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Scope and Application of Forensic Dentistry

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Abstract:
Identification of deceased persons is the most common role of the forensic dentist. Forensic physical comparison of ante mortem and post-mortem dental data is used to establish that a found body and a missing person are one and the same to high degree of certainty. Teeth are amongst the hardest structures of human body which are resistant to adverse conditions. It is also valuable source of DNA as other parts of body get destroyed or degraded in mass disasters. The unique composition of teeth and their location in jaw bone provide additional protection to DNA compared to bones making them preferred source of DNA in many cases. This article overviews the role of forensic dentistry in identification of missing or deceased persons.

Key words*: DNA, odontology, teeth*

1. Introduction

The teeth are the hardest substance in human body and depending upon ambient conditions, characteristics associated to the teeth may provide important and effective method to identify person [1].Teeth can survive in most of condition encountered at death and during decomposition even when body is exposed to extreme forces and temperature. Positive identification of found body through comparison of natural or acquired traits found in the teeth to records from dentist desk is the most common way that forensic odontologist can assist death investigator. But if ante mortem data is unavailable then exact identification becomes difficult and only the DNA profiling systems can reveal the exact identity of a person. Dental tissues exhibit resistance nature to environmental assaults such as trauma, incineration, immersion, mutilation and decomposition and represent as an excellent source of DNA material. When the conventional dental identification methods fail, this biological material can provide the necessary link to prove identity [2, 3]. Matching of the DNA extracted from the teeth of an unidentified individual with DNA isolated from known ante mortem samples such as stored blood, tooth brush, hairbrush, clothing, cervical smear, biopsy to a parent or sibling is the usual procedure in DNA analysis [1]. Forensic dentistry is an important sub speciality of forensic medicine which is contributing immensely in solving difficult criminal cases and in identification of individuals in mass disasters. The world has experienced many mass disasters like acts of terrorism, earthquakes, tsunami, bombings, air crashes, hurricanes and other transportation mishaps in the recent times [4]. In these circumstances, exact identification of an individual becomes difficult as the bodies are destroyed or charred beyond visual recognition. Dental identifications have always played a key role in natural and manmade disaster situations as the teeth and jaw resist extreme conditions and can provide a valuable clue in identification of an individual. Teeth are preferred skeletal source of DNA for several reasons. Due to their unique composition and location within the jaw bones they are largely protected from the environmental and physical conditions that act to accelerate the processes of post mortem decomposition and DNA decay [5, 6]. Therefore, DNA extracted from teeth is often of higher quality and is less prone to contamination than DNA extracted from bones [7]. Human identification is a complex problem that is usually addressed by a multidisciplinary team including odontologist, anthropologists, chemists and biologists. Most of these disciplines require teeth in their investigations if the possibility of achieving a positive identification is to be maximised. For example identification of human remains via dental comparison with ant mortem records requires the teeth in situ for visual and radiographic analysis. Dental comparison is commonly utilised for forensic investigation of identity with a high rate of success especially in mass disaster situations [8]. Chemical analysis of teeth can indicate not only the year of birth (post 1943) but also in some instances the year of death of an individual by radiocarbon dating of tooth enamel [9, 10]. Tooth enamel provides the most accurate measure as unlike bone it does not undergo continual remodelling.

2. Historical Background

The pattern, size and form of the human bite can be forensically extremely more important if the pattern of the dentition is sufficiently distinctive such as a missing tooth or a wide gap between teeth as this can then be positively matched to their assailant. Identification of bite marks can be either by the actual skin bite mark itself or from the impression on any item of hard food found at the scene of the crime. Today swabs of the bite marks for DNA can be matched either from a suspect at the time or by a later speculative search. Bite wounds are common from dogs, cats and human. Any subsequent infection depends on the degree of tissue damage and the delay in seeking treatment. Antibacterial therapy is either oral or parenteral depending on the degree of infection with surgical wound debridement if necessary. The risks of HIV, Hepatitis B, Tetanus and Rabies all need to be considered. The organisms from cats and dogs are P. Multocida, Staphylococcus Aureus, Streptococcus species, Prevotella and Fusobacteriae. Those from human bites are Streptococcus species, staphylococcus aureus, Prevotella species, Peptostrep. Species and Fusobacterium species especially Vincent's organisms, F. Fusiforme and occasionally Borrelia vincenti. Human bites are said to be more toxic than dog bites and suggested antimicrobial therapy for all of these infections is amoxicillin clavulanic acid, ampicillin or penicillin for 5-7 days. If infected then for 2 weeks. The site, severity, chronic illness such as diabetes, cirrhosis and immunity of the victim should be considered. If allergic to penicillin then erythromycin and metronidazole can be used even if under 12 years of age.

The first recorded medico legal identification of a body using dental means is that of Dr. Joseph Warren who was killed at the battle of Bread Hill more often referred to as the battle of Bunker Hill in New England in 1775. Dr. Warren was killed during one of the earliest battles of the American Revolution by a musket ball through skull and was buried by the British redcoats in a mass grave and then reburied with another American. When this grave was exhumed 10 months later the body was identified by Paul Revere (renowned silversmith, amateur dentist and revolutionary) from the dental work that had done on Dr. Warren in 1773 and in 1775. As one of the leaders of the Americans revolution, Dr. Joseph Warren was given a more fitting revolutionary hero's reburial on April 8, 1776 [11].

Mass forensic identification by dentition was first used at Paris in the aftermath of the fire of bazaar de la charite that occurred on afternoon of May 4, 1897. About 1200 people were there at that time. This led to 126 deaths with more than 200 seriously injured. By noon the following day only 30 remained unidentified. What was different was that the bazaar had been held by the "greatest ladies of France "to help "the poor and needy". These Countesses, Duchesses and other ladies had the money to have the best dentistry available at that time. Their dentists: Dr. Burt, Brault, Ducorneau and Gordon were called in. All 30 bodies were eventually identified through dental record keeping of amalgam fillings, gold repairs, crowns and evidence of extraction spaces noted in the mandibles and maxillae of the victims. Dr. Oscar Amoedo, a Cuban born dentist and professor at the dental school at Paris University at the time subsequently wrote a short article describing the identification procedures used in this disaster and postulated a methodology to be used as a basis in future. This was published in dental cosmos in 1897, the year of dreadful fire. Dr. Amoedo can truly be said to be the father of forensic odontology. His seminal work "L'Art dentaire en medicine legale" was published by Masson at Paris in 1898 [11].

The importance of forensic odontology and its development over the years has resulted in its use in a number of famous cases of murder, a guilty verdict and the death penalty being imposed and sometimes but not always of the subsequent hanging of the person found guilty.

The first case in Britain when a murderer was identified by his teeth marks was in 1948 in a case of the 1947 new year's eve dance murder at Tonbridge wells examined by the late dr. Keith Simpson. Dr. Simpson was able to match the tooth marks on Phyllis Lucy right breast with the dentition of her husband, Robert Gorringe. He was convicted, sentenced to death but later reprieved with a life sentence [12].

The sack murder at Luton of Mrs. Manton in 1943 where the body was only identified after 3 months by the retained roots. It was almost a perfect murder but her husband Bertie was found guilty and sentenced to hang [13].

In the Ruxton case of 1935 when Dr. Buck Ruxton, an Indian was found guilty of the murders of his wife Isabella and Mary Rogerson. Despite being dismembered, both bodies were identified by their past dental histories. Dr. Ruxton was hanged at HMP Strange ways on May 12, 1936.

The pajama girl case of 1934 in New South Wales, Australia showed how simple errors made by the local dentist in charting the dental work delayed identification of the body for 10 years. This case became famous because it showed the fallibility of a dentist who had no forensic training in odontology [14].

In Acid bath murder by John George Haigh at Crawley, Sussex in 1949, it was the dentures that proved the identity of Mrs. Durand Deacon [15]. Although people often only remember the three pure cholesterol gall stones. Only humans produce pure cholesterol stones but the gall stones were not unique enough to allow identification. What Haigh did not know was that the acrylic dentures dissolve only very slowly in concentrated sulphuric acid. Found guilty of murder after only 18 minute deliberation, he was hanged at HMP Wandsworth on August 10, 1949.

In Dobkin case in London in 1942, the body of Rachel Dobkin was identified by her dentist Mr. Barnet Kopkin. He made dental sketch before viewing body and recognized his fillings that he had performed. Harry Dobkin, her then husband was found guilty of murder and hanged at HMP Wandsworth in January 1943 [16].

Badly burned bodies of Adolf Hitler and Eva Braun were discovered on May 1, 1945 by Russian troops and subsequent identification of the remains was done solely on dental evidence. Dr. Hugo Johannes Blaschke who was trained in USA was Hitler's dentist and it was from his records and evidence of frontal sinus X-rays which can be as unique as finger prints and the

surviving crown and bridge work made by Fritz Echtmann that the bodies of Hitler and his wife were eventually formally identified [17].
Although DNA has become almost ubiquitous in clinical forensics and medical jurisprudence, it would be wrong not to record the debt that we owe to forensic odontology over the years and foolish not to continue to use evidence that it provides allowing truth to be found.

3. Teeth as Source of DNA

DNA the molecule that carries genetic information from one generation to the other was discovered by James Watson and Francis Crick in 1953. They presented the structure of the DNA helix for the first time and shared the Nobel Prize with Maurice Wilkins nine years later in 1962 for solving one of the most important biological riddles [18].
DNA is preserved in the teeth and bones for a very long period and thus is a valuable source of information. Ancient DNA (aDNA) analysis can be carried through extraction of the tiny amounts of DNA remaining in samples that are hundreds to tens of thousands of year's old [19]. Teeth are resistant to adverse conditions degrading the DNA such as humidity, high temperature and the microbial action [20, 21]. In the tooth, dentin and pulp are rich sources of DNA which can be successfully extracted [22]. Sufficient quantity of DNA can be extracted from the crown body, root body and root tip. However, the root body is the region which yields highest quantities of DNA [23]. Not only is the quantity of DNA available for the laboratory important but also the quality and purity. Furthermore, an abundance of quality DNA can be extracted from tooth which is an important advantage in DNA analysis [24].
Anatomically, human teeth can be divided into two parts: the crown which is exposed to mouth and the roots which are encased in the alveolar bone of the jaws. Tooth roots which are composed of cementum and dentine /pulp have been shown to yield more DNA than the crown that contains dentine/ pulp, is predominately composed of enamel [25, 26]. Gaytmenn and Sweet demonstrated that even in teeth with pulp present, the DNA yield from the crown is still ten times less than that retrieved from the roots [23].
Enamel which covers the crown of the tooth is the hardest tissue in the human body being 96% (by weight) mineral is acellular and contains no DNA. This tissue provides a physical barrier protecting the cells within the tooth from external environment such as heat, UV light, moisture and microbes. Although in Vivo enamel has limited permeability, the size of the pores between the mineral crystals is extremely small preventing the penetration of the molecules larger than water. This is particularly important in restricting the access of microbes and environmental contaminants into the tooth both in life and after death [27].
The dentine/pulp complex makes up the bulk of the tooth and in contrast to enamel is highly cellular. Pulp is a richly vascularised and innervated connective tissue containing numerous cell types. These include odontoblasts (cells that form dentine), fibroblasts, defence cells (e.g. Histocytes and macrophages), plasma cells, nerve cells and undifferentiated mesenchymal cells. The odontoblasts are oriented with their cell bodies along the pulp border and their long tissue processes extending rough the dentine. The cells that occur in the highest number in pulp tissue are the odontoblasts which number approximately 11,000 per mm^2 and fibroblasts which been estimated at 1000 per mm^2 [28, 29]. Thus approximately 80 diploid cells can yield the minimum DNA quantity required for short tandem repeat (STR) typing, thus showing pulp is a valuable source of DNA. Pulp interconnects with the periodontal tissues (tissues connecting tooth to the alveolar bone) via the root apex and accessory canals through which blood vessels pass [30]. Accessory canals occur predominately along the lower half of the root and in molar teeth through the floor of the pulp chamber into the furcation area. Given the relatively high cellularity of pulp tissue, this tissue provides the richest source of DNA in teeth. However, pulp may be in limited quantity or even absent in aged and diseased teeth.
Dentine is composed of 65% (by weight) mineral in the form of carbonated hydroxyl apatite, organic macromolecules (mostly collagen) and water. Generally, dentine does not contain nucleated cell bodies although odontoblastic cells can become entrapped in the dentine during the formation of tertiary dentine (common response to dental caries) [31]. Dentine is structurally unique tissue densely perforated by parallel tubules ringed by mineral running through the dentine from the pulp chamber. These tubules contain odontoblastic cell processes and nerve fibres. Odontoblastic processes have mitochondria along their length to the mineralising front of the dentine. Mitochondria are also present along the entire length of the nerve fibres making the tubules rich in mtDNA [32]. Other than incidental sources from the blood vessels in the main and accessory canals, dentine is a poor source of nuclear DNA.
Cementum covers the roots of teeth and is an avascular mineralised tissue with a laminated structure. It is composed of (by weight) 45-50% inorganic mineral (hydroxyapatite), collagen and non collagenous matrix proteins. Cementum is classified into two types based on the presence or absence of cells (cementocytes). Cellular cementum is a source of DNA as it contains cementocytes within the extra cellular matrix in spaces comparable to osteocytic lacunae [33]. Cellular cementum is similar in physical and chemical composition to bone but is structurally and functionally different.
It is avascular, has no innervation and contains less inorganic salts. Unlike bone cementum, it does not undergo continuous remodelling but does increase in thickness continuously throughout life [34]. Cellular cementum is predominantly seen on the apical (lower) portion of the roots and in the furcation area of molar teeth but may be absent on anterior teeth [35]. Cementocytes are connected by canaliculi that are directed towards the periodontal ligament, which is their source of nutrients. Additional sources of DNA associated with cementum are soft tissue inclusions, blood residue, vessels traversing in accessory canals, adherent periodontal tissues and fragments of bone trapped between the roots of molar teeth.
In summary, pulp and cementum are clearly the most valuable sources of nuclear DNA in the tooth and both these tissues and dentine are good sources of mtDNA. Enamel is important in the preservation of dentine and pulp but is devoid of DNA. Therefore, if enamel is sampled with the other tooth tissues, it will probably have a dilution effect and the high concentrations of minerals, including calcium, may complicate the extraction process.

4. Factors Influencing DNA Content

The total DNA content of teeth varies considerably between individuals and also between teeth from the same individual [25, 26]. Some of the factors indicated to have an effect on DNA content include tooth type, chronological age of the donor, and health status of the tooth. Each of these factors will influence the relative proportions of DNA present in the crown and root, and in the pulp, dentine and cementum.

4.1. Tooth type

The four types of human teeth, molars, premolars, canines and incisors differ in form and size but have a similar histological structure. Studies comparing DNA content among different tooth types have shown that teeth with the largest pulp volume provide the best source of DNA due to the presence of more pulp cells [36, 37]. It has also been shown that more DNA is retrieved from multi-rooted teeth than single rooted teeth most likely due to the larger pulp volume in multi-rooted teeth and the increase in root surface area providing more cementum. Multi-rooted teeth not only have a larger root surface area but also exhibit more cellular cementum per root surface area than single rooted teeth which in some instances have little or no cellular cementum. Thus tooth selection for sampling should target teeth with the largest pulp volume and root surface area with molars being the primary source.

The preference for molar teeth is indicated in the protocols for tooth sample selection published by Interpol in their Disaster Victim Identification (DVI) Guide 2009 and by the DNA Commission of the International Society of Forensic Genetics (ISFG) recommendations for DVI [38]. In the absence of molars, premolars would be expected to have more cellular cementum than anterior teeth, but canines would have a larger pulp volume. When selecting a tooth, tooth retention in the socket should also be a consideration, with multi-rooted teeth (i.e. molars and premolars) less likely to be lost than anterior teeth during post-mortem breakdown. Teeth retained in their sockets are afforded protection by the alveolar bone and have less chance of contamination [6].

4.2. Chronological age

There are a number of changes that occur with increasing chronologic age that affect the DNA content of teeth. The most obvious negative change is the decrease in pulp volume that occurs as a result of the normal continuous deposition of dentine. Pulp not only decreases in volume over time but also decreases in cellularity and becomes more fibrous [39, 40]. As a part of the ageing process, dentine increases in volume and also becomes progressively sclerotic. The process of sclerosis involves occlusion of the dentinal tubules with calcium phosphate crystals and the subsequent degeneration of the odontoblastic processes and associated nerve fibres [41]. It is believed even after occlusion of the tubules and degeneration of their content, some mtDNA remains trapped in the dentine. Although the relationship between DNA and dentine is not understood the preservation of mtDNA in completely sclerotic dentine has been demonstrated [32]. A positive change is that the amount of cellular cementum increases with age although it has been noted that cementocytes are only viable to a limited depth (approx. 60 μm) as they rely on the periodontal ligament for nutrients that must diffuse through cementum to the cells. Other changes that occur with increasing age may not affect DNA content but may affect post mortem DNA preservation. For instance, enamel becomes more heavily mineralised over time although it is also lost through natural attrition and dentine decreases in porosity due to occlusion of the tubules [42]. Hence, advancing age leads to a decrease in DNA content and a change in the distribution of DNA throughout the tooth [26].

4.3. Dental disease

Dental diseases have a negative impact on the human DNA content of teeth. Dental caries, a microbial disease results in localized dissolution and destruction of the calcified tissues of the teeth. This facilitates entry of bacteria to the pulp both directly and via empty dentinal tubules resulting in cell death [43]. In response to caries, the pulp retracts and tertiary dentine is deposited. This tertiary dentine exhibits a less organized structure than primary and secondary dentine and as deposition occurs at a rapid rate, the odontoblast cell bodies can become encapsulated in the mineralised tissue [44]. Ultimately caries can lead to a complete loss of pulp or tooth loss. Cellular cementum can only be affected by caries if it is exposed to the oral cavity, which may occur in advanced periodontal disease. As cementum receives its blood supply from the periodontium rather than from the pulp, it becomes an especially important tissue for recovery of DNA from these pulpless teeth [45]. Periodontitis or periodontal disease is a plaque-induced inflammatory disease affecting the supporting structures of the tooth (alveolar bone, periodontal ligament and cementum). The accumulation of bacterial laden plaque on the tooth surfaces leads to chronic inflammation and the production of toxins that destroy the supporting tissues and disrupt the attachment of the tooth. During the progress of the disease, alveolar bone is destroyed leading to a loss of attachment a reduction in the height of the gingiva around the tooth and exposure of cementum to the oral cavity. Cementum affected by periodontal disease loses attachment to the periodontal fibers and becomes coated with plaque and calculus [46]. Only in advanced periodontal disease is the lower half of the root compromised, so cellular cementum may be largely unaffected. Advanced periodontal disease affecting cellular cementum can lead to a reduction in the availability of DNA due to physical loss of cementum (as a result of dental treatment aimed at removing affected tissue) and to death of cementocytes. Dental diseases not only reduce the amount of DNA available but also increase the potential for contamination. Thus teeth selected for DNA analysis should be intact and free from disease. It is important to note that diseased teeth and those that have received dental treatment may still yield sufficient DNA for extraction and amplification but careful consideration of the extent of disease and specific tissues to target is required. For example, if the tooth selected is affected by caries, pulp tissue could still be present unless the disease is extensive and the cementum would be unaffected and could be specifically targeted. A dentally restored molar tooth that has been involved in mild to moderate caries is likely to still be of more value than an anterior tooth. Dental radiographic analysis would be helpful for tooth selection as this would allow an assessment of involvement in disease as well as determination of root and pulp size.

5. Conclusion

Identification of humans using the unique features of the teeth and jaws has been used since Roman times. Throughout history, various interesting stories have been recorded in which a person's unusual smile, crowded or fractured teeth or a single darkened tooth have been used to identify a corpse to the exclusion of all other people. Today, dentists are widely respected as a source of valuable data that they can provide to answer questions that arise during a death investigation. By examining the dental traits and characteristics of one or more bodies, it is possible for the odontologist to provide the corpse with its identity and satisfy a basic societal need.

6. References

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