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DNA Profiles Obtained from Molars with Caries Lesions

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Abstract

Introduction: Teeth can be used to obtain a DNA profile, such as for identifying human remains. The recommendation is to use teeth without carious lesions or other changes; however, this complicates tooth-based DNA profiling and related human identification in countries such as Mexico, where 93.3% of the adult population has caries.

Objective: To analyze whether the degree of caries correlates with the amount of DNA extracted from a tooth and/or the ability to obtain a complete DNA profile. Methodology: Thirty-five molar teeth with dental caries were collected from private clinics located in the center of the state of Veracruz, Mexico. DNA was extracted and autosomal STRs were amplified using an Investigator 24plex GO! kit.

Results: The mean DNA concentration was 6.08 ng/µL. There was no significant difference in DNA concentration with respect to tooth type ($p = 0.113$), caries size ($p = 0.104$), or location ($p = 0.718$). Twenty-one complete DNA profiles were obtained (60.0%), one partial profile was obtained (2.9%), and 13 cases failed to amplify (37.1%). The obtained DNA concentration showed a significant difference ($p = 0.023$) between teeth that yielded a complete profile (9.503 ng/µL) and those that failed to amplify (1.009 ng/µL) but did not differ with respect to caries size ($p = 0.816$).

Conclusion: The damage caused by the carious lesion does not affect the amount of DNA obtained, and therefore does not affect the likelihood of obtaining of a complete DNA profile. The use of caries-bearing teeth for the identification of human remains should be investigated.

Keywords: Forensic Genetic; Human Identification; Forensic Odontology; DNA profile

Abbreviations: DNA: Deoxyribonucleic acid; STR: Short Tandem Repeat; PCR: Polymerase Chain Reaction

Introduction

The process of forensic human identification involves assigning a correct identity to an unknown individual. Human remains may be identified by the matching of ante- and post-mortem information on physical and/or genotypic characteristics, which are compiled and analyzed by a multidisciplinary group of forensic specialists [1,2]. Dactyloscopy, forensic odontology, and genetics are considered primary methods of human identification, since each itself is sufficient to establish an identity. Alternatively, secondary methods involving the gathering of medical, anthropological and criminalistic knowledge, among others, can contribute to the biological characterization, contextual analysis, and integration of relevant elements leading to identification [1,3]. Mexico has experienced a very high crime rate for more than 15 years, and there has thus been a continuous increase in the number of unidentified humans remains. It is currently estimated that approximately 52,000 unidentified deceased persons are represented in forensic services, mass graves in public cemeteries, and other holdings [4]. In response to this situation, governmental and civil institutions have been working on legislation, infrastructure, personnel specialization, protocols,

etc., related to human identification. However, there is still much to be done [5].

The genome of an organism consists of all the Deoxyribonucleic Acid (DNA) that contains genetic information for its development and function. The human genome consists of the 23 chromosome pairs in the nucleus and the extranuclear DNA in the mitochondria. Variations in the DNA sequence give rise to human genetic diversity and render the genomic sequence unique to each human individual, except for monozygotic twins [3,6]. In forensic genetics, human remains are identified through analyzing DNA profiles of validated genetic markers, such as Short Tandem Repeats (STR), which vary in the number of repeated sequences and exhibit Mendelian inheritance [3,7]. In forensic odontology, analyses of morphological characteristics and dental treatments can lead to the identification of an unknown person [1,2,8]. In certain situations, such as natural disasters, fires, or the discovery of partial or decomposed human remains, teeth are one of the most viable sources of DNA. Their composition and location make them highly resistant to natural decomposition, trauma, environmental factors, microbiological factors, and anthropogenic actions aimed at hindering identification process [2,8].

International protocols and national regulations recommend that two or three healthy teeth, preferably free of carious lesions, without dental work be collected for forensic genetic studies in the process of human identification [1,3,9]. However, this could be a problem in certain countries: The World Health Organization (WHO) reports that dental caries in permanent teeth is the most common disease worldwide, and estimates that it affects approximately 2 billion adults, mainly in low- and middle-income countries that do not have effective oral disease control and prevention programs [10]. In Mexico, the prevalence of dental caries in the population over 20 years of age is estimated to be 93.3% [11]. The use of teeth with carious lesions could therefore benefit human identification, especially in countries with a forensic situation such as Mexico. The use of decayed teeth to obtain DNA profiles has not been extensively analyzed [12-14]. Alia-García [12], evaluated different types of healthy and carious tooth, and the influence of bacterial DNA in obtaining good quality genetic profiles [12]. Meanwhile, Shbair [14], compared two techniques for accessing and obtaining pulp tissue in decayed teeth [14]. Here, we set out to analyze whether the degree of caries correlates with the amount of DNA extracted from a tooth and the likelihood of obtaining a complete DNA profile.

Methodology

Dental samples

The donation of extracted teeth was verbally requested and written informed consent was obtained from patients of private clinics in the municipalities of Veracruz, Boca del Río, and Alvarado, all of which are in the center of the state of Veracruz, Mexico. Tooth samples were collected over the period from 2020 to 2022. Teeth with carious lesions were used; those with external or internal root resorption, open apices, alveolar bone fenestrations, endodontically treated teeth, root fractures, and healthy teeth were excluded. The presence of carious lesions was diagnosed visually or by radiographic techniques by the treating dentist. Carious lesions were classified by location and size based on the strategy proposed by Mount and Hume and modified by Lasfargues [15].

The lesion location was classified into three categories: (1) occlusal face, (2) mesial and/or distal side, and (3) cervical region. The size of the lesion was divided into four categories: (1) minimal surface cavitation with involvement of dentine, (2) moderate involvement of dentine – enamel, (3) enlarged cavity after caries removal, the remaining tooth structure is weakened, and (4) extensive caries or bulk loss of tooth structure has occurred. The collected teeth were rinsed with saline, dried, and stored in paper envelopes at 4°C until processing.

DNA extraction

A method that preserves most of the tooth structure (conservative method) was used to extract DNA from each tooth [14,16]. Briefly, the #1 carbide ball burr was inserted into the cavity created by the carious lesion and the powder was removed from the pulp chamber through the root canal of the tooth. The dental pulp powder was collected within 10 days post-extraction. It was then placed in a 1.5-ml microcentrifuge tube at room temperature until processing. Once the pulp was extracted, the average time for DNA extraction was 45 days (range 3-160 days). The pulverized tooth powder weighed less than 100 mg in all cases, so extraction was performed using a QIAamp DNA Investigator kit (Qiagen) in accordance with the manufacturer's specifications. The obtained DNA was suspended to a final volume of 20 µL and stored at -20°C until processing.

DNA Profiling. For DNA quantification, the Investigator® Quantiplex kit (Qiagen) and a real-time thermocycler (7500 Real-Time PCR system, Applied Biosystems) were used. Genetic profiling was performed using an Investigator® 24plex GO! kit (Qiagen), which amplifies the following STRs: TH01, D3S1358, vWA, D21S11, TPOX, DYS391, D1S1656, D12S391, SE33, D10S1248, D22S1045, D19S433, D8S1179, D2S1338, D2S441, D18S51, FGA, D16S539, CSF1PO, D13S317, D5S818, D7S820, and amelogenin. Fragments were resolved by capillary electrophoresis on a 3500 series genetic analyzer (Applied Biosystems) and further analyzed using the GeneMapper ID-X v 1.5 software (Thermo Fisher Scientific). Considered a complete profile where all 21 STR loci markers, amelogenin gene and DYS391 were present in the electropherogram, a partial profile where one or more STR loci markers could not be identified and no amplification where no STR loci markers were identified.

Descriptive and inferential statistics were applied using the IBM SPSS Statistics V.25 software

This study was conducted in accordance with international

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standards of research ethics. The protocol and informed consent were approved by the Ethics and Research Committee of the Institute of Forensic Medicine, Universidad Veracruzana (002-IMFCEI-2022). Informed consent was obtained from all study participants. Informed consent for the use of the genetic profile for publication was given by all participants [17].

Results

Thirty-five molar teeth with caries were collected, comprising mostly the second (45.7%) and third molars (34.3%). Most of the teeth (29, 82.8%) had the carious lesion in Zone 1 (occlusal face). The most frequent lesion sizes were size 4 (12, 34.3%), followed by size 1 (10, 28.6%) (Table 1).

Table 1: Characteristics of the 35 carious lesion-bearing molar teeth.

Molars		Caries location		Caries size	
First	7 (20.0)	Zone 1 (occlusal face)	29 (82.8)	1	10 (28.6)
Second	16 (45.7)	Zone 2 (mesial and/or distal side)	3 (8.6)	2	7 (20.0)
Third	12(34.3)	Zone 3 (cervical and/or root third)	3 (8.6)	3	6 (17.1)
				4	12 (34.3)

Data are shown as n (%).

The average weight of collected pulp tissue powder was 41.60 mg (range 12.4 mg to 89.6 mg). DNA was obtained from each sample and quantified (Table 2). The average concentration of the obtained DNA was 6.08 ng/µL (range 0.002 ng/µL to 48.477 ng/µL). Interestingly, there was no significant correlation between the weight of pulp tissue powder and the obtained DNA concentration (r = -0.132, p = 0.448) (Figure 1).

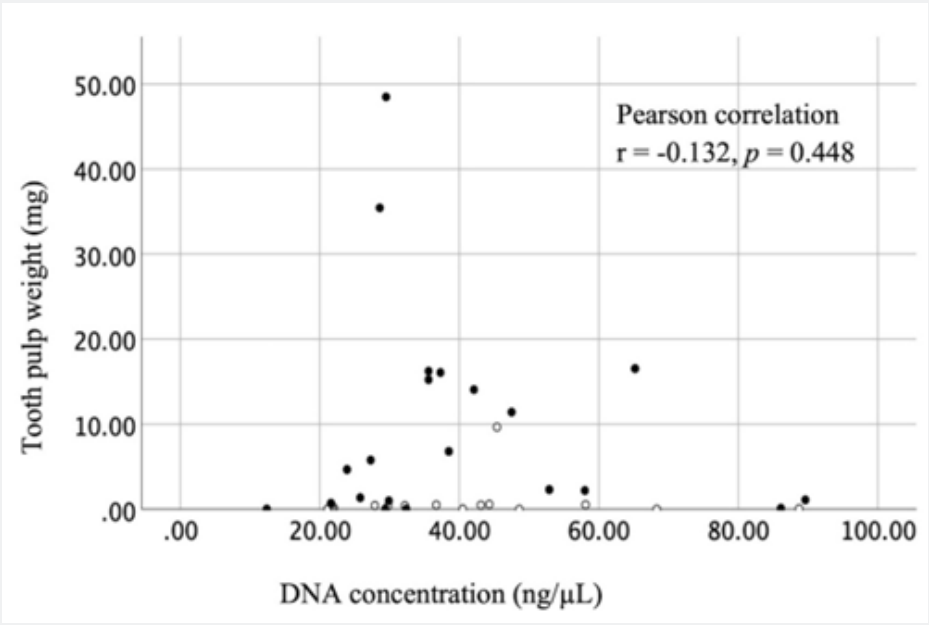

Figure 1: Pulp tissue powder weight is not correlated with DNA concentration (p = 0.448). Black dots indicate teeth from which complete DNA profiles were obtained; gray dot indicates the tooth from which a partial DNA profile was obtained; white dots indicate teeth from which no DNA profile was obtained.

There was no significant difference in the concentration of DNA obtained according to caries location (p = 0.718), molar type (p = 0.113) and carious lesion size (p = 0.104). From the 35 teeth, we obtained 21 complete DNA profiles (60.0%) and one partial profile (2.9%); in 13 cases, no DNA profile was obtained (37.1%) (Figure 1 and Table 2).

Regarding the type of molar tooth used as a source of DNA, more complete DNA profiles were obtained from second molars than from the other types (7, 38.1%), but this difference was not significant (p = 0.331). Similarly, no significant difference was observed between the number of complete DNA profiles and the location of the carious lesion (p = 0.964). More complete DNA profiles were obtained from teeth classified as having size 4 lesions (8, 38.1%), followed by size 1 (6, 28.6%), but this difference was not significant (p = 0.804) (Table 2). In contrast,

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the DNA concentration was significantly higher (p = 0.023) among teeth that yielded complete DNA profiles (9.503 ng/μL, 95% CI 3.763-15.243) compared to teeth that did not yield amplification products (1.009 ng/μL, 95% CI -0.566-2.584).

Table 2: DNA profiles obtained from 35 teeth with carious lesions of various sizes.

Caries size	No profile	Partial profiles	Complete profiles	*p*
1	4 (30.7)	-	6 (28.6)	
2	3 (23.1)	-	4 (19.0)	0.816
3	3 (23.1)	-	3 (14.3)	
4	3 (23.1)	1 (100)	8 (38.1)	
Total	13 (37.1)	1 (2.9)	21 (60.0)	

Data are shown as n (%).

Discussion

In efforts to identify human remains, it may be necessary to rely upon biological materials that are resistant to natural or induced degradation as sources of biological and DNA profiles. In this context, teeth are highly relevant both for their individualizing characteristics and as an important source of DNA [1-3]. However, there are certain limitations when using teeth to obtain a DNA profile. First, many of the methods used to extract the pulp tissue content (the source of DNA) will irreversibly damage the tooth and thus interfere with investigations carried out by other forensic disciplines; therefore, such investigations must therefore be carried out prior to DNA sampling [1-3]. Here, we used a conservative method in which the dental pulp is accessed from the cavity created by the carious lesion, and therefore the surrounding structures remain intact. Shbair et al. [14] initially described this method and found no significant difference in the DNA concentration obtained using this method versus a non-conservative method in which the dental pulp is accessed via a transverse cut [14].

The second limitation is related to the reported risk that the obtained DNA may be contaminated by microorganisms or other exogenous DNA when carious lesion-bearing teeth are sampled [1,3,12]. However, it can be difficult to obtain lesion-free teeth in some settings: According to WHO estimates, untreated dental caries in permanent teeth is the most frequent health disorder worldwide [10], and particularly affects middle- and low-income countries. One such country is Mexico, where the average prevalence in adults is reported to be 93.3%, and the prevalence is high even from an early age: The prevalence of carious lesions is estimated to be 75-80% in children and adolescents aged 6-19 years, 84-88% in those aged 20-29 years, and more than 90% in those aged over 30 years [11].

In this context, the analysis of teeth with carious lesions is very relevant and could be used to optimize the identification of human remains. Alia-García et al. [12] reported the presence of bacterial DNA in 66.7% (40/60) of teeth with cavities, but not in any of the healthy teeth, although this does not significantly affect the obtaining of genetic profiles [12]. In this study, contamination by bacterial DNA was not evaluated. In the present study, 21 autosomal STRs, DYS391, and amelogenin were amplified from the DNA of teeth with caries, and complete DNA profiles were obtained from 60% of the samples (21/35). This proportion is in line with that reported by Alia-García [12], who amplified 16 autosomal STRs and reported obtaining 56.6% adequate profiles (34/60 carious teeth; with 'adequate' defined as more than 10 amplified STRs plus amelogenin), but lower than that reported by Shbair et al. [14] who amplified 19 autosomal STRs and obtained complete DNA profiles from 71.66% (43/60) of their carious tooth samples using a conservative extraction method like the one used in this study. Meanwhile, Corte-Real et al. [13] did not report the percentage of complete STR profiles; instead, these authors indicated that they obtained mitochondrial DNA profiles from all 30 carious tooth samples [5,12,14].

The minimum amount of DNA required to obtain a DNA profile is reported to be ~ 50 pg (0.05 ng) (Sense about Science, 2019). In the present study, eleven samples yielded DNA with concentrations below or equal to 0.05 ng/μL; in these cases, the number of amplification cycles was increased from 28 to 33, and four complete DNA profiles were obtained. Similarly, Alia-García [12], reported that they had obtained several profiles from DNA amounts ≤0.001 ng/μL, while Shbair et al. [14] reported repeating the amplification step two or three times to obtain profiles of samples that did not amplify the first time [12,14].

The results obtained herein suggest that, despite the limitations of the small sample size and using teeth donated by living patients, it is feasible to obtain DNA profiles from teeth with carious lesions, even those with extensive caries damage (size 4 lesions) and/or a sub-optimal DNA concentration.

Conclusion

The damage caused by carious lesion does not significantly affect the amount of DNA obtained, and thus does not affect the completeness of profiles, making decayed teeth an alternative source of DNA for human identification purposes.

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