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Identification of calcinated human bodies through dental analysis

Review Article

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SUMMARY

In the present article an analysis of the information of forensic odontological aspect and methodology for the identification of human bodies in massive disasters or isolated corpses is carried out; we emphasize on obtainment of DNA in the dental remains and the behavior of teeth at different temperatures, with the help of new and quick methods for their identification; with this new mechanism it is intended to recover and replicate DNA.
Keywords: DNA; Forensic Odontology; ID; bodies, burned; teeth; calcination

INTRODUCTION

Keizer-Neilson defined the Forensic Dentistry in 1970 as "the branch of forensic medicine that, in the interests of justice, deals with proper management and examination of dental evidence and with the evaluation and proper presentation of dental procedures."

The identification is based on the comparison between the known characteristics of a missing person (called antemortem) with characteristics recovered from an unknown body (called postmortem). The purpose of the postmortem profile is to provide information to researchers that will restrict the search to a smaller population of individuals. Forensic dentists can usually determine sex, race and age (at the time of death) from the careful study of the teeth, their anatomical disposition and the osteological features of the skull [1].

The application of Dentistry in the identification processes is not new; Its importance is extraordinary when the corpses are charred and when fire elements do not allow the accurate identification of the human remains available, or by the own limitations presented by other methods. In addition, it is admitted by all specialists that "there are not two equal arcades" and that "even the teeth of identical twins have variations"; additionally, the resistance of teeth to destruction by fire is known, which demonstrates its high value for the correct identification of burned or charred individuals; it is based on the number of teeth present, pathological processes detectable, restorations, dental materials used and prostheses and implants that make infinite the number of possible combinations. For this reason it is evident that if there are no teeth remaining from a corpse, it will be difficult to have other data of value in the identification [2].

The identification of a body requires the comparison of radiographs or dental records antemortem and postmortem; tests of deoxyribonucleic acid (DNA) can be carried out [3]. Teeth often represent the only evidence available for forensic analysis. Teeth are the hardest structures in the human body, and their pulp is well protected by dentin, enamel and cement, explaining their frequent use to obtain DNA from extremely damaged bodies or degraded human remains [4].

The DNA extracted from the burned bone fragments can be highly degraded, which makes it difficult or even impossible to amplify the genetic markers. In addition, heavily burned bones are very prone to contamination with external DNA [5]. The maintenance of the integrity of fragile structures is crucial for the successful confirmation of identity [6].

The comparison of dental records is used for the identification in situations with great destruction or prolonged exposure of tissues of human body to the environment such as fires, severe traumas and / or massive disasters, where other methods are not enough. Teeth play an important role in the establishment of unequivocal identity, by virtue of their characteristics of uniqueness and high physical and chemical resistance. The interference of DNA analysis methods in the forensic context is known. The use of

DNA profiles in Forensic Dentistry represents a valid alternative in human identification, as it contains the genetic material, the individual's distinctive property. Currently available DNA tests are highly reliable and are accepted as legal evidence in court [7]. The rate of postmortem degradation of DNA in teeth depends on the postmortem interval and the temperature of the soil. In short to medium periods, even small changes in soil temperature can have a substantial impact on DNA preservation. Cement (the mineralized outer layer of the tooth root) is particularly important for the recovery of nuclear DNA, since its structural integrity is maintained for prolonged periods, possibly providing additional protection to the cellular material trapped within the mineral matrix [8]. DNA techniques involve the detection, quantification and analysis of genomic DNA and mitochondria. Nuclear DNA is representative of paternal and maternal inheritance. Mitochondrial DNA (mtDNA) is derived from the ovule and, therefore, is purely maternal. Especially in the teeth, the dentin consists of cellular extensions (fibers of Tomé) rich in mitochondria. Dentin powder is, therefore, presumably a good source of mtDNA [9].

Objectives

The general objective is to disseminate clearly and precisely the results of the forensic investigation, as well as to know the possible techniques to identify a corpse through forensic dentistry; in this way, we can understand why each procedure is used.

Knowing that Forensic Odontology is a great tool for the identification of corpses, it is very important to understand what the limitations are, as well as to show the public the parameters that would not allow identification mainly in burned bodies.

As an additional objective we seek to encourage research for the creation of new methods and techniques in forensic identification.

METHODS

The search for articles of forensic dentistry in the PubMed database was carried out with the following keywords: Identification of calcined bodies, Identification with teeth in calcined bodies, dental identification, report of cases, Ancient pathogen DNA in human teeth and petrous bones, effect of various temperatures, Capillary electrophoresis applied to DNA, determination and taking advantage of the sequence and the structure to bioanalysis; around 40 articles related to the topic were reviewed, and an article was taken from the Semantic scholar database.

RESULTS

In large-scale disasters associated with fire, damage caused by heat can make it difficult to legally identify human remains. The teeth, the restorations and the prostheses are resistant to quite high temperatures and can be used as an aid in the identification process (figure 1) [10].

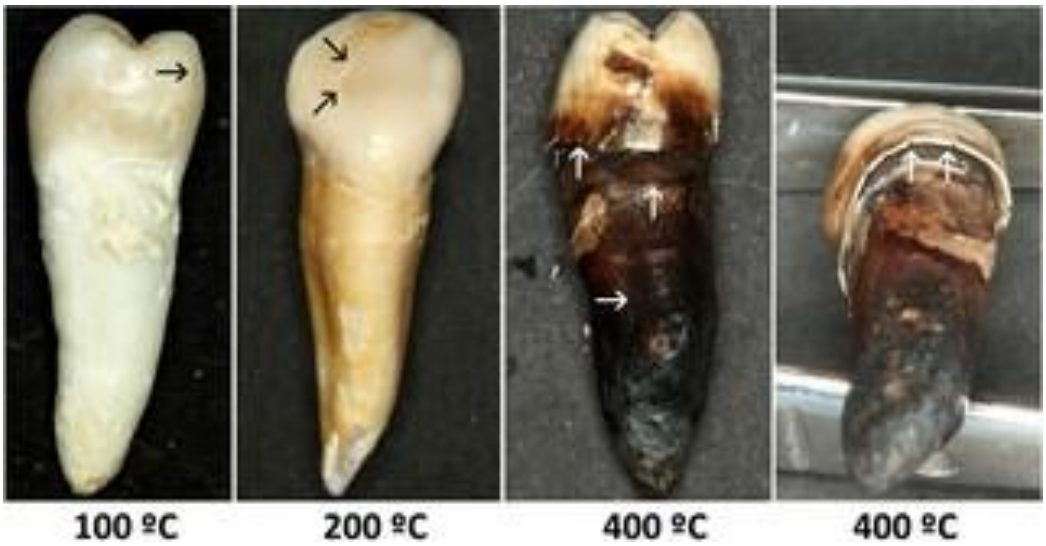

Figure 1. Dental morphological changes in relation to exposure to high temperatures

Effects of heat on dental restoration materials

Anatomical features include the morphology of the crown, the shape of the root, the size and curvatures, and the spatial relationship between the teeth. The preservation of the characteristics will depend on the duration, temperature and the degree of exposure to direct flame (figure 2) [11].

Figure 2. On the left side: Restoration of glass ionomer at 800 ° C: only macroscopically a portion of the crown structure together with the filling was maintained. the filling remained intact inside the crown that had become opaque white as had the root. Right side: Zinc oxide eugenol to 600: macroscopically dark gray with Cracks and dimensional contraction due to water loss, agglomerated zinc particles [10].

The following table (table 1) mentions the effects of fire at the level of the skull and its behavior in dental organs:

N° muestras*	Temperatura en rados centigrado	Duración en minutos	Tamaño de la muestra	Cambios morfologicos	Cambios radiograficos
1	100	30	12	Lustre de la superficie retenida de color amarillo pálido a claro	No hay cambios apreciables
2	300	30	12	Gris claro con manchas gris oscuro Raices-marrón amarillento	Fisuras entre esmalte y dentina
3	500	30	12	Esmalte:gris azulado claro a oscuro parches Raíces: marrón grisáceo Pérdida del brillo de la superficie	Fracturas entre esmalte/ dentina y dentro de dentina
4	700	30	12	Gris azulado claro a gris azulado oscuro oscuro Desintegracion en pequeños fragmentos	Fracturas entre esmalte/ dentina y dentro de dentina
5	1000	30	12	Blanco neutro con parches gris azulado claro y gris verdoso	Grandes fracturas que se extienden a traves de la dentina y el aplastamiento de la corona

Table 1. Morphological and radiological changes according to the temperature at which the teeth were exposed.

The changes presented by the skull with a comparative study at different temperatures in a given time can be summarized in Table 2.

Hora	Günther y Schmidt [4]	Richards [5]	Estudio actual
	1000 ° –1100 ° C	680 ° C	670 ° –810 ° C
8–10 min	tejidos blandos de la cara carbonizados		cráneo-tapa libre de tejidos blandos,tejido blando de la cara carbonizado
13–16 min	frente y vértice libre de tejidos blandos, huesos faciales que sobresalen calcinados	mostrando huesos de la cara	
20 minutos		mostrando cráneo	Queda escaso tejido blando en la cara, Fracturas por calor del cráneo-capuchón.
20–25 min	Contracción severa de los tejidos blandos en el cráneo, roturas de calvaria cerebro superficialmente carbonizado, destrucción de partes prominentes de el cráneo facial		
30 minutos			Tabula externa de la calvaria desmoronada
40 min			cerebro mostrando, los huesos de la cara comienzan a desintegrarse
50 min			huesos del rostro en gran parte destruidos, mostrando la base del cráneo
45–75 min	La base del cráneo sigue intacta, la cabeza a veces se parada del tronco		

Table 2. Morphological changes in skull bones

Any type of organism can be identified by examining unique DNA sequences of that species. Each cell of an individual carries a copy of the DNA. The

order of the base pairs in the DNA of each individual is different, except identical twins [14].

Chelex 100

Procedures have been developed that use Chelex 100 chelating resin to extract DNA from forensic samples to be used with PCR. The procedures are simple, fast, do not involve organic solvents and do not require multiple transfers of tubes for most types of samples [15]. It has been reported that extraction of DNA from dental pulp using this method is efficient compared to K protein and phenol-chloroform extraction. The extraction, amplification and typing of DNA based on Chelex 100 are possible in incinerated teeth [16].

Electrophoresis

Repetitive sequences within the genome are used to identify specific biomarkers for a series of applications relevant to the detection of pathogens, human diseases and especially human identification. DNA analysis is critical for forensic laboratories around the world. Human identification methods are based on the analysis of DNA sequences known as STR, which contain two to five basic repeats. An individual will have two different sets of this repetition, one copy of each parent. Thirteen different STRs are used in the combined DNA index system of the Federal Bureau of Investigation (FBI), also known as CODIS, for comparing the probability of a DNA sample with a specific individual. In a single forensic analysis, a minimum of 16 markers are separated and classified [17].

Comparison of DNA in bones and teeth

The quality of DNA extracted from teeth is generally higher than DNA of bones. The quality of DNA obtained is considered long-standing. It has been demonstrated that high amounts of microbes in the DNA (μg) can interfere with the specific hybridization of human sequences in a slot-blot format, with false negative results in the quantification of human DNA [18]. One study investigated whether the outer layer of roots of teeth contained higher proportions of endogenous DNA than dentin, which represents the inside of tooth. For 11 of 14 teeth, they observed a higher fraction of human DNA on the root surface compared to dentin [19].

Bone tissue is a dense and mineralized connective tissue consisting mainly of a mineral component (hydroxyapatite) and an organic matrix composed of collagens, non-collagenous proteins and proteoglycans (PG). The proteins of the extracellular matrix and the PG bind strongly to hydroxyapatite, protecting these molecules from the destructive effects of temperature and chemical agents after death. PGs have recently been analyzed in human and archeological bones and teeth. PGs play an important role in bone morphogenesis, homeostasis and degenerative bone disease; the ability to isolate and characterize the PG content of archaeological skeletons could reveal valuable information [20].

Nuclear DNA of bones in different stages of degradation can be isolated by three methods: classical extraction with phenol and organic chloroform, DNA extraction of aggregates of crystals and extraction by total demineralization. Total

demineralization is the best method for most cases of DNA extraction from bones, although it does not provide pure DNA. The extraction of DNA from the aggregates eliminates the inhibitors much better and is also a good method of choice when it is necessary to determine the identity of the exhumed remains. In the case of unburied bones, the total demineralization or phenol-chloroform protocols are more efficient for successful DNA extraction [21].

When there are minimal thermal changes in debris, normal identification procedures can be followed. Extensive heat-related disturbances can seriously disrupt normal procedures, but enough evidence often survives to facilitate identification. Contraction, fragmentation, and shape alterations can affect skeletal analysis and should be considered in the analysis [22].

DISCUSSION

Identification is based on the comparison between the known characteristics of a lost individual (called antemortem data) with characteristics recovered from an unknown body (called postmortem).

The result of our research with collaborators is important for the identification of calcinated human bodies through the analysis of teeth, taking into account the importance of DNA in different tissues; over time teeth have been resistant to environmental changes so it means that they are a reliable method for this type of research; the reason for this research is to obtain information on a single tooth such as sex, age, blood type of a person; this result has some limitations, such as collecting more samples of the research that could be expanded; This will lead us to study beyond identification and expedite the recognition of human bodies.

As a first parameter we chose to study about the identification of human bodies, with the help of teeth DNA, since this medium can not be altered; teeth are formed by dentin, which contains several cell extensions called Tomé fibers, which are highly rich in mtDNA that will allow identification. As a second parameter, information about PCR is collected as a high fidelity method to determine sex. As a third parameter, information is obtained about the materials and methods of obtaining DNA; although the method of phenol chloroform was widely used in its time and had good results, there are currently other methods with better results and in less time and without being so laborious; this would be the case of the extraction of DNA based on silica, since it allows very well the rescue of DNA in very old teeth or bones. Another method is with Chelex 100 resin as it reduces work in a shorter procedure and facilitates the extraction of K protein from DNA in dental organs exposed to high temperatures.

It is convenient to implement tools and expand the development of research that will help us create a DNA bank to facilitate the comparison, between DNA from burned bodies and people who are suspected of a relationship or people from the DNA bank.

Conclusion

One of the objectives of forensic odontology is to take charge of using dental knowledge in the identification of bodies that have been subjected to high

temperatures and can not be recognized. The purpose of this discipline is to collect, manage, evaluate and present information about bodies burned by analyzing the dental evidence that has been able to withstand the effects of high temperature heat.

Teeth play a very important role in the identification processes, since they are formed by enamel and cement, hard tissues and resistant to biological, chemical and mechanical stimuli that protect the soft tissues such as dentin and pulp; they form a complex from which it can obtained information specific to the individual, as it is, for example; sex, race and age from the genetic material contained in the DNA. The amount of dental pieces found, anatomy, pathological processes, restorations, prostheses and implants are also valued data when identifying a person.

Different methods of isolation are used for the forensic analysis of DNA samples obtained from teeth. It has been demonstrated that amplification and DNA typing of incinerated teeth is possible thanks to the use of chelating resins since it does not contain organic solvents besides being a fast and simple procedure.

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