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The Uses of Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM/EDS), Stable Isotope Analysis and Dental DNA in Forensic Archaeology: A Review

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ABSTRACT:

Analyzing human skeletal remains discovered at archaeological sites can be challenging, and several methods can be utilized to gather substantially accurate information. The presence of bone and/or dental tissue can be specified utilizing scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM/EDS). DNA analyzing on the teeth can determine the species that are present. While working with skeletal remains at historical sites, Forensic Odontologists should be aware that DNA analysis can help determine a person's sex and age at death in addition to positively identifying them. Stable isotope analysis, on the other hand, can assist in clarifying postmortem intervals. The distribution of stable isotopes in food webs enables the reconstruction of ancient diets as well as the geographical origin and migration of humans.

KEYWORDS: Skeletal remains, Archaeology, SEM/EDS, DNA, Stable isotope analysis.

I. INTRODUCTION

Teeth have the highest mineralization of any tissue in the body. Furthermore, teeth are the hardest and most chemically stable structure, allowing them to persist long after the supporting framework has been damaged. Teeth, unlike bone, can provide information on the diet and health of deceased individuals throughout their lifetime because they interact directly with the environment during the chewing process. While bones can decompose over time, teeth are usually well-maintained enough for visual and scientific analysis [1].

Teeth may be crucial to the analysis of archaeologically found materials. Because teeth are thought to be capable of determining indicators of human behavior, their involvement in archaeology is used as a contributing factor in archaeological investigations. These indicators are then utilized to highlight patterns of incidental dietary implementation from individual cultures[2, 3]. According to research by Hu et al. (1999) and Kim et al. (2000) in Min-Kyu (2017), teeth discovered in archaeological contexts also play a significant role in physical anthropology, dietary patterns in prehistoric humans, and stages of human cultural evolution can all be examined by incorporating genetic analysis of human skeletal remains in dental anthropology[4]. Moreover, the morphological complexity of human teeth is a valuable clue for assessing an individual's genetic lineage since it reflects complex aspects of evolution and the genetic footprint of ancestors [5].

When human skeletal remains are discovered at archaeological sites, not only cultural elements or human evolution must be evaluated, but an expert must also be able to analyze and prove whether the finds are actually human skeletal remains or not. If the discovery is already in the form of fragments or small fragments, the fragments must be analyzed and proven to be from bones or teeth. So, special techniques are needed during the analysis and proving process [2, 4, 6].

Various analytical techniques have emerged to extract information from teeth that can be related to the lifestyle of human ancestors; in addition, several techniques can be used to analyze and prove the findings of skeletal remains attributed to humans or non-humans; these various techniques are widely used and continue to expand.

This paper discusses some of the techniques that can be used in the analysis and evidence related to the discovery of skeletal remains at archaeological sites to provide information to forensic odontologists, forensic anthropologists, and forensic archaeologists in carrying out their duties, and it can be hoped that these experts have skills that can help them identify skeletal remains or teeth that may be found during exhumation at archaeological sites. This paper is also expected to better grasp the techniques for examining skeletal remains for various sorts of taphonomy examination to track evidence.

II. MATERIALS & METHODS

Literature searches are conducted using search databases such as PubMed, Google Scholar, Semantic Scholar, Web of Science, Science Direct, and Scopus. We looked for clinical trial articles, review articles, systematic reviews, and meta-analyses. Furthermore, the type of research requested was observational research and case studies completed between 1989 - 2023. Keywords included in the search included skeletal remains, forensic archaeology, SEM/EDS, DNA, and stable isotope analysis. The search included all randomized controlled studies published in English between January 1989- December 2023. Titles, authors, abstracts, case studies, and systematic reviews are identified and printed before being independently assessed by three reviewers based on keywords, titles, and reviews to see whether they fulfill the review objectives.

III. DISCUSSION

Forensic archeology uses archeological methodologies to seek for, discover, document, and map human bodies or structures in a medical-legal setting. Forensic archaeology is responsible for preserving evidence and maintaining the site intact (Potential hazard). Forensic relics, including artifacts and prehistoric fossils, are frequently discovered by accident, usually as a result of construction or erosion.When human skeletal remains are discovered at archeological sites, an expert must not only assess cultural components or human evolution, but also examine and show whether the artifacts are human skeletal remains. If the discovery consists of bits or small fragments, the fragments must be studied to determine whether they are from bones or teeth. Therefore, unique procedures are required during the analysis and proving phase:

Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM/EDS): If archaeological site finds are found in the form of minuscule fragments, SEM/EDS can be utilized to determine if they are bones or teeth. SEM/EDS analysis of unidentified specimens yields X-ray spectra that show compositional content. The elements that are present and their relative abundances can be seen on the spectrum. Moreover, SEM/EDS provides visual pictures that can be used to recognize the components of the bone's or tooth's structural component. SEM/EDS provides elemental analysis for recognizing specimens through spectrum analysis. High concentrations of calcium and phosphorus and low concentrations of other elements can be found in the tissues of the bone and teeth [6].

In an endeavor to create a prototype database for X-ray spectra for comparative research, the Federal Bureau of Investigation (FBI) started its work in 1994. To aid identification, The system allowed comparison between the database and the percentage of X-ray counts of each element in a sample that was submitted. Notwithstanding the fact that data entry is manual and the system has other flaws, this endeavor demonstrates the possibilities of this kind of SEM/EDS analysis for database applications[7].

In addition to effectively identifying bone or teeth tissue, SEM/EDS analysis is advised since it is less invasive, leaving enough sample residue for subsequent analysis to clarify the species and potentially collect the necessary molecular evidence for identification [6].

Stable Isotope analysis: It is a useful technique for reconstructing ancient diets, establishing human origins and migrations, and researching the history of human weaning. Carbon, nitrogen, oxygen, and strontium isotopes are the most commonly studied. Meanwhile, the structural protein analyzed is collagen derived from bone or dentin [8]. Geologists, archaeologists, and anthropologists have long utilized this technique to obtain data on sociocultural questions about patterns of material durability in teeth. In archaeological excavations, fragments of human teeth, which consist of the remains of tissues recovered from archaeological sites, can be isotopically analyzed[2].

Teeth are resistant to physical changes caused by environmental factors over time. Teeth have potential changes in mineralization that can accrue throughout time, which can be utilized to analyze features of geographical location, the origin of nutrients, and past behavior[9]. Teeth are particularly useful since they have good biogenic element preservation in their structure and are resistant to diagenesis. Stable carbon isotopes are the most extensively used palaeodietary probes, and this technique uses the carbon content of carbon dioxide occurring in the atmosphere, primarily in two stable isotopic forms, 13C and 12C isotopes. Carbon isotopes are found in both plant and animal structures. However, the levels of 13C and 12C vary between animal and plant groups. During photosynthesis, carbon diffuses into plant pores as carbon dioxide, with different plant groups obtaining carbon from carbon dioxide differently. Different groups of plants incorporate different numbers of carbon isotopes into their tissues. When these plants are consumed, the isotope of carbon is incorporated into the hydroxyapatite of bones and teeth in different amounts. Using mass spectrometry techniques, the primary components of the diet may then be identified by measuring the relative amounts of each isotope in the hydroxyapatite [8].

The isotope ratios of strontium and oxygen in hydroxyapatite have been used to study ancient peoples' geographical origins and movements. Different rocks and soils have varied strontium isotope ratios; consequently, plants and animals living in different geological regions have different strontium isotope ratios [4]. Moreover, radiocarbon analysis offers the most precise method for evaluating the time since death. The 5,730-year half-life of carbon-14 used in conventional radiocarbon analysis, can validate the skeletal remains' antiquity if they predate AD 1950. The radiocarbon value should be evaluated in light of the bomb-curve if fragments are discovered after 1950 AD [10].

In the 1950s, massive amounts of artificial radiocarbon were created by thermonuclear devices designed to test the atmosphere. These high concentrations of radiocarbon were digested by living organisms, including humans. Until the beginning of the 1960s, atmospheric levels climbed rapidly; after that, atmospheric examining came to an end, levels declined progressively. As a result, people born after 1950 AD had substantial radiocarbon levels integrated into their tissues, including bones and teeth. It is critical in forensic analysis first to examine tissues that are most likely to contain modern radiocarbon [11].

If teeth fragments are discovered, and they are modern human fragments, enamel analysis can explain the estimated birth period. Enamel on teeth does not change shape. Thus, radiocarbon discovered in enamel reflects the level of diet at the time the teeth were formed. If the teeth fraction found is wide enough, it makes it possible to analyze samples from two separate formation areas, allowing for placement in the bomb-curve. Ubelaker& Wu's (2020) study provides an example: The occlusal part's cusp forms earlier or prior the crown base. Varying results from the analysis of the radiocarbon samples from these diverse crown areas will enable placement on the bomb-curve. If the crown base values exceed the occlusal surface values, teeth formation will correspond to an early period before 1964. Conversely, When the crown base values are less than the occlusal surface values, it means that the time was formed after 1964 [6].

Mostly in archaeology, human teeth enamel relies on bulk sampling methods where enamel is collected from the occlusal margin to the junction of enamel junction elements across the mesial bucco-lingual and distal teeth crown surfaces [2]. In fact, isotope analysis revealed that the most resistant phosphate groups were preferred over plentiful digenetic carbonates. When applied to food webs, this gives potential evidence of ancient cultures, allowing direct estimations of tropical dietary levels and subsistence [12].

DNA:DNA is the genetic material of living cells, and its information regulates the body's structure, development, and metabolism. The recovery of genetic material from skeletal tissue has become an important research tool in various scientific domains. DNA analysis makes it possible to determine sex when conventional identification methods are unreliable. The most plausible approach for determining sex is DNA. Despite its flaws, amelogenin gene research provides compelling proof of sex [13]. Additionally, DNA analysis can help predict the origins, relationships, and movements of human populations [1].

Despite the fact that the amount of cellular DNA in teeth is less than that in bone, teeth are frequently used as samples because the outer acellular enamel protects dentin from environmental damage [14]. Typically, many methods used by molecular researchers are destructive, such as cutting, crushing, or grinding the morphological structure of the teeth, which causes curatorial issues among anthropologists and museum curators. However, Alakoç and Aka (2009) described a method to obtain DNA from fossilized molars, which involves preparing an entrance cavity on the occlusal surface of the teeth and extending into the pulp chamber. The pulp and dentin tissues are removed with a K-file, and the teeth are restored with composite fillings. This technique allows samples to be acquired for DNA analysis while preserving the morphological structure of the teeth and protecting rare archaeological specimens from damage [15].

In forensic archeology, DNA analysis can also reveal certain details about species, ancestry, and death date. Even though molecular analysis using protein-based DNA samples provides specific information regarding species, ancestry, and age at death, DNA samples taken from bones and teeth are frequently utilized to identify human skeletal remains by comparing their DNA profiles [16].

DNA types developed from skeletal bones and teeth are incredibly discriminatory between people, which helps to rule out potential connections between various samples. Short tandem repeats (STR) are now being used instead of restriction fragment length polymorphism (RFLP) to measure variation in DNA isolated from bone fragments and teeth. Within particular areas of the human genome, the probes count the quantity of variable tandem repeats (VNTR) polymorphisms[17]. In forensic identification, genomic DNA from human bones can sometimes produce some results, although it is frequently too deteriorated to produce data that is useful [18].

Because to their prevalence in samples of deteriorated DNA and low-abundance DNA templates, STRs are the most widely used forensic markers. Since the number of tandemly repeated DNA sequences varies greatly between people, STRs are polymorphic. As a result, it could tell apart those who are related (by kinship) [19]. There have been numerous studies on the utility of STRs in forensic identification. DNA was gathered from numerous bone fragments found in mass graves that originally belonged to Yugoslavia, and STR typing was done on those DNA by Milo et al. (2007). Femur samples had the highest success rate for DNA typing. Moreover, samples collected from whole teeth had very high success rates [20].

In addition to nuclear DNA analysis, mtDNA examination may be useful for forensic identification. Cells have numerous copies of the genome in their mitochondria as opposed to just one copy in the nucleus. Hence, the examination of samples that have been tainted can benefit greatly from the utilization of MtDNA. The success of DNA analysis depends on the amount of DNA extracted, hence DNA extraction is essential for determining the outcome of a forensic investigation. Even though DNA molecules are more stable in hard tissues, DNA extraction is still a difficult process. Occasionally, low-quality, scant genetic material can be obtained from fractured teeth and bones [21].

Advances in DNA extraction and purification are being used to increase DNA recovery and decrease damage and inhibitors [22, 23]. Studies comparing various extraction methods discovered that total demineralization works best on severely degraded human skeletal remains [24–26]. Other investigations examined how recently developed commercial DNA kits with better sensitivity and other technologies like acoustic energy could be used to optimize the DNA extraction process the DNA extraction procedure using newly created commercial DNA kits with improved sensitivity and other technologies including acoustic energy [27]. Moreover, a number of recent publications have demonstrated methods to obtain DNA from skeletal and dental remains that have been heated to a high temperature [28].

IV. CONCLUSION

Applying more modern scientific techniques to the analysis of skeletal remains at archaeological sites can result in greater accuracy and specificity. Such techniques are an essential part of forensic archaeological investigations as they can provide information that may not be retrievable from the archaeological record and can aid in promoting a better understanding of prior populations. Skeletal remains analysis is riddled with challenges. Nonetheless, using suitable techniques will make the analyzing procedure easier.

The SEM/EDS method can be used to distinguish between the fragments found represent bone or teeth. Radiocarbon analysis, with a focus on modern bomb-curves, can examine the essential issue of time since death. DNA analysis offers the maximum information that can be obtained despite the small size of the fragments. It is critical to note that non-destructive testing should come first, followed by genetic testing or other destructive testing.

REFERENCES

[1] R. Forshaw, “Unlocking the past: The role of dental analysis in archaeology,” Dent. Hist., vol. 60, no. 2, pp. 51–62, 2015, [online]. Available on: https://www.researchgate.net/publication/290251601%0AUnlocking.

[2] M. Hilal and A. Basit, “An Application of Odonto-Metric Analysis in Archaeology,” J. Odontol., vol. 2, no. 1, pp. 2–4, 2018.

[3] S. Kumar, S. Rathore, A. Pandey, and A. Verma, “Role of dental expert in forensic odontology,” Natl. J. Maxillofac. Surg., vol. 5, no. 1, pp. 2, 2014, doi: 10.4103/0975-5950.140147.

[4] P. Min-Kyu, “A method for studying human teeth excavated in archaeological sites: A focus on recent research sites,” Anthropol. Notebooks, vol. 23, no. 2, pp. 5–19, 2017.

[5] M. Yaşar İşcan, “The emergence of dental anthropology,” Am. J. Phys. Anthropol., vol. 78, no. 1, pp. 1–1, Jan 1989, doi: 10.1002/ajpa.1330780102.

[6] D. H. Ubelaker and Y. Wu, “Fragment analysis in forensic anthropology,” Forensic Sci. Res., vol. 5, no. 4, pp. 260–265, 2020, doi: 10.1080/20961790.2020.1811513.

[7] D. H. Ubelaker, D. C. Ward, V. S. Braz, and J. Stewart, “The Use of SEM/EDS Analysis to Distinguish Dental and Osseus Tissue from Other Materials,” J. Forensic Sci., vol. 47, no. 5, pp. 15518J, 2002, doi:

10.1520/jfs15518j.
[8] M. A. Katzenberg and A. L. Waters-Rist, "Stable Isotope Analysis: a Tool for Studying Past Diet, Demography, and Life History," in Biological anthropology of the human skeleton, John Wiley & Sons, Inc., 2019, pp. 467-504.
[9] A. K. Carlson, "Lead isotope analysis of human bone for addressing cultural affinity: A case study from Rocky Mountain House, Alberta," J. Archaeol. Sci., vol. 23, no. 4, pp. 557–567, 1996, doi: 10.1006/jasc.1996.0052.
[10] D. H. Ubelaker, "Artificial Radiocarbon as an Indicator of Recent Origin of Organic Remains in Forensic Cases," J. Forensic Sci., vol. 46, no. 6, pp. 15147J, Nov 2001, doi: 10.1520/JFS15147J.
[11] D. H. Ubelaker, "Radiocarbon analysis of human remains: A review of forensic applications," J. Forensic Sci., vol. 59, no. 6, pp. 1466–1472, 2014, doi: 10.1111/1556-4029.12535.
[12] J. Montgomery, "Passports from the past: Investigating human dispersals using strontium isotope analysis of tooth enamel," Ann. Hum. Biol., vol. 37, no. 3, pp. 325–346, 2010, doi: 10.3109/03014461003649297.
[13] E. Tekeli, T. Gültekin, M. E. Doksanaltı, S. H. Öztaner, and C. Elma, "Accurate sex determination using ancient DNA analysis for human skeletal remains from different historical archeological sites in Turkey," Mediterr. Archaeol. Archaeom., vol. 20, no. 1, pp. 93–106, 2020, doi: 10.5281/zenodo.3605672.
[14] C. J. Adler, W. Haak, D. Donlon, and A. Cooper, "Survival and recovery of DNA from ancient teeth and bones," J. Archaeol. Sci., vol. 38, no. 5, pp. 956–964, 2011, doi: 10.1016/j.jas.2010.11.010.
[15] Y. D. Alakoç and P. S. Aka, "'Orthograde entrance technique' to recover DNA from ancient teeth preserving the physical structure," Forensic Sci. Int., vol. 188, no. 1–3, pp. 96–98, 2009, doi: 10.1016/j.forsciint.2009.03.020.
[16] J. M. Butler, Advanced Topics in Forensic DNA Typing: Methodology. London (UK): Academic Press, 2012.
[17] E. S. M. Iwamura, J. A. Soares-Vieira, and D. R. Muñoz, "Human identification and analysis of DNA in bones.," Rev. Hosp. Clin. Fac. Med. Sao. Paulo., vol. 59, no. 6, pp. 383–388, 2004, doi: 10.1590/S0041-87812004000600012.
[18] K. E. Latham and J. J. Miller, "DNA recovery and analysis from skeletal material in modern forensic contexts," Forensic Sci. Res., vol. 4, no. 1, pp. 51–59, 2019, doi: 10.1080/20961790.2018.1515594.
[19] M. V. Emery et al., "Reconstructing full and partial STR profiles from severely burned human remains using comparative ancient and forensic DNA extraction techniques," Forensic Sci. Int. Genet., vol. 46, no. June 2019, pp. 102272, 2020, doi: 10.1016/j.fsigen.2020.102272.
[20] A. Miloš et al., "Success rates of nuclear short tandem repeat typing from different skeletal elements," Croat. Med. J., vol. 48, no. 4, pp. 486–493, 2007.
[21] V. C. Tucker et al., "Developmental validation of the PowerPlex® ESI 16 and PowerPlex® ESI 17 Systems: STR multiplexes for the new European standard," Forensic Sci. Int. Genet., vol. 5, no. 5, pp. 436–448, 2011, doi: 10.1016/j.fsigen.2010.09.004.
[22] T. Siriboonpiputtana, T. Rinthachai, J. Shotivaranon, V. Peonim, and B. Rerkamnuaychoke, "Forensic genetic analysis of bone remain samples," Forensic Sci. Int., vol. 284, pp. 167–175, 2018, doi: 10.1016/j.forsciint.2017.12.045.
[23] D. C. Vinueza-Espinosa, C. Santos, C. Martínez-Labarga, and A. Malgosa, "Assessing DNA recovery from highly degraded skeletal remains by using silica-based extraction methods," Forensic Sci. Int. Genet. Suppl. Ser., vol. 7, no. 1, pp. 810–812, 2019, doi: 10.1016/j.fsigss.2019.10.204.
[24] J. Jakubowska, A. Maciejewska, and R. Pawłowski, "Comparison of three methods of DNA extraction from human bones with different degrees of degradation," Int. J. Legal Med., vol. 126, no. 1, pp. 173–178, 2012, doi: 10.1007/s00414-011-0590-5.
[25] O. M. Loreille, T. M. Diegoli, J. A. Irwin, M. D. Coble, and T. J. Parsons, "High efficiency DNA extraction from bone by total demineralization," Forensic Sci. Int. Genet., vol. 1, no. 2, pp. 191–195, 2007, doi: 10.1016/j.fsigen.2007.02.006.
[26] A. Uzair, N. Rasool, and M. Wasim, "Evaluation of different methods for DNA extraction from human burnt bones and the generation of genetic profiles for identification," Med. Sci. Law, vol. 57, no. 4, pp. 159–166, 2017, doi:

10.1177/0025802417723808.

[27] P. Cavalcanti, H. Freitas, E. F. Carvalho, and D. A. Silva, "Optimized DNA extraction method from skeletal remains using different typing methodologies in forensics," Forensic Sci. Int. Genet. Suppl. Ser., vol. 5, pp. e223–e224, 2015, doi: 10.1016/j.fsigss.2015.09.089.

[28] T. Federchook, J. Pokines, K. Crowley, and C. Grgicak, "Recovery of DNA from Teeth Exposed to Variable Temperatures," Forensic Anthropol., vol. 2, no. 3, pp. 5744, 2019, doi: 10.5744/fa.2019.1029.