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MINI REVIEW

Review of tooth enamel isotope analysis in forensics

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ABSTRACT

Isotope analysis has become crucial in forensic anthropology, particularly over the last decade. This method integrates multi-isotope profiles such as C, N, O, H, S, Sr, and Pb, along with isotopic landscapes ("isoscapes") from various tissues like teeth, bone, hair, and nails. These analyses predict the potential region-of-origin of unidentified human remains, offering additional evidence for human identification, including birth region, adult residence, travel history, and dietary habits. The principles of isotope analysis involve using natural variations in isotopic ratios to infer geographic origins and behavioral patterns of individuals. Instruments like mass spectrometers are pivotal for precise measurements, ensuring accuracy in data collection. Analytical standards and quality measures are implemented to maintain reliability across analyses. Sample selection is critical, focusing on tissues that best reflect an individual's life history and environmental exposure. Future research aims to refine isotope analysis techniques, improve interpretative frameworks, and expand databases of isotopic signatures from diverse populations. This ongoing development underscores the utility of isotope analysis as a dynamic tool in forensic science, continually enhancing its potential to uncover critical information in complex cases of human identification and geographical tracing.

KEYWORDS

Forensic anthropology; Isotopes; Body remains; Environmental exposure; Human identification

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Introduction

Forensic anthropologists assess human skeletal remains for legal purposes, focusing on medicolegal relevance, creating biological profiles, evaluating antemortem conditions, estimating postmortem interval, and analyzing both perimortem trauma and postmortem changes [1]. They crucially aid in identifying individuals by determining sex, age-at-death, ancestry, and stature, and noting any anomalies, trauma, medical interventions, or pathologies. While helpful in narrowing searches, definitive identification typically relies on fingerprint comparison, DNA profiling, dental records, and radiographic analysis. Advances in these methods have significantly improved the identification of unknown decedents [2-5]. Traditional forensic tools require antemortem records for comparison with postmortem data. In longstanding unidentified remains cases, newer scientific methods like isotope analysis have become promising over the past decade for generating investigative leads in human identification.

Human dental enamel retains isotopic signatures from the diet during its formation, providing forensic insights into an individual's geographic origin and dietary habits based on enamel analysis [6-8]. In forensic science, enamel's isotopic signature reflects an individual's diet and geographic location during childhood. Enamel's high mineral content and low porosity prevent remodeling and resist diagenetic changes, making it a reliable indicator for analyzing childhood dietary and locational history [9-13]. Strontium, oxygen, and carbon isotope analysis are key forensic tools used to trace human origins and geographical movements. Isotopic differences in human tissues are key for diet and migration analysis. Despite significant enamel geochemical diversity, the correlation between individual and population-level variation in isotopes remains insufficiently quantified in humans [14-17]. Variability in isotopic inputs during enamel formation affects both intraindividual variations. Additionally, intraindividual variations can result from differences in sampling location, method, or enamel damage, such as caries, which can impact forensic analysis outcomes.

Thermal ionization mass spectrometry (TIMS) for strontium analysis and isotope ratio mass spectrometry (IRMS) for oxygen and carbon isotopes are widely used in archaeology and forensics for their high precision and accuracy in isotopic measurements [18]. In forensic science, the absence of standardized enamel sampling methods for isotopic analysis in human teeth leads to variability within individuals and reduces comparability across studies. Current practices typically involve sampling a single location across the tooth's inner enamel without considering different enamel growth phases, impacting the reliability of results [19]. This sampling method doesn't consider how different parts within a tooth might vary in isotopic composition. It's unclear if sampling from one spot accurately reflects the overall Sr-O-C isotopic makeup of the entire tooth, which is crucial in forensic analysis. Enamel is typically collected in forensic science using handheld drills equipped with tungsten or diamond bits or saws [20,21]. Some research indicates that environmental factors, like saliva and burial conditions, can introduce Sr contamination into surface tooth enamel post-mineralization (~0.1 mm) [22-24]. To mitigate this, forensic practices involve mechanically removing the outer enamel layer before sampling to ensure accurate analysis [25-28].

The study investigates how variations in strontium, oxygen, and carbon isotopes within non-migratory individuals' dental enamel can provide a baseline for

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interpreting mobility and diet in forensic and archaeological contexts. It examines intra-tooth isotopic differences, the impact of dental caries, and regional influences on isotopic patterns to refine sampling protocols for discerning dietary shifts and mobility.

Basics of Enamel Isotopic Analysis

Enamel, a critical component of calcified tissues in animals, primarily consists of hydroxyapatite, composed of calcium, phosphate, and water. It is distinguished by its exceptionally high inorganic content, averaging around 98% [29]. During development, enamel includes proteins like amelogenin and enamelin, with amelogenin being predominant and gradually replaced as enamel matures through calcification. This process involves the removal of over 90% of the initially abundant amelogenin. This chemical composition and protein evolution are crucial in forensic investigations involving dental analysis, aiding in age estimation and identification processes [30,31]. In chemistry, elements vary in isotopic composition due to differing neutron counts. Among all periodic table elements, only 21 are monoisotopic, meaning they naturally occur in a single isotopic form, crucial for analytical precision in forensic investigations. Elements usually have a main isotope that is common and one or more rare isotopes with distinct stable forms [32].

Isotopic compositions of non-metal elements like hydrogen, carbon, nitrogen, oxygen, and sulfur are described using ratios of rare to common stable isotopes. These ratios, represented as δ-values, indicate deviations from a standard in parts per thousand. For instance, δ^2H expresses hydrogen isotopic composition, while $\delta^{18}O$ denotes oxygen isotopic composition Despite small natural abundance differences, δ-notation is crucial for precise forensic analysis of samples, helping to identify origins or detect alterations based on isotopic signatures [32].

Major stable isotopes for strontium (Sr) and lead (Pb) include those formed naturally and through radioactive decay. For instance, ^{87}Sr is formed from ^{87}Rb decay, while ^{206}Pb, 207Pb, and ^{208}Pb originate from the decay of ^{238}U, ^{235}U, and ^{232}Th, respectively. These isotopes play crucial roles in tracing geographical origins and dating materials in forensic investigations [33].

Isotopes are incorporated into enamel during its formation through the diet and environmental sources. As enamel develops, these isotopes from ingested foods and water are deposited in the hydroxyapatite crystalline structure. The ratio of isotopes in enamel can provide insights into an individual's dietary habits and geographical origin, making isotopic analysis of enamel valuable in forensic investigations for tracing migration patterns.

Methodology

Sample collection

In forensic investigations, collecting enamel samples involves meticulous procedures. First, dental records are reviewed to identify suitable teeth for sampling. After securing legal permissions, forensic experts carefully extract teeth using sterile tools to avoid contamination. Enamel samples are then collected from the tooth's surface using drills or abrasive tools, ensuring minimal damage to the structure. These samples are stored in sterile containers to preserve integrity and prevent external influences. Chain of custody protocols are strictly followed to maintain evidentiary standards. Subsequent analysis, including isotopic and DNA testing, provides critical information for identifying individuals and contributing to criminal investigations [34].

Sample preparation

The enamel is extracted perpendicular to the enamel-dentine junction (EDJ) using a dental microdrill equipped with a diamond-tipped rotary burr and blade that had been cleaned with acid. Samples were taken away from the EDJ itself due to challenges in isolating the thin enamel layer there. Occlusal fissures were deliberately avoided because they are difficult to clean and sample mechanically, and are known to have lower mineral content, which could potentially lead to alterations over time [35-37].

Analytical techniques

In forensic science, isotope ratios of bio-elements are commonly measured using Isotope Ratio Mass Spectrometry (IRMS). To analyze these ratios, samples are converted into simple gases: hydrogen as H_2, carbon as CO_2, nitrogen as N_2, oxygen as CO, and sulfur as SO_2. Traditionally, this conversion was done "off-line," where purified sample gases were alternately introduced into the mass spectrometer with a reference gas of known isotopic composition, a method known as dual inlet-IRMS. Nowadays, continuous flow-IRMS (CF-IRMS) is more prevalent. In CF-IRMS, samples are converted into gases immediately before being introduced into the mass spectrometer, using various peripherals and helium as a carrier gas. In this method, the working gas is analyzed only once, either just before or just after the sample gas. This advancement in technology has streamlined the process, allowing for more efficient and timely isotopic analysis in forensic investigations [38].

Isotope ratios of trace elements are measured using thermal ionization mass spectrometry (TIMS) or multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS). TIMS is the traditional method, but MC-ICP-MS became more popular about 20 years ago. MC-ICP-MS offers the ability to analyze a wider range of elements' isotope ratios more quickly and cost-effectively, with comparable or superior precision. This technique involves ionizing a liquid sample using a high-energy plasma discharge. The ability to quickly and accurately measure isotope ratios is crucial in forensic science for sourcing and identifying materials [39].

Bio-elements

For oxygen and carbon isotope analysis, powdered enamel samples (0.3-0.8 mg) are weighed into exetainer vials. These vials, along with calibration and control standards VICS and IAEA-603, are flushed with helium. Samples are then acidified with anhydrous H_3PO_4 (100%) at 45°C for 24 hours. The resulting gas is analyzed using a Thermo Finnigan Delta plus IRMS equipped with a GasBench II. Isotopic ratios are expressed as δ values in per mil (‰), normalized to IAEA-603 ($\delta^{18}O = -2.6 \pm 0.17$, $\delta^{13}C = 2.5 \pm 0.04$) and referenced to the Vienna Peedee Belemnite (VPDB) standard [40,41].

Trace elements

To extract strontium from enamel samples, they are dissolved in 3 N nitric acid (HNO_3), followed by chromatographic

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separation conducted in a highly controlled clean laboratory environment. All perfluoroalkyl (PFA) laboratory equipment underwent extensive precleaning procedures [36,37]. Quality control involved using aliquots of an in-house synthetic tooth standard (TSTD), containing 0.05 mL, 500 ng of Sr, and 5 mg of calcium hydrogen phosphate ($CaHPO_4$) [36]. The blanks and strontium concentrations were quantified through isotope dilution with an ^{84}Sr spike. Strontium isotope analyses were carried out using a Thermo Scientific Triton Plus thermal ionization mass spectrometer (TIMS) equipped with 1011Ω resistors. Samples and standards were loaded onto out-gassed, annealed rhenium filaments in 1-2 µL of 10% HNO_3, along with 1.5 µL of tantalum pentachloride ($TaCl_5$). The strontium isotope ratios are adjusted to $^{86}Sr/^{88}Sr = 0.1194$ using the exponential mass-fractionation law [42].

Statistical analysis

Statistical analyses in the study is conducted using GraphPad Prism7 software. Normality of the data is checked using the D'Agostino & Pearson test [37]. For the ultrahigh-density analysis (n=20) of dental samples, statistical significance is evaluated via one-way ANOVA followed by Tukey's post hoc test. To facilitate comparison of results, both the range of isotopic values (Δmax–min) and the average intraindividual isotopic variation (avg_isovar) is reported [41].

Case Study

In 1921, the body of a young boy, later dubbed "Little Lord Fauntleroy," was discovered in a pond in Waukesha, Wisconsin. Despite numerous attempts to identify him, his identity remained unknown for many years. In 2014, forensic scientists employed isotopic analysis on the boy's tooth enamel to uncover clues about his origins, providing a new direction in this long-standing mystery [43].

The first step in the process involved collecting samples of the boy's tooth enamel. Tooth enamel is a highly resilient tissue that preserves isotopic signatures, which can reveal information about the geographical regions where a person resided during their tooth development. This characteristic makes enamel particularly valuable in forensic investigations. The scientists then conducted an isotopic analysis focusing on the ratios of oxygen and strontium isotopes in the tooth enamel. The composition of these isotopes varies across different geographical areas due to environmental factors such as water sources and the underlying geology of the region. By analyzing these variations, scientists can deduce the likely locations where a person lived during specific periods of their life [44].

The isotopic signatures obtained from the enamel were compared to existing isotopic maps of various regions. These maps provide detailed information on the isotopic composition of different areas, allowing researchers to narrow down the boy's potential places of origin. Through this comparison, the scientists concluded that the boy probably spent his early years in the southeastern United States. This finding was significant as it offered new leads for investigators, directing the search for the boy's identity towards a specific region. While the boy's identity has yet to be definitively established, the isotopic analysis provided critical insights that were previously unavailable through conventional forensic methods [45].

This case underscores the valuable role of isotopic analysis in forensic science. By analyzing tooth enamel, forensic scientists can uncover vital geographical information about an individual's early life, which can be instrumental in solving cases where other methods have been exhausted. The "Little Lord Fauntleroy" case illustrates the potential of isotopic studies to provide crucial information, helping to piece together the life history of unidentified individuals and offering new avenues for investigation.

Conclusions

Forensic scientists and police face significant challenges in identifying unknown bodies due to pressure from judicial, civil, and societal sectors. Identification typically involves comparing post-mortem data with available antemortem records, such as dental and medical radiographs, DNA profiles, and unique personal features like birthmarks or surgical scars. However, these traditional methods often fall short when dealing with decomposed or deliberately mutilated bodies. In such cases, investigators must rely on missing persons registries for potential matches. Enhancing identification efforts with additional data like birth year, gender, and residential patterns can be beneficial. To further aid identification, forensic experts are turning to techniques like isotope analysis of human tissues—hair, nails, and tooth enamel. These methods help reconstruct models that predict the life history of the deceased, based on geographic origin and socioeconomic factors, thereby improving the chances of establishing the deceased's identity.

Disclosure statement

No potential conflict of interest was reported by the authors.

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