



Comparison of spectroscopic and molecular methods for estimating age and sex for medico-legal purposes: protocol for a systematic review

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ABSTRACT

Introduction: In forensic contexts, accurate estimation of age and sex is fundamental for the identification of human remains. Spectroscopic methods, offering non-destructive and precise data insights, have emerged as promising alternatives/complements to traditional morphological and genetic techniques.

Objective: This protocol outlines a systematic review aimed at evaluating the effectiveness of spectroscopic and molecular methods for estimating age and/or sex based on dental samples in medico-legal settings.

Methodology: This review will be conducted in accordance with the PRISMA 2020 guidelines. Databases to be searched include PubMed, Web of Science, ScienceDirect, LILACS (via BVS), Cochrane Library (Protocols), MEDLINE (via EBSCOhost), Academic Search Complete (via EBSCOhost), CINAHL (via EBSCOhost) and grey literature. The eligibility criteria follow the PICOS framework. Risk of bias will be assessed using the QUIN tool for in vitro studies. Data synthesis will involve both quantitative and qualitative approaches. The protocol of this review has been registered in PROSPERO (CRD420250650858).

Results: This review is expected to identify a range of spectroscopic techniques applied to dental samples for age and sex estimation, including mass spectrometry, Raman spectroscopy, FTIR spectroscopy, and X-ray fluorescence, as well as molecular methods such as amelogenin analysis, DNA methylation, and telomere length assessment. The evidence is anticipated to vary regarding study

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design, sample characteristics, analytical protocols, and reported outcome measures. The review will synthesize the available evidence on the accuracy, applicability, and methodological characteristics of these approaches within medico-legal contexts.

Conclusion: This systematic review will provide a comprehensive overview of the available evidence regarding the use of spectroscopic and molecular methods for age and sex estimation from dental samples. By identifying the methodologies currently employed, their reported outcomes, and existing research gaps, the review may contribute to future research directions and support evidence-based decision-making in forensic and medico-legal practice, particularly in contexts where non-destructive analytical approaches are desirable.

RESUMO

Introdução: Em contextos forenses, a estimativa precisa da idade e do sexo é essencial para a identificação de restos mortais. Os métodos espectroscópicos, que fornecem dados não destrutivos e precisos, têm-se revelado alternativas ou complementos promissores às técnicas morfológicas e genéticas tradicionais.

Objetivo: Este protocolo descreve uma revisão sistemática destinada a avaliar a eficácia dos métodos espectroscópicos e moleculares na estimativa da idade e/ou do sexo com base em amostras dentárias em contextos médico-legais.

Metodologia: Esta revisão será conduzida de acordo com as diretrizes PRISMA 2020. As bases de dados a serem pesquisadas incluem PubMed, Web of Science, ScienceDirect, LILACS (via BVS), Cochrane Library (Protocolos), MEDLINE (via EBSCOhost), Academic Search Complete (via EBSCOhost), CINAHL (via EBSCOhost) e literatura cinzenta. Os critérios de elegibilidade seguem a estrutura PICOS. O risco de viés será avaliado utilizando a ferramenta QUIN para estudos in vitro. A síntese de dados envolverá abordagens quantitativas e qualitativas. O protocolo desta revisão foi registado no PROSPERO (CRD420250650858).

Resultados: Espera-se que esta revisão identifique um conjunto de técnicas espectroscópicas aplicadas a amostras dentárias para a estimativa da idade e do sexo, incluindo espectrometria de massa, espectroscopia Raman, espectroscopia FTIR e fluorescência de raios X, assim como métodos moleculares, tais como a análise da amelogenina, a metilação do ADN e a avaliação do comprimento dos telómeros. Prevê-se que as evidências variem no que diz respeito ao desenho do estudo, às características das amostras, aos protocolos analíticos e aos indicadores de resultados relatados. A revisão sintetizará as evidências disponíveis sobre a precisão, a aplicabilidade e as características metodológicas destas abordagens em contextos médico-legais.

Conclusão: Esta revisão sistemática proporcionará uma visão abrangente das evidências disponíveis relativas à utilização de métodos espectroscópicos e moleculares para a estimativa da idade e do sexo a partir de amostras dentárias. Ao identificar as metodologias atualmente utilizadas, os resultados relatados e as lacunas de investigação existentes, a revisão poderá contribuir para as futuras orientações de investigação e apoiar a tomada de decisões baseadas em evidências na prática forense e médico-legal, particularmente em contextos em que são desejáveis abordagens analíticas não destrutivas.

Introduction

Forensic sciences often rely on accurate methods to estimate age and sex from human remains, particularly dental specimens, which are highly resistant to post-mortem (PM) changes.¹⁻³ Traditional methods, including morphological assessment and genetic analysis, have well-established roles in forensic identification.^{1,4} However, these approaches may present limitations, including observer-dependent interpretation, challenges associated with degraded remains, the need for destructive sampling in some analyses, and variability in performance according

to the condition of the specimens and the methods employed.^{1,4}

In recent years, spectroscopic techniques such as Raman spectroscopy⁵, vibrational and infrared spectroscopy², and mass spectrometry⁷ have emerged as promising alternatives or complementary tools for forensic investigations.

These techniques may provide rapid analyses, objective measurements, and reduced sample destruction while enabling the assessment of biochemical and mineral changes in dental tissues.^{2,5,7} Similarly, molecular methods have undergone significant development, with amelogenin analysis demonstrating reliable sex estimation even in degraded

dental samples⁸⁻¹⁰, while DNA methylation¹¹ and telomere length assessment¹² have shown potential for chronological age estimation.

Despite the growing number of studies on these techniques, the available evidence remains dispersed across different methodological approaches and forensic outcomes. Recent reviews have focused on specific biochemical, genetic, or epigenetic methods for age estimation⁴, amelogenin-based approaches for sex estimation^{9,10}, or individual analytical technologies. However, to the best of our knowledge, no systematic review has specifically compared spectroscopic and molecular methods applied to human dental specimens for both age and sex estimation within a medico-legal context. A comprehensive synthesis of the available evidence is therefore needed to evaluate the methodological characteristics, reported outcomes, applicability, strengths, limitations, and research gaps associated with these approaches. This systematic review aims to address this gap by comparing spectroscopic and molecular methods for estimating age and sex using human dental samples and by identifying areas that may require further methodological development and future research. Unlike previous reviews that have focused on individual techniques, biomarkers, or a single forensic outcome, this review will provide a direct comparison between spectroscopic and molecular methods for age and sex estimation using dental samples. By integrating evidence across these methodological categories, the review aims to identify their respective strengths, limitations, diagnostic performance, and current evidence gaps, thereby providing a novel contribution to forensic odontology and helping to guide future research and medico-legal applications.

Methodology

Study protocol

The research question to be addressed in this research protocol is: *What scientific evidence is available on the effectiveness of spectrometry, compared to other methods, in estimating age and sex using human dental specimens in medico-legal contexts?*

The following PICO criteria (Population, Intervention, Comparison, Outcome) is the focus of this systematic review to address the research question:

- Population: Human dental specimens (whole teeth or fragments) analysed for forensic, archaeological, or clinical age/sex estimation.
- Intervention: Spectroscopic techniques (e.g., mass spectrometry, infrared spectroscopy, Raman spectroscopy, or atomic absorption spectrometry).
- Comparison: Molecular methods
- Outcomes: Accuracy, sensitivity, specificity, predictive values (PPV, NPV), AUC (ROC curve), and applicability.

The methodology to be applied will focus on search of scientific articles published in Portuguese, English and Spanish, without time restrictions. To fulfil the goal of this systematic review, a comprehensive bibliographic search will be conducted across multiple scientific databases, including PubMed, Web of Science, ScienceDirect, LILACS

(via BVS), Cochrane Library (Protocols), MEDLINE (via EBSCOhost), Academic Search Complete (via EBSCOhost), CINAHL (via EBSCOhost) and Grey Literature.

The articles will be selected through Rayyan, after being retrieved from the databases mentioned above. For the final review, the items identified in the reports prepared for the guidance of systematic reviews and the extension of meta-analyses (PRISMA 2020) will be used. This research protocol has been registered in PROSPERO (<https://www.crd.york.ac.uk/PROSPERO2/view/CRD420250650858>).

Inclusion and exclusion criteria

Inclusion criteria: Studies using spectroscopy for the analysis of teeth focusing on age and sex estimation; Studies with human samples that employ techniques applicable to humans; Studies published in English, Portuguese, Spanish. Exclusion criteria: Studies that do not use spectroscopic methods as main analysis method; Studies that work exclusively on other dentistry areas without focusing on age and sex estimation.

Search strategy

For this systematic review, searches will be conducted in: PubMed, Web of Science, ScienceDirect, LILACS (via BVS), Cochrane Library (Protocols), MEDLINE (via EBSCOhost), Academic Search Complete (via EBSCOhost), CINAHL (via EBSCOhost). Grey literature and manual screening of reference lists will also be included.

A preliminary search was carried out in these databases to identify the keywords and index terms used in the publications concerning the theme. This allowed the development of the search strategy for each database (Table 1).

The reference lists of all included articles will be reviewed for the possibility of including additional articles. After the search, the identified articles will be deposited in the ENDNOTE program. The electronic search results will be exported to Rayyan[®],¹³ and duplicates will be eliminated. The developer of the software is Rayyan Systems Inc. from Cambridge, MA, USA. Software AI-driven will not be used to select the articles. Rayyan software will be used as a support tool, only to gather all the articles found in the different databases described above and identify duplicates.

Study selection

Two reviewers, independently, will collect the data from the articles to decide their inclusion in this systematic review. Doubts and disagreements will be discussed using a third reviewer according to the peer review of the Electronic Search Strategies (PRESS) checklist.¹⁴

The preliminary test will be carried out by two independent reviewers, that will start by the analysis of title/abstract of articles and then, the full text. Following this analysis, 5% of the total articles will be used to get at least 75% consensus between reviewers. In the next step of the research, 2% of the full-text articles will be used to obtain the same level of agreement.

Studies that include the key goal of this review will be obtained through the identification, selection, eligibility and

application of the inclusion and exclusion criteria and research limiters.

Table 1. Bibliographic research strategy.

Database	Articulation of keywords	Number of articles
<i>Pubmed</i>	(((((teeth) OR (tooth)) AND ((Spectro*) OR (genetic*))) AND (((Age estimation by Teeth[MeSH Terms]) OR (Gender estimation)) OR ((age estimation) OR (sex estimation)))) AND (forensic*))	90
<i>Web of science - clarivate</i>	(((((ALL=(teeth)) OR ALL=(tooth)) AND ALL=(spectro*)) OR ALL=(genetic*)) AND ALL=(age estimation by teeth)) OR ALL=(sex estimation)) AND ALL=(forensic)) NOT ALL=(odontometric)	1,812
<i>Science Direct</i>	(((((teeth) OR (tooth)) AND ((Spectro) OR (genetic))) AND (((Age estimation by Teeth[MeSH Terms]) OR (gender estimation)) OR ((age estimation) OR (sex estimation)))) AND (forensic)	1,300
<i>Medline (via EBSCO)</i>	TI teeth OR AB teeth OR SU teeth OR TI tooth OR AB tooth AND TI spectro* OR AB spectro* OR TI genetic* OR SU genetic* OR AB genetic* AND SU (sex estimation) OR TI sex estimation OR AB sex estimation OR TI age estimation OR SU age estimation OR AB age estimation AND TI forensic* OR SU forensic* OR AB forensic*	49
<i>Academic search complete (via EBSCO)</i>	TI teeth OR AB teeth OR SU teeth OR TI tooth OR AB tooth AND TI spectro* OR AB spectro* OR TI genetic* OR SU genetic* OR AB genetic* AND SU (sex estimation) OR TI sex estimation OR AB sex estimation OR TI age estimation OR SU age estimation OR AB age estimation AND TI forensic* OR SU forensic* OR AB forensic*	32
<i>Cochrane Library (protocols)</i>	teeth OR tooth AND spectro* OR genetic* AND sex estimation OR age estimation AND forensic*	12
<i>Cinahl (via eb-sco host)</i>	TI teeth OR AB teeth OR SU teeth OR TI tooth OR AB tooth AND TI spectro* OR AB spectro* OR TI genetic* OR SU genetic* OR AB genetic* AND SU (sex estimation) OR TI sex estimation OR AB sex estimation OR TI age estimation OR SU age estimation OR AB age estimation AND TI forensic* OR SU forensic* OR AB forensic*	6
<i>Lilacs (via bvs)</i>	teeth OR tooth AND Spectro* OR genetic* AND sex estimation OR age estimation OR sex estimation AND forensic*	4
<i>Grey literature</i>	teeth OR tooth AND spectro* OR genetic* AND sex estimation OR age estimation AND forensic*	0

Data analysis and presentation

The data extraction form presented in Table 2 was developed by the authors specifically for this review. Its content was informed by the review objectives, the PICOS framework, and PRISMA 2020 recommendations, and includes the variables considered necessary to characterize the evidence sources and answer the review question.

After reading the full articles included in this review, data will be extracted according to goals and research questions of the present review. The tool that will be used for that, will include the following relevant information: title, author(s), year of publication, country of origin, type of study, objective(s), sample size, methodology used and main evidence relevant to the objective of this review, as shown in Table 2.

The collected data will be presented in narrative form using a qualitative assessment tool and corresponding coding.

Table 2. Form used for data collection.

Systematic Review Title	Comparison of spectroscopic and molecular methods for estimating age and sex for medico-legal purposes: protocol for a systematic review
Review objective(s)	Investigate if spectroscopic methods are comparable with molecular methods in estimating sex and age in forensic context.
Review Question(s)	Are spectroscopic techniques comparable to molecular methods for sex estimation and age estimation in forensic cases?
Inclusion/ Exclusion Criteria	Inclusion criteria: Studies using spectroscopy for the analysis of teeth focusing in age and sex estimation; Studies with human samples that employ techniques applicable to humans; Studies published in English, Portuguese, Spanish. Exclusion criteria: Studies that do not use spectroscopic methods as main analysis method; Studies that work exclusively on other dentistry areas without focusing on age and sex estimation.
Population	Human dental specimens (whole teeth or fragments) analysed for forensic, archaeological, or clinical age/sex estimation.
Intervention	Spectroscopic techniques (e.g., mass spectrometry, infrared spectroscopy, Raman spectroscopy, or atomic absorption spectrometry).
Comparison	Molecular methods
Outcome	Accuracy, sensitivity, specificity, predictive values (PPV, NPV), AUC (ROC curve), and applicability.
Types of Evidence Sources	
Evidence Source Details and Characteristics	
Author(s)	
Year of Publication	
Origin/Country of Origin (where the source was published or conducted)	
Aims/Purpose	
Population and Sample size	
Details/Results extracted from the Source of Evidence	

To map the available evidence and complement the information of each of the articles, a table will include the above and relevant information. The identification, characterization, and synthesis of knowledge that this review will bring, will be related to the proposed objective and review question.

Eligibility criteria

The methodological quality assessment of the studies to be included in this review will be conducted using the QUIN tool (Quality Assessment Tool for In Vitro Studies) according to Sheth et al.¹⁵ for in vitro studies. The QUIN tool consists of twelve questions, each with four possible answers: "adequately specified" (2 points), "inadequately specified" (1 point), "not specified" (0 points), and "not applicable" (excludes the criterion from the calculation). The points are then added to obtain a total score for a given in vitro study. The final QUIN score for an in vitro study is calculated using the following formula: Final Score = (Total Score x 100) / (2 x the number of applicable criteria)

The 12 different criteria evaluated by the QUIN tool are as follows: 1. clearly defined objectives, 2. detailed explanation of sample size calculation, 3. detailed explanation of sampling technique, 4. details of the comparison group, 5. detailed explanation of the methodology, 6. details of the operator, 7. randomization, 8. outcome measurement method, 9. details of the outcome assessor, 10. blinding, 11. statistical analysis, and 12. presentation of results. A score higher than 70% indicates a low risk of bias, between 50% and 70% indicates a medium risk, and below 50% indicates a high risk of bias.

Results

Based on the preliminary literature search, this systematic review is expected to identify a range of spectroscopic and molecular techniques applied to human dental tissues for forensic age and sex estimation, including mass spectrometry⁷, amelogenin analysis⁸⁻¹⁰, Fourier-transform infrared (FTIR) spectroscopy⁶, Raman spectroscopy⁵, X-ray fluorescence², DNA methylation analysis¹¹, and telomere length assessment.¹²

The included studies are expected to vary in terms of study design, population characteristics, sample type, analytical protocols, and reported outcome measures. The review will examine how these techniques have been applied in forensic contexts and will synthesize the available evidence regarding their methodological characteristics, reported outcomes, and potential applicability for age and sex estimation using dental samples.^{2,5-12}

The synthesis of the collected evidence may contribute to a better understanding of the current state of research in this field, including areas of methodological consistency, sources of heterogeneity, and existing knowledge gaps⁴. Furthermore, the review will explore the range of spectroscopic and molecular approaches currently reported in the literature and assess how these methods have been investigated for forensic identification purposes using human dental specimens.¹⁻¹²

Discussion

This systematic review aims to assess and compare the available evidence regarding spectroscopic and molecular methods used for age and sex estimation from human

dental tissues in medico-legal contexts. By synthesizing the existing literature, the review seeks to provide a comprehensive overview of the analytical techniques currently available, their methodological characteristics, reported outcomes, and potential applicability in forensic practice.

Accurate estimation of age and sex remains a fundamental component of human identification, particularly when traditional identification methods are unavailable or when human remains are fragmented, decomposed, or otherwise compromised. Dental tissues represent one of the most valuable sources of forensic information due to their resistance to post-mortem degradation and environmental influences. Consequently, a better understanding of the analytical approaches available for age and sex estimation using dental specimens may support evidence-based decision-making and contribute to the selection of appropriate methodologies according to the characteristics of each forensic case.

In recent years, both spectroscopic and molecular techniques have attracted increasing interest in forensic science. Spectroscopic approaches offer the potential advantage of analysing dental tissues with minimal destruction to the specimen, while molecular methods have expanded the range of biological markers available for forensic identification. As research in this field continues to evolve, a structured synthesis of the available evidence is needed to clarify how these methods have been applied, which outcomes have been evaluated, and the methodological considerations associated with their use.

The proposed review may also contribute to the identification of areas where evidence remains limited or inconsistent. Mapping the available literature may help clarify trends in the application of different analytical techniques, reveal methodological challenges, and highlight opportunities for future research. Furthermore, by directly comparing spectroscopic and molecular approaches within a single evidence synthesis, this review seeks to overcome the fragmented nature of the existing literature. The identification of methodological gaps, areas of agreement, and unresolved challenges may contribute to the development of more robust forensic protocols, support methodological standardization, and inform future research priorities. Such information may be particularly valuable in forensic disciplines, where reproducibility, reliability, and transparency are essential requirements.

Some limitations are anticipated during the review process. Variability in study design, sample size, population characteristics, dental tissues analysed, analytical protocols, and outcome measures may result in considerable methodological heterogeneity among the included studies. Differences in reporting practices and study quality may also affect comparability across studies and may limit the feasibility of quantitative synthesis. Additionally, the rapidly evolving nature of both spectroscopic and molecular technologies may contribute to variations in analytical performance and reporting standards across the literature.

To address these challenges, the methodological quality of the included studies will be assessed using the QUIN tool, allowing a structured evaluation of potential sources of bias and methodological limitations. This approach is expected

to strengthen the interpretation of the evidence and facilitate a more transparent assessment of the current state of knowledge in this field.

Overall, this systematic review aims to provide an updated and comprehensive synthesis of the evidence regarding spectroscopic and molecular methods for age and sex estimation using dental samples. By identifying methodological characteristics, reported outcomes, and current knowledge gaps, the review may contribute to future research directions and support the advancement of forensic odontology and medico-legal practice.

Conclusion

This protocol establishes a transparent and replicable methodology to systematically assess the diagnostic value of spectroscopic techniques, including Raman spectroscopy, FTIR spectroscopy, mass spectrometry, and X-ray fluorescence, as well as molecular markers such as amelogenin, DNA methylation, and telomere length, for age and sex estimation using human dental tissues.

By applying a structured and comprehensive search strategy, predefined eligibility criteria, and a standardized methodological quality assessment, this review aims to provide a robust synthesis of the available evidence regarding the application of these methods in medico-legal contexts.

Furthermore, the direct comparison of spectroscopic and molecular approaches within a single systematic review may contribute to a better understanding of their methodological characteristics, reported outcomes, strengths, limitations, and current evidence gaps. This approach has the potential to reduce fragmentation within the existing literature and to support future research priorities in forensic odontology.

Ultimately, the findings of this review may contribute to evidence-based decision-making in forensic practice and support the development of more standardized and scientifically informed approaches for age and sex estimation from dental samples.

The data supporting the results will be provided on request.

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