Postmortem human identification: Role of dentist

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Abstract

Human identification is currently one of the most outstanding areas of Forensic Sciences. Forensic anthropology is a branch of the Forensic Sciences concerned with the application of general anthropological knowledge and methods to the process of law. Postmortem human identification is one of the major areas of study and research in Legal Medicine and Forensic Dentistry and both sciences work with the same type material and the human body in several conditions.

Keywords: Dental identification, Genomic DNA, dental records.

Introduction

As we enter a new millennium, society is faced with fresh challenges in every conceivable area. Despite leaps in modern technology, medical breakthroughs and the geographical changes that the last century has brought, crime still persists in all aspects of our lives. The apprehension and subsequent prosecution of the perpetrator(s) is essential to maintain law and order. Every human being has an identity in life, which should be recognized after death both for family consolation and for juridical purposes¹. Through the specialty of forensic odontology, dentistry plays a small but significant role in this process. By identifying the victims of crime and disaster through dental records, dentists assist those involved in crime investigation. The most common role of the forensic dentist is

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the identification of deceased individuals². Dental identification takes two main forms. Firstly, the most frequently performed examination is a comparative identification that is used to establish (to a high degree of certainty) that the remains of a decedent and a person represented by antemortem (before death) dental records are the same individual. Information from the body or circumstances usually contains clues as to who has died. Secondly, in those cases where antemortem records are not available, and no clues to the possible identity exist, a postmortem (after death) dental profile is completed by the forensic dentist suggesting characteristics of the individual likely to narrow the search for the antemortem materials³. The forensic dentist produces the postmortem record by careful charting and written descriptions of the dental structures and radiographs. If the antemortem records are available at this time, postmortem radiographs should be taken to replicate the type and angle of these⁴.

Molecular Biology has been recently applied in Forensic Anthropology. Until the 1980's, the

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49

science of identification of criminal cases was based only on serological analyses of protein polymorphism, blood groups and some genetic markers. Jeffreys et al⁵. created radioactive molecular probes that could recognize certain highly sensitive regions of DNA and determine specific patterns of each individual, which were named DNA "fingerprints". The currently performed DNA profile tests are very reliable, being accepted as legal proofs in courts for investigation of paternity and human identification.

There are many acceptable methods for human identification, each one with its limitations. Fingerprints have historically been used to determine people's identity. Although the anthropologic analysis can provide useful information about stature, race and gender, it does not determine someone's identity⁶.

A range of conclusions can be reached when reporting a dental identification. The American Board of Forensic Odontology recommends that these be limited to the following four conclusions - Positive identification, Possible identification, Insufficient evidence, and exclusion⁷.

Role of DNA in dental identifications

The resistant nature of dental tissues to environmental assaults, such as incineration, immersion, trauma, mutilation and decomposition make teeth represent an excellent source of DNA material⁸. When conventional dental identification methods fail, this biological material can provide the necessary link to prove identity⁹. With the advent of the polymerase chain reaction (PCR), a technique that allows amplification of DNA at pre-selected, specific sites, this source of evidence is becoming increasingly popular with investigators. Comparison of DNA preserved in and extracted from the teeth of an unidentified individual can be made to a known antemortem sample (stored blood, hairbrush, clothing, cervical smear, biopsy, etc) or to a parent or sibling¹⁰.

Genomic DNA

Genomic DNA is found in the nucleus of each cell and represents the DNA source for most forensic applications, (there are no nuclei, and hence there is no DNA, in red blood cells.) Teeth represent an excellent source of genomic DNA. Indeed, the authors have found that even root-filled teeth supply sufficient biological material for PCR analysis⁹.

Polymerase chain reaction (PCR) procedures permit reliable replication of thousand of copies of a specific DNA sequence, in vitro, and have been described and improved in recent years, allowing the study of small amounts of DNA even when degraded. These procedures are therefore extremely useful in the analysis of forensic samples. Several lociare especially suitable for PCR analysis¹¹.

In addition to genomic DNA, cells contain mitochondrial DNA (mtDNA), the sequence of building blocks of which can be determined to assist in identification. The main advantage of mtDNA is that there is a high copy number in each cell caused by the high number of mitochondria present in most cells. This infers that in cases where genomic DNA cannot be analyzed, possibly because it is too degraded, mtDNA may be present in sufficient quantity. In addition to its higher copy number, mtDNA is maternally inherited¹². This maternal inheritance pattern confers the same mtDNA sequence, barring mutations, upon siblings and all their maternal relatives. This has important implications for the identification of individuals for which there is no antemortem comparison sample.

Other methods of dental identification

Comparative identification and postmortem profiling, represent the most common methods of dental identification. However, in some instances more novel and innovative techniques have been applied¹³. The NHS provide a fee for dentists who label their patients dentures, although this is often only used in instances where the wearer is a resident in a care home or other establishment with a central sterilizing system for dental prostheses. Labelled dentures can be of great assistance in the identification of individuals¹⁴.

Unlabelled dentures have been recovered from patients and then fitted to casts retained by the treating dentist or laboratory, and this has been an accepted method of identification¹⁵. Other dental appliances, such as removable orthodontic braces have also been used for identification purposes.

Whittaker describes a case where a removable orthodontic appliance was used to identify a victim of a house fire¹⁶. Authors have also described the use of palatal rugae patterns rendered on dental casts to compare with found remains. Positive identifications have resulted from this technique¹⁷.

Dental materials have provided clues to assist identification. One of the authors (DS) has used SEM-EDX to identify the composition of a glass-ionomer restoration and then traced this back to a prison where the filling was placed. Dental records secured the identification of the individual. In another case, it was possible to identify Kevlar fibers that had been placed within a lower denture to reinforce it. This rare procedure enabled an identification of the wearer who was a victim of homicide.

Guidelines for obtaining Dental DNA

The application of this DNA technology to forensic odontology cases is already a fact. DNA has been isolated and characterized from the dental pulp and saliva. This success provides a basis for reassociation of body parts that might not be otherwise possible because of decomposition. There is particular interest in mitochondrial DNA analysis because of its availability, especially in skeletal material remains and its successful characterization by PCR method. Using the DNA analysis' methods, DNA testing can be a powerful method of human identification.Determine if there is any soft tissue or blood adherent to the tooth that should be sampled. Debride the tooth of any plaque or calculus with a curette and wash thoroughly with hydrogen peroxide followed by ethanol.If the tooth is intact (unrestored, non-carious, unbroken) and is believed to have been removed from the alveolus recently, a conventional endodontic access and instrumentation can be conducted.Sectioning the tooth provides a greater access to the pulp chamber (vertical axis sectioning). Once the tooth is opened, the walls of the pulp chamber can be curetted or instrumented with a slow-speed rotary bur. Pulp tissue and powder can be collected over a wide-mouthed sterile container. In dried specimens, the pulp may be mummified, parchment-like or consist of wispy strands of tissue contracted against the chamber wall. After instrumentation, the chamber is best irrigated with TE buffer. Subsequent ultrafiltration of the liquid at the laboratory will remove the cellular material needed for analysis.Finally, crushing the tooth may be necessary. The odontologist then reports the findings he obtained.

Dentist should complete the following procedures to collect the evidence

Documentation

Make a record of the injury, including descriptive, narrative notes that document the physical appearance, colour, size and orientation of the injury¹⁸.

Photographs

Take extensive orientation and close-up photographs using an intra-oral camera with a macro lens and both colour and black-andwhite film. A reference scale, such as a ruler, should be placed in the same plane as the injury and visible in the photographs to enable subsequent measurements

Saliva swabs

Saliva will have been deposited on the skin during biting or sucking and this should be collected and analyzed. Use the double swab technique¹⁹ first, a cotton swab moistened with distilled water is employed to wash the surface that was contacted by the tongue and lips using light pressure and circular motions. Then, a second swab that is dry is used to collect the remaining moisture that is left on the skin by the first swab. Both swabs are thoroughly air-dried at room temperature for at least 45 minutes before they are released to police authorities for testing. The two swabs must be kept cool and dry to reduce the degradation of salivary DNA evidence and the growth of bacteria that may contaminate the samples and reduce their forensic value. Then they should be submitted to the laboratory as soon as possible for analysis.

Impression

Fabricate an accurate impression of the bitten surface to record any irregularities produced by the teeth, such as cuts, abrasions, etc. Use vinyl polysiloxane, polyether or other impression material available in the dental office that is recommended for fixed prosthetic applications²⁰. Dental acrylic or plaster can be used as a rigid support for the impression material. This will allow the impression to accurately record the curvature of the skin.

Clinical examination

The extra-oral and intra-oral structures are examined and significant findings are noted on a dental chart. Special attention is focused on the status of the general dental health, occlusion and mandibular articulation²¹. Results of a specific examination of such things as tooth mobility, periodontal pocketing, dental charting of restorations, diastemata, fractures, caries, etc., and the function of masticatory muscles are documented.

Photographs

Full facial and profile photographs are produced in addition to intra-oral exposures to depict the upper and lower dental arches and frontal and lateral views of the teeth in occlusion²².

Radiograph images

Forensic dentistry involves the identification of people based on their dental records, mainly available as radiograph images. Method involves three stages: Radiograph segmentation, pixel classification and contour matching.

Limitations in forensic odontology

The concept of using dental evidence in forensic investigation has kindled so much interest in the recent past that forensic odontology is even suggested as the single positive identification method to solve certain forensic cases. Discrepancies associated with various methods are to be weighed cautiously to make forensic odontology a more accurate, reliable, and reproducible investigatory science. The various methods employed in forensic odontology include rugoscopy, cheiloscopy, bite marks, tooth prints, radiographs, photographic study, and molecular methods.

There are shortcomings in applying rugoscopy as a definitive tool in forensic odontology are many. Postmortem identification is not possible without the antemortem records. Complex rugae patterns (patterns that cannot be classified under one particular group) can cause intra or interobserver errors. Further, Thomas et al²³ have stated that rugae patterns are genetically determined, and so can be rather used in population differentiation than individual identification.In a situation involving fire, palatal rugae are often destroyed, and also since decomposition and skeletonization can occur in less than six weeks in summer and four months in winter, rugoscopy does not have application after this stipulated period²⁴. Various factors can alter lip print recording. Lip prints have to be obtained within 24 hours of the time of death to prevent erroneous data that would result from postmortem alterations of lip²⁵. Lip print pattern depends on whether the mouth is opened or closed. In closedmouth position lip exhibits well-defined grooves, whereas in open position the grooves

are relatively ill defined and difficult to interpret. Any pathology of the lip such as mucocele or any postsurgical alteration of the lip can change the lip print pattern. Also, loss of support due to loss of anterior teeth can cause changes in lip prints. Any debris or fluid on the lip surface, application of a thick layer of lipstick, or over stretching of cellophane tape can alter lip print recording²⁶. Although lip prints are unique to an individual, when the lines are not clear, individual identification based on this trace is extremely difficult unless the trace contains more individual characteristics like scars, clefts, etc. The science of identification of bite marks is relatively new and potentially valuable in the field of forensic investigation. Due to inherent alterations, the shape and clarity of bite marks found on the skin of the victims change in a relatively short duration (10-20 minutes) both in living and dead, and this necessitates their recording at earliest possible time. Though the photographed immediately, the threedimensional bite marks on the twodimensional photograph will be associated with changes in color and spatial relations. Skin not only is associated with curved surfaces but also is a poor medium for impression²⁷. Further, it has the intrinsic property of distortion leading to considerable variability in the precision of representation of bite marks. Thus, bite mark recording of skin has to be weighed with caution. Also, the site of bite mark on skin is of prime importance²⁸. As curved surfaces tend to distort more than flat surfaces and also the accuracy of various impression materials employed has to be analyzed. Bite marks are associated with hemorrhage²⁹ and postinjury edema, which together can alter bite marks evidence. Also, there are instances in which two sets of teeth can match identically with the bite marks. Sometimes ECG electrode application can resemble bite marks and are to be differentiated.As dental features change over time, changes can occur after obtaining antemortem records. Extraction, trauma, exfoliation, periodontal disease, caries, and prosthesis work can change the configuration of teeth. Thus bite marks are considered less

reliable than other biometric methods. Ameloblasts lay down the enamel rods in an undulating and intertwining path. This is reflected on the outer surface of the enamel as patterns of the ends of a series of adjacent enamel rods. This study of the enamel rod end patterns is termed as ameloglyphics by Manjunath et al³⁰ and could aid as an identification tool in decomposed or burned bodies as enamel can resist decomposition. Though enamel is the hardest mineralized substance in human body, the enamel surface is usually subjected to micro and macrowearing. Fractured, decayed, attrited, abraded, and eroded teeth cannot be included in this method. Various morphological and pathological alterations can be studied from the radiographs. In morphology based studies, root morphology comparatively aids better in identification than crown morphology.(31) Apart from routine findings, like decayed, missed, filled, and fractured teeth, various stages of wound healing in extraction sockets, degree of root formation, and bone trabecular pattern in the jaws aid in identification.

Antemortem records are scant and if available are either incomplete or improper. There are difficulties in matching the viewing angles (identical projection, angulation), exposure, and similar magnification in postmortem radiographs to those taken antemortem. Also, the state of dental remains may entirely preclude the possibility of taking certain types of postmortem radiographs. Photographs are sometimes associated with parallax errors. Lighting, camera orientation, close-up capability, and stability are extremely critical factors while taking photographs. Tripod should support the camera perpendicular to the long axis of the object to be photographed. Photographs without a scale or any circular reference devices may be inherently inaccurate³². Molecular methods are highly accurate, reproducible, and unique, and are extremely reliable in forensic science. Drawbacks however do exist in this relatively new methodology. Errors may develop in sample collection, processing, and interpretation. Any bacterial contamination and second person's DNA can alter the

interpretation. While processing, too little amount of DNA can produce less intense bands which can cause misinterpretation of results. Also, degraded samples can produce very scant amount of high molecular weight DNA.

Discussion

In order to verify heat resistance, Remualdo evaluated the PCR amplification of DNA retrieved from teeth subjected to heat (200°C/ 392°F, 400°C/752°F, 500°C/932°F, and 600°C/1,112°F) .during 60 minutes, testing 3 different DNA extraction methods (organic; ammonia acetate/ isopropanol and silica). The authors concluded that amplification of all samples at all temperatures was possible using the ammonium/isopropanol acetate method, which gives a high credibility to the use of teeth in DNA-based forensic investigations, regardless of tooth conditions. (33).In order to evaluate the different dental tissues as DNA sources in forensic analyses, Malaver and Yunis conducted a study in which 20 teeth were obtained from unidentified bodies buried in 1995 and exhumed in 2000, providing 45 DNA samples (5 from the pulp, 20 from dentin and 20 from cementum). The pulp produced the strongest PCR amplification signals, while dentin and cementum signals were very similar to each other.(34) There is a good possibility of using teeth as sources of DNA material, even in cases where DNA extraction and retrieval seem impossible. If there is a structure in the human body that can resist the most different environment conditions and still provide material for analysis, this structure is the tooth. Forensic dentists use DNA analyses to identify recovered children. Significant quantities of DNA can be recovered from saliva and teeth, but although DNA analysis is a powerful and accurate tool for identifying humans, the methods for recovering DNA from teeth have not been efficient or cost-effective. In a study by colleagues, however, Sivagami and ultrasonication of tooth samples yielded enough DNA to use in polymerase chain reaction (PCR) analysis to be able to determine the sex of the study subjects appropriately. The authors concluded that DNA could be obtained by using this method from any tooth, regardless of the age of the patient. The QuantiT PicoGreen (Invitrogen, Carlsbad, Calif.) assay is another method used to quantify DNA. It is a nonspecific method that relies strictly on the total amount of DNA present rather than the presence of a specific gene. DNA has been isolated and characterized from the dental pulp and saliva. This success provides a basis for reassociation of body parts that might not be otherwise possible because of decomposition. There is particular interest in mitochondrial DNA analysis because of its availability, especially in skeletal material remains and its successful characterization by PCR method. Using the DNA analysis' methods, DNA testing can be a powerful method of human identification.

Conclusion

Postmortem human identification is one of the most important areas of study in Forensic Dentistry, as it can work with the human body in several conditions (quartered, lacerated, carbonized, macerated, putrefied, in skeletonization process and skeletonizated), pursuing to establish human identity. The advent of molecular biology has introduced new methods, equipments and perspectives to the contemporary Forensic Dentistry.

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