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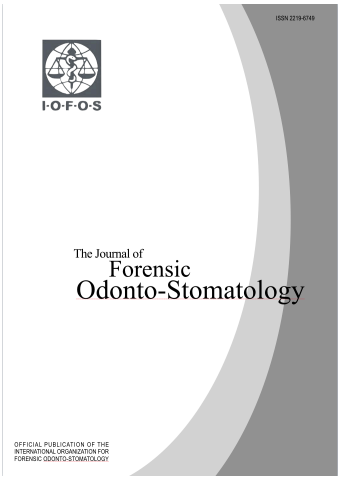

THE JOURNAL OF FORENSIC ODONTO-STOMATOLOGY

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I.O.F.O.S. Recommendations for Quality Assurance: Dental Age Assessment in Living Individuals

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KEYWORDS

Age Estimation, Dental Age Assessment, Living Individuals, Recommendations, IOFOS

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I.O.F.O.S. Recommendations for Quality Assurance: Bone Age Assessment in Living Individuals - Working Group on Dental Age Assessment in Living Individuals:

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IOFOS recommendations reviewed January 2026.

ABSTRACT

Age assessment in living individuals is an important question of legal and humanitarian decision-making when reliable identification documents are unavailable.

The *IOFOS Recommendations for Quality Assurance: Dental Age Assessment in Living Individuals* were developed to provide standardized guidance for forensic dental age assessment and have undergone successive revisions over time in response to scientific, methodological, ethical, and legal developments. The most recent revision, completed in January 2026, reflects updated evidence, accumulated practical experience, and evolving international standards.

These recommendations define minimum requirements for case documentation, clinical and radiological examination, method selection, use of population-appropriate reference data, uncertainty estimation, and reporting practices. Particular emphasis is placed on transparency, quality assurance, protection of individual rights, and the legal implications associated with age thresholds, especially the determination of minority or majority.

The revised recommendations are intended to serve as an international reference framework for dental age assessment in living individuals, to be adapted and integrated into national legal and regulatory systems in accordance with jurisdiction-specific requirements.

1. INTRODUCTION

Assessment of chronological age in living individuals is a critical function in many forensic, legal, and humanitarian contexts, especially when reliable birth documentation is absent or disputed.[1-7] In such cases, dental age assessment serves as one of several methods for human age assessment.[2,3,6-13]

As human biology advances through growth and maturation, the dentition offers measurable and broadly consistent markers that, when properly interpreted, help approximate age with forensic relevance.[9,14-21]

Yet this task is inherently problematic: individual variation in developmental timing, genetic and environmental influences, and population heterogeneity all contribute to uncertainty.[17,20-30] Most published methods will only give a mean or median age and use of and interpretation of statistics are crucial in assessing age.[2,29-36] No method yields a perfect point estimate; rather, appropriate method(s) must be acknowledged and quantified.[3,4,29,34,37-42] Therefore, the practice of dental age assessment must prioritize methodological rigor, transparency about error margins, and a cautious interpretive framework.[3,4,7,35,42-48]

In living individuals, additional constraints arise. Ethical and legal aspects demand that imaging, especially involving ionizing radiation, must be justified and minimized.[7,35,46-48,49-53] The dental examiner must balance the need for information with the principle of "do no harm".[49,52] Moreover, in many jurisdictions, age classification holds legal consequences (e.g. minority vs. majority), so the margins of error carry weighty implications.[3,4,6,48-50] Because in some cases the cost of wrongly classifying a minor as an adult is far greater than the converse.[7,49,52] In other cases of disputed age, the question may be if the person old enough. This issue is not confined to the binary determination of child versus adult.[3,4] Age assessment may also be required in other legally and socially relevant contexts, including school grade placement, eligibility for age-grouped sports, estimation of the age of criminal responsibility, and access to social security rights. In certain sporting disciplines, individuals may intentionally misrepresent their age-often claiming to be older than their true chronological age, as reported in sports such as gymnastics.[6,45,48,53-58]

Several independent dental age estimation markers are available (e.g. stages of tooth formation, root development, pulp/tooth ratios, regressive features), each of which can be applied using validated methods.[9,14-17,59-67] Although more than one marker may be assessed in a given case, there is currently no clear evidence that combining different appropriate dental age estimation methods necessarily improves the accuracy of age assessment.[29,33,34,42,45,68] When multiple markers are used, their contribution should be supported by statistical evidence and transparently reported.[3,33,34] Statistical approaches that explicitly quantify uncertainty, rather than relying solely on deterministic staging, enable the expert to report a most probable age together with associated age range and, where appropriate, minimal-age bounds.[2-4,23,33,34,40,41] This document sets forth the IOFOS Recommendations for Quality Assurance in Dental Age Assessment in Living Individuals.[3,4,42,43] Its purpose is to define baseline methodological standards, ethical safeguards, error quantification practices, and interpretive principles so that dental age assessments in living persons are scientifically defensible, transparent in their limitations, and respectful of individual rights. [3,4,43,48,52,69-71]

2. SCOPE

This document presents updated recommendations of IOFOS for Dental Age Assessment in Living Individuals

3. BACKGROUND

- IOFOS recommendations reviewed July 2018 with advice from: Patrick Thevissen (Belgium) – Coordinator of the Working Group; Ashith Acharya (India), Jannick De Tobel (Belgium), Sigrid I. Kvaal (Norway), Sang-Seob Lee (South Korea), Andreas Schmeling (Germany), Tore Solheim (Norway). [74]

- IOFOS recommendations edited (February 2008) with advice from: Guy Willems (Belgium), K.Mesotten (Belgium), K. Gunst (Belgium), Bernard Knell (Switzerland), Anastasia Mitsea (Greece), Ouvehand (Netherlands), Birgitte Sejrsen (Denmark), Sigrid I. Kvaal (Norway). [73]

4. TERMS AND DEFINITIONS FOR DENTAL AGE ASSESSMENT

4.1 Chronological Age

Actual time elapsed since birth.

4.2 Age assessment (Estimated age)

Is the inferred value (or range) of chronological age derived from biological markers and scientific models when the date of birth is unknown or disputed.

4.3 Age classification

Categories reflecting an individual's relative maturity or developmental stage, used for age assessment purposes.

4.4 Age distribution
Statistical concept describing the pattern of ages within a specific developmental stage.
4.5 Age markers
Physiological features that pass through distinguishable stages, each associated with a specific chronological age range.
4.6 Age range (Age interval)
A continuous interval between two defined age limits, within which an individual's true chronological age is estimated to lie.
4.7 Age assessment database
Information processed to generate reference distributions or percentile data for age estimation.
4.8 Bias
Variance of measured results influenced by human perceptions or systemic factors.
4.9 Biological age
Reflects the level of physiological maturity or development of an individual, inferred from biological markers (e.g. dental, skeletal) relative to normative reference data.
4.10 Blinding
Withholding information that may bias the forensic dental age assessment.
4.11 Dental age
Age inferred from tooth development stages or structural dental changes, used to approximate chronological age.
4.12 Dental age range (interval) assessment
Processes using dental information and relating it to chronological age.
4.13 Dental age assessment technique
Method used for dental age assessment.
4.14 Dental data
Analysis of an individual's dental condition in their current or previously documented state.
4.15 Expression of uncertainty
How the uncertainty around an estimated age is communicated (e.g. probability intervals, confidence bounds, or minimum-age statements).
4.16 Legal age threshold
Age defined by law for a person to acquire specific legal rights and responsibilities.
4.17 Range of uncertainty
The maximum and minimum values of the estimate, given measurement error and variability.
4.18 Maximum probable age
Highest probable value inferred from a particular developmental or regressive stage.
4.19 Minimum probable age
Lowest probable value inferred from a particular developmental or regressive stage.
4.20 Most likely age
Value that best corresponds with available scientific and medical indicators.
4.21 Most probable age interval
Value or range with the highest likelihood of representing an individual's true time since birth, based on biological indicators.
4.22 Probability
A numerical measure of the plausibility that a given biological developmental marker corresponds to a specific chronological age, considering the inherent uncertainty of the assessment.
4.23 Reference population
Group of individuals against which an assessment is compared, often having known developmental data.
4.24 Reported age
The age value declared by the individual.

5. DENTAL PROCEDURES AND STEPS

Note:
The procedures and steps described in this document are recommendations. They may be followed provided that:

1. A procedure or step may be used when it is considered suitable for adoption.
2. A procedure or step should only be used if **(a)** it complies with all applicable local legal and ethical requirements and **(b)** it is supported by the best available scientific evidence.

5.1 Dental Examination Methods

5.1.1
Dental clinical examination must include assessment of dentition status which teeth are clinically present, specific characteristics of certain teeth, according to referred scales: the degree of attrition, the occlusion, the colour of the teeth, the staining and calculus, the periodontal condition, the dental hygiene status and any oral pathology.
5.1.2
Any ionizing imaging must follow the ALARA principle (as low as reasonably achievable), with justification and dose minimization.
5.1.3
The dental imaging must be of sufficient quality and resolution to allow reliable staging of dental developmental markers.
5.1.4
The dental method(s) chosen should be compatible with the individual's developmental

stage (for instance, methods of tooth formation for juveniles and subadults; degenerative structural methods for adult individuals).

5.1.5
When possible, dental methods should be used in conjunction with other biological markers.
Note: Evidence supporting multimodal age assessment remains limited, with few validated approaches (e.g., BioAlder). However, emerging methodologies, including DNA methylation, are expected to further support integrated age assessment frameworks in the future.

5.2 Reference Studies and Validation

5.2.1
The reference studies underlying the dental method(s) must meet rigorous criteria: proven ages in the reference sample, adequate sample size, even age distribution, clear definitions of examined features, statistical measures of scatter and reproducibility.

5.2.2
The expert must check that the method's reference population is appropriate (in terms of ethnicity and sex) relative to the examined individual.

5.2.3
The method must have documented reproducibility (inter- and intra-observer) and have been validated in independent samples.

5.3 Dental Examination Procedure in Individual Case

5.3.1
The identity of the individual or the evidence in question should be established or verified and ideally a photograph should be taken. The ethnicity and given chronological age should be registered.

5.3.2
The following information should be obtained from the individual in question or from the evidence and should be reported: (a) Financial circumstances of the family; (b) History of previous food and water supply; (c) Current or prior systemic diseases; (d) Previous dental problems and treatment; and (e) Dental hygiene.
Note: This information is collected as part of the standard anamnesis; however, there is no scientific evidence that it influences dental development or dental age assessment.

5.3.3
Acquire the dental medical images needed ensuring correct technique and optimal image quality.

5.3.4
Confirm which teeth are visible on the images, note restorations, pathology, attrition, and anomalies compared to the clinical examination.

5.3.5
Apply the dental method(s) to assess developmental or structural change.

5.3.6
The method(s) used should be checked against: (a) The number, origin, age and sex distribution of the subjects included in the reference sample used; (b) The reproducibility of the used parameter registration technique; (c) The scientific soundness of the statistics used; (d) Its/their reproducibility; and (e) The validation of its/their outcomes.

5.3.7
Use methods as originally described in peer reviewed literature.

5.3.8
You may combine different methods.

5.3.9
If more than one dental method is used, combine results judiciously, using a transparent procedure (or expert rationale) when literature lacks a standard combining rule.

5.3.10
The report should make reference to each method used. If procedures to combine the methods used (and related parameters) are not properly described in the scientific literature, they should be performed based on the experts experience and the result should be reported with a detailed explanation.

5.4 Reporting and Interpretation

5.4.1
The expert should be able to advise the appropriate instructing authority or appropriate person/s (assignor/s) regarding the limitations of age assessment and what is possible to achieve.

5.4.2
The expert will report (oral and/or written) the age assessment findings to the assigner. The report should include clinical findings on images (radiographic/tomographic).

5.4.3
The report should contain a minimum age and/or an estimated age plus a measure of its

uncertainty, and (an) answer(s) to the request(s) in the age assessment assignment.
5.4.4
The report must state clearly for each applied dental method: the dental method, reference study used, the observed staging, and how that stage translates into an age estimate (or interval) plus uncertainty.
5.4.5
The report must discuss potential sources of error or bias (e.g. deviation from reference population, observer error, dental pathology) and, where possible, quantify their impact.
5.4.6
Differences or inconsistencies between dental and other age markers must be critically examined and explained.
5.4.7
The expert should interpret the dental age estimate in light of the overall case question (e.g. threshold age) and possibly express the probability that the individual is above or below certain age limits.

5.5 Quality Assurance and Review

5.5.1
Each dental age assessment should ideally be performed by two independent examiners. In case of disagreement(s) a consensus between both examiners should be obtained. In case of written report it should be signed by both examiners.
5.5.2
On randomly selected cases and on a yearly basis (at least) previous age assessment investigation(s) should be re-examined, on a blind basis, by the same investigators. In case of disagreement with the first result(s) the procedure used should be checked and adjusted where necessary.
5.5.3
External peer review (blind) of selected cases should be used to assess adherence to protocol and help refine methods when necessary.

6. EXPERT REPORT STRUCTURE

6.1 Front Page

6.1.1
Case number identification
6.1.2
Referring agency
6.1.3
Name of the expert
6.1.4
Name of the person examined
6.1.5
Place of the examination
6.1.6
Date of the examination

6.2 Page 2 Table of Content

6.3 Content

6.3.1 Abstract

The abstract should be composed at the beginning of the report and offer a concise summary of its contents. It must be brief yet precise, covering the essential elements: background, methods, results, and the associated statistical variation. Because many readers may only review the abstract, it must clearly convey the key findings and uncertainties.

6.3.2 Expert declaration

The expert must present their qualifications (a concise curriculum vitae evidencing education, credentials, and relevant experience). They must also state their impartiality and neutrality, clarifying that they hold no familial, professional, or other relationships that might influence their opinion.
By accepting the role, the expert commits to maintaining confidentiality regarding all personal and case-related information obtained in the course of their work. Additionally, they acknowledge a duty to the court or tribunal to provide an objective and unbiased opinion, rather than to advocate for either party.

6.3.3 Assigment

Under the assignment, the expert's report should present the case, lay out the specific questions to be answered, specify the methods to be used, and document informed consent. These elements should all be clearly integrated into the final report.

6.3.4 Background

In the background section, relevant case information should include details that may already be part of the assignment; the subject's country of origin; any declared chronological age; the subject's medical and dental history. Socioeconomic background may also be included, such financial circumstance of the family, history of previous food and water supply.

Note: This information is collected as part of the standard anamnesis; however, there is no scientific evidence that it influences dental development or dental age assessment.

6.3.5 Materials

For materials, the case to be examined must be presented. As part of that, the expert should verify the individual's identity.
Note: In accordance with applicable national regulations and legal frameworks, photographic documentation of the individual may be obtained for identification and documentation purposes.

6.3.6 Methods

In the methods section, it should be specified what has been done, including a clinical oral examination and radiographic examination (such as a panoramic OPG radiograph and other dental X-rays).
In the oral clinical examination, the expert should note teeth clinically present; specific characteristics of certain teeth, colour according to reference scales: the degree of attrition, the occlusion, the staining and calculus, the periodontal condition, the dental hygiene status and any pathology.
In the radiological examination, the report should document teeth present and absent, any pathology (such as caries, impacted/unerupted teeth, retained roots, and any other pathology), and application of the methods chosen.

6.3.7 Results

In the results section, present findings from both oral examination and radiological examination. For the radiological/tomographic component, report the dental grading counting of pixels or results and corresponding dental age assessment according to the chosen reference method. The report may contain a minimum age and/or an estimated age plus a measure of its uncertainty, and (an) answer(s) to the request(s) in the age assessment assignment.
Note: The content and scope of the reported results depend on the specific request made by the competent authorities and on the applicable national legal and procedural regulations. Reporting an estimate of uncertainty is mandatory.

6.3.8 Discussions/Considerations

In discussing the dental results, the expert should address how long carious teeth needed to be present in the mouth for sufficient time for decay to develop, thereby implying a minimal duration of presence and influencing age interpretation. The presence of significant dental caries may skew estimates, and thus its influence on the age assignment must be critically assessed. Missing teeth must also be considered: whether their absence is due to congenital agenesis or earlier removal, as that will affect which developmental markers remain available. Additional factors (e.g. enamel hypoplasia, restorations, periodontal disease) should also be evaluated with respect to their potential to bias or obscure true developmental signals.

6.3.9 Conclusion

The expert should state: "In my opinion, based on the observed dental and imaging findings, the individual's most probable age falls within the range of [X to Y] years, with a minimum age bound of [Z] years. This estimate accounts for statistical variation and uncertainty inherent to the methods applied". The report also addresses the plausibility of any reported age in light of the developmental evidence and recognizes that the "minimum-age concept" prevents overestimation, thus safeguarding against misclassification of minors.

6.3.9.1 Finalise

In the finalisation of the report, local legal conventions should be considered. The document must include the date, the signature of the expert, and, where required, a counter-signature.

6.4 Writing Report

In writing the report, is recommended to paginate all pages and structure the text into paragraphs with clear headlines and sub-headlines. It is also recommended to use short sentences to enhance readability. The language should be accessible to non-specialists; technical terms must be explained, and a glossary of technical words may be included. There is no universally mandated format, as practices may vary across countries. Wherever possible, two experts should be involved in preparing or reviewing the report to strengthen validity.

7. CONCLUSIONS

In dental age assessment, the expert's report should present a most likely age and/or a minimum age bound and critically examine the plausibility of any declared age. When at least one developmental marker has not reached maturity, this indicates ongoing growth and must be considered in the final interpretation. If the most likely age exceeds a legally relevant threshold (such as 18 years), it may be inferred with reasonable probability that the threshold has been crossed, provided the uncertainties and variation in reference data are rigorously assessed. The adoption of the "minimum-age concept" further ensures that the dental age estimate does not overstate the true age, thereby favouring a conservative approach that protects against misclassifying minors as adults.

8. SUMMARY

Forensic age assessments are often requested by judicial or administrative authorities when a person's true age cannot be confirmed. Experts in forensic odontology field have an obligation to carry out these assessments; refusal without valid justification may result in legal consequences. An age assessment report must clearly present the reliability of the methods employed so that decision-makers (judges, agencies) can properly weigh uncertainties and support the legally safer outcome for the individual when doubts remain.

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I.O.F.O.S. Recommendations for Quality Assurance: Bone Age Assessment in Living Individuals

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ABSTRACT

Age assessment in living individuals is an important question of legal and humanitarian decision-making when reliable identification documents are unavailable.

In January 2026, the International Organization for Forensic Odonto-Stomatology (IOFOS) finalized, for the first time, the IOFOS Recommendations for Quality Assurance: Bone Age Assessment in Living Individuals. This document establishes a harmonized framework for the scientific, ethical, and legal application of bone age assessment in living persons.

The recommendations define minimum standards for the selection and interpretation of bone indicators addressing imaging modalities, reference population requirements, uncertainty reporting, and integration with dental age assessment. Particular emphasis is placed on transparency, protection of individual rights, and the forensic implications of legal age thresholds.

These recommendations are intended to be applied alongside IOFOS Recommendations for Quality Assurance: Dental Age Assessment in Living Individuals and to serve as an international reference framework, to be adapted and incorporated into national legal and regulatory systems according to jurisdiction-specific requirements.

1. INTRODUCTION

Estimating chronological age in living individuals plays a pivotal role in forensic, legal, and humanitarian settings, especially when reliable birth documentation is unavailable or disputed.[1] While dental age assessment has long served as a foundational method, skeletal or bone age assessment offers valuable complementary information, particularly from the hand/wrist bones, clavicular epiphyses, knee and, in some contexts, vertebral maturation. [2-8,10]

Bone age assessment is built on observing predictable ossification and epiphyseal fusion patterns over time, which are influenced by genetic, nutritional, hormonal, and environmental factors.[6-8,11] Classical techniques, such as the Greulich & Pyle atlas and the Tanner-Whitehouse scoring system applied to hand/wrist radiographs, remain central in clinical and forensic practice.[5,6,8,9] Radiographic assessments of the medial clavicular epiphysis (e.g. via CT or high-resolution imaging) are also used for later adolescent ages, since the hand bones may have already matured.[11-18] In certain approaches, vertebral maturation (especially of cervical vertebrae) is evaluated via cephalometric radiographs as a skeletal maturity marker.[19]

Because dentists are trained in interpreting radiographic anatomy and growth of craniofacial structures, they hold a potential role in bone age assessment, provided they are familiar with skeletal imaging, ossification staging, and the anatomical landmarks of the skeletal regions in question.[7,10,20] A dentist with expertise in radiographic evaluation and skeletal biology can integrate dental and skeletal data, thereby enhancing the robustness of age assessment.[2,4,21-28]

This document proposes the IOFOS Recommendations for Bone Age Assessment in Living Persons, preserving the structural framework of the dental age guideline but tailored to skeletal methods (hand/wrist, clavicle, knee, vertebrae).[29,30] It seeks to define minimum methodological standards, ethical safeguards[20], error quantification practices, and interpretative principles, so that bone age assessments in living individuals can be scientifically defensible, transparent in their limitations, and respectful of individual rights. [20,29,31,33]

2. SCOPE

This document presents the first recommendations of IOFOS for Bone Age Assessment in Living Individuals.[29,30]

3. BACKGROUND

In many forensic, clinical, and legal contexts, relying solely on dental indicators may not capture the full picture of an individual's maturational status. Integrating dental information with skeletal (bone) data offers a more complete framework for estimating chronological age.[2-4,21,22-26] This is because ossification and epiphyseal fusion in the hand/wrist, clavicle, knee and vertebral bodies follow biologically informed sequences that complement dental maturation trends.[5,6,8,12,16,17,19,34,36-38]

When skeletal development of the hand or wrist is incomplete, this usually indicates a subadult status.[5,6,8] If the hand skeleton is fully mature, an additional evaluation of the medial clavicular epiphysis may be undertaken, as this structure continues to ossify beyond adolescence.[12-18,34,35] The clavicle provides critical information in the transition between late adolescence and early adulthood, complementing dental findings in determining whether a legal age threshold (e.g., 18 years) has been surpassed. [39-49]

In clinical dentistry, particularly in orthodontics, dentists routinely interpret skeletal maturation indicators (e.g. hand/wrist radiographs, cephalometric vertical growth patterns) to guide treatment timing, predict growth spurts, or assess skeletal stage.[5,50-54] Thus, using such information for bone age assessment in living individuals does not exceed the domain of competence of a trained dentist; rather, it leverages a dimension of expertise already present in dental practice.[39,40,43-45,55-58]

This document proposes to formalize how a dentist, working within a multidisciplinary forensic or medical team, may responsibly incorporate skeletal imaging (such as hand/wrist and clavicle) alongside dental markers to arrive at a combined, justified age assessment without overstepping professional boundaries or ethical constraints. [7,10,20,27,20,59]

4. TERMS AND DEFINITIONS FOR BONE AGE ASSESSMENT

Note: For the purposes of this document, the terms and definitions given in the IOFOS Recommendation's Document for Dental Age Assessment in Living Individuals apply. The following additional terms and definitions are specific to bone age assessment:

4.1 Bone age

Is the age inferred from bone development stages or structural bone changes, through radiographic images, used to approximate chronological age.

4.2 Bone age range (interval) assessment

Processes using bone information and relating it to chronological age.

4.3 Bone age assessment technique

Method used for bone age assessment.

5. PROCEDURES AND STEPS

Note:

1. Please consult the IOFOS Recommendations for Dental Age Assessment for Living Individuals for procedures and steps already specified there; this document adds the bone estimation components not covered in the dental guidance.
2. The use of bone age markers, imaging procedures, and bone assessment methods must comply with the legal and regulatory frameworks of the jurisdiction in question, as requirements for ionizing radiation or imaging in living individuals differ between countries.

The choice of bone assessment methods (for instances such as hand/wrist, knee and clavicle) must be grounded in the dental findings: the developmental status of the dentition guides which bone regions and imaging modalities are most informative in the given case. For the hand/wrist, the Greulich & Pyle atlas approach remains a classical method to compare radiographs of carpal and phalangeal ossification with standard reference plates. [4] For the clavicle, a staging system of medial clavicular epiphyseal fusion can be applied, in CT or high-resolution imaging, to interpret ossification progression corresponding to age thresholds.[15,16] In both domains, the relevant reference studies must be carefully selected, those that document chronological ages associated with each ossification stage, in populations comparable to the subject, to calibrate the bone age assessment meaningfully in relation to the dental age results.

The use of alternative methods or bones must be supported by appropriate reference studies, establishing valid correlations between ossification or morphological markers and chronological age in the relevant population.

6. CONCLUSIONS

In forensic bone age assessment, the expert's report should integrate the findings from skeletal development (e.g., hand, wrist, and clavicle, etc) with dental markers to provide a scientifically justified estimate of chronological age. The assessment must define both the most probable age and/or a minimum age, in accordance with the forensic purpose and national legal or regulatory context.

As in dental age assessment, the minimum-age concept may be considered in some jurisdictions and case contexts; however, its use remains debated. Where applied, it ensures that the reported age does not overestimate the true biological age, thereby safeguarding the individual from being incorrectly classified as an adult. Reference studies appropriate to sex, population, and imaging modality (such as, radiograph, CT, MRI) must be used to justify stage assignment and age inference.

Finally, the interpretation of bone age must always be contextual and integrative, combining skeletal and dental markers. Where there is evidence or a reasonable basis to suspect such effects, variations attributable to population background, socioeconomic factors, or medical conditions shall be considered and documented in the interpretation. The final forensic opinion should provide a transparent statement of uncertainty, explicitly indicating whether the evidence supports or refutes that the individual has surpassed the relevant legal age threshold.

7. SUMMARY

Forensic age assessment is frequently requested by judicial or administrative bodies when an individual's actual age is unknown or disputed. Traditionally, dental age assessment has provided fundamental indicators, but skeletal maturation offers additional, complementary insights, especially through analysis of bone development such as in the hand/wrist, clavicle and knee.

This document presents the first IOFOS Recommendations for Bone Age Assessment in Living Individuals, intended to be used alongside the existing dental age assessment recommendations.

The proposed framework includes methodology for selecting imaging modalities, staging bone maturity, integrating reference studies, documenting uncertainties, and combining skeletal and dental indicators into a coherent final age assessment. Legal, ethical, and radiological constraints must be respected per jurisdiction, and all novel methods must be justified by validated reference studies.

The goal of these recommendations is to ensure bone age assessments in living persons are transparent, scientifically defensible, and compatible with individual rights and legal rigor.

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Assessment of the coming of age using the Caggiano method based on Demirijian stages and the Cameriere method applied to third molars in a Brazilian population

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ABSTRACT

Age estimation can be carried out through dental methods to determine whether an individual has attained legal majority. The aim of the study was to apply the methods proposed by Caggiano et al. (2022) based on Demirjian et al. (1973) stages and Cameriere et al. (2008), to third molars to estimate the legal age of 18 years, through the evaluation of panoramic radiographs in a Brazilian sample. The final sample of this study comprised 270 radiographs, including 188 from females and 82 from males, belonging to individuals aged between 16.00 and 23.99 years. After data collection, the information was organized in Microsoft Excel© spreadsheets and subjected to descriptive statistical analysis. The method proposed by Caggiano et al. (2022) method based on Demirjian et al. (1973) stages, proved to be more sensitive than specific. In contrast, the method proposed by Cameriere et al. (2008) showed the opposite pattern, demonstrating greater specificity than sensitivity. It can be concluded that the results showed moderate accuracy for both methods.

INTRODUCTION

A person's age is directly related to their civil rights and responsibilities.[1] In Brazil, majority is reached upon turning 18 years of age.[2] From that age onward, individuals are eligible to obtain a driver's license, buy and sell property, get married, as well as being legally required to vote.[2] In the criminal context, the age of majority is established at the same age.[3] Therefore, estimating an individual's age is essential to ensure that penal laws and penalties are properly applied to the offender.[3]

In Dentistry, age estimation can be carried out using methods that assess the development and mineralization stages of the third molars.[4-6] The analysis of these teeth is important because, at the end of adolescence, dental development is finished except for these teeth, and that serve as the sole dental age indicator during the transition from adolescence to adulthood.[7]

In the study by Cameriere et al. (2008),[6] the third molar maturity index (I_{3M}) was created, defined as the ratio of the sum of the root apex openings to the total tooth length.[6] If by chance the root development is complete, the numerator is zero. Furthermore, the authors established a cutoff value of 0.08. Thus, a value less than or equal to 0.08 indicates that the individual is 18 years of age or older, whereas a value above this threshold suggests otherwise.[6]

On the other hand, the method proposed by Demirjian et al. (1973)[8] outlined eight stages of dental development. In this method, the seven permanent left mandibular teeth are considered, except for the third molar.[8] The stages are classified using letters (A to H) and range from crown formation to root apex closure.[8] The assessment was performed based on the scores proposed by the TW2[9] method, comparing dental age with skeletal age. Although third molars were excluded from the original study by Demirjian et al. (1973),[8] subsequent studies have used solely these teeth, in combination with the authors' development stages, to determine the attainment of 18 years of age.[7,10] Accordingly, Caggiano et al. (2022)[10] modified the method proposed by Demirjian et al. (1973),[8] establishing that, in the classification of the four third molars, an individual was considered to have reached legal age even when a lower third molar at stage G was associated with an upper third molar at stage H.

The methods proposed by Cameriere et al. (2008)[6] and Demirjian et al. (1973)[8] have been widely tested in different populations.[1,11-13] However, it is still necessary to compare their applicability, given the importance of third molars for estimating civil and criminal majority. Considering the scarcity of studies conducted in the Brazilian population, particularly those addressing adaptations of the method proposed by Demirjian et al. (1973),[8] further research is warranted to assess its applicability in this context. The aim of this study was to apply the methods proposed by Caggiano et al. (2022)[10] based on Demirjian et al. (1973)[8] stages and Cameriere et al. (2008)[6], to third molars in order to estimate the legal age of 18 years, through the evaluation of orthopantomograms (OPGs) in a Brazilian sample.

MATERIALS AND METHODS

This project was submitted to and approved by the Research Ethics Committee of the University of Ribeirão Preto, under CAAE number 45480721,3,0000,5498. This is a cross-sectional study that used a sample obtained from the dental records, in which OPGs of individuals with known age and sex were obtained and analyzed.

The OPGs were obtained from individuals who underwent dental treatment. For the image acquisition, the individual was positioned with an upright spine, feet together, and chin resting on the chin support of the Veraviewepocs 2D© radiographic device (J. Morita Corp., Osaka, Japan). The anterior teeth were positioned in the incisal guide (standard/disoccluded panoramic position), with the head secured between the device's head supports, while the tongue was kept pressed against the palate. The device was adjusted so that the Frankfurt plane was parallel to the ground.

Initially, 951 OPGs from both sexes, with ages ranging from 16.00 to 23.99 years were obtained. The inclusion criteria comprised radiographic examinations of good quality, with minimal distortion, adequate sharpness, appropriate contrast and density, allowing proper visualization of all four third molars for the accurate assessment of their development and mineralization stages. The exclusion criteria were examinations showing impacted or extracted third molars, anomalies, agenesis, endodontic treatments, pathological processes such as odontogenic tumors, extensive carious lesions, and metallic devices that could impair tooth visualization.

After applying these criteria, the final sample reduced to 270 radiographs, 188 from females and 82 from males. Then, the samples were randomized and renamed so that the examiners were blinded to the individuals' information, avoiding cognitive bias in the analysis. ImageJ® software (National Institutes of Health, Bethesda, Maryland, USA) was used in a low-light environment for image analysis.

For the method proposed by Cameriere et al. (2008),[6] a measurement was taken of the width between the internal walls of open apexes, and another measurement of the total tooth length. The first measurement of the internal width of the apices was related to the development process, understood as the sum of the measurements of each open apex or the measurement of a single apex (Fig. 1). The index was calculated as the ratio between the width measurements and the total tooth length, with a cutoff value of 0.08 (Fig. 1). The results were then put in a spreadsheet: values greater than or equal to 0.08 indicated individuals younger than 18 years, and greater values assigned individuals otherwise (18 years old or older).

The same classification system developed by the authors was used For the method proposed by Demirjian et al. (1973),[8] (Fig. 2). However, to

determine the legal age of 18 years, the Caggiano et al. (2022)[10] method was used, which considers all four third molars present in an individual's dental arches. The legal age was assigned to the subject based on the presence of a mandibular third molar in stage G and a maxillary third molar in stage H.[10] The results were coded in the same manner as for the Cameriere et al. (2008)[6] method.

Statistical Analysis

The samples were independently analyzed by two examiners for both methodologies. The data collected were organized in Microsoft Excel© spreadsheets (Microsoft Corp., Redmond, WA, USA) and subjected to descriptive statistical analysis using the same software.

To assess the intra- and inter-examiner reliability, the intraclass correlation coefficient (ICC) was calculated for the Cameriere et al. (2008)[6] method. For the Caggiano et al. (2022)[10] method based on Demirjian et al. (1973)[8] stages, Cohen's Kappa coefficient was used. Agreement analyses were performed on 10% of the total sample (n = 27), with a fifteen-day interval between evaluations.

After the sample analysis, a confusion matrix was constructed to assess the metrics of the methods to distinguish whether individuals were above or below 18 years of age. The accuracy, sensitivity, and specificity were then calculated for each method.

Figure 1. Representation of the application of the Cameriere et al. (2008)[6] method in the sample.

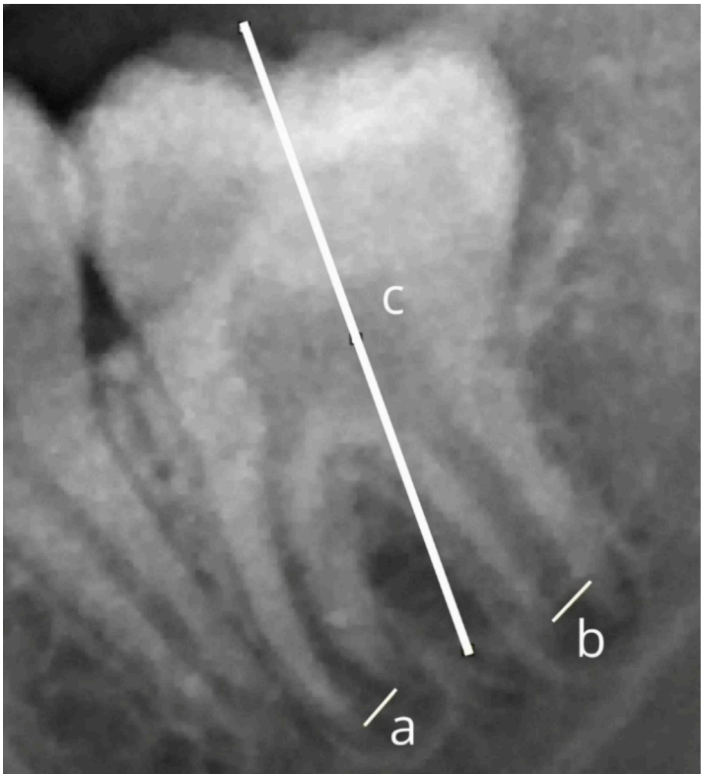
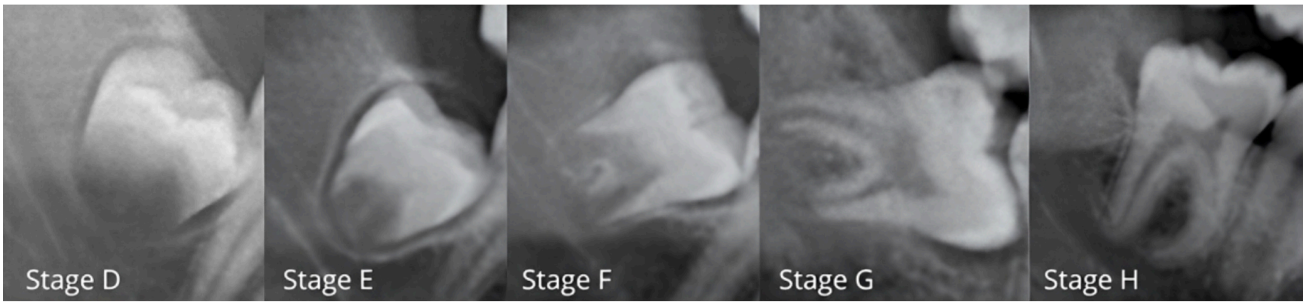


Figure 2. Application of selected Demirjian et al. (1973)[8] stages according to the approach of Caggiano et al. (2022)[10] in the sample.



RESULTS

A total of 270 radiographic examinations were analyzed. Table 1 shows the distribution of the sample according to sex and age group.

For the Cameriere et al. (2008)[6] method, an ICC of 0.95 was obtained for intra-examiner agreement, considered excellent, and 0.76 for inter-examiner agreement, indicating good reliability.[16] For Caggiano et al. (2022)[10] method based on Demirjian et al. (1973)[8] stages, Cohen's Kappa coefficient was calculated for each analyzed tooth. For intra-examiner reliability, the values obtained for the upper third molars were considered substantial (0.65 and 0.66), while for the lower third molars they were considered almost perfect (0.87 and 0.93)[17] (Table 2). On the other hand, the inter-examiner coefficients were considered moderate for the upper left third molar (0.49) and the lower right third molar (0.55), while for the upper right third molar and lower left third molar they were considered substantial (0.65 for both)[17] (Table 3).

The data were organized using confusion matrices for the Cameriere et al. (2008)[6] method (Table 4) and the Caggiano et al. (2022)[10] method based on Demirjian et al. (1973)[8] stages (Table 5). Thus, the Cameriere et al. (2008)[6] method obtained an accuracy of 0.52, a sensitivity of 0.37, and a specificity of 0.78 in the total sample (Table 6). For the Caggiano et al. (2022)[10] method, the total sample obtained an accuracy of 0.65, a sensitivity of 0.76, and a specificity of 0.46 (Table 6).

Table 1. Descriptive statistics of the total sample grouped by sex and age.

Age groups (Years)	Female	Male	Total
16.00 – 16.99	25	12	37
17.00 – 17.99	41	18	59
18.00 – 18.99	25	28	53
19.00 – 19.99	26	6	32
20.00 – 20.99	29	5	34
21.00 – 21.99	19	4	23
22.00 – 22.99	14	4	18
23.00 – 23.99	9	5	14
Total	188	82	270

Table 2. Intra-examiner agreement coefficients obtained using Cohen's Kappa for the Caggiano et al. (2022)[10] method based on Demirjian et al. (1973)[8] stages.

Tooth	Cohen's Kappa Coefficient
18 (upper right third molar)	0.65
28 (upper left third molar)	0,66
38 (lower left third molar)	0.87
48 (lower right third molar)	0,93

Table 3. Inter-examiner agreement coefficients obtained using Cohen's Kappa for the Caggiano et al. (2022)[10] method based on Demirjian et al. (1973)[8] stages.

Tooth	Cohen's Kappa Coefficient
18 (upper right third molar)	0.65
28 (upper left third molar)	0,49
38 (lower left third molar)	0.65
48 (lower right third molar)	0,55

Table 4. Confusion matrix evaluating the ability of the Cameriere et al. (2008)[6] method to distinguish individuals according to the legal age of 18 years.

		Real Age	
		>18 years	<18 years
Estimated Age	>18 years	66	21
	<18 years	108	75

Table 5. Confusion matrix evaluating the ability of the Caggiano et al. (2022)[10] method based on Demirjian et al. (1973)[8] stages to distinguish individuals according to the legal age of 18 years.

		Real Age	
		>18 years	>18 years
Estimated Age	>18 years	133	51
	<18 years	41	45

Table 6. Presentation of the likelihood ratio (LR+), negative predictive value (VP-), positive predictive value (VP+), accuracy (Acc), sensitivity (Sens), and specificity (Spec) for both the Cameriere et al. (2008)[6] and Demirjian et al. (1973)[8] methods.

Method	LR+	VP-	VP+	Acc	Sens	Spec
Cameriere et al.[6] (2008)	1.73	0.4	0.75	0.52	0.37	0.78
Caggiano et al. (2022)[10]	1.43	0.52	0.72	0.65	0.76	0.46

DISCUSSION

The stage classification developed by Demirjian et al. (1973)[8] can be applied to third molars for assessing the legal adulhood.[18] Mincer et al. (1993)[7] demonstrated that the development of upper third molars occurs slightly earlier than that of the lower ones. Conversely, Zandi et al. (2014)[19] found no significant difference in the development of these teeth. In the present study, the application of the Caggiano et al. (2022)[10] method revealed a higher concentration of stage H classifications in the upper third molars compared with the lower ones. Similarly, Gaêta-Araujo et al. (2021)[20] reported that, when the method proposed by Demirjian et al. (1973)[8] was applied to a Brazilian sample, the mean ages corresponding to each stage showed no significant differences between the upper and lower arches. Pinheiro et al. (2023)[21] also observed approximately a 90% agreement between the developmental stages of maxillary and mandibular third molars in the Brazilian population.

In the present study, the Cameriere et al. (2008)[6] method showed excellent intra-examiner agreement and good inter-examiner agreement.[16] These values were not substantially different compared to the study by Sartori et al. (2024),[1] who applied the method to a sample from the southern Brazilian population, obtaining an ICC of 0.92 for intra-examiner and 0.85 for inter-examiner reliability.

On the other hand, for the Caggiano et al. (2022)[10] method based on the Demirjian et al. (1973)[8] stages, intra-examiner agreement was considered substantial for the upper third molars and almost perfect for the lower third molars.[17] Meanwhile, for inter-examiner reliability, the agreement values ranged from moderate to substantial.[17] These values were lower than those reported in other studies. In the study by Caggiano et al. (2022),[10] values of $\kappa = 0.86$ and weighted $\kappa = 0.76$ were obtained for intra- and inter-examiner evaluations, respectively. For Marrero-Ramos et al. (2020),[22] who considered only the "D" and "H" development stages of the Demirjian et al. (1973)[8] method, a weighted Kappa index of 0.78 was reported.

The fact that the inter-examiner agreement ranged from low to moderate for the stages of the Caggiano et al. (2022)[10] method based on Demirjian et al. (1973)[8] stages, did not necessarily imply disagreement regarding the determination of legal adulthood. This is because, overall, the outcome was consistent between the evaluators;

that is, both reached the same conclusion when classifying the individual as either above or below 18 years of age. In such cases, the legal threshold (i.e., whether the age of criminal responsibility has been reached) holds greater relevance for judicial authorities than the variation in the stages assigned by each examiner.

The discrepancies observed among the reported values in different studies may be attributed to variations in the age range of the samples, as younger individuals (9 to 21 years old) allow for a more accurate assessment of third molar developmental stages.[10] In addition, factors such as agenesis, impaction, and the timing of third molar eruption can reduce the reliability of the analysis of this dental element.[10] Furthermore, the morphology of third molar roots may limit visualization, thus requiring greater examiner experience.[23-25] Moreover, biogeographical differences and sample size can directly influence the results, as pointed out by Shi et al. (2024).[26]

After applying the methodologies, it was observed in the present study that using the Demirjian et al. (1973)[8] method in combination with the analysis by Caggiano et al. (2022)[10] for third molars, the total sample showed higher sensitivity than specificity, with respective values of 0.76 and 0.46. Thus, when considering legal adulthood, the method better identifies individuals who are over 18 years of age. The same was observed in the original study by Caggiano et al. (2022),[10] although with a higher sensitivity value of 96.7%.

In the present study, an accuracy of 0.65 was found, whereas Caggiano et al. (2022)[10] reported an accuracy of 90.2%. In the study by Upalananda et al. (2025),[12] although a different methodological approach was used, a similar accuracy of approximately 0.60 was observed for stage H in both sexes and on both the right and left sides.

In contrast, for the Cameriere et al. (2008)[6] method, this study found a specificity of 0.78 in the total sample, indicating a good performance in estimating individuals under 18 years of age. Given that the method is more specific than sensitive, this value was similar to that reported by Sartori et al. (2024),[1] which was 0.84. However, when comparing the specificity results with the study by Cameriere et al. (2008),[6] the value in the original study was higher, at 0.95 although the same condition was observed,. In the forensic context, this means that the method is more reliable for underestimating the individual, which is important considering the civil and criminal implications for an adult individual.[27]

Overall, when analyzing the results obtained in the present study, it can be observed that for both methods the performance was lower than that reported in previous studies in the scientific literature.[10,28-30] This may have occurred due to the need to select all four third molars in the radiographic examination in order to standardize the sample for both methods analyzed. However, it is important to note that the maxillary third molars were used only for the assessment of the Caggiano et al. (2022)[10] method based on the Demirjian et al. (1973)[8] stages.

However, third molars are subject to agenesis, which is related to a human evolutionary processes involving changes in dietary habits and a reduction in jaw size, resulting in decreased functionality of these teeth.[31-33] Moreover, third molars are commonly extracted as they are often misplaced or impacted.[32-34] Therefore, it is important that future studies consider using other teeth, such as second molars, to estimate legal adulthood, as has already been proposed by some authors.[35,36]

The study also analyzed a somewhat uneven sample, both in terms of sex and in the distribution of individuals above and below 18 years of age. As the sample is defined as a representation of a greater population, any hurdles in sample collection directly affect the inferences and results of the study.[37] This is a limitation that should be observed in the present study.

The use of Artificial Intelligence (AI) for age estimation through radiographic analysis has emerged as a promising alternative. Considering its performance comparable to that of human evaluators and advantages such as faster methodological processing and a lower risk of bias.[12,38] Murray et al. (2024)[39] highlighted that convolutional neural networks performed better in age estimation and in pattern recognition when compared with manual methods. Therefore, it is worth emphasizing that investing in AI-based research within forensic fields is essential.[12] However, in diverse contexts, such as regions with limited access to advanced technological resources, techniques, and methods previously applied to other populations should be considered and adapted according to the socioeconomic conditions of each region.[40]

CONCLUSION

The application of the methods proposed by Caggiano et al. (2022)[10], based on Demirjian et al. (1973)[8] stages, and Cameriere et al. (2008)[6] to third molars, using orthopantomograms in a Brazilian sample, showed moderate accuracy in estimating the legal age of 18 years. However, some limitations were observed, particularly related to the distribution of age groups and sex. Future research should explore the use of additional teeth to enhance the accuracy of age estimation in this population.

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Evaluation of post-mortem interval based on gingival tissue hypoxia inducible factor-1α gene expression

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The authors declare that they have no conflict of interest.

KEYWORDS

Post Mortem Interval, Hypoxia Inducible Factor-1, Gene Expression, Histopathological Analysis

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ABSTRACT

Purpose: In the realm of forensic science, the Post Mortem Interval (PMI) is a critical component that determines the time that has passed since the person's physiological death. Although techniques exist to precisely determine the PMI, the results are often unreliable. Hypoxia inducible factor-1 (HIF-1) is a transcriptional factor, and in hypoxic conditions, HIF-1α protein is expressed after proteosomal degradation and ubiquitination pathway involving von Hippel-Lindau protein (pVHL). The aim of the study was to assess HIF-1α mRNA expression in human gingival tissues at different PMIs.

Methods: Gum tissues were collected from cadavers at three definite intervals, namely short PMI (SPMI), medium PMI (MPMI), and long PMI (LPMI). The relative fold change in gene expression of HIF-1α was studied by RT PCR. Histopathological analysis of the tissue samples was done to determine the PMI.

Results: In the case of short PMI (SPMI), the relative fold change in gene expression of HIF-1α is 26.90 ± 23.62. However, in the medium PMI (MPMI) and long PMI (LPMI), the relative fold change in gene expression decreased to 6.32 ± 10.90 and 5.33 ± 8.12, respectively. Histopathological analysis of the post mortem samples revealed less necrosis in SPMI than LPMI. Inflammatory cell infiltration is more in SPMI than MPMI, with their notable absence in LPMI. Ulceration was prominent in LPMI. Destructive vasculitis was visible in SPMI and MPMI. Cystic changes were increased in MPMI and LPMI.

Conclusion: Combined gene expression of HIF-1α and histopathological analysis is a good option for determination of PMI.

INTRODUCTION

Post Mortem Interval (PMI) is one of the crucial factors in the field of forensic science as it determines the time that has elapsed from the physiological death of the person. The estimation of the PMI is considered to be a fundamental subject in the standard forensic practice.[1-3] In current practice, several factors are considered to determine the PMI accurately such as physical, biochemical, physicochemical, entomological, botanical and microbiological investigations.[4] According to the standard forensic practice, short PMI refers to the initial 24-hour period after death and it is evaluated based on the gross

post-mortem physical changes which include the temperature of the body, muscular and neuro-reactivity and livor mortis. However, the longer PMI (several days to years) is estimated depending on the stage of decomposition of the body, growth of the fly larvae (known as entomological analysis) along with the proper assessment of the bone radioisotope concentration.[5] Though these methods are utilized to determine the PMI accurately, there are several problems associated with the existing protocol. As the method gets influenced by the gender, age, pathological and physiological states of the deceased the results are often inaccurate.[6] Therefore, there is a need to develop a certain protocol of measurement based on the time-reliant degradation of the biologically available markers such as DNA or RNA and also the proteins. It was believed initially, that DNA is considered to be much more stable than RNA as it degrades rapidly based on temperature, ribonuclease which is ubiquitously present, growth of microorganisms, along with the environmental factors such as humidity and sunlight.[7] However, during the last few years, several literatures have reported works on RNA which could remain stable for a longer period of time and therefore, the quantification of the degradation of mRNA can be used as an excellent marker for the accurate estimation of PMI.[8] According to the study conducted by researchers Bauer et al., 2003 there is a significant correlation with the degradation of the fatty acid synthase-messenger RNA (FASN mRNA) with the PMI up to 5 days in the autopsy cases.[9] Several studies have also been conducted on the RNA integrity numbers (RIN) and quantitative PCR analysis with several tissues and cell states of the rat and humans. The findings of the study highlighted that β-actin, GAPDH, HIF-1α and 18S rRNA are the potential markers that can be utilized to estimate PMI upto several numbers of days. There are other studies also which pointed out that there is no such correlation between the degradation of RNA and the PMI estimation up to several years.[9, 10]

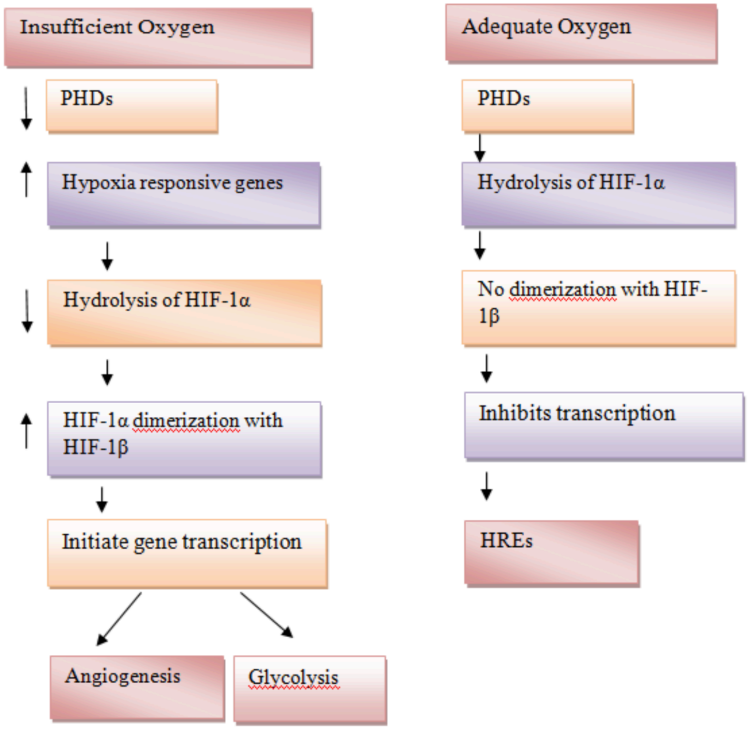
The study conducted by Tu et al.(2018) highlighted the stability of the circular RNAs (circRNAs) and miRNAs in mice models and also suggested certain markers that are suitable for the estimation of PMI miR-133a, miR-122, and 18S in heart tissue, circ-AFF1, LC-Ogdh, and miR-122 in liver and circ-AFF1, miR-133a and LC-LRP6 in skeletal muscle tissue.[7] Additionally, the study of Zhang et al. reported that U6, 18S RNA and GAPDH are considered to be the most accurate PMI markers of human tissues like the heart, kidney, skin, and brain [11]. The sample size of the study was 40 cadavers and the duration of PMI was between 1 to 72 hours. However, the study also emphasized that miRNA cannot be considered to be a stable endogenous control as mRNA because it lacks the cap structure at the 5' end and the 3' poly A tail. One of the most novel approaches in the field of forensic science is the study of the human thanatotranscriptome which determines the time and cause of death.[11] The approach includes all RNA transcripts from the awakened or functional internal parts of the dead body. In relation to this approach, the study of Javan et al. preliminary findings on the thanatotranscriptome study highlighted that RNA remains in a stable state within the internal organs of the cadavers whereas the pro-apoptotic genes such as the caspases gene expression were upregulated and the anti-apoptotic genes *BAG3* and *BCL2* remained downregulated in liver samples of humans.[11,12] As stated earlier, the existing approaches for the measurement of the PMI give controversial and sometimes inconclusive reports, therefore several strategies have been explored by the researchers. In addition to these existing methods, the histological and the immunohistochemical analyses of the varied post mortem tissues have become very important as they provide added insights in determining the PM time span.[13]

The key regulator of the hypoxic environment cellular adaptation is the transcription factor HIF1α protein. HIF-1 is composed of 2 sub units, HIF-1α and HIF-1β, whereas HIF-1α is an oxygen-sensitive subunit. Under hypoxic condition the HIF-1α protein is expressed after proteosomal degradation and ubiquitination pathway involving von Hippel-Lindau protein (pVHL) [14]. After death, the absence of blood flow leads to rapid cellular oxygen deprivation. So to estimate the time since death, the immune histochemical marker should be the one which is absent in physiologic condition and appears with hypoxia.[14] In HIF-1α, there are three domains. Near to the N terminal, there is Basic Helix Loop Helix DNA binding domain. Next to that is the Proline residue, which is necessary for stability of the factor. This domain is also known as Oxygen-Dependent Degradation Domain (ODD).

Oxygen -Dependent Degradation Domain is also the substrate for the enzyme Prolyl Hydroxylase enzyme (PHD). Among all these enzymes, PHD2 is most prominent. Beside it, near the C terminal, there is Arginine residue which regulates transcriptional activity. When PHD acts on Proline residue of HIF-1α under a normoxic condition, an oxygen molecule is utilized in oxidative decarboxylation of α-ketoglutarate (part of TCA cycle).[15] The products are hydroxyl prolyl residue, carbon dioxide and succinate. When both proline residues are decarboxylated, pVHL (Von Hippel Lindau) is attached to HIF-1α. In the next process under a normoxic condition, ubiquitin gets attached to the complex of HIF-1α and pVHL. Consequently, process of ubiquitylation takes place. When ubiquitylation process is complete, the complex is degraded by 26S proteosome[15] (Fig. 1).

On the basis of these observations, the aim of the study was to assess the HIF-1α and HIF-1α mRNA expression in human gingival tissues at different PMIs (Post Mortem Interval).

Figure 1. The fate of HIF-α in presence or absence of oxygen.



MATERIALS AND METHODS

Place of study

The autopsy samples were collected from Department of Forensic Medicine and Toxicology, N.R.S. Medical College & Hospital. The biopsy samples (as control) were collected from the Department of Periodontics, Dr. R. Ahmed Dental College and Hospital, Kolkata. The collected samples were sent to R&D Unit, Department of Biotechnology Heritage Institute of Technology, Kolkata for further processing and molecular biology study.

Period of study

The duration of the study was one year and six months.

Cadaver selection criteria

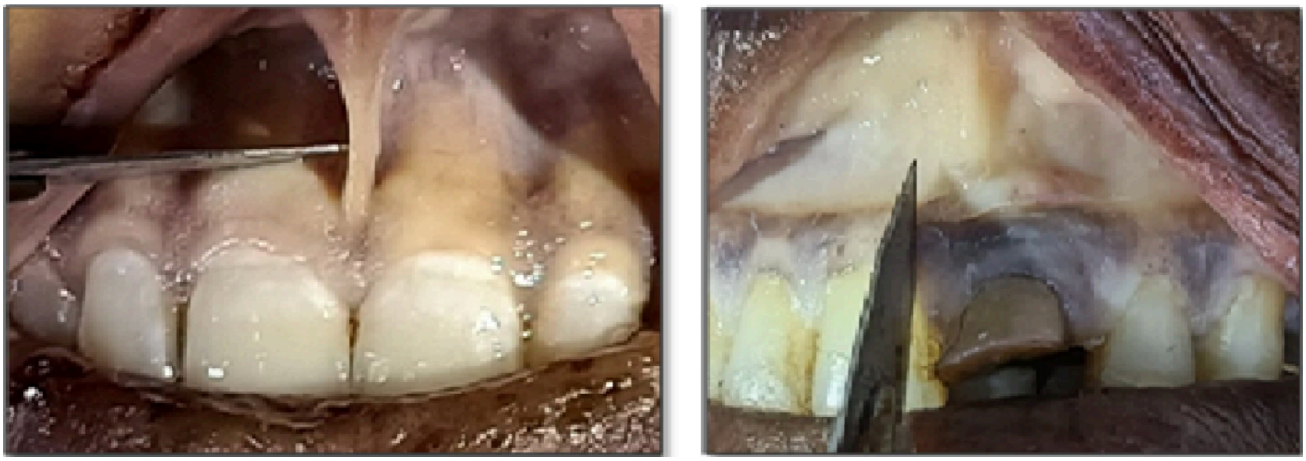
All samples were collected by a qualified dentist (SB) and a forensic expert (SC) from cadavers with no known medical history of periodontitis. Furthermore, only individuals without obvious oropharyngeal pathological lesions were selected. Consequently, cases of periodontitis were excluded, as significant alterations in HIF-1α levels-notably increased levels in diseased periodontal tissues-are typically associated with this condition. Biological changes—such as algor, livor, and rigor mortis, along with early autolysis (SPMI), putrefactive changes (MPMI), and advanced decay or mummification (LPMI)—occur significantly. Therefore, specific inclusion/

exclusion criteria were followed for each group, in addition to the general criteria provided below:
General Inclusion and Exclusion Criteria:
Inclusion Criteria: Cadavers aged 18 to 60 years, with a post-mortem interval ranging from 0 to 216 h.
Exclusion Criteria: Advanced putrefaction; direct, severe damage to the oral cavity, mucosa, or gingiva (e.g., due to acid burns or poisoning); premorbid history of lung-related disorders (e.g., tuberculosis, COPD).
Short Post-Mortem Interval (SPMI):
Inclusion Criteria: Existence of fresh symptoms, such as early secondary flaccidity, rigour in the muscles, and algor mortis. Death from trauma or natural causes does not substantially change the biochemistry of the body right away.[16]
Exclusion Criteria: Extended exposure to extremely high or low temperatures can skew cooling rates and biochemical indicators; prolonged resuscitation or medication therapy can alter early metabolic profiles.[16]
Medium Post-Mortem Interval (MPMI):
Inclusion Criteria: There should be enough soft tissue / insect colonisation (such as blowfly larvae) and obvious putrefactive alterations (such as bloating, marbling, and skin slippage).[16]
Exclusion Criteria: Primary tissue markers which were destroyed by animals; bodies recovered from water.[16]
Long Post-Mortem Interval (LPMI):
Inclusion Criteria: indications of mummification, skeletonization, or severe deterioration.[16]
Exclusion Criteria: insufficient context or environmental information on soil interaction.[16]

Sample grouping
The samples were divided into several groups according to the PMI (Post Mortem Interval, or time since death): The first group had a known PMI ranging from 0 to 72 h (short PMI – SPMI); the second group had a known PMI for 73-120 h (medium PMI–MPMI); the third group had a known PMI ranging from 121-216 h (long PMI – LPMI). Gingiva taken from healthy people during the frenectomy and crown lengthening procedure served as the control sample. There were no patients with gingival inflammation in this control group.

Collection of Gum tissue sample
Samples of gingival tissue of approximate size (0.5 – 1 cm2) were collected with sterile 22 no. surgical bard parker (b.p.) blade from the maxillary gingiva neighbouring to the central incisor at the time of medico-legal autopsies from the cadavers within the age range from 18 years to 60 years (Fig. 2).
All the bodies were stored in a government tertiary care hospital and medical college hospital morgue maintaining standard operating procedures. The storage system was comprised of specialized, refrigerated units designed to maintain body temperatures between 1°C and 5°C for 10 days with digital systems manage, display and monitor the temperature with alarm systems for power failure. Cases were mostly belonging to sudden death group excluding cases mentioned in exclusion criteria. After collection all the tissues were stored in homogenized conditions at -80°C.

Figure 2. Collection of gingival tissue from the cadavers.



Tissue Processing and RNA extraction
The reagent RNA isoplus (TAKARA, USA; Product code: 9108) is used to extract total RNA from the tissue sample that was collected. RNA isoplus makes it simple to extract total RNA from 50–100 mg of tissue sample. RNA isoplus reagent must be used to homogenise the tissue in a mortar and pestle treated with diethyl pyrocarbonate (DEPC) to make it endonuclease free. Chloroform is added to the RNAiso Plus solution after the tissues homogenization and the solution is then carefully mixed and centrifuged to separate it into three layers. Proteins, polysaccharides, fatty acids, cell debris, and a small amount of DNA are all present in the red organic solvent that makes up the lowest layer. A clear liquid containing RNA makes up the top layer, which is followed by a semi-solid containing DNA. An isopropanol precipitation method is used to extract the entire RNA. Following the RNA extraction, it is stored for the night at 4°C to dehydrate.[15]

Synthesis of cDNA and RT PCR study
After overnight dehydration, the dried RNA pellet was mixed with 60µl of de-ionized, nuclease-free water. To dissolve the RNA, it was then incubated in a hot water bath set at 56°C for ten minutes. Ten microlitres of RNA were removed from the solution and placed in a PCR tube in order to measure absorbance and determine the OD260/OD280 ratio. A nano-drop was used to measure the amount of RNA yield of each sample. By comparing the optical density (OD) values of various wavelengths (A260 and A280), the samples' purity was further examined. We determined the amount of water and RNA required to produce 1µg/ml of complementary DNA (c-DNA) using the OD260/OD280 ratio. 4 µL of the reverse transcriptase master mix (BIO-RAD, USA, Cat No. 1708841) was added within each PCR tube, along with calculated amount of RNA and water and it is mixed together in accordance with the kit's instructions. The PCR tubes were then put in PCR (T100 BIORAD, USA) to produce complementary c-DNA. After that, the c-DNAs were stored for RT PCR at -20°C.[15]
The iTaq SYBR green kit (BIO-RAD, USA; Cat No.: 1725121) was utilised to perform gene expression analysis of the gene HIF-1α using the synthesised cDNA in accordance with the comprehensive manufacturer's protocol. The RT-PCR (CFX-96 model, BIO-RAD, USA) was carried out using two microlitres of cDNA combined with eighteen microlitres of PCR master mix.[3] The gene expression of HIF-1α was determined by calculating the relative fold change in the gene expression of the housekeeping gene β-actin using RT-PCR (CFX-96 model, Bio-Rad, USA).[16]

The primer sequences of HIF-1α:
Forward Primer:
TATGAGCCAGAAGAACTTTTAGGC
Reverse Primer:
CACCTCTTTTGGCAAGCATCCTG

The PCR reaction condition followed is given below:
Stage 1: pre-heat: 95 °C for 10 min; Stage 2: Denaturation: 95 °C for 15 sec, Stage 3: Annealing: 60°C for 1 min.
Using RT-PCR (CFX-96 model, Bio-Rad, USA), the relative fold change in gene expression of the housekeeping gene β-actin was used to calculate the gene expression of HIF-1α. Using formula $2^{-(\Delta\Delta Ct)}$, the relative fold change in gene expression was determined. Since $\Delta\Delta Ct = \Delta Ct1 - \Delta Ct2$ in this case, meaning that ΔCt = Ct (target gene) - Ct (reference gene), $\Delta\Delta Ct = \Delta Ct$ (target sample) - ΔCt (Reference Sample). Cycle threshold is denoted here by Ct; because $\Delta\Delta CT$ equals 0 and 2^0 equals 1, the reference sample's gene expression is typically standardized to 1.[17]

Histopathological Study
The tissue samples were processed and stained with Hematoxylin and eosin (H&E) staining. The slides were observed under microscope.

Statistical analysis
The RT PCR analysis was done in triplicates and mean ± SD was calculated of the gene expression. The Statistical analysis was done as nonparametric data, Mann-Whitney U test one tailed (https://www.socscistatistics.com/tests/mannwhitney/) was performed and *p*-values were calculated. To confirm the results, the histopathological examinations were also conducted in duplicate.

RESULTS

RT PCR assay and statistical analysis:
In SPMI group we collected samples from 10 cadavers, similarly samples from 4 cadavers and

samples from 6 cadavers were collected in MPMI and LPMI groups respectively.

The 'Control sample' for the histopathological analysis was gingiva taken from healthy people during the frenectomy and crown lengthening procedure collected instantaneously served as the control set. From the same tissues we have extracted RNA using Trizol and synthesized cDNA and analyzed gene expression of HIF-1α. The gingiva taken from healthy people served as the molecular control to establish the basal expression. The gene expression was calculated using the formula $2^{-(\Delta\Delta Ct)}$, the relative fold change in gene expression was determined. Since ΔΔCt = ΔCt1 - ΔCt2 in this case, meaning that ΔCt = Ct (target gene) - Ct (reference gene), ΔΔCt = ΔCt (target sample) - ΔCt (Reference Sample). Cycle threshold is denoted here by Ct; because ΔΔCT equals 0 and 2^0 equals 1, the reference sample's gene expression is typically standardized to 1.

The relative fold change in gene expression of HIF-1α was calculated against the housekeeping gene β-actin, the relative fold change in HIF-1α gene expression among the control samples is 1 ± 0.32 (Mean ± SD); In case of short PMI (SPMI) the Mean ± SD of relative fold change in gene expression of HIF-1α calculated by $2^{-(\Delta\Delta Ct)}$ is 26.90 ± 23.62. However with the deterioration of tissue state and the unit cellular level as observed in the medium PMI (MPMI) and long PMI (LPMI) the mean relative fold change in gene expression came down to 6.32 ± 10.990 and 5.32 ± 8.12 respectively. Specific availability of cadavers at different time periods following our ethical guidelines and inclusion and exclusion criteria was extremely difficult and due to this samples size was relatively less. However, if we compare the number of cases of only one published paper so far available on this issue, we found they could get total of 10 cases throughout the different time-periods.[23] High standard deviations are common in gene expression studies, requiring the analysis of non-parametric data. This occurs because PCR fold changes in *HIF-1α* gene expression typically follow a log-normal distribution rather than a normal distribution, resulting in a long, skewed tail when plotted on a linear scale. While logarithmic transformation can normalize the data, the high raw variability—ranging from low-fold to over a hundred-thousand-fold changes -means a single high value can skew the mean, leading to a substantial standard deviation compared to the average. Statistical analysis was done as nonparametric data, Mann-Whitney U test (https://www.socscistatistics.com/tests/mannwhitney/) was performed and the *p*-values are given in the Table 1 and Fig. 3.

Table 1: Showing mean, standard deviation (SD), standard error of mean (SEM) of HIF-1α gene expression fold changes along with U, Z, *p*-values between different groups. * Represents distribution of two groups are significantly different, at the 0.05 significance level Null hypothesis is rejected.

In post mortem samples the surface keratin layer is largely absent, there was no hyperplasia, vascularity is indistinct but oedema degeneration and necrosis were markedly increased when compared with control samples (Fig. 4). In SPMI, necrosis is less (Fig 5) and in LPMI necrosis is more (Fig 7). Although inflammatory cells are more in SPMI but it was mild in MPMI (Fig 6) or may be absent in LPMI. Ulceration was extremely prominent in LPMI. Destructive vasculitis was only visible in SPMI and MPMI. Cystic changes were increased in MPMI and LPMI. The changes which usually occur in chronic cases like fibrosis, fibrinous deposits, giant cells, mononuclear inflammatory cells, granulomatous change, metaplastic change and calcification are absent in all intervals.

Table 1. Mean, standard deviation (SD), standard error of mean (SEM) of HIF-1α gene expression fold changes along with Mann-Whitney U, Z, Test Value *p*-values between different groups

Different groups	Mean ± SD ± SEM	Mann-Whitney U Test Value			
	HIF-1α gene expression fold changes	Group comparison	U	Z	p-value
SPMI	26.8934±23.6186±7.4688	SPMI:MPMI	6.00	-1.9799	0.0238*
MPMI	6.3238 ± 10.9907 ± 5.4953	SPMI:LPMI	7.00	-2.4947	0.0063*
LPMI	5.3278±8.1179±3.3141	MPMI:LPMI	6.00	-1.2792	0.1004

Figure 3. Mean of relative fold change in HIF-1α gene expression among the different samples collected at three different PMIs.

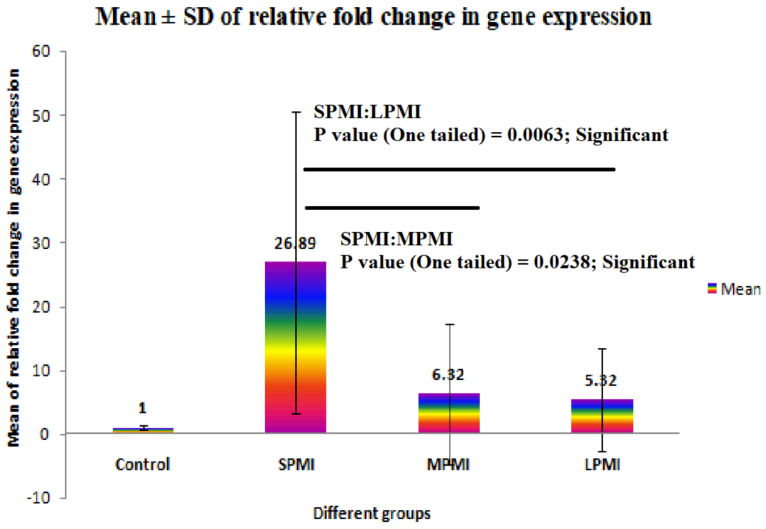


Figure 4. Histopathological Findings - Control Set: Normal (control) gingival tissue with normal keratin layer (a), normal stratified squamous layer (b) (400x), few inflammatory cells (c)(400x) and normal fibrovascular layer (d)(100x).

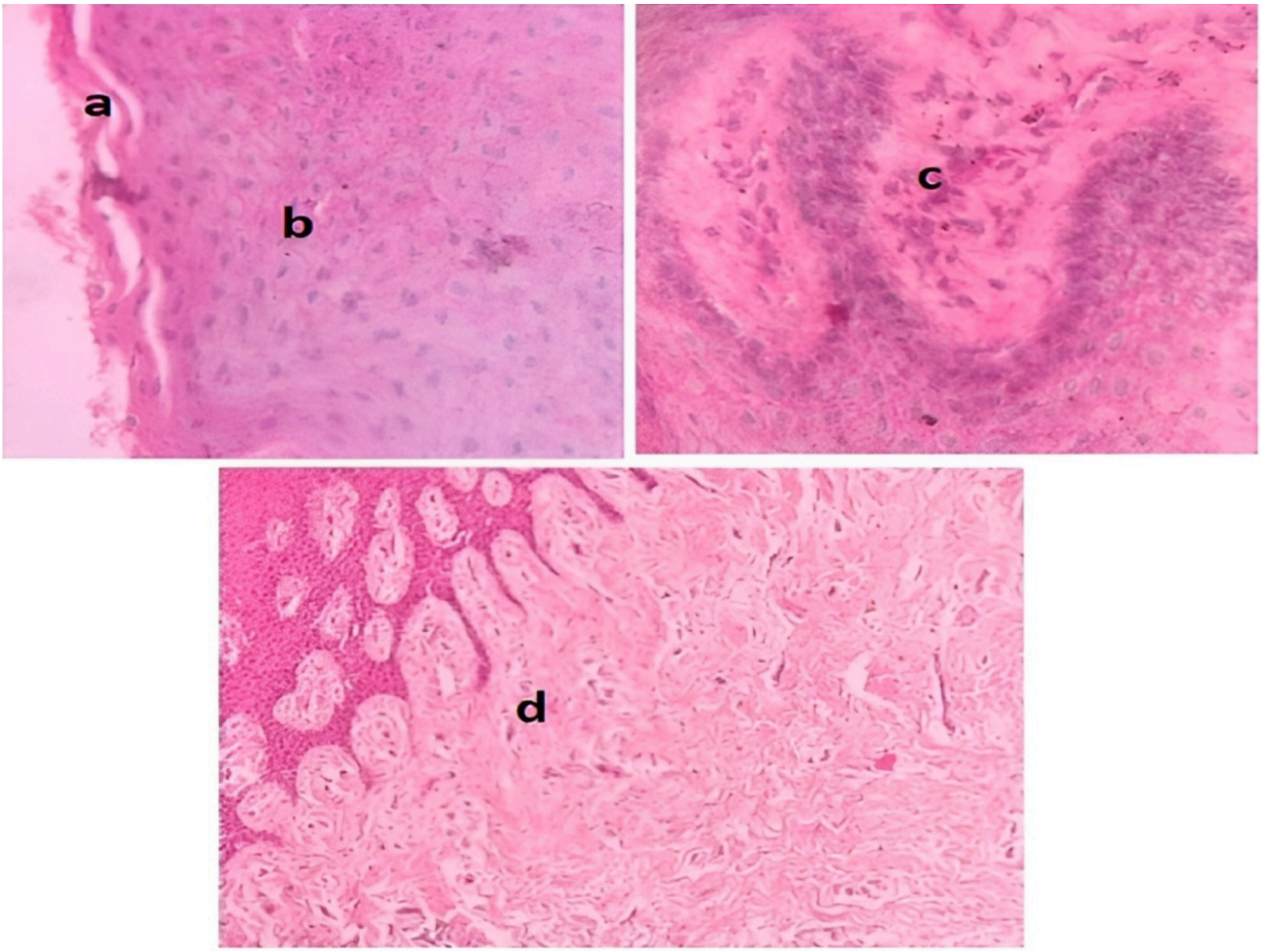


Figure 5. SPMI Group - SPMI: In SPMI, necrosis is less, cell is without nucleus (a), inflammatory cells (b) are more, destructive vasculitis (c) was visible in SPMI.400x

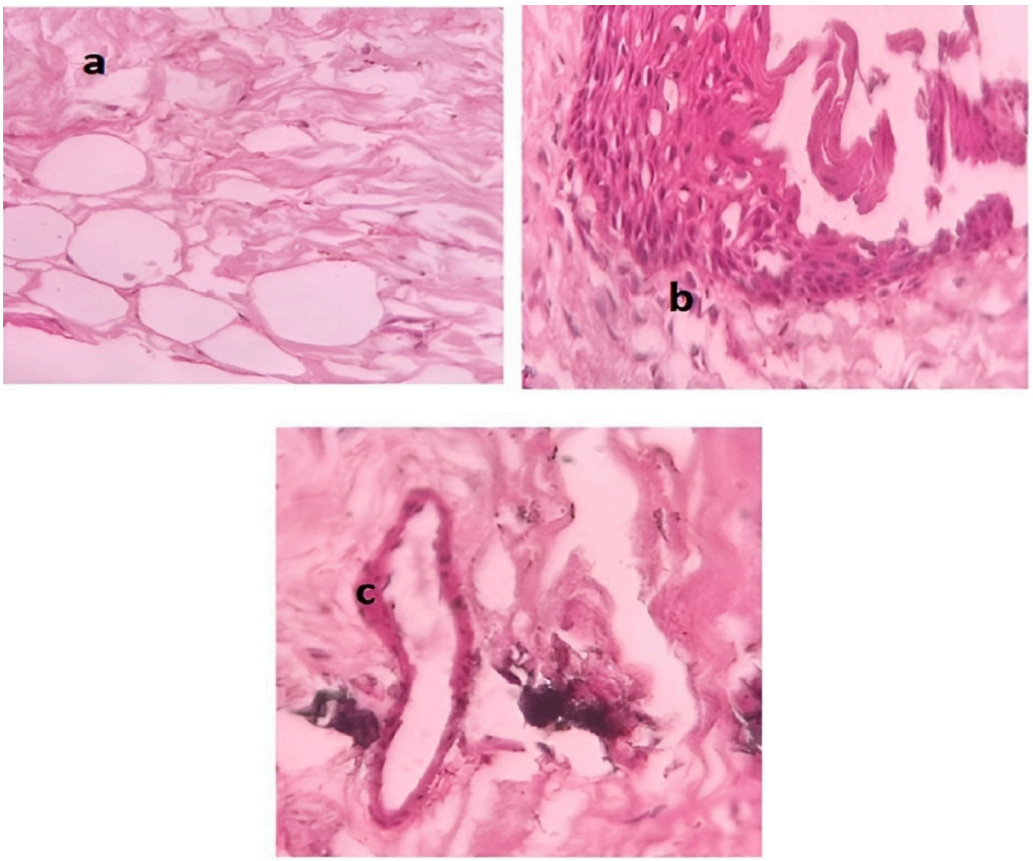


Figure 6. MPMI Group - MPMI: Inflammatory cells (a) was mild in MPMI, destructive vasculitis (b) was also visible in MPMI and cystic changes (c) were increased 400x

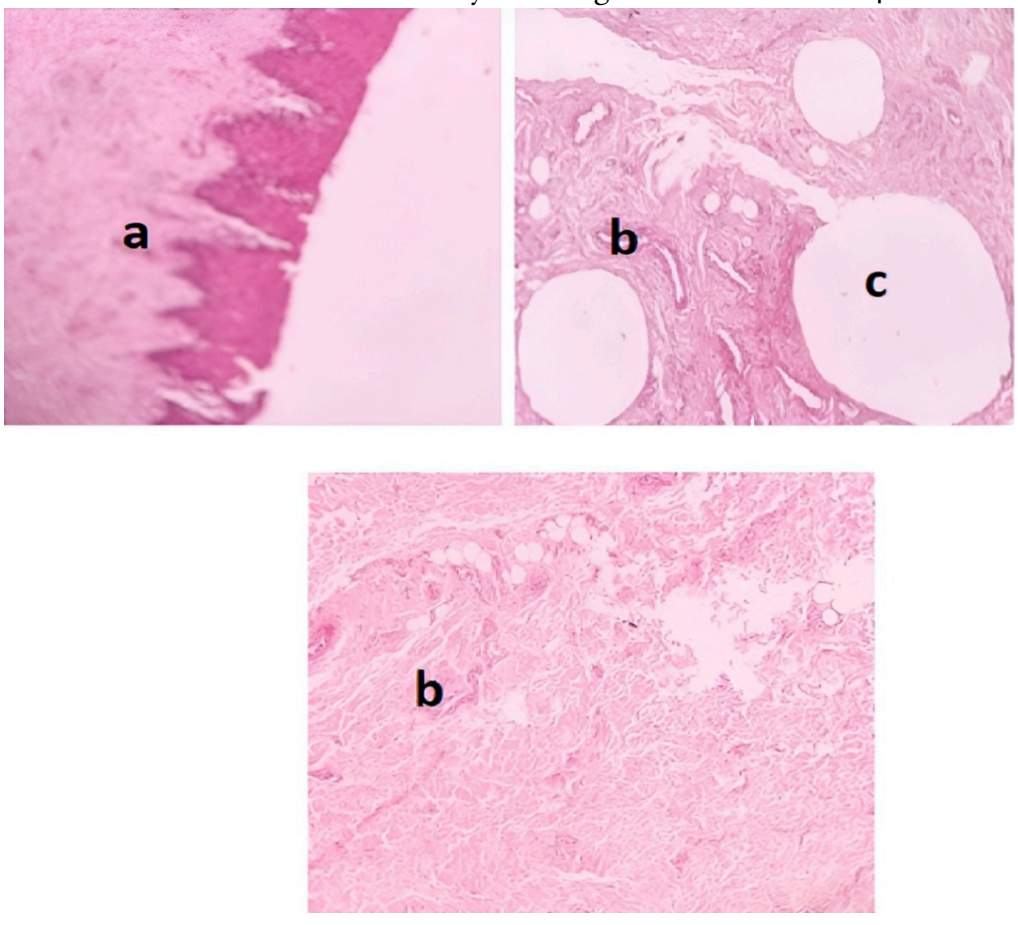
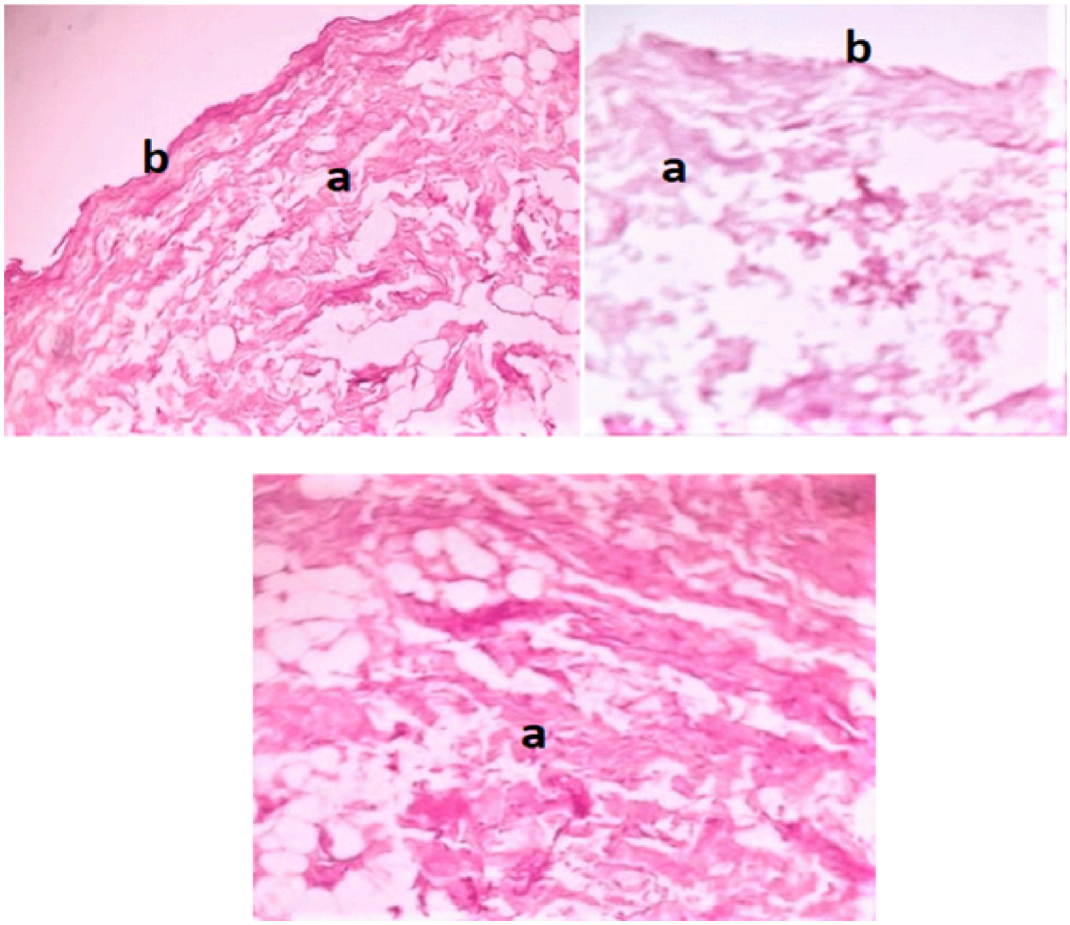


Figure 7. LPMI Group - LPMI: In LPMI necrosis is more (a), inflammatory cells are absent, ulceration (b) was extremely prominent in LPMI. 400x



DISCUSSION

The invention of hypoxia-inducible factor (HIF) in the mid-nineties of the last century by Gregg L. Semenza opened a new horizon of explaining pathophysiology of numerous pathological conditions and physiological adaptation in new dimensions [18]. This not only helped us to understand the pathological basis of a disease but also gave future direction for treatments. HIF was incidentally discovered during investigation on the regulatory mechanism of erythropoietin (EPO) gene which encodes EPO protein to promote erythropoiesis.[18] Hypoxia- inducible factor-1 (HIF-1) is a transcriptional factor of a dimer consisting of two proteins, HIF-1α and HIF-1β.[19] Under normal oxygen concentration (10-30 μ mol), HIF-1α is present in low quantity in THE cytoplasm and HIF-1β is present in high level in the nucleus of a cell. Evidence also suggests that HIF-1α also plays a vital role in the pathogenesis of cancer.[20] With an increase in the size of tumour, the inner part of it lacks perfusion favouring tumour cell death. But once HIF-1α is expressed in the hypoxic core of solid tumours where the partial pressure of oxygen may be less than 10 mm Hg, HIF-1α is not degraded by proteosomal enzymes but a varied number of transcriptional factors like vascular endothelial growth factor (VEGF) is released which not only helps for the survival of the tumour but also facilitates its growth.[20]

In our study, we have walked into a path not traversed by many researchers. Unnatural deaths, homicides, genocides, natural calamities and not to mention the mammoth loss of precious human lives in war necessitate post mortem investigations [21]. Time lapsed from death to the autopsy table, in other words the post mortem interval, is an integral part of post mortem investigations. These include post mortem changes like algor mortis, rigor mortis, livor mortis, nomogram methods, forensic entomology, thanatochemistry and many others. But no single methodology can clearly identify the time since death and it remains as elusive as before.[22, 23]

In 2018, Paolo Fais et al. in their landmark study postulated the gene expression of HIF-1α as a

potential biomarker for the post mortem interval.[24] The study aimed to investigate the immunohistochemical distribution and mRNA expression of hypoxia-inducible factor (HIF-1α) in post mortem gingival tissues to establish a correlation between the presence of HIF-1α and the time since death. They collected gingival tissues from 10 cadavers at different PMIs (1–3 days, 4–5 days and 8–9 days) and 3 controls were processed for immunohistochemistry with quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The results showed a time-dependent correlation of HIF-1α protein and mRNA with different times since death, which suggested that HIF-1α is a potential marker for post mortem interval (PMI) estimation.

In our study, we have taken samples of different known PMI. Among those, 10 samples of short PMI (0-3 days), 4 samples of medium PMI (4-5 days) and 6 samples of long PMI (6-9 days).They were compared with 5 biopsy samples of healthy gingival tissue collected during crown lengthening and gingival depigmentation surgeries in our department. We had been unable to maintain uniformity in sample size in all groups because medium and long PMI cadavers were scarce and patients with healthy gingiva reporting to our department for crown lengthening surgeries were inadequate in the stipulated time frame of our study. Samples of gingival tissue of approximate size (0.5 – 1 cm^2) were collected. The gene expression of HIF-1α was determined by calculating the relative fold change in the gene expression of the housekeeping gene β-actin. The relative fold change in gene expression of HIF-1α was calculated against the housekeeping gene β-actin, because the gene expression of β-actin is constant in all cell types across the organisms and its expression is not subjected to change in any experimental condition. We have done the relative fold change in gene expression of HIF-1α from the gingival tissue. Gingivae were used to investigate HIF-1a and HIF-1a mRNA expression due to the rapid, easy, and non-mutilating nature of the sampling technique. Furthermore, gingivae are typically shielded from labial tissues, reducing the influence of external elements that may cause tissue deterioration to occur quickly. Gingival tissue is therefore the most suitable option for protein and mRNA analysis since it is a more stable area for study. Gingival mRNA and immuno histochemical markers can be successfully analyzed within a range of 1 to 10 days (up to 240 hours), according to research on human cadavers. Signals are highest during the first 3 days (72 hours) and then gradually decline thereafter.[24]

The relative fold change in gene expression of HIF-1α was calculated against the housekeeping gene β-actin, the relative fold change in HIF-1α gene expression among the control samples is 1 ± 0.32 (Mean ± SD); In case of short PMI (SPMI) the Mean ± SD of relative fold change in gene expression of HIF-1α calculated by $2^{-(\Delta\Delta Ct)}$ is 26.90 ± 23.62. However with the deterioration of tissue state and the unit cellular level as observed in the medium PMI (MPMI) and long PMI (LPMI) the mean relative fold change in gene expression came down to 6.32 ± 10.990 and 5.32 ± 8.12 respectively (Fig. 3).

This is because immediately after death the value of HIF-1α gene expression increases due to the swift start of the hypoxic condition throughout the body. The cells within the body are experiencing a severe and sudden hypoxic state which can be considered as the last-ditch effort to adapt to the state of oxygen deprivation. However, with the deterioration both at the tissue and cellular level as observed in the medium PMI (MPMI) and long PMI (LPMI), the mean relative fold change in gene expression came down to 6.32 ± 10.990 and 5.32 ± 8.12 respectively. In the study by Paolo Fais et al**.** (2018), the medium PMI group had the highest elevation of gene expression of HIF-1α but in our study the short PMI group had the highest elevation in gene expression of HIF-1α.[24] Histopathological analysis of the post mortem samples revealed that the surface keratin layer is largely absent, there was no hyperplasia, vascularity is indistinct but oedema degeneration and necrosis were markedly increased. In SPMI, necrosis is less and in LPMI necrosis is more. Inflammatory cells are more in SPMI, mild in MPMI or may be absent in LPMI. Ulceration was extremely prominent in LPMI. Destructive vasculitis was only visible in SPMI and MPMI. Cystic changes were increased in MPMI and LPMI. The changes which usually occur in chronic cases like fibrosis, fibrinous deposits, giant cells, mononuclear inflammatory cells, granulomatous change, metaplastic change and calcification are absent in all intervals (Fig. 4 - 7; Table 2). However, the study has a few limitations. Firstly, the sample size was not

uniform because of the limited availability of samples in the stipulated time frame. Secondly, time frame and economic constraints had caused hindrance in increasing the sample size but the sample was anyway larger than that in the Paolo Fais et al**.** study (2018).

Table 2. Histopathological changes within the gingival tissues of different PMIs (in duplicate)

Sl. No.	Parameters	Con	Con	SPMI	SPMI	MPMI	MPMI	LPMI	LPMI
1	Epithelial keratin	++	++	+	-	-	-	-	-
2	Epithelial hyperplasia	+	+	-	-	-	-	-	-
3	Fibrosis	-	-	-	-	-	-	-	-
4	Vascularity	++	++	+	+	+	+	+	+
5	Oedema	-	-	+++	+++	++++	+++	+++	+++
6	Degenerative changes	-	-	++	+++	++++	+++	+++	+
7	Necrosis	-	-	+	+	+++	++	+++	++++
8	Fibrinous deposits	+	-	-	-	-	-	-	-
9	Inflammatory cells	+	+	++	+++	+	+	+	-
10	Giant cells	-	-	-	-	-	-	-	-
11	Ulceration	-	-	+	+	+	+	++++	++++
12	Large stellate fibroblast	-	-	-	-	-	-	-	-
13	Celullar stroma of mononuclear cells	-	-	-	-	-	-	-	-
14	Destructive vasculitis	-	-	++	++	++	+	-	-
15	Granulomatous change	-	-	-	-	-	-	-	-
16	Cystic change	-	-	+	+	+	+++	+	++
17	Metaplastic change	-	-	-	-	-	-	-	-
18	Calcification	-	-	-	-	-	-	-	-

The high time-dependent post-mortem stability of Hypoxia-Inducible Factor 1-alpha (HIF-1 α) in tissues such as the myocardium and gingiva justifies its inclusion as a marker for short PMI estimation (usually 1–3 days), which permits and frequently enhances the accuracy of conventional forensic techniques.[24] A molecular-level biomarker of the cellular response to post-mortem oxygen deprivation is HIF-1 alpha, whereas classical approaches are frequently impacted by environmental conditions. In the early stages (1–3 days), HIF-1 α gene expression is strongly correlated, observable in gingiva tissue even when outward body indications are unclear. Nonetheless, nomogram-based rectal temperature (mostly the Henssge nomogram) in conjunction with supravital responses and rigour mortis is also regarded as a very effective and trustworthy technique for calculating the PMI in the early post-mortem period, usually within 24 hours.[25] However, there are a few drawbacks; including the fact that its accuracy is impacted by environmental influences (such as fluctuating ambient temperature) and that precise body weight estimation and "cooling conditions" (correction factors) are necessary. After 12 to 15 hours, it becomes less accurate. Although it is subjective and less accurate, rigour mortis is a decent measure of PMI.[25] The "rule of 12" usually applies: it shows up in the first 12 hours, lasts for

12 hours, and then goes away in the following 12 hours.[26] The technique is less accurate than rectal cooling since it is greatly impacted by temperature, metabolic condition, and body muscle mass. It works best when used with other techniques, such AS the nomogram, to validate and refine the projected time range. Tissue responses that continue beyond a person's physical death are known as supravital reactions. For example, skeletal muscle may be electrically or mechanically excitable. During the first 12 to 15 hours after death, they are useful for narrowing down the PMI.[27] Thus, it can be said that the molecular approach to forensic odontology adds value by using Hypoxia-Inducible Factor 1-alpha (HIF-1 α) as a marker for short Post-Mortem Interval (PMI) assessment.

CONCLUSION

In this pilot study HIF-1α gene expression in gingival tissue along with histological changes allows preliminary indication for determination of PMI particularly in SPMI period. The findings are exploratory and require validation using a larger, better-controlled dataset. Future work incorporating larger sample numbers, environmental covariates and quantitative modelling of gene expression decay could substantially enhance the scientific impact and forensic applicability.

Key Points (3 key points)

This study was based on both HIF-1α gene expression in gingival tissue and its histological changes over time for determination of PMI.
Combined HIF-1α gene expression in gingival tissue and its histological changes appears to be a good option for determination of PMI.
These two parameters may be considered for accurate determination of PMI along with other conventional methods.

Authors' Contribution

Author SB carried out the experimental work along with DC. Author DC has written the first draft of the manuscript. Author SB and SC collected the gingival tissue from the cadavers. AR and SD guided the entire work. Author KP gathered the resources for the experimental work.

Funding

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Ethics approval and consent to participate

The study was approved by the Institutional Ethical Committee of Dr. R. Ahmed Dental College & Hospital, Kolkata, India dated 10th April 2023 and the study was completed with complete anonymization of the data in accordance with the Ethical Committee guidelines.

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CBCT assessment of maxillary sinus sexual dimorphism: morphometry and sex-classification performance in Peruvian adults

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KEYWORDS

Maxillary Sinus,
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ABSTRACT

The maxillary sinus exhibits sexual variation that is useful for forensic identification. Cone-beam computed tomography (CBCT) enables precise quantification of linear and volumetric dimensions; however, evidence regarding the most discriminative parameter remains heterogeneous. This study aimed to determine whether CBCT-derived morphometry of the maxillary sinuses discriminates biological sex in adults and to quantify classification performance. We conducted a retrospective observational study and randomly selected 108 CBCT scans from 150 eligible records (age 20–70 years, permanent dentition). Height, width, length, and volume were measured bilaterally from standardized multiplanar reconstructions; normality was assessed and sexes were compared using Student's t test. Males showed larger sinus volumes than females (right: 17.41 ± 1.61 vs 15.38 ± 1.79 cm^3; left: 17.46 ± 1.37 vs 15.86 ± 2.02 cm^3; $p < 0.001$). In side-specific univariate linear discriminant analysis with 10-fold cross-validation, width was the best single predictor: right width AUC = 0.80 (95% CI: 0.72–0.88) and accuracy = 0.72 (95% CI: 0.63–0.79); left width AUC = 0.74 (0.64–0.83) and accuracy = 0.68 (0.59–0.76). In multivariable models using width + age, linear discriminant analysis achieved AUC = 0.771 (95% CI: 0.676–0.858) and accuracy = 0.722 (95% CI: 0.631–0.798), with sensitivity = 0.729 (95% CI: 0.590–0.834) and specificity = 0.717 (95% CI: 0.592–0.815); logistic regression performed similarly (AUC = 0.771; accuracy = 0.713). Conclusions: CBCT-based morphometry of the maxillary sinus discriminates sex with moderate performance; width is the most informative single metric in this cohort. For forensic application, population-specific external validation and multivariable models integrating shape and volume descriptors are recommended.

INTRODUCTION

Sex determination is a core component of the biological profile in human identification because it narrows the search spectrum and guides subsequent stages of forensic analysis. Although the pelvis and skull are the most reliable references, remains are often incomplete or fragmented in the context of disasters, conflicts, or accidents. In this scenario, the paranasal sinuses preserve radiologic features that are useful due to their anatomic robustness and individual variability.[1]

Cone-beam computed tomography (CBCT) provides

reproducible three-dimensional measurements of the maxillary sinus at reduced radiation dose and has become a complementary technique for sex determination and population studies; recent syntheses concur that males tend to present larger dimensions and that classification accuracy is typically moderate when linear and/or volumetric measures are combined in discriminant functions.[2-4] Even so, the evidence is heterogeneous owing to differences in 3D segmentation, software and thresholds, as well as sample composition (age, dentition, skeletal pattern) and population specificity.

Internationally, studies from India, Brazil, Egypt, Turkey, and the United States have shown the usefulness of linear and volumetric dimensions to predict sex, with discrepancies regarding the "best" predictor (height, width, or volume) and accuracies typically in the 70–80% range.[5-8] Craniofacial factors - such as sagittal pattern - modulate sinus volume and may explain part of the variability across studies.[9]

From a forensic perspective, the anatomical uniqueness of the maxillary sinus supports 3D–3D comparison, with calls to standardize protocols and to conduct local validation to ensure transportability.[10-11] Advances in automated CBCT segmentation are beginning to improve inter-center reproducibility, although adoption in forensic series remains incipient.[12-14]

In Latin America, research is more limited, yet consistent results have been reported in countries such as Peru and Colombia; in Peru, recent studies confirm sexual dimorphism with moderate performance.[15] Given the region's ethnic and geographic diversity - which underscores the need to expand databases to ensure representativeness—and the inter- and intrapopulation variability of maxillary sinus dimensions,[2,3] the objective of this study was, using discriminant analysis, to determine whether linear and volumetric morphometric measurements obtained from CBCT of the maxillary sinuses allow sex determination in a Peruvian adult population, thereby contributing evidence that can be integrated into the global map of forensic anthropology.

MATERIALS AND METHOD

Study design and population

Observational, cross-sectional, analytical, retrospective study based on CBCT scans obtained at a single radiology center in Lima. The sampling frame comprised 150 examinations performed between August 2023 and September 2024 at the "Instituto de Diagnóstico Maxilofacial" (IDM). From this population, a sample size of 108 scans was calculated and selected by simple random probabilistic sampling. Inclusion criteria were age 20–70 years and complete permanent dentition; scans were excluded if they showed maxillary sinus pathology, deciduous dentition, or artifacts/defects precluding anatomical assessment. The final sample included 48 males and 60 females.

CBCT acquisition and image processing

CBCT examinations were acquired using a Planmeca ProMax® 3D Plus unit (Planmeca Oy, Helsinki, Finland) and exported in DICOM format. Acquisition and reconstruction parameters were retrieved from DICOM metadata using PointNix RealScan 2.0-CDViewer-3D (PointNix Co., Ltd., Seoul, Republic of Korea), registering 90 kVp, 5 mA, and 31.895 mAs (≈ 6.38 s), with an approximate voxel size of 0.25 mm (250 µm).

Patient data acquisition

Before measurements, sex and age were recorded for each CBCT, and compliance with the eligibility criterion of complete permanent dentition was verified.

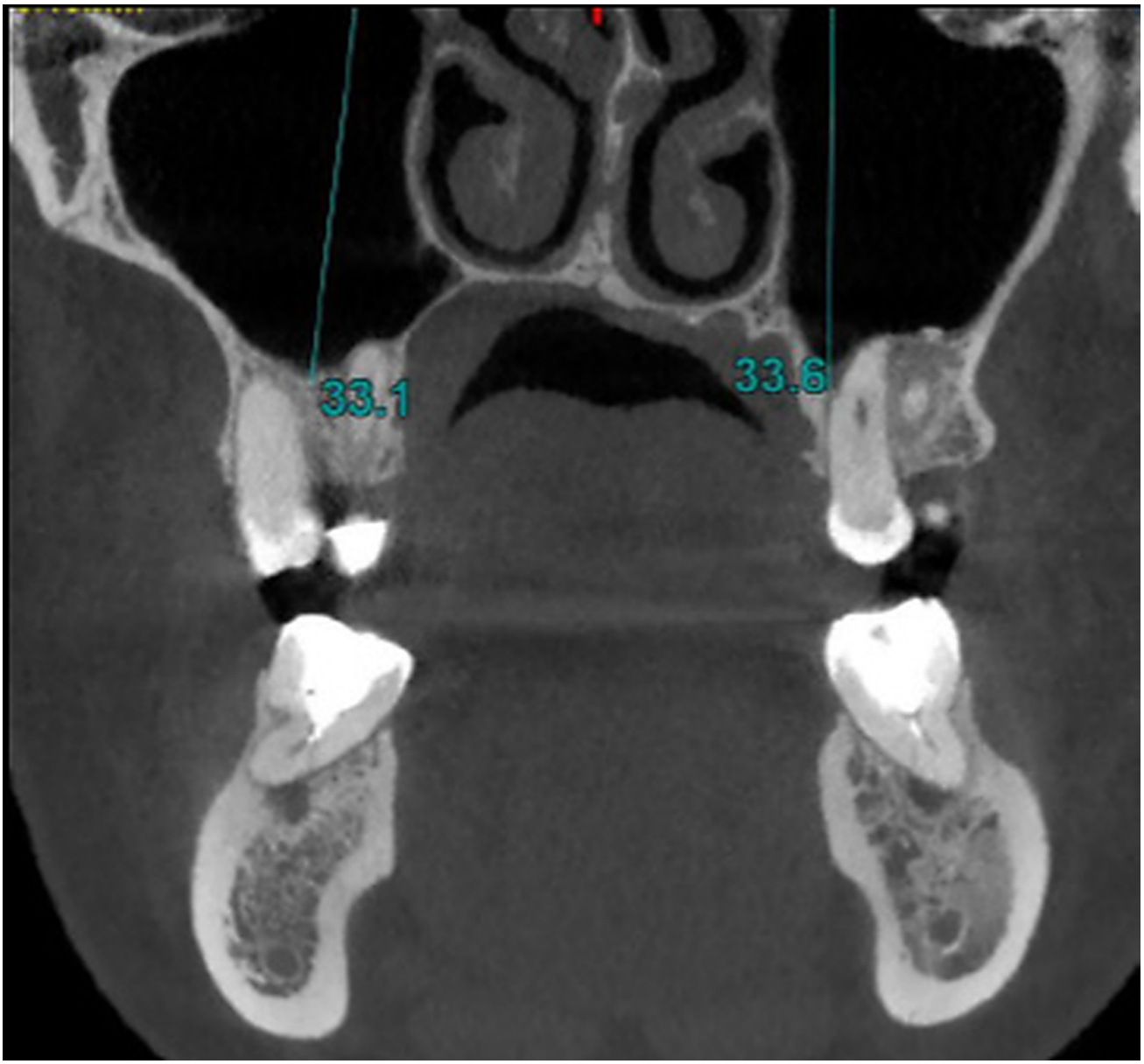
Selection of anatomical landmarks and measurement protocol

Images were analyzed in PointNix RealScan 2.0-CDViewer-3D using multiplanar reconstructions (MPR) in maximum intensity projection (MIP) mode with 2-mm slab thickness. All measurements were performed with the head oriented in a standardized fashion and recorded bilaterally in millimeters. To minimize variability due to slice selection, a standardized rule was applied for all linear measurements. Using MPR views (MIP mode, 2-mm slab thickness), the observer first scrolled through the dataset to identify the axial slice where the maxillary sinus showed its maximum mediolateral extent; width and length were measured on that slice. Height was then measured on the coronal slice that provided the maximum superoinferior dimension between the orbital floor and the sinus floor, aligned to the axis of the greatest axial width. When similar maximum values were observed across adjacent slices, the middle slice among

those candidates was selected to ensure consistency. This slice-selection rule was applied bilaterally and consistently across all scans.

Height was defined as the maximum superoinferior distance from the orbital floor to the floor of the maxillary sinus (see Figure 1).

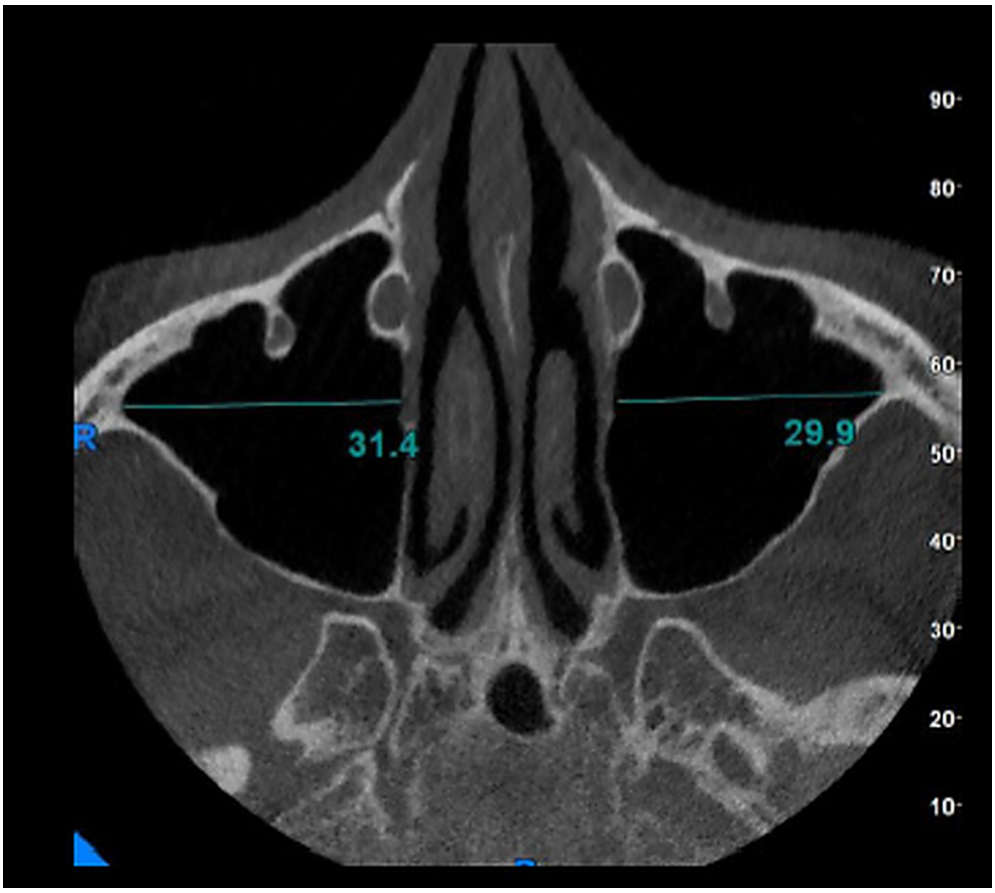
Figure 1. Maximal supero-inferior height of the maxillary sinus on a coronal slice (bilateral measurement, mm). Coronal image obtained after multiplanar reconstructions (MPR) in maximum-intensity projection (MIP) with a 2-mm slab. The lines indicate the maximal supero-inferior distance between the superior and inferior cortical walls of the right and left maxillary sinuses.



Width was defined as the maximum mediolateral distance between the lateral and medial sinus walls, and length as the maximum anteroposterior distance between the anterior and posterior walls, following the protocol described by Urooge and Patil (see Figure 2).

From these linear dimensions, derived variables were calculated using predefined geometric approximations: perimeter = 2×length + 2×width (mm); area = length × width (mm^2); and volume = (length × width × height)/2 (mm^3). Where appropriate, results were expressed in cm or cm^3, preserving traceability to the original measurements in mm.

Figure 2. Maximal mediolateral width of the maxillary sinus on an axial slice (bilateral measurement, mm). Axial image in the plane of greatest mediolateral expansion; the lines show the distance between the medial and lateral walls of each sinus.



Statistical analysis

Data were tabulated and analyzed in R v4.5.1 (R Foundation for Statistical Computing, Vienna, Austria). An independent-samples Student's t test was used to compare sexes. To evaluate classification ability, we applied side-specific univariate linear discriminant analysis (LDA) and, complementarily, multivariable models (LDA and logistic regression) with width and age as predictors. Performance was estimated using stratified 10-fold cross-validation (positive class = male, decision threshold = 0.50), reporting AUC with 95% CIs by bootstrap (1,000 replicates), accuracy, sensitivity, and specificity with Wilson 95% CIs, as well as balanced accuracy and the confusion matrix. ROC analysis was additionally performed using pooled out-of-fold predicted probabilities from the stratified 10-fold cross-validation, and the optimal decision cutoff was determined using the Youden index.

Calibration and reliability

An expert trained the investigator in applying the measurement protocol. Intraobserver reproducibility was assessed using the intraclass correlation coefficient (ICC), yielding high values across all dimensions (ICC 0.862–0.956).

Ethical considerations

The study was conducted with anonymized images under guaranteed confidentiality, with authorization from the Instituto de Imágenes Maxilofacial for secondary data use, and approval from the Institutional Ethics Committee of Universidad Privada Norbert Wiener (Exp. No.: 0822-2023).

RESULTS

Sample characteristics and reliability

A total of 108 CBCT scans were evaluated (48 males, 60 females; 20–70 years). Variable distributions were compatible with normality (Kolmogorov–Smirnov, all $p > 0.05$), so parametric tests were used. Intraobserver reproducibility was high to excellent (ICC = 0.862–0.956, $p < 0.001$), supporting the stability of the measurement protocol.

Between-group comparisons of tomographic measurements

Bilateral maxillary-sinus measurements were systematically larger in males. For perimeter, means (±SD) were 12.60 ± 0.47 cm (right) and 12.60 ± 0.38 cm (left) in males versus 12.07 ± 0.56 cm and 12.20 ± 0.59 cm in females (Student's t test, both $p < 0.001$). For area, males averaged 9.71 ± 0.73 cm^2 (right) and 9.69 ± 0.54 cm^2 (left), whereas females averaged 8.87 ± 0.88 cm^2 and 9.07 ± 0.91 cm^2 (both $p < 0.001$). For volume, differences were larger: 17.41 ± 1.61 vs 15.38 ± 1.79 cm^3 (right) and 17.46 ± 1.37 vs 15.86 ± 2.02 cm^3 (left), corresponding to reductions of ~2.0 cm^3 and ~1.6 cm^3 in females relative to males (both $p < 0.001$). Full estimates and ranges are provided in Tables 1–3.

Table 1. Distribution of maxillary sinus perimeter (cm) from CBCT morphometric analysis in Peruvian adults.

Maxillary sinus	Sex	n	x̄	sd	Min.	Max.	p value*
Right perimeter	M	48	12.6	0.47	11.42	14.1	<0.001
	F	60	12.1	0.56	11.14	14.5	
Left perimeter	M	48	12.6	0.38	11.76	13.6	<0.001
	F	60	12.2	0.59	10.74	14.1	

M: male, F: female, n: sample size, x̄: arithmetic mean, SD: standard deviation, Min.: mínimum, Max.: máximum, p value*: Student's t test.

Table 2. Distribution of maxillary sinus area (cm^2) from CBCT morphometric analysis in Peruvian adults.

Maxillary sinus dimension	Sex	n	x̄	s	Min.	Max.	p value*
Right area	M	48	9.71	0.73	7.61	12.3	<0.001
	F	60	8.87	0.88	7.47	12.9	
Left area	M	48	9.69	0.54	8.4	11.1	<0.001
	F	60	9.07	0.91	7.06	12.2	

M: male, F: female, n: sample size, x̄: arithmetic mean, SD: standard deviation, Min.: mínimum, Max.: máximum, p value*: Student's t test.

Table 3. Distribution of maxillary sinus volume (cm^3) from CBCT morphometric analysis in Peruvian adults.

Maxillary sinus dimension	Sex	n	x̄	s	Min.	Max.	p value*
Right volume	M	48	17.41	1.61	14.04	22.3	<0.001
	F	60	15.38	1.79	12.42	21.2	
Left volume	M	48	17.46	1.37	14.6	20.1	<0.001
	F	60	15.86	2.02	11.22	21.5	

M: male, F: female, n: sample size, x̄: arithmetic mean, SD: standard deviation, Min.: mínimum, Max.: máximum, p value*: Student's t test.

Univariate linear discriminant analysis by dimensión

In side-specific univariate LDA, width was the most discriminative metric. Right width achieved AUC = 0.80 (95% CI: 0.72–0.88) and accuracy = 0.72 (95% CI: 0.63–0.79), with sensitivity = 0.66 (95% CI: 0.52–0.78) and specificity = 0.76 (95% CI: 0.64–0.85); left width showed AUC = 0.74 (0.64–0.83) and accuracy = 0.68 (0.59–0.76). Heights exhibited intermediate performance (AUC = 0.66–0.67; accuracy = 0.67), whereas lengths were the least informative (AUC = 0.62–0.63; accuracy = 0.63–0.64), penalized by sensitivity ≈ 0.50. Between-sex comparisons were significant for all dimensions (Welch's t test: $p < 0.001$ for widths and heights; $p = 0.005$ and $p = 0.022$ for lengths). Overall, widths consistently outperformed heights and lengths and were therefore prioritized as predictors in the multivariable analysis. Details are summarized in Table 4.

Table 4. Side-specific univariate linear discriminant analysis (LDA) of the maxillary sinus on CBCT for sex determination.

Dimension (side)	Right width (mm)	Left width (mm)	Right length (mm)	Left length (mm)	Right height (mm)	Left height (mm)
AUC (95%CI)	0.80 (0.72–0.88)	0.74 (0.64–0.83)	0.63 (0.53–0.75)	0.62 (0.51–0.73)	0.67 (0.57–0.78)	0.66 (0.56–0.76)
Accuracy (95% CI)	0.72 (0.63–0.79)	0.68 (0.59–0.76)	0.63 (0.54–0.72)	0.64 (0.55–0.73)	0.67 (0.58–0.75)	0.67 (0.58–0.75)
Sensitivity (95% CI)	0.66 (0.52–0.78)	0.66 (0.52–0.78)	0.50 (0.36–0.63)	0.50 (0.36–0.63)	0.68 (0.54–0.80)	0.68 (0.54–0.80)
Specificity (95% CI)	0.76 (0.64–0.85)	0.70 (0.57–0.80)	0.75 (0.62–0.84)	0.76 (0.64–0.85)	0.66 (0.54–0.77)	0.66 (0.54–0.77)
TN	46	42	45	46	40	40
FP	14	18	15	14	20	20
FN	16	16	24	24	15	15
TP	32	32	24	24	33	33
p (Welch's t test)	<0.001	<0.001	0.005	0.022	<0.001	< 0.001

AUC: área under the ROC curve, TN: true negatives (females correctly classified), FP: false positives (females misclassified as males), FN: false negatives (males misclassified as females), TP: true positives (males correctly classified).

Performance of multivariable models (logistic regression and linear discriminant analysis) with cross-validation
Using width and age as predictors with cross-validation, both models showed comparable, moderate performance. Linear discriminant analysis (LDA) achieved AUC = 0.771 (95% CI: 0.676–0.858), accuracy = 0.722 (95% CI: 0.631–0.798), sensitivity = 0.729 (95% CI: 0.590–0.834), and specificity = 0.717 (95% CI: 0.592–0.815), with balanced accuracy = 0.723. Logistic regression yielded AUC = 0.771 (95% CI: 0.675–0.858), accuracy = 0.713 (95% CI: 0.621–0.790), sensitivity = 0.729 (95% CI: 0.590–0.834), and specificity = 0.700 (95% CI: 0.575–0.801), with balanced accuracy = 0.715. The confusion matrices were TN/FP/FN/TP = 43/17/13/35 for LDA and 42/18/13/35 for logistic regression. Overall, LDA showed a slight advantage in accuracy, specificity, and balanced accuracy and was therefore considered the reference model (see Table 5).

Table 5. Performance of multivariable models (logistic regression and linear discriminant analysis) to estimate sex from maxillary-sinus CBCT.

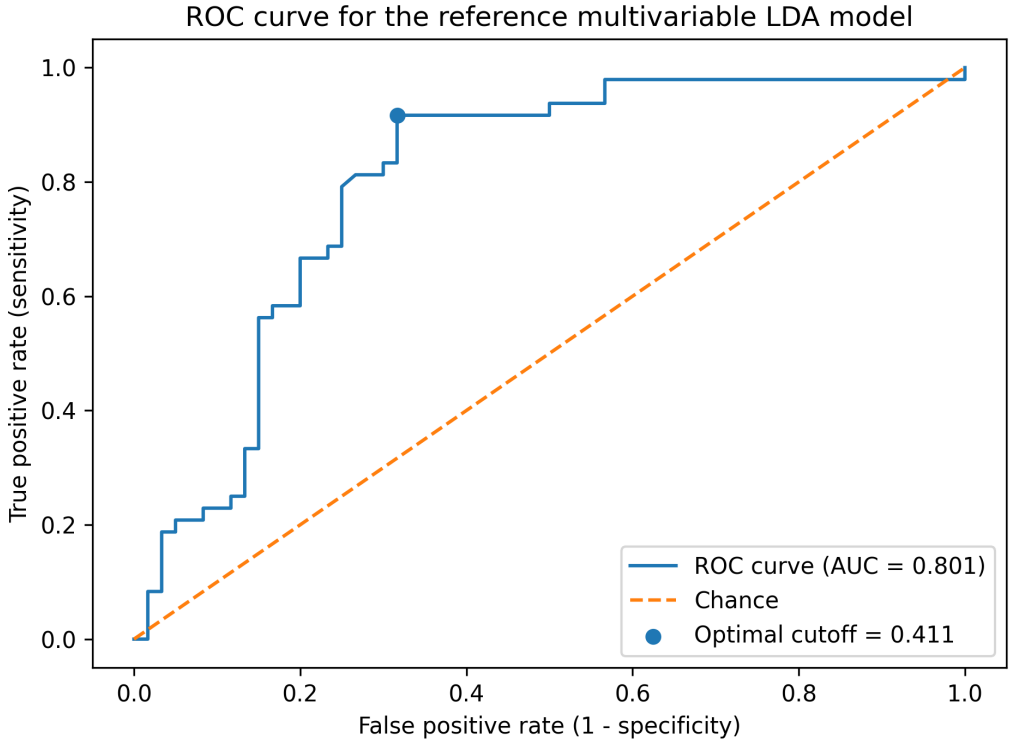
Model	Logistic	Linear
AUC (95%CI)	0.771 (0.675–	0.771 (0.676–
Accuracy (95%	0.713 (0.621–	0.722 (0.631–
Sensitivity (95%	0.729 (0.590–	0.729 (0.590–
Specificity (95%	0.700 (0.575–	0.717 (0.592–
Balanced	0.715	0.723
TN	42	43
FP	18	17
FN	13	13
TP	35	35

AUC: area under the ROC curve, TN: true negatives (females correctly classified), FP: false positives (females misclassified as males), FN: false negatives (males misclassified as females), TP: true positives (males correctly classified), Balanced accuracy = (sensitivity + specificity) / 2.

ROC analysis and optimal cutoff

ROC analysis of the reference multivariable model (linear discriminant analysis using right maxillary-sinus width and age) yielded an AUC of 0.801 based on pooled out-of-fold predicted probabilities from the stratified 10-fold cross-validation. The optimal decision threshold for sex classification (positive class = male), defined by the Youden index, was 0.411, corresponding to a sensitivity of 0.917 and a specificity of 0.683 (Figure 3).

Figure 3. ROC curve for sex determination using the reference multivariable model (linear discriminant analysis with right maxillary-sinus width and age; positive class = male). AUC = 0.801 (stratified 10-fold cross-validation, pooled out-of-fold probabilities). Optimal cutoff (Youden) = 0.411 (sensitivity 0.917, specificity 0.683).



DISCUSSION

In this study, we demonstrated sexual dimorphism of the maxillary sinus on CBCT, with statistically significant differences across all dimensions and moderate performance of univariate classifiers; in this cohort, width was the best predictor. Indeed, in side-specific univariate discriminant analysis with cross-validation, right width reached an AUC of 0.80 and accuracy of 0.72, outperforming heights and lengths; and in the multivariable analysis (width + age), linear discriminant analysis yielded an AUC of 0.77 and accuracy of 0.72, with performance comparable to logistic regression. This pattern is consistent with recent reviews supporting the forensic utility of the maxillary sinus and the dependence of performance on population, parameter, and protocol.[2-4] Our results also align with reports from India and Brazil and exceed the accuracy reported by previous studies in Peru, reinforcing the value of three-dimensional CBCT for this purpose. The reliability of linear CBCT measurements described in the literature supports the use of these metrics in applied research.[16] In contemporary series, the "most informative" metric varies - height, width, or volume - with typical accuracies of 60–80% in simple analyses.[17,18]

Part of the heterogeneity among studies is attributable to clinical factors that modify pneumatization (e.g., tooth loss, age), thereby affecting morphometry and its anatomic context.[19] Methodologically, cross-validation tends to reduce the optimism of apparent classification percentages, which helps explain differences with series that do not apply validation procedures, even when the ranking of metrics - with the predominance of width - remains stable. Segmentation choices and

acquisition parameters also influence estimates; comparisons between automated and manual/semi-automatic segmentation show operational advantages of the former.[20] In parallel, craniofacial determinants - such as sagittal pattern and cranio-maxillary relationships - modulate sinus volume and may account for population-level discrepancies.[21] AI-based standardization (U-Net/nnU-Net) reduces operator variability and improves pipeline traceability,[22-24] while 3D models of the paranasal sinuses offer a more robust framework for identification than isolated linear descriptors.[23] In the same vein, in forensic odontology, sinus metrics should be employed as auxiliary indicators, prioritizing externally validated multivariable models and reporting uncertainty with explicit decision thresholds.[2-4,23] Population differences have also been documented (e.g., U.S. series with 3D-CT),[24] as well as effects of age/tooth loss[24] and facial asymmetry.[25] Inter-sinus integration (frontal/sphenoidal) is an additional avenue to strengthen estimation,[26] and nasal septal deviation may act as a relevant anatomic covariate.[27]

This study contributes three elements: an explicitly three-dimensional methodological approach; the quantification of performance with cross-validation and confidence intervals in both univariate and multivariable settings; and the inclusion of an urban Peruvian population that complements high-Andean series, thereby expanding the Latin American evidence base, which remains limited relative to other regions. The reproducibility of our protocol was high, consistent with CBCT reliability assessments.[16] Nevertheless, univariate models constrain accuracy; the literature favors multivariable models and 3D shape/volume descriptors to improve sex separation.[18,23] Limitations include the retrospective, single-center design, lack of external validation, and potential confounders (granular age, tooth loss, asymmetries, septal deviation) not modeled explicitly.[19,24,25,27] The urban character of the sample restricts generalization yet ensures technical homogeneity; multicenter studies will allow broader testing and contrast of these findings.

Future work should include: development and validation of population-specific multivariable functions integrating linear, volumetric, and 3D shape measurements;[18,23] multicenter evaluation of the impact of automated segmentation on classification accuracy;[20.22-24] modeling of clinical confounders - age, tooth loss, facial asymmetry, septal deviation - through stratified analyses;[19,24,25,27] exploration of inter-sinus combinations (maxillary, frontal, sphenoidal) to increase discriminative capacity;[26] and standardization of uncertainty reporting with promotion of external validation for disaster victim identification applications.

Overall, this study provides additional local evidence that 3D CBCT–based morphometric measurements of the maxillary sinus - particularly width - show sexual dimorphism and can achieve moderate discriminatory performance in an urban Peruvian adult sample. However, given the single-center retrospective design, the sample size, and the lack of external validation, these findings should be interpreted cautiously and should not be assumed transferable to other Latin American populations. Further multicenter studies with larger samples and external validation are needed before broader forensic implementation.

CBCT-based morphometry of the maxillary sinus demonstrated sexual dimorphism in adults. Width was the most discriminative single parameter, yielding classification rates above 70%, and area, perimeter, and volume showed consistent between-sex differences. Taken together, these findings support CBCT as a complementary tool for sex determination in forensic contexts; however, the moderate performance of univariate models argues for its use in conjunction with other forensic indicators.

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Accuracy and confidence in human dental identification using panoramic radiographs: the role of observer experience

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ABSTRACT

Swift and accurate disaster victim identification (DVI) is essential for medicolegal closure and repatriation of remains. Dental comparison is one of three primary identification methods recognized by The International Criminal Police Organization (INTERPOL), and is conducted by forensic odontologists, often by matching antemortem and postmortem intraoral radiographs. However, obtaining postmortem intraoral images can sometimes be challenging (e.g., from burn victims) and in those cases extraoral methods, especially computed tomography (CT), could be an alternative. This study uses conventional panoramic radiographs (PRs) to simulate CT-reconstructed PRs which are increasingly used in forensic scenarios, where traditional intraoral postmortem imaging is challenging. The aim is to assess the accuracy in comparative dental identification by different professionals when comparing antemortem intraoral radiographs to postmortem PRs.

In this retrospective study, intraoral radiographs and PRs from 30 patients were used to simulate a closed disaster event with 25 deceased and 30 possible missing persons. Twenty-five observers from four professional groups—eight oral radiology specialists (ORs), three forensic odontologists (FOs), six dentists trained in the basics of DVI (DVI-D), and eight dental students (DS) - matched PRs, serving as simulated postmortem radiographs, to antemortem intraoral radiographs using a comparative method. Statistical analysis was performed using chi-square and Kruskal-Wallis tests.

FOs and ORs achieved 100% accuracy, while DS and DVI-D had accuracy rates of 98.5% and 94.7%, respectively. Fillings were the most commonly used radiographic feature for matching. Confidence levels, ranging from "no match" to "established", differed, with 96% of ORs and 92% of FOs selecting established matches compared with 67% of DS and 51% of DVI-D.

Conventional PRs can be used for accurate matching in dental identification, particularly when interpreted by experienced observers. FOs and ORs had significantly higher matching accuracy and confidence, emphasizing the critical importance of observer experience; these findings support that ORs may be an asset in DVI operations.

INTRODUCTION

Death may occur due to natural causes or unexpected events such as accidents, mass disasters or crimes, which often result in extensive and unpredictable injuries. In all such cases, establishing the identity of the deceased is essential before issuing death certificates. The International Criminal Police Organization (INTERPOL) recognizes three primary identifiers: dental comparison, DNA analysis, and friction ridge analysis such as fingerprints.[1] Compared with the other two, dental structures are both tough and well protected, resisting both decomposition and extreme temperatures.[2–4] This, combined with the uniqueness of each person's teeth, makes them valuable for postmortem (PM) identification.[2,5]

When there are multiple fatalities and when the police deem it necessary, a disaster victim identification (DVI) operation is initiated, and a DVI team, which follows INTERPOL's guidelines, is activated.[1] In many cases, these identifications must be performed hastily to aid criminal investigations and/or provide crucial answers for relatives. [6,7]

The DVI team includes forensic odontologists (FOs).[1] In Sweden, the Swedish National Board of Forensic Medicine (Rättsmedicinalverket) is the sole employer of FOs and currently has three tenured FOs.[8] These FOs acquire their expertise through national and international postgraduate education, continuing professional development courses, and practical training. More than 250 dental identifications are conducted yearly by FOs in Sweden. When a DVI incident occur, the FOs can be supported by an additional nine intermittently employed dentists trained in the basics of DVI (DVI-D). The DVI-D consists of general dental practitioners or dental specialists who have undergone basic DVI training focusing on PM examination methodology and AM data transcription. Together, FOs and DVI-D constitute the core dental personnel for dental identification in Swedish DVI operations.

Oral radiology specialists (ORs), whose specialty requires proficiency in human identification and age assessment [9], represent another group with relevant competence; however, they do not perform identifications or participate in DVI operations. Although forensic odontology is not formally recognized as a dental specialty in Sweden, it is taught to dental students (DS) at all four dental schools, providing general dental practitioners with a very basic understanding of the field.

Regardless of professional background, all those involved in dental identification rely on the same fundamental methods. Intraoral radiography is considered the gold standard for radiological examination because it captures detailed images of tooth and bone morphology (including anatomical outlines e.g., maxillary sinuses) and dental restorations.[10,11] When performed correctly, intraoral radiographs provide high-resolution images that are essential for identification.[12–14] Furthermore, the images can also be captured under challenging conditions at disaster sites, using portable equipment.[15,16] FOs compare PM dental status, including radiographic images, with AM data such as dental records, radiographic images and other dental information linked to the missing person. Using a comparative method analyzing structures and patterns unique to the individual in the AM and PM material, the FOs assess whether the findings correspond to the same person.[5,17]

More advanced imaging techniques, including computed tomography (CT), cone-beam computed tomography (CBCT), and panoramic radiography have become more common both in clinical practice and for identification.[11,18–21] CT/CBCT provides 3D images for detailed assessments, while panoramic radiographs (PRs) offer an overview of the teeth and jaws. CT/CBCT images can also be transformed into PR reconstructions for easier interpretation and comparison with intraoral images. All these techniques can depict the teeth and other unique anatomical structures.[22] PM intraoral radiographs can sometimes be challenging or impossible to obtain (e.g., from burn victims).[4] Extraoral methods can then be preferred, particularly CT/CBCT images reformatted to PRs for easier interpretation.[11,15,21,23] The use of conventional PRs is often not practical due to the need for precise positioning and specialized equipment.[19,24] However, reformatted CT/CBCT images can in some cases be comparable and even provide benefits compared to conventional PRs, such as wisdom teeth staging, and have shown promising results for identification. [19,21,24] On the other hand, reformatted CT/CBCT images also provide their own challenges, particularly with metal artefacts.

In Sweden, CT/CBCT interpretation is part of the required competence for ORs [9], but falls outside the scope for general practitioners and other odontological specialties.

Given the complexity of these advanced imaging modalities, the ability to accurately interpret them may vary between professionals. [21] Previous studies have shown that FOs and ORs achieve greater matching accuracy than general practitioners and other odontological specialists, especially in complex cases e.g., edentulous individuals or those with unrestored dentition.[14,25–27]

The difference in identification accuracy may be more pronounced when using more advanced modalities such as PRs.[25] Previous findings indicate a high degree of variation in identification accuracy between professions when advanced modalities are used.[21] This means that FOs, DVI-D, and other dental professionals who may be recruited for identification work will likely require additional training to utilize these imaging modalities.[21]

Against this background, the aim of this study was to assess the accuracy of comparative human dental identification using conventional PRs serving as PM and intraoral dental radiographs as AM in a simulated medium-scale closed-disaster event (i.e., an incident where the victim pool is limited and presumptive identities are known, such as an aircraft crash with a passenger list). Additionally, we aimed to analyze the extent to which the experience of the observer affects the ability to perform accurate identification, which radiographically detectable identifiers were used to determine identity, and the degree of certainty.

MATERIALS AND METHODS

Ethical considerations

Ethical approval was obtained (Dnr 2023-08010-01), and the Swedish Ethical Review Authority has raised no objections to the research project. Furthermore, a review by the Västerbotten Region was conducted and approved on March 11, 2024.

All radiographs were retrospectively collected from patient records and fully anonymized. No identifiable personal information was accessible to the observers. The data were handled in accordance with the General Data Protection Regulation (GDPR, EU 2016/679) and institutional data protection guidelines. Given the retrospective and anonymized nature of the study, informed consent was waived by the Swedish Ethical Review Authority.

The study complies with the principles outlined in the Declaration of Helsinki[28] and relevant Swedish regulations for research involving human data.

Study design

This study simulated a medium-scale closed-disaster scenario to assess identification accuracy when comparing PM PRs with AM intraoral radiographs across four different professions; FOs, ORs, DVI-D, and DS.

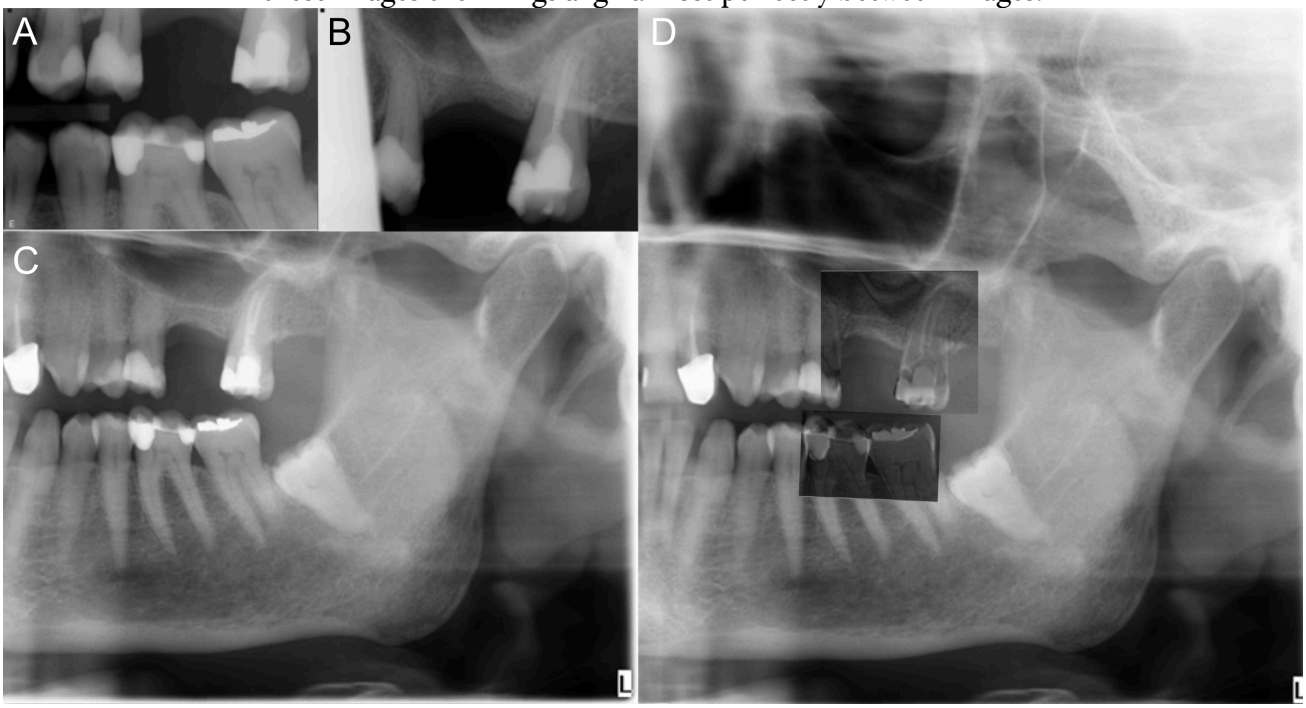
Conventional PRs were used as surrogates for CT-reconstructed PRs, as they provide comparable anatomical overviews and present similar interpretative challenges [23,29], however conventional PRs are more readily available and easier to organize for reading sessions.

Retrospective Collection of Intraoral and Panoramic Radiographs

This study is part of a larger project in which intraoral radiographs and conventional PRs from 100 adult patients were collected from the Oral Diagnostic Radiology Department, Västerbotten Region, during 2023–2024. The examinations were full mouth examinations performed on odontological indications and were included sequentially as they appeared in the image archive. Examinations were excluded if they depicted highly distinguishing features, such as fixation plates or large pathologies, or if no prior examinations were available. All available examinations, including both bite-wings and apical radiographs, from the preceding 10 years were downloaded and anonymized by an oral radiologist (NG). The date of each examination was recorded, and the date of birth and sex were extracted from the social security number.

Once the radiographs were collected, the 100 cases were assessed for inclusion in the present study by two dental students (TT and IL). Thirty-seven patients met the inclusion criteria of having both a PR examination and at least one prior intraoral radiographic examination.

Figure 1. shows representations of antemortem intraoral radiographs (A, B) and a corresponding postmortem panoramic radiograph (C), with superimposed comparison (D) highlighting matching features in teeth 27, 36, and 37, according to FDI World Dental Federation or ISO 3950 notation. In these images the fillings align almost perfectly between images.



Simulated Disaster Scenario

A medium-scale disaster scenario was then simulated by the same dental students, who randomly selected 30 patients from the pool of 37 eligible cases and organized these into presentations (Microsoft PowerPoint). To prevent observers from using the process of elimination, 25 simulated deceased individuals were presented for identification against 30 potential matches in randomized order. The observers were tasked with matching PRs, representing PM material, with intraoral images, representing AM material (Figure 1).

For the 25 simulated deceased individuals, the mean interval between the PR and the most recent intraoral examination was 1.8 years (range: 0.3–6.4 years). The antemortem material consisted of examinations conducted between 2017 and 2024. The average age was 54 years (range: 15–83 years), with 16 males and 14 females.

The prevalence of radiographically detectable identifiers visible on both AM and PM radiographs was 24/25 (96%) for dental fillings, 1/25 (4%) for dental implants, 18/25 (72%) for prosthodontic restorations, and 12/25 (48%) for root canal fillings. In all cases, tooth and bone anatomy were depicted, with no edentulous individuals included. Additionally, 8/25 (32%) exhibited distinctive bone features beyond trabecular anatomy, such as socket sclerosis, extraction socket remnants, or idiopathic sclerosis. Visible tooth anatomy included root morphology, crown morphology, and pulp chamber dimensions, among others.

Observers and Matching

The study included 25 observers: eight ORs, three FOs, six DVI-D, and eight DS. They were also grouped according to experience: eleven with a high degree of experience interpreting complex image data (ORs and FOs) and fourteen with less experience (DVI-D and DS).

The DS were in their final semester and had completed their radiology and forensic odontology training. The DVI-D had 15–30 years of experience as dentists. Further, some have participated in real DVI incidents involving PM examinations and AM data transcription, but none had performed matching during the reconciliation phase. The ORs had practiced solely in radiology for 5–30+ years, and two had experience performing dental identifications. The FOs had 5–20+ years of experience and regularly perform dental identifications.

Observers accessed the material at the Department of Odontology, Umeå University, or

at the Swedish National Board of Forensic Medicine. Conditions varied slightly across groups; most used a single screen, while ORs and one FO used multiple screens. DVI-D and two FOs performed their assessments in parallel under identical conditions. All matching was performed individually without discussion. A maximum of 2.5 hours was allotted, which DS and DVI-D nearly fully utilized, whereas ORs and FOs used approximately half of that time. To minimize recall bias, the oral radiologist (NG) performed the matching assessment more than one year after the collection of radiographs, and the dental students who constructed the exercise (TT and IL) were excluded from participation.

Observers were informed that each of the 25 PM cases had a corresponding match among the 30 AM cases, and were permitted to zoom and adjust contrast and brightness. During the assessment, they specified which identifiers supported their match, including dental fillings, tooth morphology, prosthodontics, and bone anatomy. For features such as root fillings or amalgam fragments, the category "other" was used.

Based on the findings, the observers then classified identification outcome according to the INTERPOL DVI scale[1,30]: *identity excluded (no match)*, when PM radiographs were clearly inconsistent with all AM records; *possible identification*, when there were similarities and no excluding features but the available data were limited; *probable identification*, when specific corresponding features were observed despite limited AM or PM material; and *established identification*, when there was absolute certainty that the PM and AM radiographs belonged to the same individual. [1,30]

No formal calibration session was conducted. The observers relied on training inherent to their professions and all received detailed written instructions outlining the assessment procedures.

Statistical Analysis

Power calculations prior to inclusion indicated that with 25 cases at least 3 participants per group were required to detect differences similar to those reported by Fridell and Ahlqvist [26], with 80% power at a significance level of $\alpha = 0.05$, including adjustment for Bonferroni correction.

The data were not normally distributed according to the Shapiro-Wilk and Kolmogorov-Smirnov tests; therefore, group differences were analyzed using cross-tabulations, the chi-square test, and the Kruskal-Wallis test for pairwise comparisons. Bonferroni correction was applied for multiple comparisons, and all reported p-values were adjusted accordingly.

Statistical analyses were performed and charts created using Jamovi project (version 2.6; https://www.jamovi.org), which is based on the R statistical environment (version 4.4).

A *p*-value of less than 0.05 was considered significant.

RESULTS

Accuracy of matching

Overall, 25 observers from the four different professional groups completed the matching of 25 cases each, resulting in 625 assessments in total. Across all groups 98.2% (614/625) of cases were correctly matched. There were differences between the groups: ORs and FOs were correct in 100% of cases, while DS were correct in 98.5% and DVI-D were correct in 94.7% ($p = 0.013$; Table 1).

When the participants were grouped by level of experience, highly experienced observers (ORs and FOs) demonstrated 100% accuracy, while the less experienced observers (DVI-trained dentists and DS) achieved 96.8% accuracy (p = 0.012; Table 2).

Table 1. The count and percentages of correct, no match, and incorrect assessments by oral radiology specialists (ORs), forensic odontologists (FOs), dentists trained in the basics of disaster victim identification (DVI-D), and dental students (DS).

Match	FOs (N=3)	ORs (N=8)	DVI-D (N=6)	DS (N=8)	p-value
Correct (%)	75 (100%)	200 (100%)	142 (94.7%)	197 (98.5%)	0.013
Not found (%)	0 (0%)	0 (0%)	2 (1.3%)	1 (0.5%)	
Incorrect (%)	0 (0%)	0 (0%)	6 (4.0%)	2 (1%)	

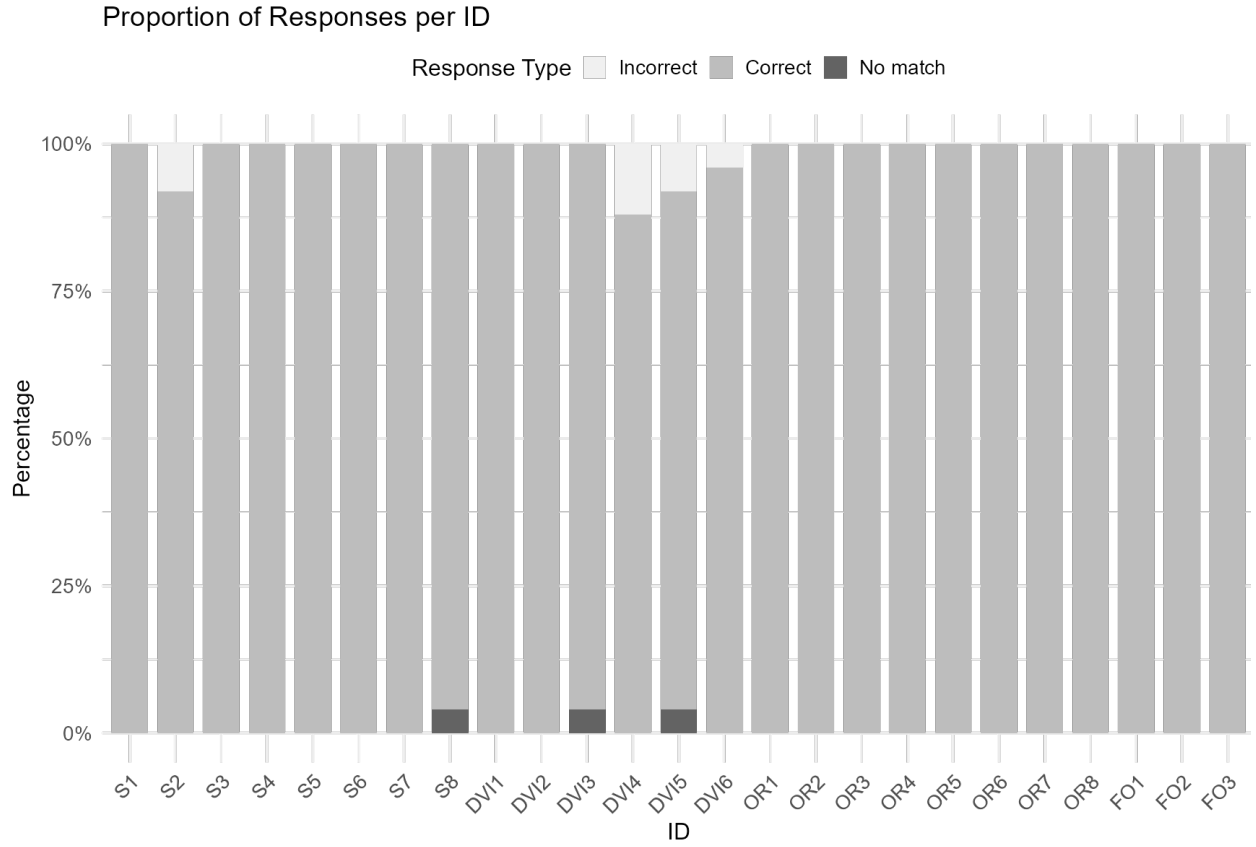
N = number of observers

Table 2. The count and percentages of correct, no match, and incorrect assessments for the highly experienced (oral radiology specialists and forensic odontologists) and less experienced (dentists trained in the basics of disaster victim identification and dental students) observers.

Match	Highly experienced (N=11)	Less experienced (N=14)	p-value
Correct (%)	275 (100%)	339 (96.8%)	0.012
Not found (%)	0 (0%)	3 (0.9%)	
Incorrect (%)	0 (0%)	8 (2.3%)	

N = number of observers

Figure 2. Bar chart visualization showing the percentage distribution of correct matches, incorrect matches, and no matches, with each column representing an individual observer identified by professional group. The three forensic odontologists are labeled FO1-FO3, the eight oral radiology specialists are labeled OR1-OR8, the six dentists trained in the basics of disaster victim identification labeled DVI1-DVI6 and the eight dental students are labeled DS1-DS8.



The incorrect and not found matches were distributed as follows: among the DVI-D, four out of six (66%) had either errors or cases without matches; among the DS two of eight (25%) had errors or cases without matches. Out of the eight incorrect matches, six (75%) were classified as probable and the remaining two (25%) as possible. None of the ORs and FOs (0%) committed any errors (Figure 2).

To explore whether case characteristics influenced matching accuracy among the less experienced groups, cases correctly matched by DVI-D and DS were compared with those resulting in errors. No significant differences were found regarding simulated victims age, interval between examinations, visible radiographically detectable identifiers nor sex.

Radiographically detectable identifiers used for matching

Fillings were the primary radiographically detectable identifier used for matching across all groups, with slight but significant differences between them ($p < 0.001$). The highest use was

seen among ORs (94.0%), followed by FOs (90.7%), DVI-trained dentists (79.3%), and DS (73.5%).
Prosthodontics were used more frequently by ORs (61.5%) than by DVI-D (51.3%), FOs (49.3%), and DS (40.5%) (p = 0.004).
Tooth anatomy was used significantly more often by DS (58.5%), ORs (52.5%), and FOs (42.7%) than by DVI-D (18.7%) (p < 0.001). A similar trend was noted for the "Other" category, where ORs and DS reported use in 69.0% and 48.0% of cases, respectively, compared with 21.3% for FOs and 15.4% for DVI-D (p < 0.001).
The most pronounced difference between groups was observed for bone anatomy: ORs used this characteristic in 75.5% of cases, compared with only 10.7% for FOs, 2.7% for DVI-D, and 1.0% for DS (p < 0.001). A complete breakdown of matching characteristics by group is presented in Table 3.

Table 3. The count and percentage of each radiographically detectable identifiers used for matching by the oral radiology specialists (ORs), forensic odontologists (FOs), dentists trained in the basics of disaster victim identification (DVI-D), and dental students (DS).

Match using	FOs (N=3)	ORs (N=8)	DVI-D (N=6)	DS (N=8)	p-value
Bone anatomy	8 (10.7%)	151 (75.5%)	4 (2.7%)	2 (1.0%)	< .001
Fillings	68 (90.7%)	188 (94.0%)	119 (79.3%)	147 (73.5%)	< .001
Tooth anatomy	32 (42.7%)	105 (52.5%)	28 (18.7%)	117 (58.5%)	< .001
Prosthodontics	37 (49.3%)	123 (61.5%)	77 (51.3%)	81 (40.5%)	0.004
Other	16 (21.3%)	138 (69.0%)	23 (15.3%)	96 (48.0%)	< .001

N = number of observers

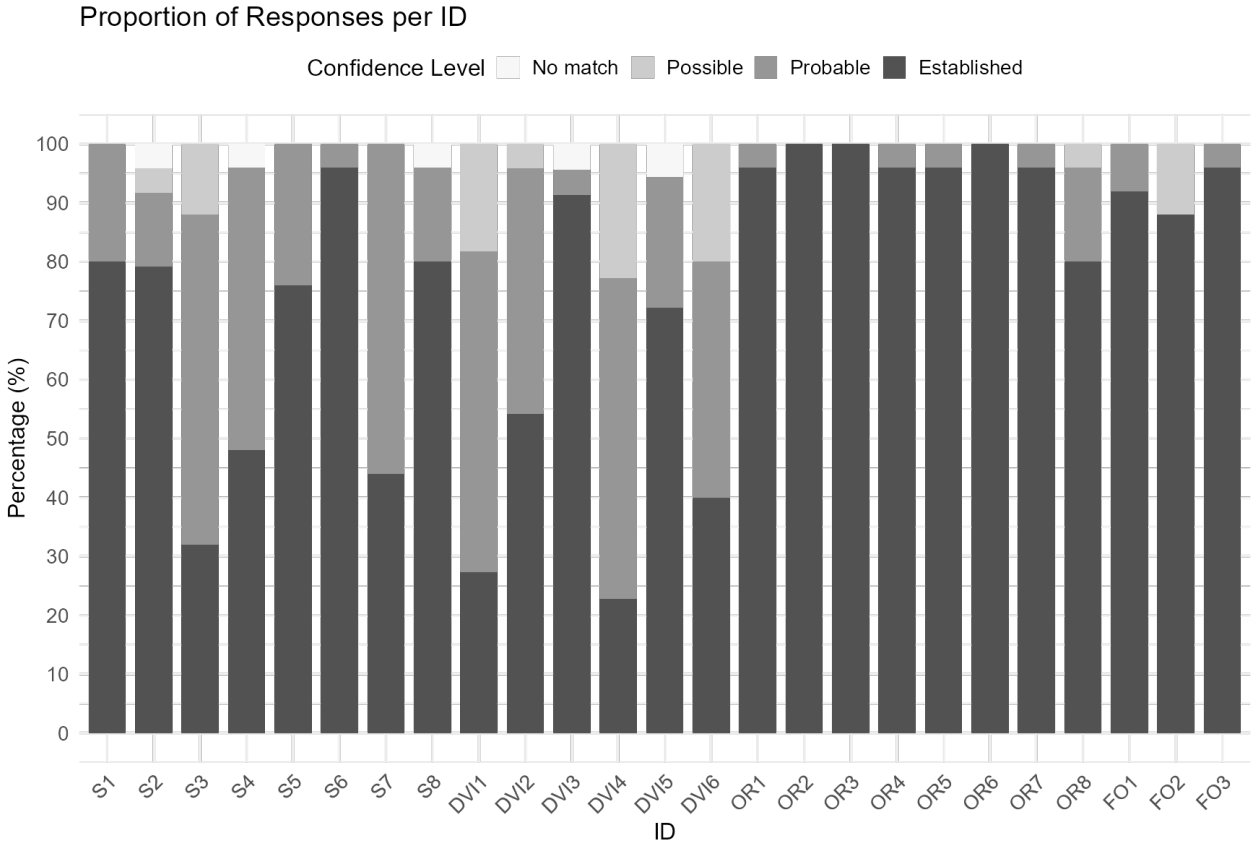
Matching confidence
The matching confidence varied greatly between the groups, with the ORs selecting "established" in 95.5% of cases, compared with 92.0% for the forensic odontologists, 50.7% for the DVI-D, and 66.8% for the DS (p < 0.001). Pairwise comparisons revealed significant differences between ORs vs DVI-D (p < 0.001), ORs vs DS (p <0.001), FOs vs DVI-trained dentists (p < 0.001), and FOs vs DS (p < 0.001). The difference between DVI-D and DS was also significant (p = 0.005), while there were no significant differences between ORs and FOs (p = 0.63). For detailed distribution see Table 4.
There was also great individual variation among DS and DVI-D, with some almost matching the confidence of the ORs (Figure 3). Overall, the ORs and FOs demonstrated significantly higher confidence than the DVI-D and DS.

Table 4. The count and percentage of confidence classifications for oral radiology specialists (ORs), forensic odontologists (FOs), dentists trained in the basics of disaster victim identification (DVI-D), and dental students (DS).

Confidence	FOs (N=3)	ORs (N=8)	DVI-D (N=6)	DS (N=8)	*p*-value
No match	0 (0.0%)	0 (0.0%)	2 (1.5%)	3 (1.5%)	< .001
Possible	3 (4.0%)	1 (0.5%)	15 (11.2%)	4 (2.0%)	
Probable	3 (4.0%)	8 (4.0%)	49 (36.6%)	59 (29.6%)	
Established	69 (92.0%)	191 (95.5%)	68 (50.7%)	133 (66.8%)	

N = number of observers

Figure 3. Bar chart visualization showing the percentage distribution of match confidence levels, with each column representing an individual observer identified by professional group. The three forensic odontologists are labeled FO1-FO3, the eight oral radiology specialists are labeled OR1-OR8, the six dentists trained in the basics of disaster victim identification labeled DVI1-DVI6 and the eight dental students are labeled DS1-DS8.



DISCUSSION

The present study demonstrates that the four professional groups differed significantly in both accuracy and confidence when performing matching between PM PRs and AM intraoral radiographs. FOs and ORs correctly matched all cases—the only acceptable outcome in real disaster scenarios—compared with 94.7-98.5% among the less experienced groups. Confidence levels had a similar pattern, with FOs and ORs classifying 92–96% of matches as established, compared with 51–67% for the less experienced groups.

The significantly higher matching confidence among ORs and FOs compared with the less experienced groups was expected and can be attributed to their specialized expertise, as they routinely analyze and interpret a wide variety of radiological images, consistent with previous findings.[14,25–27] That ORs, despite not performing dental identifications, achieved identical matching accuracy as FOs suggests that their expertise may be an asset in DVI scenarios. The high proportion of established matches in this study reflects that most cases contained multiple distinctive identifiers, making established matches achievable. However, real-life scenarios, with fewer distinguishing radiographically detectable identifiers, may warrant greater caution in confidence to reduce the risk of false identifications.[11,25,26,31,32]

The groups also differed in their use of radiographically detectable identifiers. As expected, fillings (74-94%) and prosthodontics (41-62%) were the most commonly used radiographically detectable identifier for matching, consistent with previous findings.[11,21,27,32,33] Fillings are relatively easy to compare between PR and intraoral radiographs and tend to vary in terms of placement, material, outline, and condition between individuals. Similarly, prosthodontics can be highly distinctive in appearance, placement, and materials. [5,27] As restorations become less prevalent, features such as tooth morphology, rotation and placement, as well as jawbone structure, pathologies, and any

injuries or alterations become more valuable for identification. [5,22,26,31,34]

The largest discrepancy was regarding the use of bone anatomy, with ORs using bone identifiers to a very high degree (75.5%), compared with 10.7% for FOs, 2.7% for DVI-D, and 1.0% for DS, despite only 32% of cases having distinguishing bone identifiers. This pattern was specific to ORs rather than a general trend among experienced observers, which likely reflects that they are trained in comprehensive radiographic assessment and apply that methodology to matching, while FOs are trained to focus on the most relevant identifiers to establish an identification. [9,21,31,32] While both approaches produced accurate results, the extensive use of bone anatomy among ORs in this study was not warranted and could represent a less efficient allocation of attention and time. The results also suggest that each group could benefit from complementary training in the other's area of expertise. Training in assessing bone anatomy (e.g. sinus outlining and trabecular bone patterns) could be particularly important for preparing for future scenarios where dental restorations may be fewer or less distinctive. [5,27,35]

Another notable finding was that the DVI-D had the lowest accuracy (94.7%) and confidence (50.7% established matches) compared to all the other groups, including DS. Although DVI-D receive annual training, often provided by FOs, this training primarily focuses on PM examination and AM transcription rather than the radiographic comparison. This combination of infrequent practical application and long time between training sessions affects knowledge and skill retention and might explain this finding. [36] However, in a real DVI operation the DVI protocols specify that quality control should be performed which would improve matching and identification accuracy.[1,37] These results suggest that matching in the reconciliation phase should be performed by experienced FOs or possibly ORs. Further, this suggests that DVI training programs should incorporate more radiographic comparison and matching exercises to ensure that DVI-D are well-prepared to carry out all stages of dental identification in future DVI incidents [21,25–27,32], while being efficient and accurate.[38]

In both single-case identifications and DVI incidents, time and accuracy are of the essence. [38,39] In this study, less experienced dentists (DS and DVI-D) generally required more time to perform matching yet produced more errors and expressed lower confidence in their matches, making them less suited for matching tasks. Furthermore, long working hours and intense external pressure during DVI operations [39], can increase the risk of errors, which suggests that the most experienced personnel should assume primary responsibility for matching and quality assurance.

Although less experienced personnel performed inferiorly in matching tasks, they may contribute valuable support in other aspects of DVI operations. Real-life incidents involve more than just matching: PM examinations, AM data transcription, and report writing also need to be performed, e.g. a general dental extensive clinical experience can be particularly beneficial for conducting PM examinations. [40] These considerations highlight the importance of maintaining a network of highly trained and experienced personnel who can lead and support their less experienced colleagues, while simultaneously handling the most challenging tasks in DVI incidents such as performing matching, especially with complex images such as PRs and CT/CBCT. [11,21]

Conventional PRs present challenges due to positioning requirements, distortions, and overlapping anatomical structures. [11,23,33] While CT/CBCT-reconstructed PRs can be more easily obtained postmortem and configured to better match AM images, they are more susceptible to artifacts from metal restorations and often have lower spatial resolution. [23] Nevertheless, conventional and reconstructed PRs are generally considered comparable in clinical and forensic contexts [11,21,33], and our findings may therefore be applicable to CT/CBCT-based identification, though this warrants further evaluation.

A limitation of the study is that conditions were not fully standardized across the participant groups, especially regarding the use of multiple screens. The study design also imposed a time limit on the observers which may have been a source of stress, potentially leading to errors. [38] However, if time pressure were a significant factor, one would expect it to affect all groups. The fact that FOs and ORs achieved the highest matching accuracy while using the least amount of time suggests that experience, rather than time constraints, determined performance. Less experienced observers performed inferiorly

despite utilizing all available time, and while additional time might have improved their results [38], real-world disaster scenarios rarely afford such flexibility.

The use of more AM cases (30) than PM cases (25) is a strength of this study, as it prevented observers from using a process of elimination to match the final cases, which would have artificially inflated accuracy rates. By including five AM cases without a PM match, observers were required to actively identify matches based on radiographically detectable identifiers rather than simply pairing remaining cases by exclusion. The study design could have been strengthened by including PM cases without an AM match. Building on this, future studies could also evaluate all phases of the DVI process across professions, including transcription of AM and PM examinations using INTERPOL dental codes.

Overall, our results emphasize the importance of experience in detecting subtle details and indicate that additional training is required for less experienced personnel to improve both accuracy and efficiency. Beyond reducing errors, such experience may also mitigate the post-traumatic stress and risk of burn-out commonly associated with DVI incidents. [41,42]

CONCLUSION

Conventional PRs could be used for identification with high accuracy, particularly when interpreted by experienced observers. FOs and ORs had significantly higher matching accuracy and confidence than the less experienced groups, emphasizing the critical importance of observer experience. The findings support that ORs may be an asset in DVI operations. Fillings were the most commonly used identifier, while ORs notably utilized bone anatomy extensively. PRs reconstructed from PM CT could be valuable for initial screening and for narrowing potential matches in comparative dental identification, but this requires further investigation.

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Digital radiography and GIMP software in mandibular sex estimation: implications for forensic anthropology

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ABSTRACT

Background: Forensic anthropology frequently utilizes mandibular features to establish biological sex. The mandible, being the most resilient cranial bone, demonstrates marked sexual dimorphism. Among its indices, the gonial and antegonial angles have emerged as reliable parameters for sex differentiation. With digital imaging widely available, open-source tools such as GIMP allow precise, reproducible, and cost-effective morphometric analysis of orthopantomograms (OPGs).
Materials and Methods: This cross-sectional study analyzed 500 archived OPGs (250 males, 250 females; age 20–40 years) from AIIMS Nagpur. In orthopantogram, gonial and antegonial angles were measured bilaterally using GIMP (v2.10.34). Statistical analyses were conducted with Jamovi (v2.6).
Results: Females exhibited significantly larger gonial (130.80° ± 5.35) and antegonial (167.85° ± 2.15) angles compared to males (123.03° ± 5.43 and 162.69° ± 2.12, respectively; $p < 0.001$). Regression analysis confirmed significant dimorphism, even after adjusting for age. Discriminant function analysis demonstrated that both angles contributed positively to sex classification, yielding a robust predictive model. No significant sex-related differences were observed in age group distribution ($\chi^2 = 0.812$, df = 2, $p = 0.666$).
Conclusion: Mandibular gonial and antegonial angles measured from OPGs using GIMP demonstrate significant sexual dimorphism in the 20–40-year age group. Females consistently displayed larger angular values, supporting their diagnostic value for sex estimation. These findings underscore the relevance of digital radiography and open-source analysis in forensic anthropology, orthodontics, and maxillofacial surgery.

INTRODUCTION

Forensic anthropology involves the utilization of the expertise and techniques of anthropology, particularly in the areas of biological anthropology and archaeology, to address legal and medical matters [1]. This involves various measurements based on the requirements of the case. Many researchers have attempted somatometry, physical anthropometry, and computerized evaluation of radiological investigations to confirm the age and sex of individuals.
The mandible is the most robust section of the skull, owing to its thick layer of compact bone. Its significance lies in its sexual dimorphism and radio-morphometric features, even though it

experiences size changes and remodelling as it develops until it reaches a certain age. Mandibular dimorphism is characterized by differences in size and shape, with male mandibles generally being larger and stronger than those of females. It is the last bone in the skull to finish growing and grows significantly during the teenage years. The way the lower jaw develops, how fast it grows, and how long it takes are different for males and females. This helps in estimating biological sex. Among the various structures of the mandible, the ramus is the most sexually dimorphic. [2,3,4]

The gonial and antegonial angles of the mandible have garnered attention as dependable indicators of sexual dimorphism, offering valuable insights into the estimation of biological sex. [5] In the era of digital medicine, X-ray image of individuals are readily available. An orthopantomogram (OPG) is a type of X-ray that provides a panoramic view of the lower face, jaw, and teeth. Dental practice often relies on radiography to deliver crucial insights into teeth, including their inner core structure, developmental stages, and surrounding tissues. [6,7,8] The OPG are used instead of lateral cephalogram in this study because it provides simultaneous view of both sided angular parameter of mandibular bone which eases the measurement process. Also because of its lower radiation dose OPG were used abundantly in the dental OPD, which reproduces its utility in large population based study. Given the significance of the mandible in forensic anthropology for identifying sexual dimorphism, this study focuses on leveraging digital tools such as the GIMP software, which facilitates precise measurements of gonial and antegonial angles from OPG images.

The software provides tools for tracing lines and measuring angles, enabling quantification of the morphometric indices. Using GIMP, researchers can efficiently and cost-effectively analyze large samples of OPGs to study mandibular sexual dimorphism and other anatomical variations. The open-source nature of GIMP makes it accessible for forensic anthropology and dental research. [9] GIMP was used because of its precise nature of pixel based measurement tool. It is having advantage over other freely available software's by having linear and angular measurements without requiring additional plugins. Unlike ImageJ, GIMP provides simpler workflow, better usability for non-technical users, and consistent reproducibility across system. Proper calibration and standardized measurement protocols are essential when using GIMP to ensure the reliability and reproducibility of morphometric analysis across different OPG samples.

In a similar vein, the GIMP software, which is freely accessible, can significantly contribute due to its accurate predictions and ability to correct image errors. Digital radiography offers benefits in the analysis of facial features, allowing for the precise measurement of facial structures without the need for further dissection. This study focused on predicting sex by calculating mandibular morphometric indices from orthopantogram utilizing the GIMP software.

MATERIALS AND METHOD

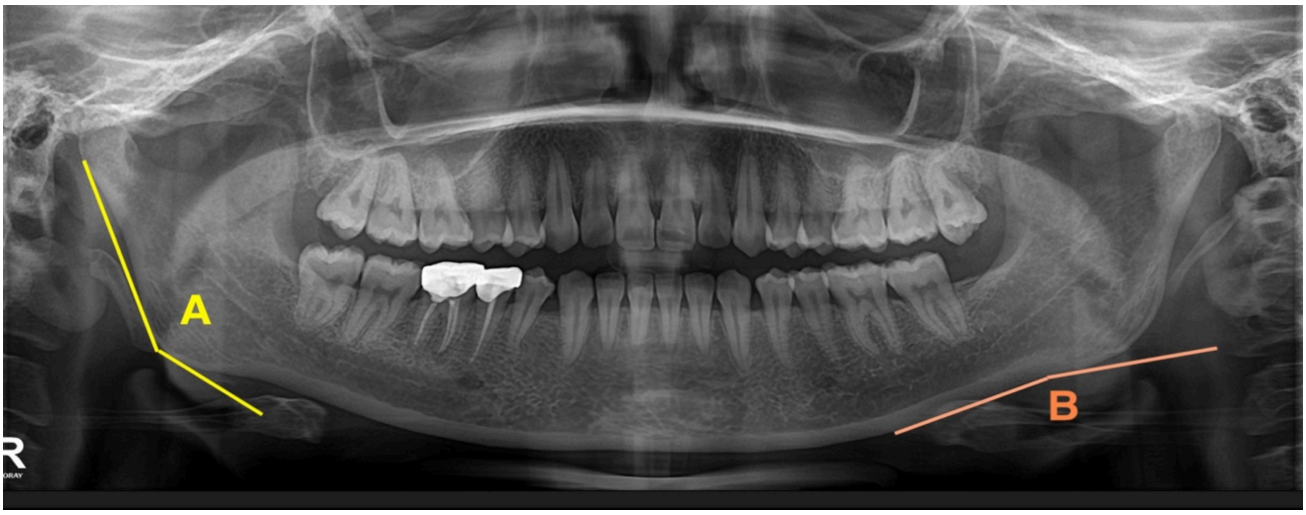
A cross-sectional study was carried out in the Department of Forensic Medicine, AIIMS Nagpur, Maharashtra, utilizing archived dental records. From these, 500 orthopantomograms were randomly chosen, consisting of 250 male and 250 female individuals aged between 20 and 40 years. The present age group were selected taking into consideration of completed skeletal maturity with minimal bone remodelling with fewer age related wear and tear. Only those radiographs with confirmed sex, high resolution and sharpness, proper head positioning, complete permanent dentition, and without any evidence of mandibular pathology or traumatic changes were included. Images with artifacts or bone abnormalities were excluded. All orthopantogram were obtained using a Papaya Genoray unit with exposure parameters of 74 kV, 12 mA, and 14.3 seconds. The selected OPGs were processed and analyzed with GIMP software. The morphometric assessments were conducted bilaterally using GNU Image Manipulation Program [GIMP version 2.10.34 (revision 3)].

As shown in Fig 1, the Gonial Angle can be estimated by the intersection of a tangent line along the lower border of the mandibular body and angle with another tangent drawn to the posterior border of the ramus and condyle. [10] Similarly the antegonial angle is formed by two lines drawn parallel to the antegonial region, intersecting at the deepest portion of the antegonial notch. [11]

Each digital orthopantomogram (OPG) was opened as a new file in the macOS version of GIMP. Subsequently, a new transparent layer was created to delineate anatomical reference lines. The gonion was identified at the intersection of

the posterior border of the ramus and the inferior border of the mandible. Utilizing the Measure Tool, a line was drawn along the posterior border of the ramus, and a second line was drawn along the inferior border of the mandible. The gonial angle was determined by activating the angle mode (SHIFT key) and measuring the angle formed at the gonion. The angle value displayed in the GIMP status bar was recorded. Measurements were conducted on both sides, with each measurement being performed three times, and the mean value was used for analysis. To ensure objectivity, the observers were blinded to the sex of the radiographs. GIMP software was advantageous as it reduced magnification errors and minimized measurement bias.

Figure 1. OPG with Angular measurements of mandibular parameters



Data analysis was carried out using Jamovi (version 2.6, The Jamovi Project, 2025), a free and open-source statistical software. Independent t-tests were employed to compare the measurements between groups, and discriminant function analysis was performed on the recorded variables.

All archived OPG of known sex and age range of 20-40 years were included in the study, except radiographs with artifacts, fractures, and trauma. To achieve a statistical power of 95% and a significance level (alpha error probability) of 0.05, the sample size was determined using G*Power software (version 3.1.9.4, Düsseldorf, Germany). The required sample size was calculated to be 482; however, a total sample of 500 OPGs was included.

RESULTS

In the present study, the archival data of OPGs from the Department of Dentistry were carefully screened, and cases were selected after applying the above-mentioned inclusion criteria and those were considered for the study. After taking measurements using GIMP software, the data were entered in MS Excel format. Data analysis using the Jamovi software yielded the following results.

Table 1 presents the comparison of angular parameters between males and females. The mean gonial angle was significantly higher in females compared to males. Similarly, the antegonial angle showed higher values in females than in males and the difference was statistically significant. These findings indicate notable sexual dimorphism in both gonial and antegonial angles. Females exhibited larger angular measurements (gonial and antegonial angles), suggesting a different mandibular shape than males.

The linear regression analysis outlined in the table 2 compares different craniofacial measurements between males and females. Each entry represents a specific parameter, reported separately for the right and left sides of the mandible, along with the mean value calculated as (R+L/2).

Additionally, a chi-square test was performed to examine the distribution of age groups across sexes. The contingency table 2 summarizes the number of male and female participants within the categories of young, middle, and old age groups.

The chi-square test results were as follows: χ^2 = 0.812, df = 2, p = 0.666, indicating no statistically significant difference in age group distribution between males and females.

Table 3 and 4 indicates the standardized coefficients of the six measurements. The positive coefficients for gonial angle, and antegonial angle suggest their relative contribution to the discrimination between the male and female groups.

Figure 2 visual comparison of the standardized and unstandardized coefficients, along with the t-values and p-values annotated above each bar. The antegonial angle had a strong positive contribution to sex classification. All variables were statistically significant ($p < 0.05$, most of them with $p < 0.001$).

Table 1. Comparison of Mandibular Measurements Between two sexes

S.N.	Parameter	Male (in mm)		Female (SD)		P value
		Mean	SD	Mean	SD	
1	Gonial Angle	123.03	(5.43)	130.80	(5.35)	<0.001
2	Antegonial Angle	162.69	(2.12)	167.85	(2.15)	<0.001

Table 2. Linear regression analysis of outcomes with gender (Female compared to Male)

S.N.	Parameter	URC (95% CI)	p-value	ARC (95% CI) *	p-value
		(R+L/2)		(R+L/2)	
1	Gonial Angle	7.77 (6.82, 8.71)	<0.001	7.77 (6.82, 8.72)	<0.001
2	Antegonial Angle	5.16 (4.79, 5.54)	<0.001	5.16 (4.78, 5.54)	<0.001

URC; Unadjusted Regression Coefficient, ARC; Adjusted Regression Coefficient, *; Age adjusted

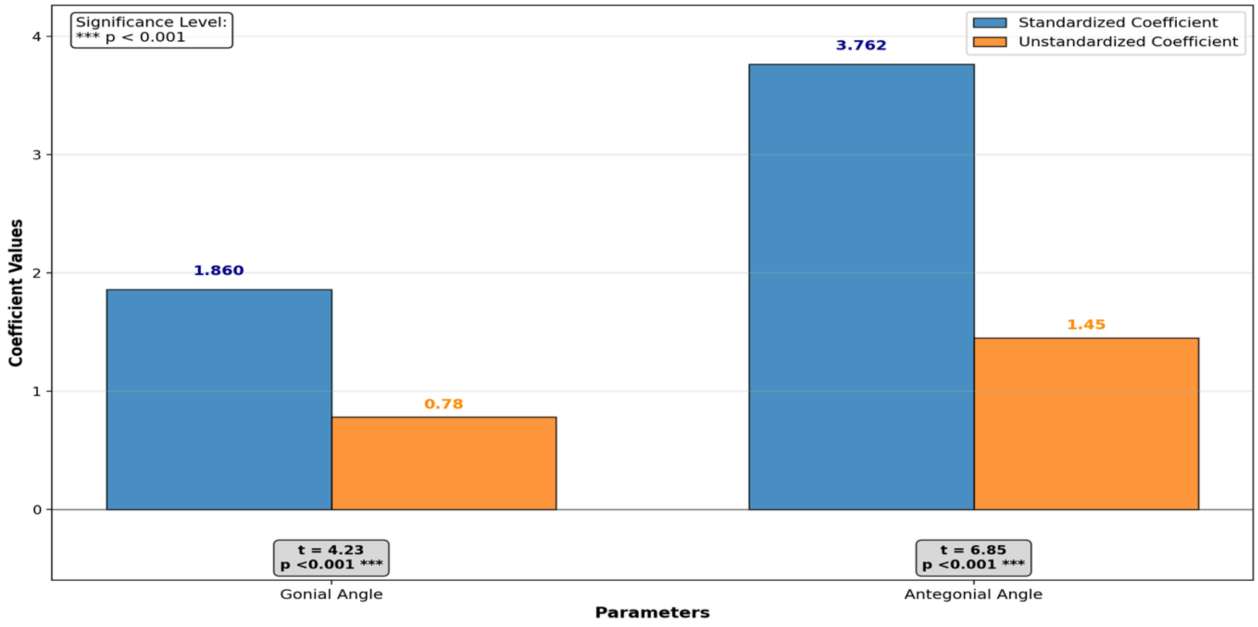
Table 3. Standard canonical discriminant function coefficients

Function coefficient	Function
Gonial Angle:	1.8595916302188826
Antegonial Angle:	3.7624129016981236

Table 4. Standardized and unstandardized coefficients

Parameter	Standardized Coefficient	Unstandardized Coefficient	t-value	p-value
Gonial Angle	1.86	0.78	4.23	<0.001
Antegonial Angle	3.762	1.45	6.85	<0.001

Figure 2. Standardized vs standardized coefficients with statistical significance (All parameters: p<0,001)



DISCUSSION

This study sought to estimate sex by analyzing mandibular morphometric indices obtained from digital orthopantomograms (OPGs) using the GIMP software. The strengths of this study lie in its age range of 20–40 years and its sample size of 500 participants, which offered a solid dataset for analysis. This approach enhances previous research that had either smaller sample sizes or broader age ranges, which may have introduced age-related variations. The results indicated significant sexual dimorphism in all evaluated mandibular parameters, with males generally having larger linear dimensions and females displaying larger angular measurements. These outcomes align closely with those of previous studies on mandibular sexual dimorphism.

The larger gonial angle in females (130.80° vs. 123.03° in males) corroborates the findings of Shahabi et al [12] in the Iranian population and Belaldavar et al [13] in Indian population. However, this finding contrasts slightly with that of Arthnari et al [14], who found minimal differences between the sexes. This variation may be due to differences in the populations.

The significantly larger antegonial angle in females (167.85° vs. 162.69° in males) aligns with the findings of Arthnari et al [14], confirming its utility in sex estimation.

In their research on the gonial angle using CT Scan images, Bulut et al [15] discovered no statistically significant differences in gonial anthropometric measurements among individuals aged 20–39 and 40–59 years. Nevertheless, it identified statistically significant sexual dimorphism in the older adult group aged 60–80 years. Despite this observation, the results suggest that the gonial angle is not a dependable indicator for estimating sex from the cranium and should not be used as the sole method for such identification. [15] In other study using similar CT images but using vastly sixteen parameters Senol et al [16] found that the accuracy rates of sex determination from mandibular measurements were 89.5% in females and 76.1% in males.

In his research, Ayoub et al [17] found that there is no notable difference in the mandibular angle for estimating sex in a young Lebanese population. The variation in the reliability of measuring either the gonial or antegonial angle for identifying sexual dimorphism is primarily influenced by differences in age, ethnicity, and population. Additional studies involving diverse ethnic groups are required to advance research in this field.

The findings indicate that females had notably smaller measurements in several craniofacial aspects, including condylar ramus height, projective ramus height, maximum ramus breadth, and minimum ramus breadth, compared to males. Conversely, the gonial and antegonial angles were significantly larger in women than in men. All p-values in the table were less than 0.001, indicating that the differences observed for each craniofacial parameter between males

and females were statistically significant. Regression analysis consistently demonstrated notable distinctions between females and males in various craniofacial measurements, highlighting the necessity of accounting for sex when assessing craniofacial structures. Another parameter, such as ramus flexure, was examined on OPG by Premkumar et al [18], who found a significant sex difference in this measure. In his study of the Italian population, Nuzzolese et al [19] highlighted the importance of various landmarks on OPG, which exhibit significant sexual dimorphism.

The canonical discriminant function was used to categorize individuals into groups by analyzing their craniofacial measurements. The coefficients indicate which variables play a more significant role in differentiating between the groups. A higher score on the discriminant function suggests a greater probability of being part of a specific group (likely females, as indicated by positive coefficients), whereas a lower score suggests membership in the other group (likely males).

This analysis holds particular value in fields like forensic anthropology, where distinguishing between male and female remains through craniofacial measurements is essential. The canonical discriminant function offers a mathematical framework for categorizing individuals by sex based on their craniofacial characteristics. These coefficients can assist clinicians and researchers in identifying patterns of craniofacial development, sexual dimorphism, and variability among different populations. Furthermore, they can be applied in personalized treatment approaches in orthodontics and maxillofacial surgery, where such anatomical features may influence the treatment planning.

The use of the GIMP software for morphometric measurements in digital radiographs represents a significant advancement over previously employed methodologies. Traditional studies, such as those conducted by More et al [20], utilized Kodak Master View software, whereas Ojha et al [21] relied on ImageJ for their assessments. Although these tools are effective, they have limitations, such as cost, platform restrictions, and steep learning curves. In contrast, GIMP is an open-source, cross-platform image editing software that is freely available and user-friendly, making it accessible to a broader range of users. Furthermore, its customizable interface and wide range of measurement tools offer the potential to minimize user-induced errors, thereby reducing measurement bias and enhancing reproducibility across studies. Various dental imaging software options are available for measurement and analysis in forensic odontology, including DentaScan, ImageJ, ModelMatch3D, and Kodak Master View. These tools facilitate tasks such as the comparison of antemortem and postmortem images, odontometric analysis, and three-dimensional (3D) model comparison. These sophisticated proprietary software solutions offer high efficiency in image analysis for diagnostic and treatment purposes. However, forensic odontology typically requires only basic image analysis, which can be effectively conducted using freely available software such as GIMP.

The findings reinforce the role of angular mandibular indices in sexual dimorphism. While several studies like Shahabi et al [22] and Bulut et al [23] question the reliability of these angles in older populations, this study confirms their effectiveness in the 20–40 age group. Differences in ethnicity and age influence diagnostic accuracy, emphasizing the need for population-specific standards. Additionally, linear regression analysis confirmed the presence of sexual dimorphism, revealing significant differences even after adjusting for age related factors. This effectively addresses a limitation found in earlier studies that failed to consider age-related changes in the mandibular morphology.

CONCLUSION

This study demonstrated that mandibular morphometric indices derived from digital orthopantomograms can reliably distinguish between sexes in the 20–40-year age group, with males showing larger linear dimensions and females exhibiting larger angular values. Canonical discriminant analysis further reinforced the diagnostic utility of these measurements for sex estimation, underscoring their potential applications in forensic anthropology, orthodontics, and maxillofacial surgery. The use of GIMP software proved advantageous due to its accessibility, flexibility, and reproducibility, offering a practical alternative to proprietary imaging tools in forensic odontology. Future studies should aim to test these findings in diverse populations, incorporate three-dimensional imaging

techniques, and validate predictive models with independent datasets.

In conclusion, mandibular morphometric analysis using digital radiographs and open-source software provides a cost-effective and efficient approach for sex estimation. This approach enhances the precision of forensic investigations while advancing the integration of digital tools in anthropological and clinical practice.

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