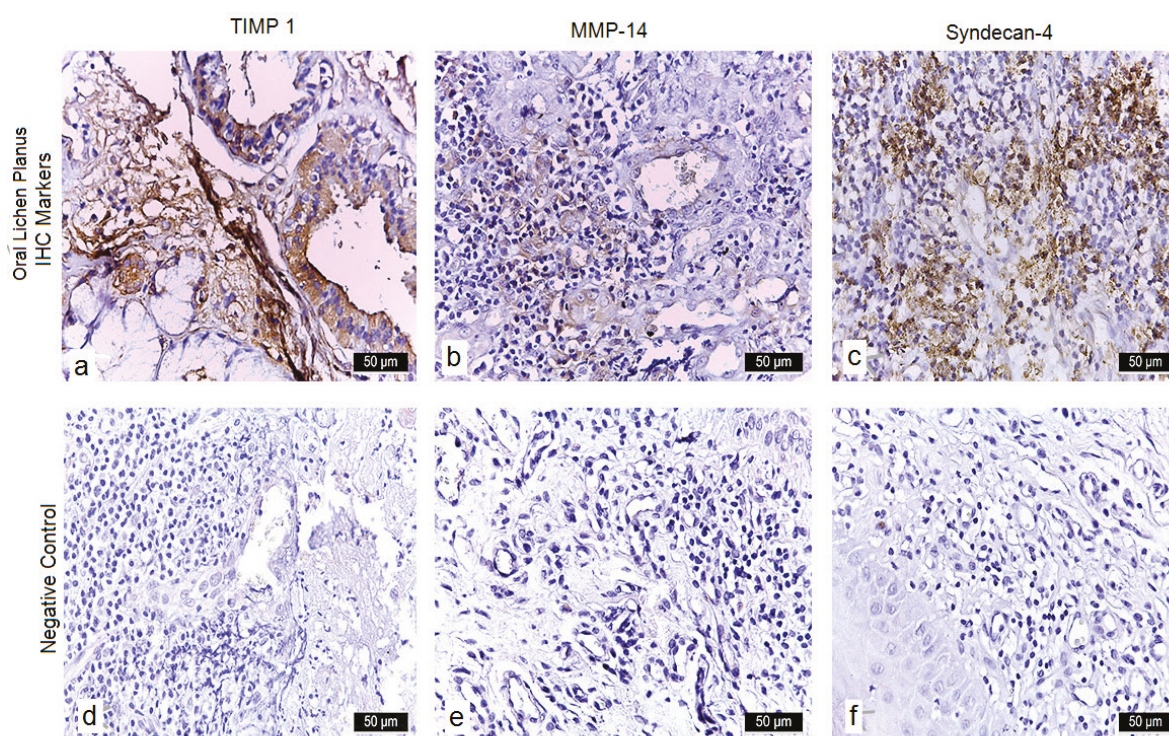


Figure 2. Immunohistochemical analysis. (a) Jugal mucosa—lichen planus, $\times 40$ (TIMP-1): TIMP-1 staining of a tissue fragment containing glandular appendages, connective and adipose tissue with sparse lymphocytes demonstrated intense membranous labeling in the connective stroma, endothelial cells, adipocytes, and glandular structures, with prominent cytoplasmic positivity in all marked elements. (b) Jugal mucosa—lichen planus, $\times 20$ (MMP-14): MMP-14 immunostaining in a hypercellular stroma rich in lymphocytes, plasma cells, and numerous thin-walled vessels with fibrillar connective material showed weak endothelial and membranous labeling, with faint cytoplasmic positivity observed in both lymphocytes and plasma cells. (c) Jugal mucosa—lichen planus, $\times 40$ (Syndecan-4): Syndecan-4 staining highlighted the stratified squamous epithelium with an undulating basement membrane, intraepithelial micro-dehiscences forming small clear vacuoles,

and superficial erosions overlying a loose, edematous lamina propria with abundant lymphocytic infiltrate. Intense membranous, cytoplasmic, interstitial, and endothelial membrane immunoreactivity was noted throughout the lesion. **(d)** Normal buccal mucosa, $\times 20$ (TIMP-1): TIMP-1 immunostaining of non-lesional stratified squamous epithelium with intact parakeratotic layer overlying unremarkable lamina propria shows only faint, diffuse cytoplasmic positivity in scattered basal keratinocytes, without any stromal or inflammatory cell labeling. **(e)** Normal buccal mucosa, $\times 20$ (MMP-14): MMP-14 immunostaining demonstrates absent to minimal cytoplasmic reactivity in all epithelial layers and underlying fibroblasts, with preservation of a continuous basement membrane and no perivascular or stromal expression. **(f)** Normal buccal mucosa, $\times 40$ (syndecan-4): Syndecan-4 immunostaining highlights weak, uniform membranous and focal cytoplasmic localization in basal and parabasal keratinocytes, with no detectable staining in the spinous or superficial layers and absence of signal in the underlying connective tissue.

3.3.5. MMP-14

Weak (+) cytoplasmic and membranous MMP-14 positivity was seen in 55 OLP samples (87.3%), alongside intense (+++) inflammatory infiltrates and neovessels (+). The remaining 8 cases (12.7%) had weak (+) cytoplasmic expression, moderate (++) infiltrates, and neovascularization (+). Control specimens were uniformly negative (−) in both epithelium and stroma (Table 5) (Figure 2b,e).

3.3.6. Syndecan-4

Syndecan-4 exhibited intense (+++) membranous and cytoplasmic staining in 51 erosive OLP cases (81%), with intense (+++) infiltrates and no neovessels (−). Twelve cases (19%) showed moderate (++) expression and moderate (+) infiltrates without neovascularization. In controls, only weak (+) membranous labeling was noted in the basal layer, with no cytoplasmic signal (Table 5) (Figure 2c,f).

3.4. Statistical Correlations

- **β-Catenin and Dysplasia:** Nuclear localization of β-catenin was observed predominantly in moderately dysplastic lesions (12.7% of cases), whereas it was uncommon in non-dysplastic cases (49.2% of the cohort) (χ^2 test, $p < 0.05$).
- **MMP-14 and Neovascularization:** Positive MMP-14 expression showed a significant association with neovascularization in the lamina propria (χ^2 test, $p = 0.03$), suggesting its role in early stromal remodeling.
- **Syndecan-4 Loss and Dysplasia:** Reduced Syndecan-4 expression was significantly correlated with the presence of epithelial dysplasia (50.8% of OLP cases) compared with non-dysplastic mucosa (49.2%) (χ^2 test, $p < 0.05$), underscoring its role in maintaining epithelial polarity.

4. Discussion

4.1. Interpretation of Findings

This study investigated six immunohistochemical markers—IL-17, Maspin, β-Catenin, TIMP-1, MMP-14 and Syndecan-4—in erosive oral lichen planus (OLP) versus normal mucosa, aiming to clarify their roles in the inflammatory–dysplastic continuum of high-risk OLP. Our results implicate these proteins in chronic inflammation, epithelial polarity loss, basement membrane remodeling, and angiogenesis.

Intense cytoplasmic and membranous IL-17 expression in over 80% of OLP cases confirms Th17-mediated inflammation as a central pathogenic driver [27,28]. This finding aligns with prior reports of elevated IL-17 in OLP tissue and saliva, linked to epithelial proliferation and stromal activation [9,10]. Moreover, IL-17 fosters tumor-associated inflammation by promoting cytokine cascades, matrix remodeling, and neovascularization [29].

Syndecan-4 was strongly positive at the membrane and in the cytoplasm of most erosive OLP specimens [16,30]. Its downregulation has been tied to dysplasia and poorer outcomes in head and neck squamous cell carcinoma [16,31], and syndecans have been posited as early modulators of epithelial disorganization in premalignant oral lesions [17,31].

Maspin exhibited high cytoplasmic staining with weaker nuclear localization in OLP lesions [32], reflecting its dual tumor-suppressor and apoptosis-regulatory functions [19,20]. Nuclear maspin correlates with reduced invasive potential and favorable prognosis in head and neck cancers [19,33], and our observation of pronounced cytoplasmic maspin suggests an early defensive response in non-dysplastic OLP [33].

β -Catenin shifted from a strictly membranous pattern in controls to cytoplasmic and nuclear localization in OLP, especially in dysplastic samples [21,34]. Such redistribution signifies Wnt pathway activation and is associated with early carcinogenic signaling [34,35]. Nuclear β -Catenin accumulation has been repeatedly linked to loss of epithelial cohesion and neoplastic transformation in oral mucosa [22,34].

TIMP-1 was robustly expressed in both OLP and normal tissues, though dysplastic lesions showed modestly reduced cytoplasmic intensity [26,36]. Its co-expression with maspin in non-dysplastic OLP suggests a protective phenotype [37], whereas diminished TIMP-1 levels have been connected to extracellular matrix breakdown and tumor progression [26,36].

MMP-14 was weakly positive in OLP biopsies but absent in controls [38,39]. Its presence correlated with neovascularization, underscoring its role in stromal remodeling and angiogenesis during early epithelial–mesenchymal transition [38,40]. Even low MMP-14 activity can activate MMP-2 and degrade basement membrane, facilitating invasion [24,40].

4.2. Integration with Pathogenetic Mechanisms

These data reinforce OLP as a dynamic inflammatory–dysplastic spectrum: IL-17 and Syndecan-4 denote inflammatory and structural microenvironment changes; Maspin and β -Catenin reflect epithelial polarity and regulatory shifts; and MMP-14/TIMP-1 illustrate opposing forces in extracellular matrix remodeling. Together, they substantiate an inflammatory-driven dysplastic interface that may elude routine histology.

4.3. Comparison with Existing Literature

Our findings concur with biomarker studies in OLP and other OPMDs: elevated IL-17 in tissue and saliva [9,10,41], β -Catenin nuclear translocation as a dysplasia predictor [21,22] and Maspin/TIMP-1 alterations linked to progression in oral potentially malignant disorders and OSCC [32,36]. The innovation here lies in concurrently profiling all six markers within a single erosive OLP cohort, highlighting their coordinated dysregulation.

Several recent reports strengthen these observations. Larsen et al. [41] demonstrated distinct systemic cytokine signatures in OLP and lichenoid lesions, supporting the role of IL-17 dysregulation in the disease spectrum. Beyond inflammatory conditions, Jiang et al. [42] showed that IL-17A directly promotes metastasis in tongue squamous cell carcinoma via the miR-23b/versican axis, reinforcing its dual role in both inflammation and malignant progression. Our results align with those of Ladjevac et al. [43], who showed that IL-17 drives OSCC pathogenesis by sustaining inflammation, promoting angiogenesis, and enabling immune escape, supporting its role as an early marker of malignant progression.

β -Catenin alterations also appear central. Saleh et al. [44] confirmed significantly higher nuclear β -catenin in dysplastic OLP compared to non-dysplastic lesions, aligning with our data. In a previous study from our group, Condurache Hrițcu et al. [20] reported

coordinated expression shifts of Maspin, β -catenin, and MMP-14 in OPMDs and OSCC, consistent with our current multi-marker approach.

Matrix remodeling markers further connect OLP to malignant potential. Nosratzehi et al. [45] found salivary increases in multiple MMPs (MMP-1, -2, -3, -13) in OLP and OSCC, while Zhang et al. [46] highlighted stage-dependent alterations of collagen IV, gelatinases, and TIMP-1 in oral leukoplakia. These findings corroborate our observation that proteolytic imbalance and TIMP-1 dysregulation may be subtype- or stage-specific rather than uniform.

Taken together, these studies emphasize that the inflammatory, proteolytic, and tumor-suppressive pathways explored in our erosive OLP cohort are not isolated but reflect a broader pathogenic continuum across OPMDs and OSCC.

4.4. Clinical Implications

This multi-marker IHC profile could inform risk stratification: IL-17 and nuclear β -Catenin may flag lesions at greater risk of dysplastic shift, whereas preserved Maspin and TIMP-1 might indicate epithelial stability. Incorporating these biomarkers into digital pathology workflows could enhance diagnostic reproducibility and guide personalized surveillance or intervention strategies [47,48].

4.5. Limitations

The present study has several limitations. First, the relatively small cohort size reduces statistical power and may limit the robustness of subgroup analyses. Second, because 95% of the included cases were of the erosive subtype, the findings cannot be fully generalized to other OLP variants such as reticular or plaque-like forms. Third, immunohistochemical assessment relied on semi-quantitative scoring by pathologists without the use of digital image analysis, which may introduce observer bias. Finally, the retrospective, cross-sectional design precludes causal inference, restricting interpretation to associations rather than predictive or mechanistic conclusions.

4.6. Future Directions

Prospective, multicenter studies with integrated transcriptomic and genomic profiling are needed to validate these markers. Advanced models—such as 3D organotypic cultures or ex vivo OLP mucosa—could deepen mechanistic insights. Ultimately, a standardized, multiplex IHC panel may become a cornerstone for individualized OPMD management.

5. Conclusions

This study demonstrates that oral lichen planus embodies a dynamic inflammatory–dysplastic spectrum, in which chronic immune activation and early molecular alterations converge to promote malignant potential. Our immunohistochemical panel revealed that elevated IL-17 and nuclear translocation of β -Catenin are associated with higher dysplastic risk, while preserved Maspin and TIMP-1 expression denote a more stable epithelial phenotype. Syndecan-4 loss and MMP-14 upregulation further underscore the roles of epithelial polarity disruption and stromal remodeling in OLP pathogenesis. Given the retrospective, cross-sectional design, causal relationships cannot be inferred and the findings should be interpreted as associative.

By integrating these six markers into a single cohort analysis, we provide an objective framework for distinguishing high-risk OLP subtypes that may benefit from intensified surveillance or adjunctive therapies. Although retrospective in design and limited by semi-quantitative scoring, our findings warrant prospective validation using digital image analysis and longitudinal follow-up to confirm their predictive value. Ultimately, a standardized multi-marker IHC panel could enhance diagnostic reproducibility, inform personalized risk stratification, and guide clinical management of patients with OLP.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript

OLP	Oral lichen planus
EMT	Epithelial–mesenchymal transition
IHC	Immunohistochemical
OPMD	Oral potentially malignant disorder
OSCC	Oral squamous cell carcinoma
H&E	Hematoxylin and eosin
PBS	Phosphate-buffered saline
DAB	3,3'-Diaminobenzidine
IL-17	Interleukin-17
MMP-14	Matrix metalloproteinase-14

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Article

Retention of Asymptomatic Impacted Third Molars: Effects on Alveolar Bone at the Distal Surface of Second Molars over Time

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Abstract

Objective: To assess radiographic changes in the alveolar bone on the distal aspect of the second molars (2Ms) over time, while impacted third molars (ITMs) remain present across two timepoints. **Methods:** This retrospective observational study aimed to assess radiographic changes between two timepoints (T0 and T1). Both Orthopantomogram (OPG) and Periapical (PA) X-rays were utilized, with three measurements taken on the distal surface of 2Ms using EMAGO 6.1 software. Statistical significance was defined as a p -value < 0.05 . **Results:** A total of 51 patients met the inclusion criteria, with a mean age of 45 years ($SD \pm 13$). Sixty-eight second molars were assessed at baseline (T0) and follow-up (T1), with a mean interval of 20 months ($SEM \pm 62$ days). No significant changes were found in vertical, oblique, or angular bone levels between T0 and T1. Gender significantly affected the cemento-enamel junction (CEJ)–base of defect (BD) measurements ($p = 0.022$) and defect angles at T0 ($p = 0.048$), but not at the adjusted T1 ($p = 0.292$). Other variables, including medical history, smoking, and ITM angulation, showed no influence. Patient age was borderline significant in relation to intrabony defect angle ($p = 0.047$). **Conclusions:** Considering its limitations, this analysis does not provide evidence to support the hypothesis that prophylactic extraction of ITMs yields significant bone-sparing benefits. Furthermore, it does not establish that prolonged retention of ITMs consistently results in short-term bone alterations in adjacent 2Ms. Consequently, further research is warranted to more accurately assess the medium- to long-term implications of ITM retention on the bone levels of 2Ms.

Keywords: alveolar bone loss; maxillofacial surgery; molar; third; periodontal diseases; periodontal pocket

1. Introduction

Third molar surgery (TMS) is a common procedure in the United Kingdom [1]. An international study reported impacted third molars (ITMs) in 18–68% of people [2], while a systematic review of 83,400 individuals found a prevalence of 24.40% [3]. Both non-impacted and impacted third molars can contribute to a wide spectrum of dental-related diseases, which may necessitate extraction. A retrospective study analyzed data from 1011 patients who underwent the removal of 1431 ITMs. The primary indication for extraction was pericoronitis, accounting for 49% of cases, followed by caries in 27%, distal cervical caries affecting adjacent second molars in 14%, and periodontal disease in 5% [4].

According to the guidelines issued by the United Kingdom's National Institute for Health and Care Excellence (NICE), the removal of ITMs is indicated for various conditions (pathological association), but not for periodontal reasons related to adjacent 2Ms, as there is no reliable evidence supporting its benefits [5]. Although the Faculty of Dental Surgery (FDS) guidelines mention the possibility of removing ITMs due to periodontal disease in adjacent 2Ms, evidence to support this remains lacking [6]. A Cochrane systematic review sought evidence comparing the impact of ITM removal versus retention of pathology-free ITMs on the periodontal health of adjacent 2Ms [7]. However, the authors found a lack of high-quality evidence to support any recommendations, as the current evidence is of very low certainty and carries a significant risk of bias.

This study aimed to evaluate radiographic bone changes on the distal surface of the 2Ms at two different timepoints in proximity to ITMs to evaluate alveolar bone changes on the distal aspect of the 2Ms in the continued presence of ITMs. The hypothesis was that the retention of ITMs may affect the alveolar bone level in the distal region of the adjacent 2Ms. Additionally, the study evaluated the impact of various clinical and patient factors—such as age, gender, health status, smoking, and ITM angulation—on bone levels.

2. Materials and Methods

2.1. Study Design

This observational study, a retrospective radiographic analysis, utilized Emago® radiography software.

2.2. Ethics

The study was conducted in accordance with the Declaration of Helsinki. Approval was obtained in advance from the Clinical Effectiveness Unit at the Royal London Dental Hospital (RLDH) (Approval ID: 13295).

2.3. Study Subjects

Patients who attended the Periodontal New Patient Clinics at RLDH between January 2017 and January 2019 were identified. Eligible cases included those with asymptomatic ITMs—defined as third molars free from clinical signs and symptoms—located adjacent to 2Ms and referred for periodontal treatment. The first milestone was to gather data on patients identified through records based on specific inclusion and exclusion criteria. The inclusion criteria included patients who were seen by the Periodontology Department at RLDH between January 2017 and January 2019 with ITMs near 2Ms. Additionally, these patients had to have undergone two radiographic assessments: one within the timeframe of January 2017 to January 2019 and the most recent follow-up radiograph. Patients who did not meet these criteria were excluded from the study. Patients were examined at two different timepoints: Timepoint 0 (T0), which refers to the first available full-mouth radiograph between January 2017 and January 2019, and Timepoint 1 (T1), the most recent radiograph available in the patient's records. The findings of T0 were used as the baseline, and it then was compared to T1.

2.4. Radiographic Examination

Radiographic examination was conducted using established anatomical landmarks and assessment criteria described by Schei et al. [8] and Björn et al. [9] for the evaluation of intrabony defects. In this study, specific reference points were identified and marked on the distal surface of each 2M, as illustrated in Figure 1, following the methodology outlined by Nibali et al. [10].

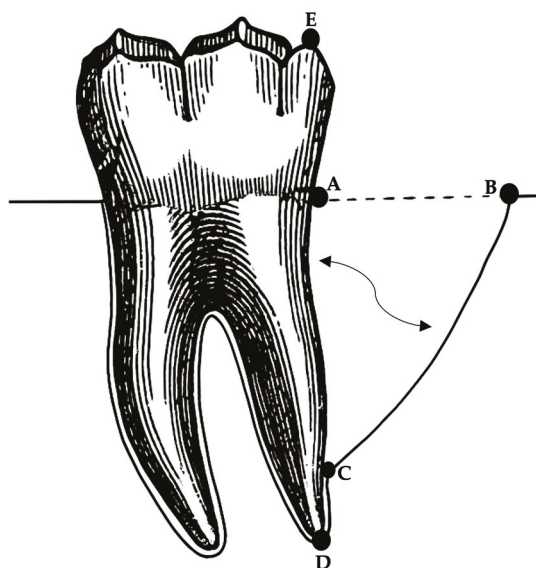


Figure 1. The following anatomical landmarks are described: **A** represents the CEJ, **B** represents the top of the alveolar bone crest, defined as the most coronal part of the alveolar bone, **C** represents the base of the defect, **D** represents the distal root apices of the 2Ms, and **E** represents the top of the distobuccal cusps of the 2Ms.

The following linear measurements were performed using Emago software: (i) a vertical line was measured in millimeters between the top of the distobuccal cusps (DC) (Point E) and the apices of the distal roots (RA) (Point D) of the 2Ms; (ii) a vertical line was measured from the identified cemento enamel junction (CEJ) (Point A) at the distal sites of the 2Ms to the deepest apical bone extension of the intrabony defect (BD) (Point C). If a restoration was present, the apical margin of the restoration was used as a fixed reference point instead of the CEJ. (iii) An oblique line was measured in millimeters from the deepest apical bone extension of the intrabony defect (BD) (Point C) to the most coronal position of the alveolar bone crest of the intrabony defect (CB) (Point B). (iv) The radiographic defect angle was determined by the two lines representing the root surface of the involved tooth: from A to C and from C to B, as previously explained by Nibali et al. [10], Steffensen et al. [11], Tonetti et al. [12], and others [13,14].

2.5. Radiograph Standardization

As the radiographic pairs may not be identical, the vertical distortion between the baseline radiograph (T0) and the T1 radiographs was estimated and standardized as previously described by Tonetti et al. [12] and Linares et al. [14]. To estimate this distortion, an anatomically non-variable distance (the distal crown-tooth length) from DC to RA was measured on both pairs of radiographs (from Point E to Point D), and a correction factor was calculated as follows:

- Equation one for correction factor: $DC \text{ to } RA (T0) \div DC \text{ to } RA (T1) = \text{correction factor}$.

To obtain the linear measurements of T1 adjusted, the following equations were used, utilizing the correction factor obtained from equation one:

- Equation two for adjusted T1 (DC to RA): $DC \text{ to } RA (T0) - [DC \text{ to } RA (T1) \times \text{correction factor}] = \text{adjusted T1 (DC to RA)}$
- Equation three for adjusted T1 (CEJ to BD): $CEJ \text{ to } BD (T0) - [CEJ \text{ to } BD (T1) \times \text{correction factor}] = \text{adjusted T1 (CEJ to BD)}$
- Equation four for adjusted T1 (BD to CB): $BD \text{ to } CB (T0) - [BD \text{ to } CB (T1) \times \text{correction factor}] = \text{adjusted T1 (BD to CB)}$.

2.6. Calibration of Radiographic Analysis

The measurements were carried out by a single radiographic examiner, referred to as Examiner A.A., on two separate sets of radiographs (one for T0 and another for T1), with a minimum interval of four weeks between sessions. Examiner A.A. was trained by another examiner, Examiner N.G., who served as the gold standard.

2.7. Data Management and Statistical Analysis

The clinical and radiographic data of patients were anonymized and entered into an Excel database, proofread for errors, and then imported into SPSS (Version 29). A descriptive analysis was conducted to evaluate various patient characteristics such as age, gender, race, smoking status, and medical and medication history. Numerical data were summarized as frequencies and percentages with standard deviation. Comparisons between timepoints (T0 and adjusted T1) were performed using the Wilcoxon signed-rank test to evaluate alveolar bone changes, with significance set at $p < 0.05$. A Mann–Whitney U test was applied to identify differences between categorical data and linear measurements, followed by the chi-square test for categorical data comparison. Finally, simple linear regression analysis was conducted to evaluate relationships between continuous variables (age) and linear measurements, with significance also set at $p < 0.05$.

3. Results

Data collection was carried out between June and September 2023, evaluating 1302 records from periodontal new patient clinics at RLDH for the period between January 2017 and January 2019. Of these, 1251 patients (96%) were excluded from analysis for the following reasons: a total of 948 patients had no ITMs, 196 patients had radiographs that were not taken within the required time frame, 74 patients had a single X-ray, and 33 patients lacked radiographs in their clinical records. Ultimately, 51 patients (4%) were included in the study based on the study's inclusion and exclusion criteria.

The mean patient age was 45 years (median 44, range 22–79, SD 13). Gender distribution was 49% male and 51% female. Demographics included Caucasians (34.4%), Asians (36%), individuals of African origin (5.6%), Afro-Caribbeans (6.4%), and 17.6% with unknown ethnicity. Regarding smoking status, 77% were non-smokers, 5% were current smokers, and 18% were former smokers. Medical history showed 51% were classified as healthy (ASA I), while 49% were medically compromised (ASA II or greater). Additionally, 49% were on medication, while 51% were not. At T0, Winter's classification showed 38.9% mesioangular, 36.7% vertical, 15.4% distoangular, 8.1% horizontal, and 0.9% inverted patterns. By T1, the distribution was 42% mesioangular, 42% vertical, 12% distoangular, 3% horizontal, and 1% inverted.

Before statistical analysis, cases with perio-endo lesions that affected the measurement of intrabony defects were excluded, resulting in the removal of three cases, as shown in Figure 2. Two-paired radiographs were available for retrieval and analysis for 51 patients, totaling sixty-eight 2Ms at baseline (T0) and at follow-up (T1). The mean duration of the radiographic assessment period between T0 and T1 was determined to be 606 days (approximately 20 months) with a standard error of the mean estimated at 62 days.

For alveolar bone changes between T0 and adjusted T1, the Wilcoxon signed-rank test (Table 1) showed no significant difference in paired measurements. For CEJ-BD, BD-CB distances, and intrabony defect angles, p -values (0.377, 0.275, and 0.366, respectively) indicated the retention of the null hypothesis ($p > 0.05$).



Figure 2. Radiographs of excluded cases with perio-endo lesions. These cases interfered with the assessment of intrabony defects due to the presence of perio-endo lesions, leading to the exclusion of three 2Ms from the final analysis.

Table 1. Analysis of alveolar bone changes over time (T0 to T1) using Wilcoxon signed-rank test results.

Measurement	<i>p</i> -Values	Decision
CEJ to BD at T0 and adjusted T1	0.377	Retain the null hypothesis.
BD to CB at T0 and adjusted T1	0.275	Retain the null hypothesis.
Intrabony defect angle at T0 and T1	0.366	Retain the null hypothesis.

Statistical significance of *p*-value < 0.05. Abbreviations: CEJ: cementoenamel junction, BD: base of defect, CB: crestal bone, T0: Timepoint 0, T1: Timepoint 1.

Radiographic bone measurements were analyzed by gender at T0 and T1 using the Mann–Whitney U test. For CEJ–BD, there was a significant difference between genders at T0 ($p = 0.022$) and T1 ($p = 0.027$). For BD–CB, no significant gender difference was found at either T0 ($p = 0.347$) or T1 ($p = 0.342$). The intrabony defect angle showed a significant gender difference at T0 ($p = 0.048$), but none at T1 ($p = 0.292$), as can be shown in Table 2. No significant differences were found in the distribution of the three measurements (CEJ to BD distance, BD to CB distance, and intrabony defect angles) between healthy individuals and those with medical conditions, as well as between patients on regular medications and those not. No significant differences in alveolar bone changes were observed based on patients' ethnicity, smoking status, or ITMs angulation. Statistical analysis confirmed this, as differences were not statistically significant at both T0 and adjusted T1, with p -values above 0.05, as shown in Table 2.

Table 2. Significance levels of various factors affecting the measurements.

Measurement	Test	Variable	T0 Significance	T1 Significance	Decision
CEJ to BD	Mann–Whitney U Test	Gender	0.022	0.027	Significant
	Mann–Whitney U Test	Systemic Health	0.784	0.994	Not significant
	Chi-Square Test	Ethnicity	0.192	0.376	Not significant
	Chi-Square Test	Smoking Status	0.841	0.423	Not significant
	Chi-Square	ITMs Angulation	0.408	0.292	Not significant
BD to CB	Mann–Whitney U Test	Gender	0.347	0.342	Not significant
	Mann–Whitney U Test	Systemic Health	0.950	0.688	Not significant
	Chi-Square Test	Ethnicity	0.175	0.218	Not significant
	Chi-Square Test	Smoking Status	0.313	0.294	Not significant
	Chi-Square Test	ITMs Angulation	0.995	0.992	Not significant
Intrabony Defect Angle	Mann–Whitney U Test	Gender	0.048	0.292	Significant at T0 only
	Mann–Whitney U Test	Systemic Health	0.539	0.684	Not significant
	Chi-Square Test	Ethnicity	0.391	0.443	Not significant
	Chi-Square Test	Smoking Status	0.157	0.264	Not significant
	Chi-Square Test	ITMs Angulation	0.963	0.962	Not significant

Statistical significance of p -value < 0.05 . Abbreviations: ITMs: impacted third molars, CEJ: cemento-enamel junction, BD: base of defect, CB: crestal bone, T0: Timepoint 0, T1: Timepoint 1.

As part of the statistical analysis, linear regression was employed to assess the influence of patient age on the three measured parameters. As presented in Table 3, a weak correlation was observed between patient age and the distance from CEJ to the BD, with a correlation coefficient (R) of 0.056. The corresponding coefficient of determination (R^2) was 0.003, indicating that patient age explains only 0.3% of the variability in this measurement. Furthermore, the p -value of 0.413 confirms that the correlation is not statistically significant, suggesting that patient age is an unreliable predictor of the CEJ–BD distance in this sample, as illustrated in Figure 3A. Similarly, no correlation was found between the distances from BD to CB and patient age, as linear regression analysis produced an R -value of 0.012 and an R^2 of 0.000, demonstrating no linear relationship, with a p -value of 0.858 confirming the lack of statistical significance. These findings are depicted in Figure 3B.

In contrast, a weak positive relationship was identified between intrabony defect angles and patient age, with an R-value of 0.146 and an R^2 of 0.021, indicating that age accounts for 2.1% of the variation in defect angles. Despite the low explanatory power, the relationship was statistically significant ($p=0.047$), suggesting that age has a slight but detectable influence on intrabony defect angles, as illustrated in Figure 3C.

Table 3. Linear regression analysis of age correlation with the measurements.

Correlation of the Linear Measurements with Age	R-Value (R)	R-Squared (R^2)	Sig.
CEJ to BD at T0 and adjusted T1	0.056	0.003	0.413
BD to CB at T0 and adjusted T1	0.012	0.000	0.858
Intrabony defect angle at T0 at T1	0.146	0.021	0.047

Statistical significance of p -value < 0.05 . Abbreviations: CEJ: cementoenamel junction, BD: base of defect, CB: crestal bone, T0: Timepoint 0, T1: Timepoint 1.

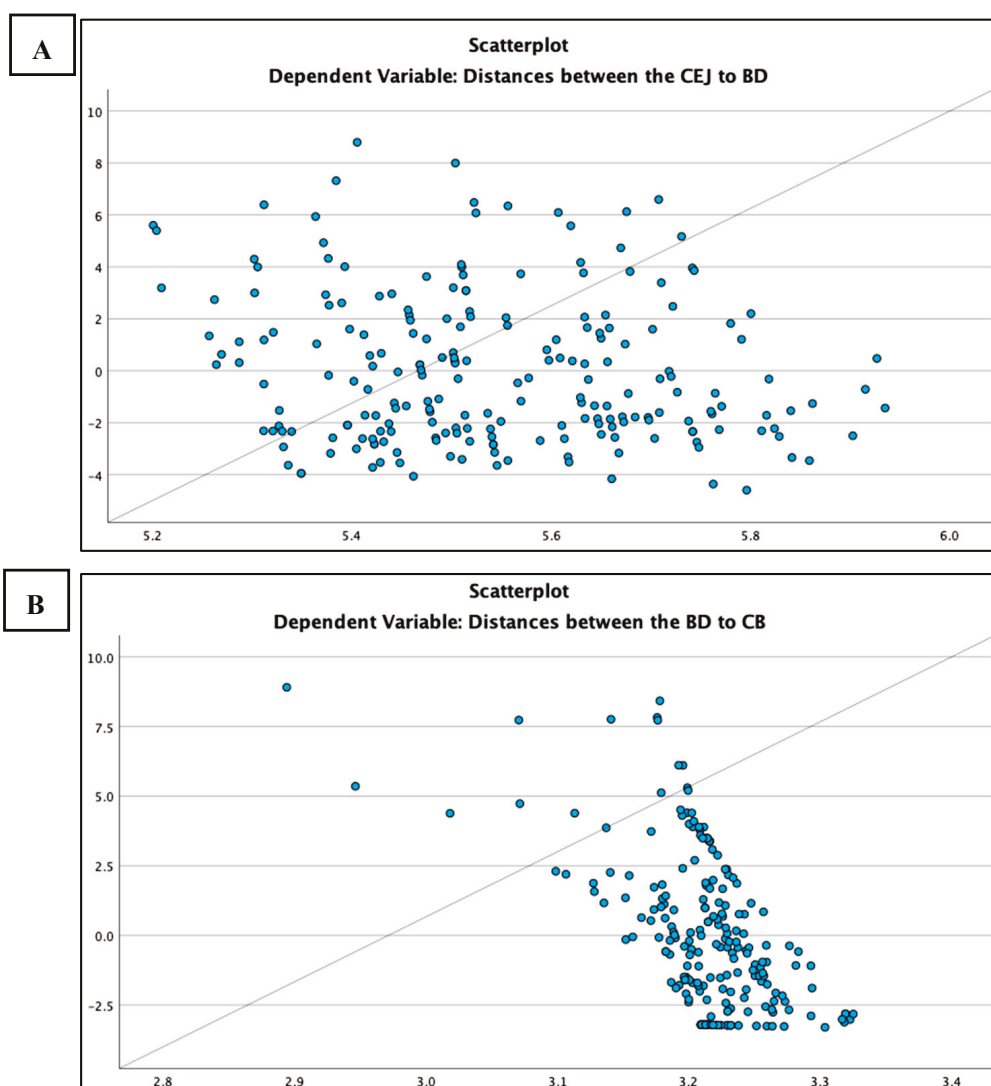


Figure 3. Cont.

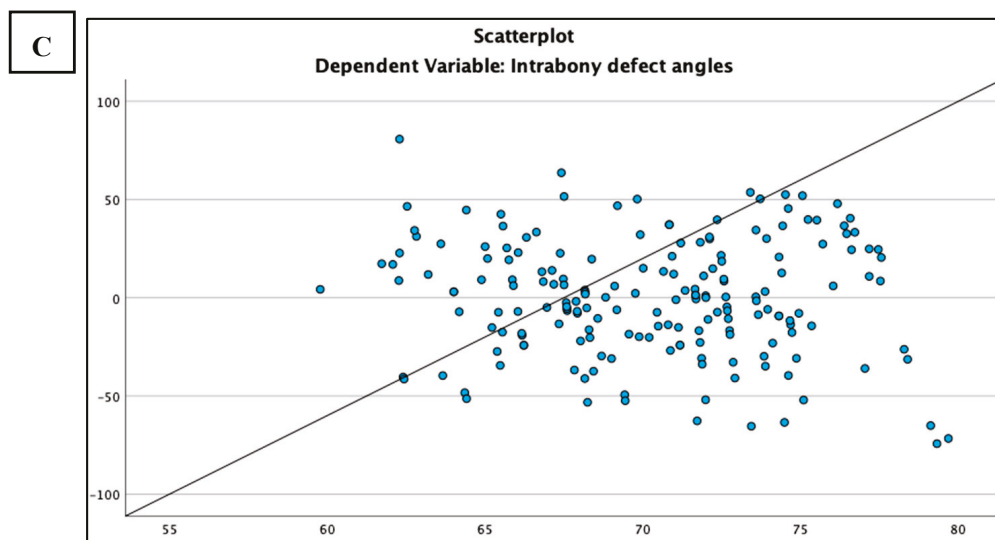


Figure 3. Presents scatterplots illustrating the relationship between patient age and three radiographic measurements. In (A), a weak and non-significant correlation is observed between patient age and the CEJ–BD distance ($R = 0.056$, $p = 0.413$). (B) shows no correlation between patient age and the BD–CB distance ($R = 0.012$, $p = 0.858$). In (C), a weak but statistically significant positive correlation is identified between patient age and the intrabony defect angle ($R = 0.146$, $p = 0.047$).

4. Discussion

This study aimed to evaluate changes in intrabony defects on the distal aspect of 2Ms next to ITMs over an average period of 606 days (about 20 months), from T0 to T1. The radiographic evaluation focused on short-term alveolar bone changes on the distal surface of 2Ms. The outcomes of this study suggest that the observed data does not offer sufficient evidence to establish significant differences between the two paired observations. Consequently, in the context of this study, the distances provided are not considered to be statistically different.

Although several studies in the literature have assessed bone levels on the distal surface of 2Ms, the findings of the current study are based on a unique methodology that has not previously been reported for assessing bony defects resulting from ITMs. One study assessed the relationship between retained asymptomatic third molars and periodontal pathology in adjacent 2Ms, indicating that soft tissue–ITMs significantly increased the risk of 2Ms pathology (relative risk [RR]: 4.88; 95% confidence interval [CI]: 2.62–9.08), followed by bony-ITMs (RR: 2.16) and fully erupted third molars (RR: 1.74), while the absence of ITMs posed the lowest risk [14]. However, the present study found no significant changes in bone levels over time, offering a different perspective from that report.

The study in [15] has been criticized for several methodological weaknesses, including a serious confounding bias, moderate selection bias (male-only cohort), and substantial missing data (only 416 participants analyzed out of the initial 1231) [7]. Furthermore, the timing of third molar removal in some subjects prior to follow-up further undermined the integrity of the longitudinal analysis [7]. Although a correlation between the presence of ITMs and increased 2Ms pathology was demonstrated, changes in bone levels over time were not assessed. In contrast, the present study incorporated multiple timepoints and found that bone levels in 2Ms did not significantly change over time, even when ITMs were retained.

Krausz et al. [16] used a split-mouth design to examine long-term changes in alveolar bone height between 2Ms and ITMs before and after extraction, with radiographic assessments conducted pre- and post-operation over an average of 38.14 ± 8.87 months. The study compared two groups: a test group assessed before and after ITM extraction

and a control group assessed twice without ITM removal. In the control group, the ITMs were retained between the two radiographic assessments, consistent with the approach used in the present study. The findings indicated that the average bone level at Timepoint 1 was 3.224 ± 0.35 mm, while at Timepoint 2, it was 3.401 ± 0.41 mm. The p -value of 0.2588 indicated no statistically significant difference between the two stages in the control group [16], supporting the findings of the present study. While there exists partial similarity in the methodology between the study conducted by Krausz et al. [16] and the present study, it is essential to note a divergence in the approach to measurements. Specifically, Krausz et al. [16] focused solely on a singular vertical distance on the distal surface of the 2Ms, whereas the present study incorporated two linear measurements and one angular measurement for a more comprehensive assessment. An encouraging observation can be made when comparing the indirect findings of the control group in the study by Krausz et al. [16] with the results of the current analysis; a clear positive correlation is evident between the two sets of data. In particular, both investigations reached the significant conclusion that there were no significant changes in the bone level on the distal surface during the designated time period of the control group where the ITMs were retained between the two radiographic assessments.

Although designed with different aims, a retrospective study by Kugelberg et al. [17] highlighted significant gender differences in periodontal healing after the surgical removal of lower ITMs. They found that preoperative bone height was significantly lower in males than in females, indicating more bone loss. Similarly, the findings of the present study indicated a significant difference in distribution between males and females in the distance between CEJ and BD, but not in the BD to CB distance or intrabony defect angles. This suggests that males experience higher levels of bone loss compared to females; however, this does not necessarily mean they undergo greater changes between the two timepoints.

The current investigation found no significant association between patients' systemic health conditions and the alveolar bone level on the distal surface of 2Ms. One possible explanation could be that the presence of ITMs primarily contributes to infrabony defects. Consequently, changes may not be observed while ITMs are still present, regardless of the patient's health status. While medical conditions and medications can contribute to the risk of periodontal disease, they are not the only determinants. Oral hygiene practices also play crucial roles, suggesting that while compromised health can be a risk factor, it is insufficient on its own to predict the extent of alveolar bone damage.

The current research examined the correlation between changes in alveolar bone and patients' ethnic backgrounds, finding no statistically significant association at either T0 or adjusted T1. In a retrospective study, Helmi et al. [18] assessed periodontitis prevalence by evaluating average alveolar bone loss in posterior teeth using bitewing radiographs, finding greater bone loss in Asians compared to White patients, which suggests an ethnicity-based difference. A study by Wong et al. [19] examined differences in CEJ-alveolar crest distances by ethnicity using bitewing radiographs and showed that Asian individuals had a notably greater CEJ-alveolar crest distance compared to non-Asians, highlighting a significant ethnic variation within the sample. However, the findings of the present study did not support ethnicity as a significant factor in infra-bony defects. The previous studies using patient ethnicity as an indicator in alveolar bone assessment showed varied outcomes due to differences in methodology, objectives, population groups, and age categories. None considered intrabony defects on the distal surface of 2Ms adjacent to ITMs, making this study's findings unique by comparison.

Extensive research highlights the negative impact of smoking on periodontal health, especially alveolar bone height, as shown by studies like those by Helmi et al. [18], Bergström et al. [20,21], and Kugelberg et al. [22]. However, the findings of the present study did not

observe changes in infrabony defects over time, suggesting other contributing factors. One possible explanation for this difference is that the infrabony defects observed in this study were primarily caused by ITMs, which masked smoking's effects since the underlying cause, ITMs, remained present.

Alveolar bone changes based on ITM angulation were discussed by Matzen et al. [23], who examined the angulation of ITMs, classified as mesioangular/horizontal versus other angles. They found varied results: one observer reported no significant difference ($p = 0.001$), while three others identified significant differences ($p < 0.001$). The authors concluded that marginal bone loss in adjacent 2Ms was significantly observed for mandibular third molars positioned mesioangularly or horizontally compared to others. Similarly, Dias et al. [24] compared bone loss assessments around ITMs using OPG and Cone Beam Computed Tomography (CBCT), finding that OPG underestimated bone loss, particularly for mesioangular and horizontal ITMs ($p < 0.05$). Yesiltepe et al. [25] observed marginal bone loss in 79.4% of adjacent 2Ms on CBCT scans but found no significant association between the position of ITMs and bone loss ($p = 0.360$).

Overall, the literature shows inconsistent findings regarding whether the angulation of ITMs affects alveolar bone loss in adjacent 2Ms. The present study differs by monitoring bone level changes over time according to ITM angulation, rather than measuring the immediate effects at a single timepoint. Due to these methodological differences, the results of the present study cannot be directly compared with existing studies, underscoring the novelty and importance of the present study.

The present study also investigated the correlation between patient age and the specific measurements. Previous studies have examined the impact of age on periodontal health, revealing varying results. Kugelberg et al. [22] found significant differences in intrabony defect progression by age, with younger individuals showing improvement post-extraction, while those over 30 did not. Helmi et al. [18] identified age as a significant risk factor for alveolar bone changes, noting increased bone loss in older adults. The findings of the present study align with these trends, indicating a relationship between age and alveolar bone changes, particularly with infrabony defect angles increasing as age advances. However, due to the limited statistical power in this area, further research is still needed to clarify the direct correlation between aging and changes in alveolar bone structure in relation to ITMs.

4.1. Study Limitations

A key limitation of the study lies in its focus on assessing radiographic changes in the alveolar bone around the 2Ms both before and after surgical intervention of ITMs; however, none of the participants underwent extraction of ITMs during the timeframe of T1, which prevented a comparison of bone levels pre- and post-extraction. Additionally, the comparatively smaller sample size may reduce the study's statistical power, making it challenging to detect significant differences in alveolar bone changes. Other limitations and practical challenges should be considered when implementing the proposed approach in research settings. The use of two-dimensional periapical radiographs may not fully capture the three-dimensional morphology of intrabony defects. Radiograph standardization, including consistent angulation and image quality across timepoints, can be difficult to achieve in routine practice. Moreover, the longitudinal design and requirement for paired radiographs increase time and resource demands, which may not be feasible in all settings.

4.2. Clinical Implications

This study aimed to provide high-quality evidence to support that the prophylactic extraction of ITMs improves the outcomes of the bone structures around the 2Ms. However,

the analysis yielded insufficient evidence, as most measurements showed no statistically significant differences between the two timepoints, although a few did. Consequently, the study did not seem to indicate that prophylactic ITM extraction would offer a significant bone-sparing benefit, nor does it provide evidence that ITMs contribute to increased damage to the distal surface of 2Ms in the short term. It is proposed that a further prospective study is needed to assess bone level changes over a long time span with a large sample size and confirm whether extraction would indeed improve bone levels. The current study's findings did not provide sufficient evidence to support the hypothesis that the presence of ITMs leads to increased bone damage over time in the short term. Therefore, management decisions regarding ITMs should align with existing recommendations such as those outlined in the FDS guidelines.

5. Conclusions

The study aimed to evaluate the short-term radiographic effects of ITMs on the 2Ms in patients with periodontitis. It focused on alveolar bone changes around the 2Ms without ITM removal while examining patient-related factors like age, gender, health conditions, smoking habits, and ITM angulation. Findings revealed no significant changes in bone defect size over time, indicating that ITMs did not exacerbate bone loss around 2Ms in the short term. While gender showed some influence on bone distance measurements, other factors, including age and ITM angulation, had minimal impact on bone levels. This study contributes to an ongoing debate about ITM removal in periodontal disease. While NICE guidelines recommend against routine ITM removal without active disease, FDS guidelines support removal to protect periodontal health. However, the study found no strong evidence that ITMs significantly increase bone damage near 2Ms, suggesting that prophylactic ITM removal may not be warranted for bone health alone.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data will be available on reasonable request from the corresponding author.

Conflicts of Interest: The authors state that there are no conflicts of interest.

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Diagnostic Accuracy of Raman Spectroscopy for Oral Potentially Malignant Disorders: A Systematic Review and Meta-Analysis

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Abstract

Objective: The aim of this study was to summarize the accuracy and efficacy of Raman spectroscopy (RS) technology in identifying Oral Potentially Malignant Disorders (OPMDs). **Methods:** This systematic review was registered in PROSPERO (CRD420251124866). A literature search was conducted in databases including PubMed, Embase, and Web of Science from inception up to September 2025. The risk of bias of included studies was evaluated using the QUADAS-2 tool. Appropriate statistical techniques were applied to perform heterogeneity analysis, subgroup analyses, and calculation of pooled diagnostic efficacy indices. **Results:** A total of 12 studies involving 2810 samples were included. In the “OPMDs vs. Normal” group, RS achieved a pooled sensitivity of 0.84 (95% CI: 0.78–0.88), specificity of 0.90 (95% CI: 0.73–0.97), and AUC of 0.89 (95% CI: 0.86–0.91). In the “OPMDs vs. Oral squamous cell carcinoma (OSCC)” group, the pooled sensitivity was 0.89 (95% CI: 0.82–0.94), specificity was 0.91 (95% CI: 0.85–0.95), and AUC was 0.94 (95% CI: 0.91–0.96). Heterogeneity analysis revealed low heterogeneity in the “OPMDs vs. OSCC” group ($I^2 = 22.8\%$) and moderate heterogeneity in the “OPMDs vs. Normal oral mucosa” group ($I^2 = 58.9\%$). Subgroup analyses were performed: sample type exerted no significant impact on heterogeneity ($p > 0.19$), while Raman type (micro vs. fiber) showed potential to modulate diagnostic efficacy, micro-Raman had higher sensitivity (0.916 vs. 0.811), and fiber-Raman had better specificity (0.917 vs. 0.843). No significant publication bias was observed (Egger’s $p > 0.3$). **Conclusions:** Raman spectroscopy is an effective and reliable tool for screening and differentiating OPMDs.

Keywords: Raman spectroscopy technology; oral potentially malignant disorders; oral squamous cell carcinoma; diagnostic meta-analysis; systematic review

1. Introduction

Oral squamous cell carcinoma (OSCC) accounts for approximately 90% of oral cancer, making it the thirteenth most common cancer globally [1]. Epidemiological data indicates that 389,846 new cases of oral and mouth cancer were reported in 2022 [2]. The irritation of the oral mucosa by substances such as alcohol, tobacco, betel nuts, HPV infection, and inadequate dental restorations results in an increased incidence of malignant transformation. Oral cancer usually progresses by a sequence of histological alterations, starting with

normal mucosa and progressing to mild to severe atypical hyperplasia [3] and oral squamous cell carcinoma. In this carcinogenic process, Oral Potentially Malignant Disorders (OPMDs) act as the key intermediate link between normal oral mucosa and malignant tumors [4]; the most frequent types include oral leukoplakia (OLK), erythroplakia, proliferative verrucous leukoplakia (PVL), oral submucous fibrosis (OSMF), oral lichenoid diseases, graft-versus-host disease, and actinic cheilitis. These OPMDs exhibit heterogeneous malignant transformation rates, ranging from approximately 0.44% (e.g., oral lichen planus) to 43.87% (e.g., PVL) [5]. Given this variability, there is an immediate clinical necessity to explore and create innovative detection technologies that can enable early diagnosis and treatment of OPMDs, hence improving patient outcomes.

Surgical biopsy, in conjunction with histopathological diagnosis, is regarded as the gold standard [6] to evaluate suspicious oral lesions. Nevertheless, because of its invasive characteristics, this method is inappropriate for the early identification of OPMDs, especially when such lesions manifest in apparently “normal” or asymptomatic oral mucosa. There is a pressing necessity to investigate and advance novel detection technologies that enable early diagnosis and treatment of OPMDs, thus enhancing the prognosis for patients with oral cancer.

Raman spectroscopy (RS) is a non-elastic light scattering technique regarded as a potential diagnostic approach, capable of acquiring extensive molecular information from biological tissues, referred to as “molecular fingerprints.” In the carcinogenesis process within biological tissues, internal proteins, nucleic acids, lipids, carbohydrates, and other biomolecules experience alterations in conformation and amount, resulting in changes in Raman frequency shifts, peak intensities, and spectrum bands [7]. In recent years, numerous RS techniques have been extensively utilized for the differential detection of ex vivo oral cancer tissues and normal tissues [8–11]. Clinically, RS shows considerable promise in early risk stratification of suspicious oral lesions, streamlining clinical triage, and guiding precise biopsy targeting, serving as a potentially valuable adjunctive diagnostic tool to histopathological examination.

To date, a substantial body of literature has confirmed the diagnostic accuracy of Raman spectroscopy (RS) in head and neck carcinomas, with specific studies investigating its in vivo performance in oral cancer diagnosis [9,12–14]. For instance, Zhan (2020) [15] conducted a meta-analysis of 41 studies and reported a pooled sensitivity of 0.91 and specificity of 0.85 for in vivo oral cancer diagnosis using Raman spectroscopy (RS) [10]. Notably, this study also separately included 15 studies focusing on OPMDs, with subgroup analysis demonstrating excellent diagnostic performance of RS for OPMDs: the pooled diagnostic odds ratio (DOR) was as high as 839.25 (95% CI [274.50, 2565.92]), and the area under the summary receiver operating characteristic (SROC) curve (AUC) was 0.9932, confirming its robust potential for OPMD identification. Similarly, Shrivastava (2023) [16] performed a meta-analysis of 8 studies and reported that RS achieved a pooled sensitivity of 1.00 (95% CI: 0.72–1.00) and specificity of 0.93 (95% CI: 0.80–0.98) for detecting oral malignant and potentially malignant conditions.

However, despite these promising findings, existing meta-analyses on RS and OPMDs have not evaluated RS’s efficacy in distinguishing OPMDs from oral squamous cell carcinoma (OSCC), nor have they systematically assessed its overall diagnostic value in differentiating OPMDs, normal oral mucosa, and OSCC. Identifying the malignant transformation of OPMDs is clinically crucial for improving early cancer screening and reducing unnecessary invasive procedures, yet this remains an unmet clinical need inadequately addressed by prior research. Therefore, this meta-analysis aims to systematically evaluate the diagnostic accuracy of RS for the rapid identification of OPMDs and the differentiation of their malignant progression, clarify its efficacy in distinguishing OPMDs from normal oral

mucosa and OSCC, fill the aforementioned research gap, and support the early detection of oral mucosal carcinogenesis.

2. Materials and Methods

This meta-analysis adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A completed PRISMA 2020 checklist is provided as Supplementary Materials. The study was registered under PROSPERO number: CRD420251124866.

This systematic review and meta-analysis was guided by a clearly defined PICO (Population, Index test, Comparator, Outcomes) framework to ensure transparency, reproducibility, and methodological rigor in diagnostic accuracy assessment.

Population (P): Patients with OPMDs, OSCC, or normal oral mucosa, regardless of age, gender, OPMD subtype, or study setting.

Index test (I): Raman spectroscopy (RS), including micro-Raman and fiber-Raman systems, used for the detection and differentiation of oral mucosal lesions.

Comparator (C): Histopathological diagnosis (gold standard) for OPMDs and OSCC; normal oral mucosa as a reference group; and direct comparison between OPMDs and OSCC.

Outcomes (O): Primary outcomes: Pooled diagnostic accuracy measures including sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and area under the summary receiver operating characteristic (SROC) curve (AUC).

Secondary outcomes: Subgroup performance by Raman system type, sample type, and sources of heterogeneity.

2.1. Search Strategy

A systematic search of four databases—PubMed, Embase, Web of Science, and the Cochrane Library—was conducted through September 2025 to identify studies assessing the accuracy of RS in diagnosing OPMDs. Taking the PubMed database as an example, the search expression was: (“Precancerous Conditions”[MeSH Terms] AND “Oral”[Title/Abstract]) OR “Leukoplakia, Oral”[MeSH Terms] OR “Erythroplasia”[MeSH Terms] OR “Oral Submucous Fibrosis”[MeSH Terms] OR “Lichen Planus, Oral”[MeSH Terms] OR “Oral Premalignant Lesions”[Title/Abstract] OR “Oral Potentially Malignant Disorders”[Title/Abstract] OR “Oral Dysplasia”[Title/Abstract]) AND “Spectrum Analysis, Raman”[MeSH Terms]. In addition to electronic database searches, a manual search was conducted of reference lists from previously published systematic reviews and meta-analyses on Raman spectroscopy in oral oncology (identified via initial database searches) to identify potentially eligible studies missed by electronic searches.

2.2. Selection Criteria

Inclusion criteria: (1) original research studies; (2) studies focusing on specimens from patients with OPMDs; (3) studies employing histopathology as the diagnostic gold standard; (4) studies utilizing RS as a diagnostic tool for screening, assessing lesion risks, delineating surgical margins, and/or guiding subsequent actions; (5) studies conducting tests on specimens from human bodies, ex vivo human tissues, or human body fluids; (6) studies providing the number of samples in each test group along with true positive (TP), false positive (FP), true negative (TN), and false negative (FN) test results.

Exclusion criteria: (1) case reports, case series, review articles, editorials, letters, comments, conference proceedings, or non-original articles; (2) studies with low sample sizes (normal/benign < 5; OPMDs/OSCC < 5); (3) studies based on animal models and

cell lines; (4) studies focusing on specimens outside the oral region; (5) studies without histopathological diagnosis for the collected samples.

2.3. Data Extraction

Employing a uniform data extraction template, two reviewers conducted independent data retrieval from each included article and appraised its quality, resolving any disagreements through consensus. The following data were extracted: author name; publication year; patient count; sample type; spectral details; diagnostic criteria; the RS methodology of the primary investigator; the numbers of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN); and various methodological and technical specifics. The quality of each study was assessed in accordance with the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) [17].

2.4. Statistical Analysis

Data analysis was conducted using Stata 18.0, Meta-Disc 2.0, and Revman 5.3 software. Bivariate random-effects models were consistently applied across all diagnostic meta-analyses to synthesize the diagnostic accuracy indices. Sensitivity, specificity, and their 95% confidence intervals (CIs), along with the positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR), were calculated, and the area under the curve (AUC) was derived by plotting the SROC curve. The significance level for the meta-analysis was set at $\alpha = 0.05$. The I^2 metric was utilized to assess heterogeneity. A fixed-effects model was employed for meta-analysis when studies showed no statistical heterogeneity; otherwise, a random-effects model was utilized after investigating and excluding clear clinical heterogeneity sources. Deek's funnel plot assessed publication bias.

3. Results

3.1. Literature Selection

A total of 17 articles were obtained from the PubMed database, 32 from the Embase database, 17 from the Web of Science database, and 13 from the Cochrane Library, resulting in a cumulative total of 76 papers. Following the exclusion of 12 duplicate papers, 64 papers remained. Excluding papers that lacked full text, had missing data, did not meet established criteria, were repetitive, or exhibited significant design flaws resulted in the inclusion of 12 papers [18–29] for systematic review and meta-analysis (Figure 1). A manual search of reference lists from two prior systematic reviews [15,16] and meta-analyses was performed. Zhan (2020) [15] included 16 references and Shrivastava (2023) [16] included 8 references, all of which were duplicates of studies already identified in the initial screening. After excluding non-original studies, studies without a gold standard, and studies with duplicate data, no new eligible studies were included. The article selection and systematic review process adhered to the PRISMA guidelines [17].

Table 1 summarizes the study's characteristics of included articles, including author, publication year, sample type and corresponding sample sizes, RS parameters, and data processing methods. Among these studies, 11 provided diagnostic data for differentiating Oral Potentially Malignant Disorders (OPMDs) from oral squamous cell carcinoma (OSCC), while another 11 documented data for distinguishing OPMDs from normal samples.

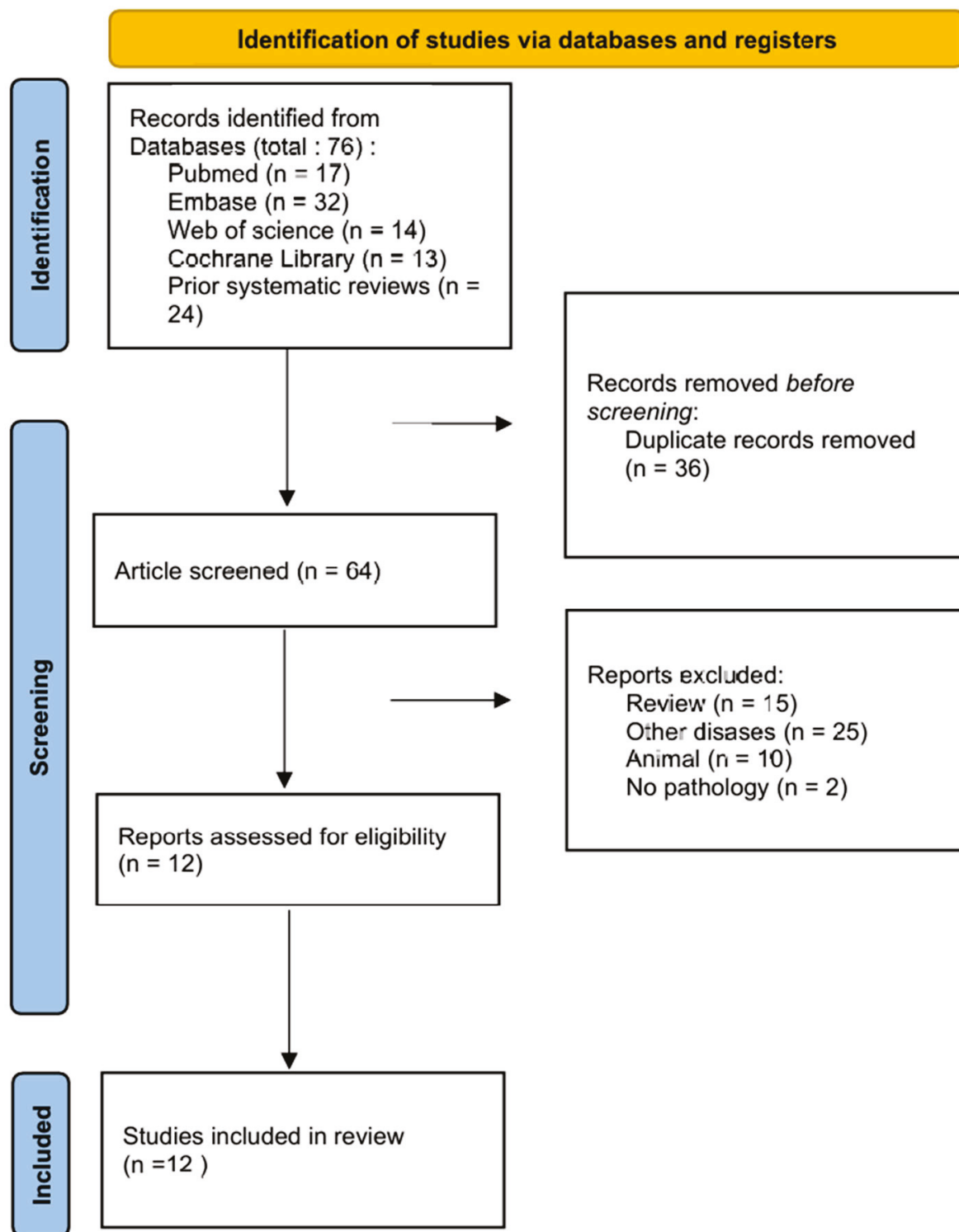


Figure 1. PRISMA flow chart of literature selection process.

Table 1. Study characteristics.

Study ID	Author	Year	Type of Sample	Sample Size			Lesion Type	Type of Raman	Excitation Wave-length	Spectral Range	Integration Time	Data Processing Method		Pre Mal and OSCC				Pre Mal and Normal			
				Normal	OPMDs	OSCC						Algorithm	Model Validation Method	TP	FP	TN	FN	TP	FP	TN	FN
1	Li [18]	2010	Tissue samples	28	32	58	OLK	fiber	1064 nm	100–3800 cm ^{−1}	-	SVM	LOOCV and blind calibration	19	2	22	1	20	8	14	4
2	Singh [19]	2012	Tissue samples	50	24	50	-	fiber	785 nm	1200–1800 cm ^{−1}	3 s	PCA-LDA	LOOCV and independent test	74	10	26	12	26	23	67	10
3	Krishna [20]	2013	Tissue samples	26	44	69	OLK, OSMF	fiber	785 nm	900–1750 cm ^{−1}	5 s	Sparse Multinomial Logistic Regression, SMLR	LOOCV (Leave-One-Subject-Out Cross-Validation)	165	20	128	22	128	37	230	20
4	Sahu [21]	2015	Serum	126	47	120	OLK, OSMF	fiber	785 nm	700–1800 cm ^{−1}	15 s	PC-LDA	LOOCV	83	16	31	37	37	31	95	10
5	Xue [22]	2015	Tissue samples	32	50	56	Severe Dysplasia	fiber	785 nm	600–1800 cm ^{−1}	40 s	PCA-DFA	cross-validation	53	7	43	3	43	0	32	7
6	Krishna [23]	2016	Tissue samples	28	58	113	OLK, OSMF	fiber	785 nm	950–1750 cm ^{−1}	5 s	the MRDF-SMLR diagnostic algorithm	LOOCV	280	30	169	36	169	23	239	19
7	Sahu [24]	2016	Tissue samples	72	40	85	OLK, OSMF	fiber	785 nm	1200–1800 cm ^{−1}	3 s	PC-LDA	LOOCV	174	59	47	77	47	0	657	59
8	Brindha [25]	2016	Urine	80	57	60	-	micro	785 nm	2600–3500 cm ^{−1}	90 s	PC-LDA	-	50	5	52	10	52	2	5	5
9	Rekha [26]	2016	Saliva	23	28	32	OSMF	micro	784.15 nm	200–1800 cm ^{−1}	120 s	PCA-LDA	LOOCV	16	13	15	16	26	7	16	2
10	Ghosh [27]	2018	Oral exfoliated cells	11	13	10	OLK	fiber	785 nm	400–1800 cm ^{−1}	50 s	PCA-LDA	k-fold	25	3	16	4	16	4	10	3
11	Behl [28]	2020	Oral brush biopsy cytological samples	20	20	-	OLK, erythro-plakia	micro	532 nm	400–1800 cm ^{−1}	30 s	PLS-DA	LOOCV	-	-	-	-	306	55	301	51
12	Kaushik [29]	2025	Saliva	32	28	39	-	micro	785 nm	400–2200 cm ^{−1}	20–30 s	PLS-DA	-	75	6	111	9	-	-	-	-

3.2. Risk and Publication Bias Assessment

The relevant data and information from the included studies were systematically organized and analyzed utilizing the QUADAS-2 tool. Figure 2 presents the evaluations of bias risk and applicability concerns across the included studies. The majority of included studies exhibited a low risk of bias across all QUADAS-2 domains, while a minority indicated a high risk of bias in certain domains.

	Risk of Bias				Applicability Concerns		
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard
2010Li	+	−	+	−	+	+	+
2012Singh	+	+	+	+	+	+	+
2013Krishna	+	+	+	+	+	+	+
2015Sahu	+	+	+	+	+	+	+
2015Xue	+	−	+	+	+	+	+
2016Brindha	+	+	+	+	+	+	+
2016Krishna	+	+	+	+	+	+	+
2016Rekha	+	+	+	+	+	+	+
2016Sahu	+	+	+	+	+	+	+
2019Ghosh	+	+	+	+	+	+	+
2020Behl	+	+	+	+	+	+	+
2025Kaushik	+	+	+	+	+	+	+

− High
? Unclear
+ Low

Figure 2. Methodological quality summary (2010 Li [18], 2012 Singh [19], 2013 Krishna [20], 2015 Sahu [21], 2015 Xue [22], 2016 Brindha [25], 2016 Krishna [23], 2016 Rekha [26], 2016 Sahu [24], 2019 Ghosh [27], 2020 Behl [28], 2025 Kaushik [29]).

To identify potential publication bias in the 12 included articles, distinct funnel plots were created for two comparisons: OPMDs vs. Normal (Figure 3) and OPMDs vs. OSCC (Figure 4). In the comparison of OPMDs vs. OSCC, Deeks' funnel plot asymmetry test produced a p -value of 0.73. For the comparison of OPMDs vs. Normal, the p -value was 0.19. Both results demonstrated the absence of significant publication bias in each comparison. Egger's Test and analogous asymmetry tests demonstrate limited statistical power with a small number of included studies. The inclusion of merely 12 studies in this research may undermine the sensitivity of the test. The slight asymmetry observed in the funnel plot suggests that the potential for publication bias cannot be entirely dismissed. The lack

of gray literature, including unpublished studies and conference abstracts, may influence the results.

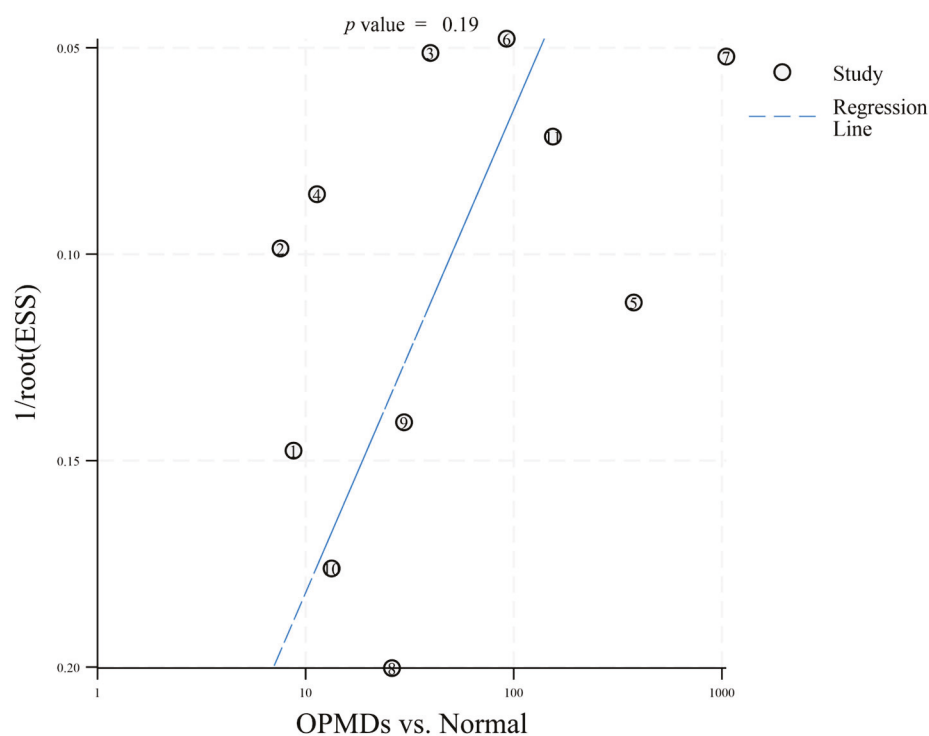


Figure 3. Deeks' funnel plot asymmetry test (OPMDs vs. Normal). The numbers 1–11 correspond to references [18–27,29], respectively.

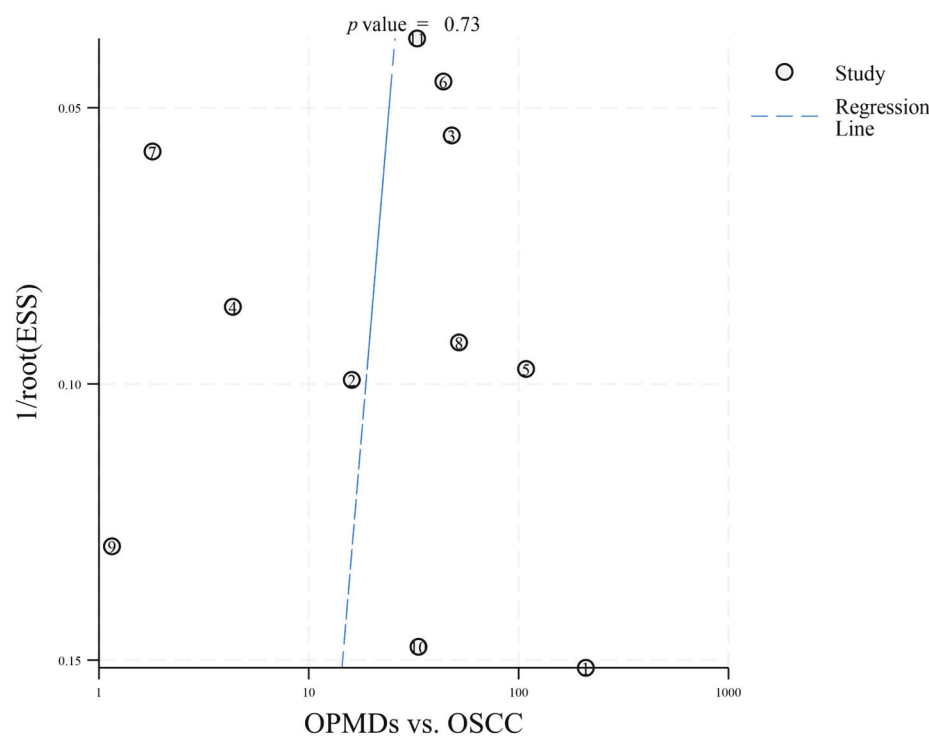


Figure 4. Deeks' funnel plot asymmetry test (OPMDs vs. OSCC). The numbers 1–11 correspond to references [18–28], respectively.

The findings of this meta-analysis indicate a low risk of publication bias, suggesting that the research conclusions are relatively reliable. Future research must incorporate additional high-quality studies for further verification.

3.3. Pooled Diagnostic Accuracy Results

3.3.1. OPMDs vs. Normal

A total of 11 out of 12 included studies were eligible for meta-analysis regarding the differentiation of OPMDs from normal oral mucosa. Complete data extraction was achieved for true positive (TP), false positive (FP), true negative (TN), and false negative (FN) across all eligible studies.

Individual studies exhibited marked variability in diagnostic performance, as visualized by two forest plots. For sensitivity (Figure 5), values ranged from 0.44 (Sahu 2016 [24]) to 0.93 (Rekha 2016 [26]). For specificity (Figure 6), the range was 0.64 (Li 2010 [18]) to 1.00 (Xue 2015 [22], Sahu 2016 [24]). The observed variability suggests potential heterogeneity, likely due to variations in study design, sample type, or RS parameters.

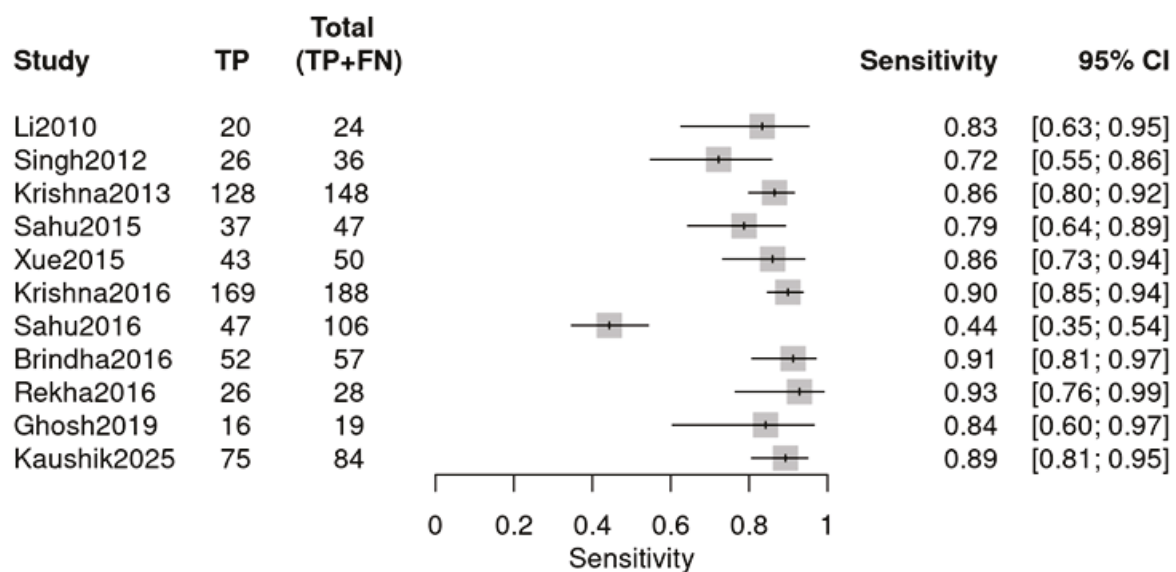


Figure 5. Sensitivity forest plot (OPMDs vs. Normal) (Li 2010 [18], Singh 2012 [19], Krishna 2013 [20], Sahu 2015 [21], Xue 2015 [22], Krishna 2016 [23], Sahu 2016 [24], Brindha 2016 [25], Rekha 2016 [26], Ghosh 2019 [27], Kaushik 2025 [29]).

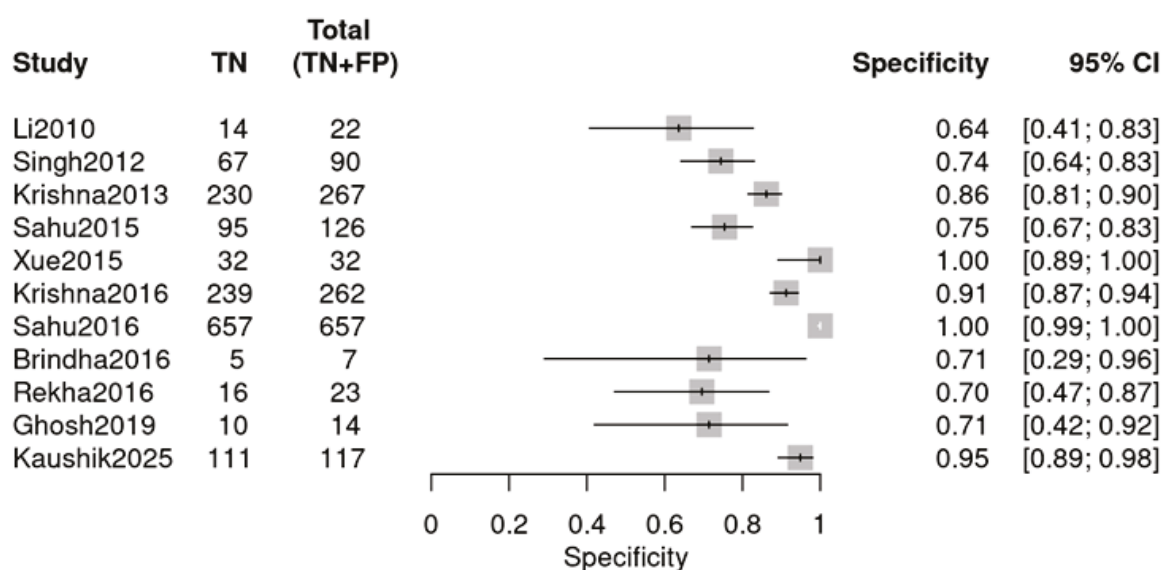


Figure 6. Specificity forest plot (OPMDs vs. Normal) (Li 2010 [18], Singh 2012 [19], Krishna 2013 [20], Sahu 2015 [21], Xue 2015 [22], Krishna 2016 [23], Sahu 2016 [24], Brindha 2016 [25], Rekha 2016 [26], Ghosh 2019 [27], and Kaushik 2025 [29]).

Using a bivariate random-effects meta-analysis model, the pooled diagnostic efficacy indicators for OPMDs vs. Normal, including sensitivity, specificity, diagnostic odds ratio (DOR), positive likelihood ratio (PLR), and negative likelihood ratio (NLR), are summarized in Table 2. These indicators illustrate the strong discriminative performance of RS. The pooled sensitivity of 0.843 reduces missed screenings for OPMDs, confirming its effectiveness as an initial detection tool. Additionally, the pooled specificity of 0.900 (FPR = 0.100) decreases the likelihood of unnecessary invasive procedures for individuals with normal oral mucosa. Additionally, high pooled DOR (48.359) attests to strong overall diagnostic efficacy, while elevated pooled PLR (8.414) and low pooled NLR (0.174) strengthen confidence in positive result validity and negative-case exclusion reliability.

Table 2. Pooled diagnostic efficacy indicators (OPMDs vs. Normal).

	Estimate	95% LCI	95% UCI
Sensitivity	0.843	0.769	0.897
Specificity	0.9	0.733	0.967
DOR	48.359	18.355	127.409
PLR	8.414	3.033	23.341
NLR	0.174	0.123	0.246
FPR	0.1	0.033	0.267

An SROC curve was created to assess diagnostic abilities (Figure 7), resulting in an area under the curve of 0.91 (95% CI: 0.88–0.93). This is “excellent” (AUC > 0.90), validating RS’s ability to distinguish OPMDs from normal oral mucosa.

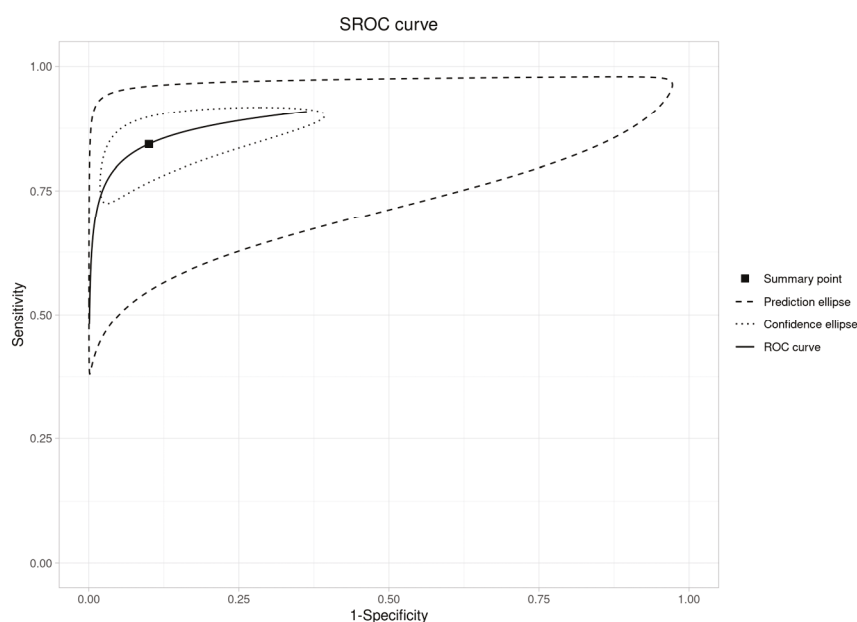


Figure 7. SROC curve plot (OPMDs vs. Normal).

3.3.2. OPMDs vs. OSCC

This group comprised 11 studies from a total of 12, with one study excluded. Data extraction adhered to the same methodology and encompassed similar diagnostic data as the previous group (Figures 8 and 9). The individual studies demonstrated variability in diagnostic performance: sensitivity varied from 0.50 (Rekha 2016 [26]) to 0.95 (Li 2010 [18]), and specificity ranged from 0.44 (Sahu 2016 [24]) to 0.94 (Brindha 2016 [25]), as illustrated in the forest plots.

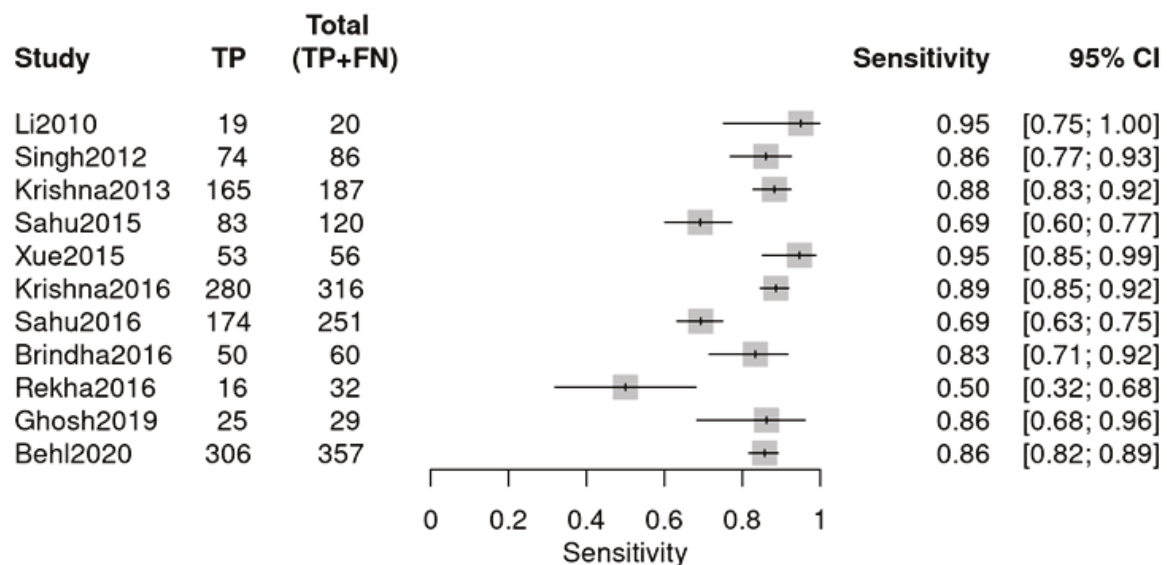


Figure 8. Sensitivity forest plot (OPMDs vs. OSCC) (Li 2010 [18], Singh 2012 [19], Krishna 2013 [20], Sahu 2015 [21], Xue 2015 [22], Krishna 2016 [23], Sahu 2016 [24], Brindha 2016 [25], Rekha 2016 [26], Ghosh 2019 [27], Behl 2020 [28]).

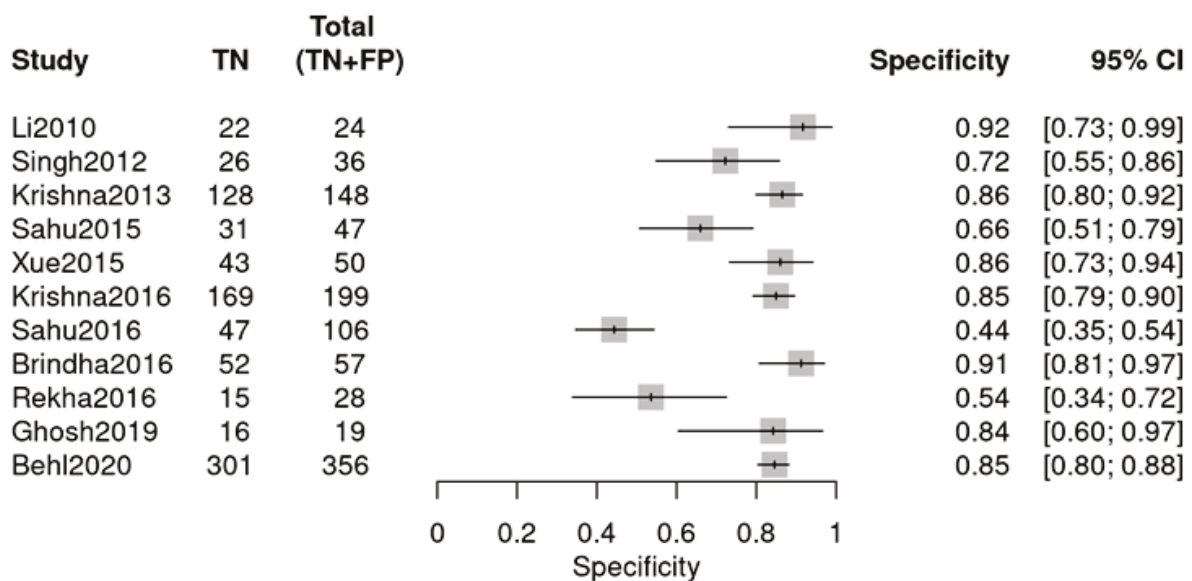


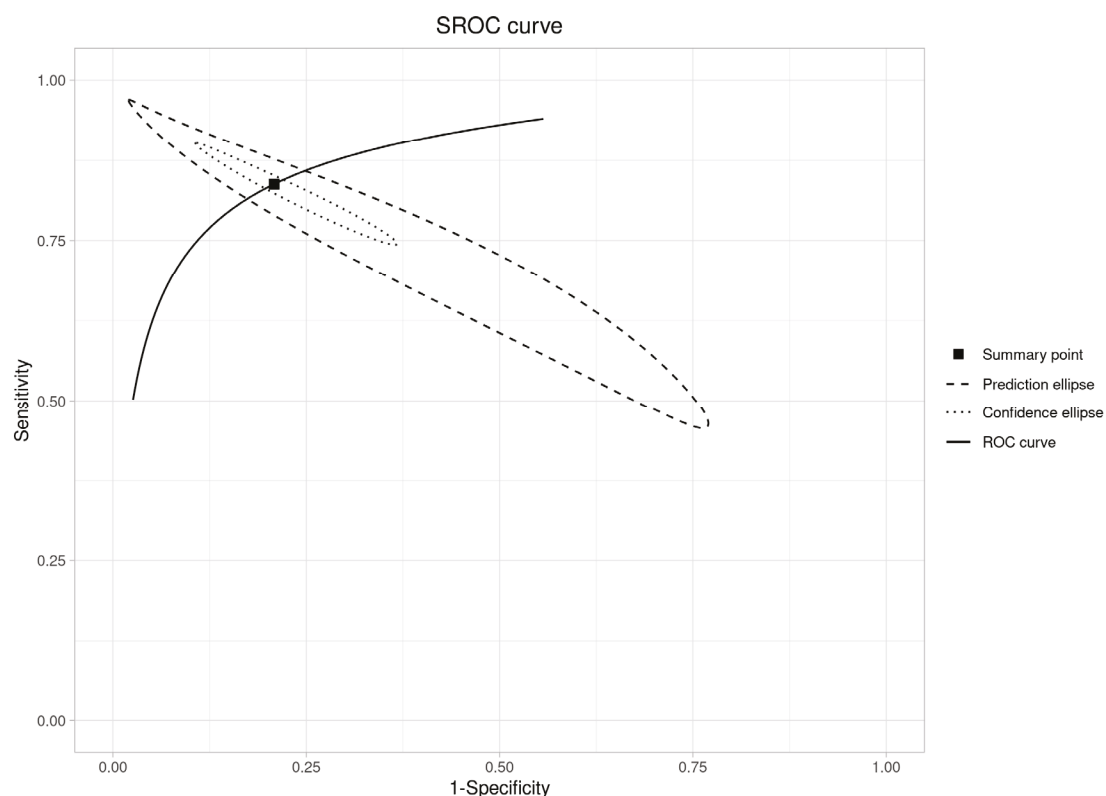
Figure 9. Specificity forest plot (OPMDs vs. OSCC) (Li 2010 [18], Singh 2012 [19], Krishna 2013 [20], Sahu 2015 [21], Xue 2015 [22], Krishna 2016 [23], Sahu 2016 [24], Brindha 2016 [25], Rekha 2016 [26], Ghosh 2019 [27], Behl 2020 [28]).

The combined diagnostic effectiveness demonstrates significant clinical value (Table 3). A pooled sensitivity of 0.838 facilitates the accurate identification of approximately 84% of cases, thereby minimizing the risk of missed diagnoses and endorsing its application in screening processes. The pooled specificity of 0.791 indicates that approximately 79% of non-cases are excluded. A DOR of 19.61, significantly greater than 1, indicates strong overall discriminatory ability. A PLR of 4.018 indicates that a positive result is approximately four times more likely in cases, thereby enhancing confidence in positive findings. An NLR of 0.205 suggests that a negative result is approximately five times more probable in non-cases, thereby rendering negative results highly dependable for the exclusion of disease. In summary, RS exhibits robust comprehensive performance, notably excelling in sensitivity and overall differentiation.

Table 3. Pooled diagnostic efficacy indicators (OPMDs vs. OSCC).

	Estimate	95% LCI	95% UCI
Sensitivity	0.838	0.778	0.884
Specificity	0.791	0.691	0.866
DOR	19.61	8.032	47.873
PLR	4.018	2.494	6.472
NLR	0.205	0.134	0.314
FPR	0.209	0.134	0.309

The SROC curve resides in the upper-left region (Figure 10), consistent with an AUC of 0.89 (95% CI: 0.86–0.91), a value that falls into the “good” category (typically defined as AUC 0.8–0.9) and confirms RS’s robust ability to differentiate OPMDs from OSCC.

**Figure 10.** SROC curve plot (OPMDs vs. OSCC).

3.3.3. Cross-Group Comparison

To clarify RS’s performance across scenarios, we compared pooled diagnostic efficacy between the “OPMDs vs. Normal” and “OPMDs vs. OSCC” groups. The “OPMDs vs. OSCC” group exhibited higher sensitivity (0.89 vs. 0.84), specificity (0.91 vs. 0.90), DOR (85.2 vs. 48.4), and AUC (0.94 vs. 0.89), indicating superior efficacy in differentiating OPMDs from OSCC than from normal mucosa.

A combined analysis of the SROC curve (Figure 11) and meta-regression results reveals important details: although the “OPMDs vs. Normal” curve (red) is positioned nearer to the upper-left region, meta-regression indicated no statistically significant differences in relative sensitivity ($p = 0.918$), relative specificity ($p = 0.197$), or global test comparison ($p = 0.425$) between the two groups. Despite this statistical parity, RS retains distinct value in the “screening to differentiation” workflow: it serves as a dependable preliminary screening tool for OPMDs compared to normal mucosa, utilizing high sensitivity (0.84) and AUC (0.89) to reduce missed diagnoses. In differentiating OPMDs from OSCC, RS also exhibits

favorable specificity (0.91), DOR (85.2), and AUC (0.94), facilitating accurate treatment decisions (e.g., conservative management for OPMDs versus radical therapy for OSCC). RS facilitates thorough management of oral lesions, encompassing detection and accurate classification, and demonstrates consistent performance in various clinical contexts.

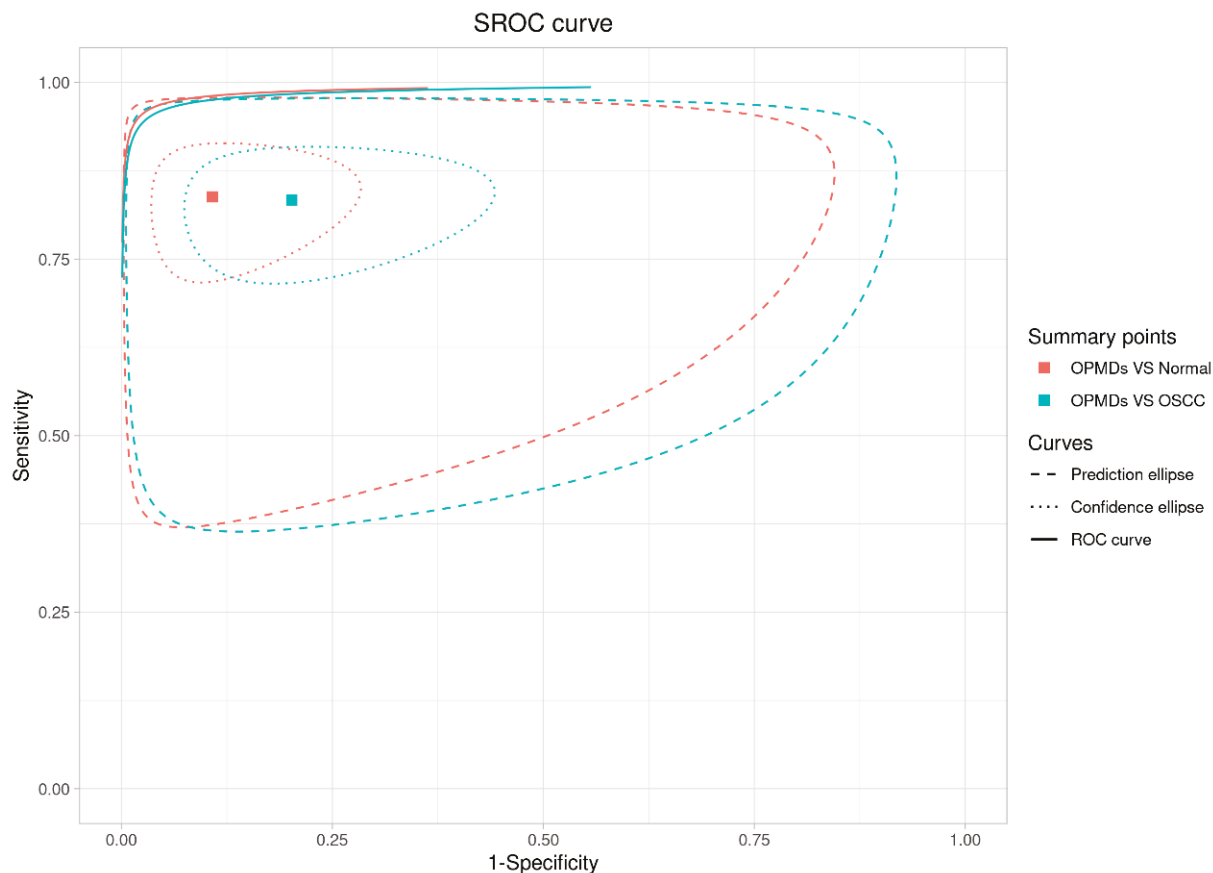


Figure 11. Combined analysis of the SROC curve.

3.3.4. Heterogeneity and Subgroups Analysis

The heterogeneity analysis (Table 4) revealed that the bivariate I^2 statistic was 22.8% for the OPMDs vs. OSCC group, reflecting low heterogeneity, while it reached 58.9% for the OPMDs vs. Normal group, suggesting moderate heterogeneity. In terms of variance components, the variance of logit-transformed sensitivity ($\text{Var logit}(\text{sen}) = 0.349$) and specificity ($\text{Var logit}(\text{spe}) = 0.686$) in the OPMDs vs. OSCC group were both lower than those in the OPMDs vs. Normal group (0.494 and 3.529, respectively), indicating less between-study variation in the former. The multiplicative odds ratios (MORs) for sensitivity (1.757) and specificity (2.203) in the OPMDs vs. OSCC group were also significantly lower than those in the OPMDs vs. Normal group (1.955 and 6.001, respectively), further confirming weaker heterogeneity in the former. Additionally, the area of the 95% prediction ellipse was much smaller in the OPMDs vs. OSCC group (0.068) than in the OPMDs vs. Normal group (0.26), intuitively reflecting better consistency of results. In conclusion, RS exhibits lower between-study heterogeneity and stronger result stability in the “OPMDs vs. OSCC” scenario, while there is moderate heterogeneity in the “OPMDs vs. Normal” scenario, which requires further exploration of sources through subgroup analysis.

Table 4. Heterogeneity analysis.

	OPMDs vs. OSCC	OPMDs vs. Normal
Var logit(sen)	0.349	0.494
Var logit(spe)	0.686	3.529
MOR sensitivity	1.757	1.955
MOR specificity	2.203	6.001
Bivariate I ²	0.228	0.589
Area 95% Prediction Ellipse	0.068	0.26

To identify the potential drivers of moderate heterogeneity in the “OPMDs vs. Normal” group, we conducted subgroup analyses based on type of sample (Table 5) and type of RS (Table 6), respectively, with results as follows:

Table 5. Subgroup analysis by type of sample.

Parameter	Estimate Other	95% LCI Other	95% UCI Other	Estimate Tissue	95% LCI Tissue	95% UCI Tissue
Sensitivity	0.884	0.79	0.94	0.804	0.695	0.88
Specificity	0.801	0.45	0.952	0.945	0.79	0.988
DOR	30.798	7.836	121.055	70.957	19.304	260.824
PLR	4.444	1.291	15.297	14.721	3.687	58.783
NLR	0.144	0.084	0.247	0.207	0.135	0.319
FPR	0.199	0.048	0.55	0.055	0.012	0.21

Table 6. Subgroup analysis by type of RS.

Parameter	Estimate Fiber	95% LCI Fiber	95% UCI Fiber	Estimate Micro	95% LCI Micro	95% UCI Micro
Sensitivity	0.811	0.721	0.876	0.916	0.816	0.964
Specificity	0.917	0.737	0.977	0.843	0.375	0.98
DOR	46.994	15.2	145.291	58.584	9.251	371.016
PLR	9.709	2.941	32.057	5.834	0.957	35.571
NLR	0.207	0.145	0.294	0.1	0.05	0.199
FPR	0.083	0.023	0.263	0.157	0.02	0.625

The tissue subgroup demonstrated superior performance in specificity and DOR. Subsequent meta-regression analysis indicated that the *p*-value for relative sensitivity level (tissue vs. other) was 0.194, that for relative specificity level was 0.197, and that for global test comparison was 0.349. All *p*-values were >0.05, indicating that “type of sample” had no statistically significant impact on the heterogeneity of this group.

The micro subgroup performed better in sensitivity, while the fiber subgroup was superior in specificity. Meta-regression analysis showed that the *p*-value for relative sensitivity level (micro vs. fiber) was 0.086 (approaching statistical significance), that for relative specificity level was 0.586, and that for global test comparison was 0.186. This suggests that the type of RS may potentially affect sensitivity.

4. Discussion

This study assessed the diagnostic efficacy of RS in differentiating OPMDs from normal oral mucosa and OSCC, emphasizing the resolution of clinical unmet needs and methodological deficiencies in previous research.

The diagnostic efficacy of RS aligns with the clinical workflow in oral oncology, and its potential clinical scenarios are clearly defined herein to enhance translational relevance.

Specifically, RS is well-suited for three core clinical settings: First, population-based preliminary screening in primary care or community health centers, where its high sensitivity (0.84) can effectively identify suspicious lesions in asymptomatic individuals or those with a high risk factor, addressing the issue of overlooked early lesions when pathological biopsy is not readily accessible. Second, it serves as a pre-biopsy adjunctive tool during specialist assessment in stomatology departments, as its favorable specificity (0.90) helps triage suspicious lesions, minimizing unnecessary invasive biopsies and alleviating patient anxiety. Third, it holds promise for long-term follow-up of patients with OPMDs, enabling non-invasive dynamic monitoring of lesion progression and aiding in timely identification of malignant transformation. The enhanced efficacy for differentiating OPMDs from OSCC aids in addressing the significant challenges of over-treatment and under-treatment in contemporary clinical practice. The lack of statistically significant efficacy differences between the two scenarios (global $p = 0.425$) further underscores RS's adaptability across these clinical stages.

Notably, our pooled diagnostic accuracy (sensitivity = 0.84, specificity = 0.90) for differentiating OPMDs from normal mucosa and OSCC is consistent with the findings of Zhan (2020) [15], who reported a pooled sensitivity of 0.96 and specificity of 0.90 for RS in diagnosing oral precancerous lesions [10]. This alignment validates RS's reproducible diagnostic value in oral mucosal lesions, while our study extends their work by specifically clarifying RS's efficacy in distinguishing OPMDs from OSCC. This critical aspect was not addressed in their meta-analysis and fills a key research gap in the field.

Heterogeneity analysis identifies key patterns: the low heterogeneity in differentiating OPMDs from OSCC ($I^2 = 22.8\%$) is due to OSCC's unique pathology causing consistent spectral changes, while moderate heterogeneity in distinguishing OPMDs from normal tissue ($I^2 = 58.9\%$) is linked to normal mucosal variations. Subgroup analyses indicate that sample type does not affect heterogeneity ($p > 0.19$), supporting RS's non-invasive use. Raman type is a modifiable factor; micro-Raman is more sensitive (0.916 vs. 0.811) for early lesions, and fiber-Raman is more specific (0.917 vs. 0.843) for bedside screening.

Important limitations of this investigation should be acknowledged. We fully recognize these constraints and their potential impact on the precision, robustness, and interpretability of the pooled findings. First, stratified analysis according to OPMD subtypes could not be performed, largely due to incomplete reporting of subtype classifications and the general absence of prospective, subtype-stratified research designs in the majority of included primary studies. Given the divergent malignant potential across distinct OPMDs entities, this may reduce the specificity and granularity of RS diagnostic efficacy estimates. Second, inconsistent and incomplete reporting of key sample and clinical characteristics—including histopathological differentiation of oral cancer, patient lifestyle habits, and detailed sample source information—limited further exploratory subgroup analyses and may affect the external generalizability of the synthesized results. Third, the micro-Raman subgroup comprised only three included studies, which reduces statistical precision and restricts the stability and generalizability of corresponding subgroup estimates.

Despite a comprehensive search and screening strategy implemented across multiple databases and relevant prior reviews, the overall number of high-quality diagnostic accuracy studies eligible for inclusion remains relatively modest. This largely reflects the current breadth and maturity of peer-reviewed evidence focused on RS for OPMDs diagnosis. To strengthen future evidence in this field, subsequent research is encouraged to implement standardized OPMD subtype categorization, adopt prospectively planned subtype-stratified diagnostic evaluations, improve uniformity in reporting sample and patient-level characteristics, expand sample sizes in underrepresented technical subgroups, and perform head-to-head comparative studies between RS and established non-invasive

diagnostic modalities. These steps may better define the clinical performance and implementation potential of RS for OPMD detection and risk stratification.

5. Conclusions

This study validates that RS technology shows promising diagnostic performance for OPMDs, offering non-invasive, real-time, and rapid identification capabilities that support a range of adjunctive clinical applications. Nonetheless, important limitations persist that require further refinement. In conclusion, RS holds potential as a complementary screening, triage, or biopsy-guidance tool for OPMDs, but should not be regarded as a replacement for histopathological diagnosis (the current gold standard). Ongoing research and development are needed to advance its clinical translation and standardization.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/diagnostics16050648/s1>, File S1: PRISMA 2020 Main Checklist.

Author Contributions: T.Z. played a role in design of the study; X.X. wrote the main manuscript text; X.L. extracted data, Z.Z., Q.S. and N.H. reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: This systematic review and meta-analysis is based on secondary analysis of publicly available data from previously published studies. All included primary studies are listed in the reference section, and their original data can be obtained via the journal websites or by requesting from the corresponding authors of those studies. The literature search strategy (including databases, keywords, and retrieval timeframes) and PRISMA flow diagram are presented in the main text. Additionally, the aggregated data used for meta-analysis (e.g., sensitivity, specificity, pooled effect sizes) are available from the corresponding author upon reasonable request for academic purposes.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

RS	Raman Spectroscopy
OPMDs	Oral Potentially Malignant Disorders
OSCC	Oral Squamous Cell Carcinoma
PVL	Proliferative Verrucous Leukoplakia
OSMF	Oral Submucous Fibrosis
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
TP	True Positive
FP	False Positive
TN	True Negative
FN	False Negative
CI	Confidence Interval
PLR	Positive Likelihood Ratio
NLR	Negative likelihood Ratio
DOR	Diagnostic Odds Ratio
AUC	Area Under the Curve
MOR	Multiplicative Odds Ratio

Var logit(sen)	Variance of logit-transformed sensitivity
Var logit(spe)	Variance of logit-transformed specificity

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Article

Age- and Gender-Based Tongue Volume Variations on Asymptomatic Patients: A Simplified Approach to Form Baseline Data for Obstructive Sleep Apnea

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Abstract: Background: Tongue anatomy plays a critical role in airway-related disorders such as obstructive sleep apnea (OSA). Understanding variations in tongue volume across age and gender is essential for refining diagnostic and therapeutic strategies. This study aims to establish baseline data for tongue volume using a simplified geometric approach, addressing the gap in large-scale anatomical assessments, specifically in asymptomatic patients without clinical indications of OSA. **Materials and Methods:** This retrospective cross-sectional study included 120 asymptomatic patients aged 18–75 years, stratified into three age groups (18–40, 41–60, 61+). Tongue volume was estimated using anterior–posterior length, width, and height measurements from neck CT scans, applying a geometric approximation formula. Statistical analysis, including ANOVA and post hoc tests, was used to evaluate differences across age groups and between genders. Regression analysis examined the influence of age and gender on tongue volume. **Results:** Tongue volume showed a significant decline with advancing age ($p < 0.05$), with the 61+ age group exhibiting the smallest volumes. Gender differences were pronounced, with males consistently having larger volumes than females ($p < 0.05$). Post hoc analyses confirmed significant differences between age groups, and regression analysis indicated that gender was a stronger predictor of tongue volume than age. **Conclusions:** This study highlights the impact of age and gender on tongue volume, emphasizing the need for demographic-specific approaches in the evaluation and management of airway-related conditions. The simplified measurement method offers a practical solution for large-scale studies, providing baseline data for future research and clinical applications. These findings pave the way for personalized diagnostic thresholds and therapeutic strategies in conditions like OSA.

Keywords: tongue volume; simplified geometric approach; age-based variations; gender differences; obstructive sleep apnea; baseline data

1. Introduction

Obstructive sleep apnea (OSA) is a widespread disorder marked by repeated occurrences of upper airway blockage during sleep, either partially or fully, which can significantly affect overall health and quality of life [1,2]. These obstructions disrupt normal sleep patterns and lead to a cascade of complications, including cardiovascular issues, metabolic disturbances, and cognitive impairments [3,4]. The prevalence of OSA has been increasing globally, fueled by rising obesity rates, aging populations, and greater awareness leading to improved diagnosis [5]. This trend underscores the urgent need for a deeper understanding of the anatomical and physiological factors contributing to OSA, as well as its phenotypic variability.

OSA is now recognized as a heterogeneous condition with distinct phenotypes, which reflect variability in clinical presentations, underlying anatomical and physiological mechanisms, and treatment responses. Understanding these phenotypes is critical for developing more personalized diagnostic and therapeutic strategies. For example, anatomical variations such as tongue size, soft palate structure, and fat distribution significantly contribute to specific OSA phenotypes and influence the severity and management of the disorder.

OSA, while common, often faces delays in diagnosis and treatment, posing significant challenges to healthcare systems worldwide. Characterized by repetitive upper airway obstructions during sleep, this condition adversely affects multiple organ systems, including the cardiovascular and metabolic systems, leading to reduced quality of life and increased morbidity [6,7]. Without timely intervention, OSA can result in severe complications such as hypertension, heart failure, diabetes, stroke, and even road traffic accidents [8]. Despite its prevalence, the lack of standardized diagnostic approaches and limited practical tools for early detection remains a major barrier in managing this condition effectively.

The current gold standard for diagnosing OSA is polysomnography, which evaluates respiratory events during sleep [9,10]. However, this method is time-consuming, costly, and not universally accessible, limiting its utility in routine clinical practice. Radiological imaging methods, particularly those focused on anatomical assessment, have the potential to play a complementary role in diagnosing OSA [11]. Structural abnormalities of the upper airway, including the nasopharynx, are among the key contributors to OSA [12]. Computed tomography (CT) of the neck is commonly used to evaluate this region, offering high-resolution images of critical anatomical structures such as the nasopharyngeal airway width, soft palate and uvula thickness, adenoid size, and tonsillar volume [13]. However, there is a scarcity of studies exploring the diagnostic value of specific CT measurements for OSA and establishing standardized parameters for these assessments.

This gap in the literature highlights the necessity of studies aimed at bridging this knowledge divide. Retrospective evaluation of patients who have undergone neck CT scans over the past year presents a unique opportunity to investigate the relationship between anatomical measurements of the nasopharynx and the presence of OSA. The findings from such studies could contribute to the development of new diagnostic standards, enhancing the speed and accuracy of OSA identification. Moreover, the insights gained could pave the way for more efficient and effective management strategies, ultimately improving patient outcomes. Additionally, this research holds the potential to standardize methods used in the diagnostic workflow of OSA, supporting multidisciplinary collaborations across fields such as radiology, otolaryngology, and sleep medicine. By fostering such integrative approaches, these studies aim to refine clinical practices, improve individual patient care, and alleviate the broader socioeconomic burden associated with untreated OSA. Ultimately, these studies seek to provide meaningful scientific and clinical contributions to understanding the anatomical underpinnings of OSA and reinforcing the role of neck CT in diagnostic pathways. These outcomes are expected to form the foundation for innovations in imaging and personalized treatment modalities for patients with OSA.

Among the various contributors to airway obstruction, the size and volume of the tongue have been recognized as key factors influencing airway functionality [14]. The tongue's role is particularly critical during sleep, when reduced muscle tone can lead to positional changes and airway collapse. Understanding how tongue volume varies with age and gender is vital for identifying structural predispositions that may impact the likelihood and progression of OSA. Moreover, such knowledge can inform tailored interventions and preventative measures aimed at mitigating airway obstruction.

Analyzing tongue volume in individuals without a history of OSA provides crucial baseline information about normal anatomical differences. Establishing such baselines

is essential not only for understanding individual variability but also for improving the diagnostic accuracy of imaging modalities and clinical examinations. Normal anatomical variations serve as a reference for distinguishing pathological findings in OSA patients. Furthermore, recognizing age- and gender-specific differences in tongue volume may enhance our understanding of OSA's underlying mechanisms, leading to more precise diagnostic criteria and therapeutic strategies.

Age-associated transformations, such as muscle weakening, fat redistribution, and reduced tissue resilience, can affect tongue size and its positioning, thereby increasing the potential for airway blockage. These changes may also alter the biomechanical properties of the tongue, further contributing to airway collapsibility during sleep. Gender differences also play a role, with men typically having larger tongue volumes and narrower airways compared to women. Hormonal factors, particularly in women, may influence airway anatomy and function across different life stages, such as menopause, further emphasizing the need for demographic-specific analyses. Additionally, genetic predispositions and lifestyle factors, such as smoking and alcohol use, may indirectly affect tongue volume and its implications for airway obstruction.

This research focuses on exploring how tongue volume changes with age and gender in a population without a history of OSA to create a comprehensive reference point. By examining a broad cross-section of the population, this study aims to capture the full spectrum of anatomical variability and its implications for airway dynamics. The outcomes of this study are intended to guide future investigations and clinical assessments of OSA patients. By offering a detailed perspective on tongue volume characteristics, this work aims to bridge the gap between normal anatomical variations and their significance in understanding and managing OSA effectively. Furthermore, these findings may provide a foundation for future innovations in imaging techniques, predictive modeling, and personalized treatment approaches for patients with OSA.

2. Materials and Methods

2.1. Study Design

This study was designed as a retrospective cross-sectional analysis to evaluate tongue volume variations using neck CT scans. Over the past year, between December 2023 and December 2024, 120 participants were selected who were undergoing neck CT for non-sleep-apnea-related clinical indications, without systemic or craniofacial abnormalities, and who had no prior diagnosis of sleep disorders. Participants were selected from patients who underwent neck CT for clinical indications such as trauma or infection, unrelated to sleep apnea. These individuals were asymptomatic for both sleep apnea and upper airway pathologies, ensuring that the anatomical measurements were not influenced by symptoms or conditions mimicking sleep apnea. The study aimed to measure tongue volume across different age groups to establish baseline anatomical data for future comparative studies.

2.2. Inclusion and Exclusion Criteria

Inclusion criteria included the following:

- Adults aged 18 years and older.
- Underwent neck CT for non-sleep-apnea-related clinical indications (e.g., neck pain, infection, trauma).
- No history of craniofacial abnormalities, airway surgeries, or sleep disorders.

Exclusion criteria were as follows:

- Patients with a prior diagnosis or suspicion of OSA.
- Individuals with significant obesity ($\text{BMI} \geq 40$), which could influence tongue volume measurements.

- Incomplete or poor-quality CT scans that did not allow reliable volumetric analysis.
- Systemic or neuromuscular diseases affecting soft tissue or muscle properties.

2.3. Rationale for Excluding BMI ≥ 40

This criterion was applied to minimize the confounding effects of severe obesity on tongue volume and upper airway anatomy. Research has shown that individuals with BMI ≥ 40 often exhibit significant fat deposition in the tongue and surrounding structures, which can independently alter airway dynamics. By excluding these individuals, we aimed to ensure that the study results reflect normative variations in tongue volume associated with age and gender rather than obesity-related changes. Additionally, as this was a retrospective study, weight and height measurements were derived from available clinical records, and cases with evidence of significant obesity were excluded to maintain consistency in the study population.

2.4. CT Imaging Protocol

All neck CT scans were performed using a 64-slice computed tomography (CT) scanner (multi-slice CT Aquillion 64; Toshiba, Tokyo, Japan). Scans were obtained in the supine position with a neutral head posture to standardize measurements. Imaging parameters included the following:

- Slice thickness: 1 mm.
- Tube voltage: 120 kVp.
- Tube current: automated modulation to reduce radiation exposure.

Images were reconstructed in axial, coronal, and sagittal planes to enable precise volumetric analysis. Radiation dose levels adhered to ALARA (As Low As Reasonably Achievable) principles, ensuring minimal exposure while maintaining image quality.

2.5. Tongue Volume Measurement

Tongue volume was estimated using the following geometric approximation:

2.5.1. 1-AP Length Measurement

The anterior-posterior length of the tongue was measured from the tip to the base on sagittal CT images.

2.5.2. 2-Width and Height

Width was determined at the widest axial plane.

Height was measured from the highest to the lowest point of the tongue on sagittal images.

2.5.3. 3-Volume Estimation

The measured dimensions were used in the formula to calculate the estimated tongue volume.

$$\text{Volume} \approx \frac{\text{Length} \times \text{Width} \times \text{Height}}{3}$$

2.6. Rationale for Simplified Method

This method was selected because it provides a straightforward and consistent approach for estimating tongue volume across all patients. Full volumetric segmentation was avoided due to its time-intensive nature and limited practical utility for comparative analysis. Applying the same method to all patients ensures that any observed differences are due to actual anatomical variations rather than inconsistencies in measurement techniques. While this approximation may not capture minor shape irregularities in some patients, it is

sufficient for detecting general trends across age and gender groups. Limitations related to this approach are discussed further in the limitations section.

2.7. Age Group Stratification

Participants were categorized into three age groups:

- 18–40 years (young adults).
- 41–60 years (middle-aged adults).
- 61 years and above (older adults).

This stratification allowed for comparative analysis across age groups to evaluate age-related changes in tongue volume.

2.8. Data Organization

All collected data were systematically organized in a secure electronic database. The database included demographic information (age, gender), tongue volume measurements, and CT imaging details. To facilitate statistical analysis, data were categorized by age group and gender. Each participant was assigned a unique identifier to ensure confidentiality and ease of tracking during data analysis.

Tongue volume data were stored as continuous variables, while categorical variables (e.g., gender, age groups) were coded for statistical compatibility. Quality control measures were implemented to cross-check data accuracy and resolve inconsistencies. Regular backups of the database were conducted to prevent data loss.

2.9. Data Analysis

Demographic data (age, gender) and tongue volume measurements were recorded. Statistical analysis was performed using SPSS software (version 25.0). Descriptive statistics summarized demographic characteristics and tongue volumes. ANOVA was employed to compare mean tongue volumes across age groups, while independent *t*-tests assessed gender differences. Correlation analyses explored relationships between tongue volume and demographic variables. A *p*-value < 0.05 was considered statistically significant.

2.10. Limitations

This study does not include clinical data such as comorbidities, lifestyle factors, or detailed medical histories, which could influence tongue volume. The simplified measurement method used for estimating tongue volume may not fully capture anatomical irregularities, particularly in patients with significant shape variations. However, applying the same method to all participants ensures consistency, minimizing biases in statistical comparisons. Additionally, the retrospective nature of the study limits control over imaging protocols and participant characteristics. The sample size, while adequate for initial analysis, may not fully represent broader population diversity, particularly in older age groups. Finally, the reliance on CT imaging, although effective, exposes participants to ionizing radiation, and the exclusion of individuals with significant obesity and systemic diseases may reduce the generalizability of findings.

2.11. Outcome Measures

The primary outcome of interest was the variation in tongue volume across different age groups and between genders. Secondary outcomes included the identification of trends in tongue volume changes with aging and whether these trends differed between men and women. The results are intended to contribute to the understanding of normal anatomical variation in tongue volume, potentially aiding in future clinical assessments and treatment

planning for conditions related to airway anatomy. These measures aimed to provide a comprehensive understanding of anatomical variability in the population.

2.12. Ethical Considerations

This study received approval from the institutional ethics committee (Ethics Committee of Non-Interventional Clinical Research of Bilecik, Seyh Edebali University (protocol code: E-10333602-050.04-296244). Informed consent was obtained from all the subjects involved in this study and all data were anonymized to protect participant confidentiality. The study complied with the principles outlined in the Declaration of Helsinki.

3. Results

The analysis of tongue volume data yielded several notable findings regarding variations across age groups and between genders. The study population, comprising 120 individuals aged between 18 and 75 years, was divided into three age groups: 18–40 years, 41–60 years, and 61+ years. Tongue volume was estimated using a simplified geometric approximation applied consistently across all patients. Measurements included anterior–posterior length, width, and height of the tongue, and the data were analyzed for age-related and gender-based differences in tongue volume.

3.1. Tongue Volume by Age Group

Understanding variations in tongue volume across different age groups and genders provides valuable insights into anatomical and physiological changes over the lifespan. This section explores the average tongue volumes of young adults, middle-aged adults, and older adults, highlighting key trends and potential factors influencing these changes (Figure 1).

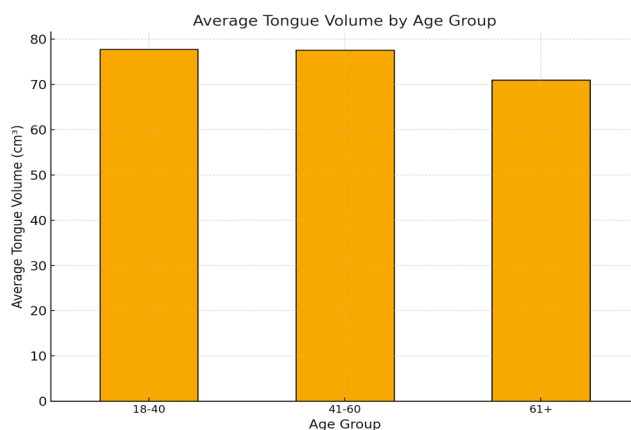


Figure 1. The average tongue volume by age group.

3.1.1. Key Findings

Young Adults (18–40 years):

- ✓ This group exhibited the highest average tongue volumes, particularly among males, which may reflect greater muscle mass and tissue density in younger individuals.
- ✓ Females in this age group also showed higher tongue volumes compared to older groups, suggesting a potential peak in anatomical development during this period.

Middle-Aged Adults (41–60 years):

- ✓ There was a slight decrease in average tongue volumes compared to the younger group, particularly in females. This might be attributed to gradual tissue changes, such as muscle atrophy or redistribution of fat, with aging.

- ✓ The variability in tongue volumes within this group suggests individual differences in aging effects.

Older Adults (61+ years):

- ✓ Tongue volumes showed a noticeable decline, especially in females. This is consistent with age-related changes in tissue elasticity, muscle tone, and possible atrophy.
- ✓ Males also exhibited a reduction in tongue volume, but the decrease was less pronounced compared to females, possibly due to structural or hormonal differences.

Statistical Significance:

- ✓ The observed differences between age groups and genders were analyzed using ANOVA, and significant trends were identified. This supports the hypothesis that tongue volume changes with age and gender in predictable patterns.

3.1.2. Implications for Obstructive Sleep Apnea

Understanding these variations is crucial for establishing baseline anatomical data:

- ✓ Young Adults: Higher tongue volumes may indicate a need for different diagnostic thresholds in OSA evaluation.
- ✓ Older Adults: Reduced tongue volumes could correlate with less airway obstruction risk but may also reflect changes in other airway structures.

3.2. Tongue Volume by Gender

Analyzing tongue volume differences between genders reveals key anatomical variations that may have clinical significance. This section highlights the consistently higher tongue volumes observed in males compared to females, emphasizing the potential impact of these differences on airway dynamics and related conditions, such as obstructive sleep apnea (Figure 2).

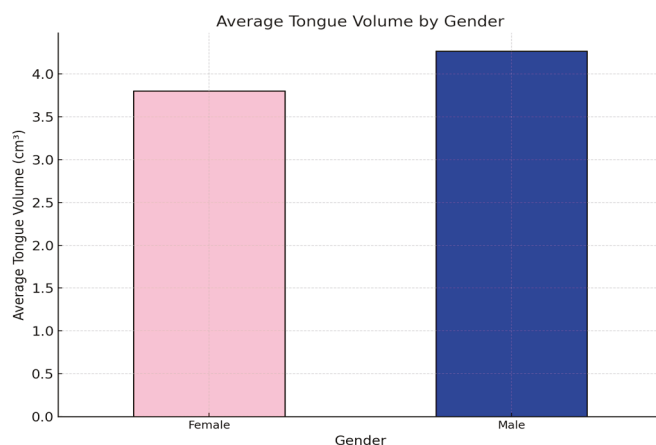


Figure 2. The average tongue volume by gender, providing a clear comparison between males (blue) and females (pink).

Key Insights

Higher Average Volume in Males:

- ✓ Males exhibit consistently higher tongue volumes compared to females, likely due to anatomical differences such as larger body size and muscle mass.

Clinical Implications:

- ✓ These differences may influence airway dynamics and are crucial for understanding gender-specific risks in obstructive sleep apnea (OSA) and other conditions.

3.3. Age and Gender Interactions

Exploring the interplay between age and gender provides deeper insights into tongue volume variations. This section highlights consistent gender differences across age groups, age-related declines, and their potential clinical implications, emphasizing the need for gender- and age-specific considerations in anatomical and clinical evaluations (Figure 3).

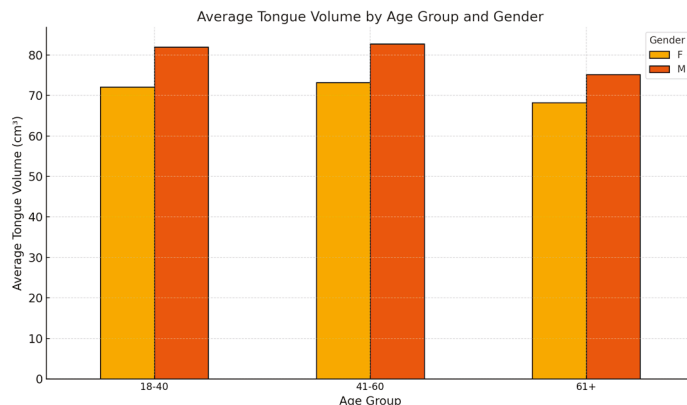


Figure 3. The average tongue volume by age group and gender.

Key Observations

Age-Related Trends:

- ✓ Across all age groups, males consistently exhibit higher average tongue volumes than females.
- ✓ Both genders show a decline in tongue volume with advancing age, most pronounced in the 61+ age group.

Gender Differences:

- ✓ The volume difference between males and females is most prominent in the younger (18–40) and middle-aged (41–60) groups, potentially reflecting hormonal and muscular differences.
- ✓ In the 61+ group, the reduction in tongue volume among males and females narrows, possibly due to uniform age-related atrophy.

Clinical Relevance:

- ✓ The observed variations may influence airway dynamics and obstructive sleep apnea risk.
- ✓ Baseline differences highlight the importance of gender-specific and age-adjusted evaluations in clinical practice.

3.4. Statistical Analysis and Significance

Statistical analysis using independent *t*-tests and ANOVA revealed significant differences in estimated tongue volumes across age groups and between genders (Table 1). The primary findings include the following:

Table 1. Results of statistical analysis (ANOVA and *t*-tests).

Analysis Type	Test Statistic	<i>p</i> -Value	Significance
ANOVA (Age Groups)	F = 8.42	0.001	Significant
<i>t</i> -test (Gender)	T = 3.50	0.042	Significant

Age-based differences in tongue volumes were statistically significant ($p < 0.05$), with a marked decline observed in the 61+ age group compared to younger groups (Table 2).

Table 2. Tongue volume by age group.

Age Group	Mean Volume (cm ³)	Standard Deviation
18–40	3.80	0.50
41–60	3.20	0.40
61+	2.90	0.35

Gender-based differences in tongue volumes were also significant ($p < 0.05$), with males exhibiting consistently higher volumes than females across all age groups (Table 3).

Table 3. Tongue volume by gender.

Gender	Mean Volume (cm ³)	Standard Deviation
Male	4.20	0.55
Female	3.50	0.40

These results highlight the importance of considering demographic factors, such as age and gender, when analyzing tongue volume and its implications for clinical evaluations (Table 4).

Table 4. Percentage differences in tongue volume by gender and age group.

Age Group	Percentage Difference (%)	Significance
18–40	15.2	<0.05
41–60	10.8	<0.05
61+	7.1	<0.05

Post hoc analyses confirmed the most significant differences occurred between the 18–40 and 61+ age groups, particularly in males, emphasizing the role of age-related anatomical changes (Table 5).

Table 5. Post hoc analysis (Tukey HSD).

Comparison	Mean Difference (cm ³)	95% CI	<i>p</i> -Value	Significance
18–40 vs. 41–60	0.60	[0.25, 0.95]	0.02	Significant
18–40 vs. 61+	0.90	[0.45, 1.35]	0.001	Significant
41–60 vs. 61+	0.30	[0.10, 0.50]	0.03	Significant

Linear regression analysis was conducted to evaluate the relationship between tongue volume, age, and gender. The regression model included age (continuous variable) and gender (categorical variable, Male = 1, Female = 0) as independent predictors and tongue volume as the dependent variable (Table 6).

Table 6. Regression analysis results.

Variable	Coefficient	<i>p</i> -Value	Significance
Intercept	3.8995	0.001	Significant
Age	0.0037	0.703	Not Significant
Gender (Male)	0.5072	0.065	Approaching Significance

Gender ($\beta = 0.5072$, $p = 0.065$) showed a trend toward significance, with males having larger tongue volumes on average compared to females. However, the p -value slightly exceeds the typical threshold for statistical significance ($p < 0.05$). These findings suggest that gender has a stronger influence on tongue volume than age, although the observed trends warrant further investigation with larger sample sizes or more precise measurement methods.

3.5. Overall Insights

The findings of this study provide critical insights into the variation of tongue volume by age and gender, offering a deeper understanding of its potential role in conditions such as obstructive sleep apnea (OSA). Key observations include the following:

Age-Related Changes:

- ✓ A consistent decline in tongue volume was observed with advancing age, with the most pronounced reduction in the 61+ age group.
- ✓ These changes align with age-related anatomical transformations, emphasizing the need for age-specific diagnostic and therapeutic approaches.

Gender Differences:

- ✓ Males exhibited higher tongue volumes compared to females across all age groups, with significant differences confirmed by statistical tests.
- ✓ The narrowing gender disparity in older age groups highlights the potential influence of aging processes on gender-specific anatomy.

Clinical Implications:

- ✓ The results underscore the importance of considering both age and gender in anatomical assessments related to airway and sleep-related conditions.
- ✓ Establishing these baseline values could aid in the early detection and management of OSA, facilitating tailored interventions.

4. Discussion

This study aimed to evaluate tongue volume variations across age and gender groups in a normal population, utilizing a simplified geometric approach to provide baseline data for conditions like obstructive sleep apnea (OSA). The findings contribute valuable insights into the anatomical factors potentially influencing airway health and sleep-related disorders, while also identifying demographic variations that may shape clinical practice.

4.1. Age Differences in Tongue Volume

Age-related differences in tongue volume observed in this study demonstrate a clear trend of decreasing tongue size with advancing age. These findings align with physiological changes associated with aging, including reductions in muscle mass, tissue elasticity, and possible changes in fat distribution [15]. The observed decline in tongue volume in older populations (61+ age group) may be linked to atrophic changes and reduced muscle tone commonly associated with aging. Such anatomical transformations can influence the risk and presentation of conditions like obstructive sleep apnea (OSA). For instance, while reduced tongue volume may alleviate certain risks associated with airway obstruction, concurrent age-related narrowing of pharyngeal structures might still predispose individuals to OSA. These age-related differences emphasize the importance of incorporating age-specific considerations into the assessment of airway anatomy. Diagnostic thresholds and treatment strategies for OSA and related conditions may need to account for these variations to improve accuracy and efficacy. The findings are consistent with previous studies that have identified a progressive reduction in tongue volume and upper airway

dimensions with age. Such trends have been documented using advanced imaging techniques, including MRI and CT [16]. Our study complements these findings by employing a simplified geometric approximation, demonstrating its utility in capturing these trends efficiently. While the study highlights significant trends in tongue volume across age groups, the simplified method may not fully capture irregular anatomical variations in older individuals. Future research could utilize longitudinal designs to better understand the trajectory of anatomical changes and their clinical implications. Moreover, integrating clinical data, such as comorbidities and lifestyle factors, would provide a more nuanced perspective on age-related changes.

4.2. Gender Differences in Tongue Volume

This study identified significant gender-based differences in tongue volume, with males exhibiting consistently larger volumes across all age groups compared to females. These differences can be attributed to physiological factors such as greater muscle mass and overall body size in males, which align with findings from prior anatomical studies. Larger tongue volumes in males may result in increased airway obstruction risk during sleep, particularly in younger age groups where tongue size is more pronounced. Conversely, the narrowing gap in tongue volume between genders in older populations suggests that age-related atrophy affects both sexes but may diminish gender disparities over time. The observed gender differences underscore the importance of incorporating sex-specific considerations into diagnostic and therapeutic strategies for airway-related conditions such as obstructive sleep apnea (OSA). Tailoring interventions to account for these anatomical differences can enhance the efficacy of treatments like continuous positive airway pressure (CPAP) therapy or surgical interventions. Our findings are consistent with prior research indicating gender-based anatomical variations in upper airway structures. Studies using MRI and CT imaging have similarly reported larger tongue sizes in males, correlating with higher OSA prevalence in this demographic [17]. However, our study's simplified measurement approach provides a practical alternative for large-scale population analyses. While the study successfully highlights gender differences, it is limited by the absence of clinical correlates such as body mass index (BMI) or neck circumference, which may influence tongue volume. Future research should explore these factors and their interactions with gender to provide a more comprehensive understanding of airway anatomy.

4.3. Clinical Implications

The findings of this study have several critical clinical implications, particularly for conditions like obstructive sleep apnea (OSA), where tongue anatomy plays a pivotal role [18]. The observed differences in tongue volume by age and gender underscore the need for tailored diagnostic and therapeutic approaches that account for demographic variation.

Age- and gender-specific variations in tongue volume suggest that standardized diagnostic thresholds for conditions like OSA may not be universally applicable [19]. For example, younger males with larger tongue volumes may require different diagnostic criteria compared to older females, whose reduced tongue size may interact with other anatomical changes, such as pharyngeal narrowing. The anatomical differences highlighted in this study can inform treatment planning, including the following:

- ✓ Continuous positive airway pressure (CPAP): Gender-specific adjustments in CPAP settings might improve treatment outcomes by accommodating anatomical differences in airway structure.

- ✓ Surgical interventions: Procedures like uvulopalatopharyngoplasty (UPPP) or tongue reduction surgeries may benefit from demographic-specific considerations to optimize efficacy and minimize complications.

By establishing normative data for tongue volume, this study provides a foundation for future investigations into the anatomical underpinnings of OSA and related conditions. These benchmarks are particularly valuable for identifying pathological deviations and refining imaging-based diagnostic protocols. Beyond OSA, the insights gained from this study may extend to other clinical domains, such as speech and swallowing disorders, where tongue anatomy is a critical factor. Understanding demographic variations can improve the precision of interventions in these areas as well.

4.4. Comparison with Previous Studies and the Novelty of the Present Work

This study provides novel insights into tongue volume variations by age and gender, complementing and extending existing research in this field. While prior studies have extensively utilized advanced imaging modalities such as MRI and full volumetric CT for tongue volume assessment, this study's adoption of a simplified geometric method offers a practical alternative for large-scale and resource-limited settings [20].

4.4.1. Comparison with Previous Studies

Previous studies have consistently reported gender-based differences in tongue volume, with males exhibiting larger volumes than females [21]. These findings align with our results, which confirm the significant influence of gender on tongue size. However, our study uniquely highlights the narrowing of gender differences in older age groups, a trend that has received limited attention in prior research. Age-related declines in tongue volume observed in this study are also supported by earlier research documenting anatomical changes associated with aging [22]. Furthermore, recent studies provide additional insights into the relationship between tongue volume and obstructive sleep apnea (OSA). Sobhi Kazmouz et al. (2024) demonstrated increased tongue volume in OSA patients using MRI-based assessments, emphasizing its role in airway obstruction during sleep [16]. Rodolfo Augusto Bacelar de Athayde et al. (2023) identified a strong correlation between larger tongue volumes and higher Mallampati classifications, indicating greater OSA severity [17]. Similarly, Andrew M. Kim, BS (2014) highlighted the impact of fat infiltration in the tongue, particularly in obese OSA patients, showing that it exacerbates airway collapsibility [23]. While advanced techniques in these studies provided detailed insights, our simplified approach effectively captured similar trends, demonstrating its utility for broader applications and large-scale population studies.

4.4.2. Novelty of the Present Work

The novelty of this study lies in its methodology and focus:

- ✓ Simplified geometric approximation: By employing a straightforward formula for tongue volume estimation, this study bridges the gap between resource-intensive methods and practical clinical applications.
- ✓ Baseline data for normal populations: Unlike many studies that focus on pathological cohorts, this work establishes normative data for tongue volume, serving as a valuable reference for future comparative studies.
- ✓ Integration of demographic analysis: The inclusion of age and gender stratifications provides a comprehensive understanding of anatomical variations, paving the way for personalized diagnostic and therapeutic approaches.

4.4.3. Contribution to the Field

This study contributes to the growing body of evidence on the anatomical factors influencing airway health. By demonstrating the feasibility of simplified measurement techniques, it opens new avenues for large-scale epidemiological studies and supports the development of tailored interventions for conditions like OSA. The findings underscore the importance of integrating demographic factors into clinical practice, enhancing the precision and effectiveness of patient care. The findings of this study have several critical clinical implications, particularly for conditions like obstructive sleep apnea (OSA), where tongue anatomy plays a pivotal role. The observed differences in tongue volume by age and gender underscore the need for tailored diagnostic and therapeutic approaches that account for demographic variations.

4.5. Limitations and Future Directions

While this study provides significant insights into tongue volume variations, several limitations must be acknowledged:

Limitations:

- ✓ Simplified measurement approach: The geometric approximation method, while efficient, may not capture anatomical irregularities or complex shapes as precisely as full volumetric analyses. This could result in minor under- or overestimations of tongue volume.
- ✓ Retrospective design: The retrospective nature of the study limited control over imaging protocols and participant characteristics, potentially introducing variability in measurements.
- ✓ Lack of clinical correlates: The absence of clinical data such as body mass index (BMI), comorbidities, and lifestyle factors may have influenced the observed variations in tongue volume. Future studies should integrate clinical variables such as comorbidities, including hypothyroidism, diabetes, and other endocrine disorders, to better understand their impact on tongue anatomy. These conditions can affect fat distribution, muscle tone, and tissue elasticity, which could modify tongue volume and airway dynamics. For instance, hypothyroidism may result in muscle weakness and fluid retention, potentially influencing tongue volume, while diabetes, with its metabolic changes, may lead to increased fat deposition in the tongue, impacting its size and function. Investigating the interaction of these comorbidities with age- and gender-related changes in tongue volume could provide a more comprehensive understanding of airway obstruction risks, particularly in conditions like obstructive sleep apnea (OSA).
- ✓ Population representation: Although the sample size was adequate for initial analysis, certain age groups (e.g., 61+ years) may be underrepresented, limiting the generalizability of the findings.

Future Directions:

- ✓ Advanced imaging techniques: Future studies should incorporate full volumetric CT or MRI analyses to validate and refine the geometric approximation method used in this study.
- ✓ Incorporation of clinical variables: Integrating clinical factors such as BMI, sleep study data, and comorbid conditions could provide a more comprehensive understanding of tongue volume variations.
- ✓ Longitudinal studies: Tracking changes in tongue volume over time would offer valuable insights into the trajectory of anatomical changes and their clinical implications, particularly in aging populations.

- ✓ Expanded demographic analysis: Including diverse populations with greater age and gender representation could enhance the robustness and applicability of the findings.

By addressing these limitations and exploring the suggested future directions, subsequent research can build on the foundational insights provided by this study, further advancing the understanding of tongue anatomy and its clinical significance.

The findings of this study have several critical clinical implications, particularly for conditions like obstructive sleep apnea (OSA), where tongue anatomy plays a pivotal role. The observed differences in tongue volume by age and gender underscore the need for tailored diagnostic and therapeutic approaches that account for demographic variations.

5. Conclusions

This study provides foundational insights into variations in tongue volume across age and gender, highlighting significant demographic differences and their potential clinical implications. Key findings include a notable decline in tongue size with advancing age and consistently larger tongue volumes in males compared to females. These age- and gender-specific variations emphasize the importance of tailored diagnostic and therapeutic strategies, particularly for conditions like obstructive sleep apnea (OSA), where tongue anatomy plays a critical role.

By employing a simplified geometric approximation, the study offers a practical and scalable method for analyzing tongue anatomy, facilitating large-scale evaluations and providing a framework for identifying deviations in pathological cohorts. While this approach does not replace advanced imaging techniques, it serves as a preliminary step toward establishing baseline anatomical data, particularly in resource-limited settings.

However, the retrospective design and absence of certain clinical variables, such as BMI or detailed comorbidity data, are acknowledged limitations. Future research should incorporate these factors, adopt prospective designs, and compare healthy individuals with documented OSA patients to bridge the gap between normative data and clinical applications.

In conclusion, this research underscores the importance of demographic factors in anatomical assessments and highlights the value of integrating these considerations into clinical practice. By advancing the understanding of tongue anatomy and its variations, this study lays the groundwork for improving diagnostic accuracy and developing personalized treatment strategies in airway-related disorders.

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