Assessing the viability of carious lesions in human identification using STR typing

Mohammed Shbair¹, Atif Adnan², Pang Hao³ and Yi Liu¹

¹School and Hospital of Stomatology, China Medical University, Liaoning Provincial Key Laboratory of Oral Diseases, Shenyang, China
²Department of Human Anatomy, School of Basic Medical Sciences, China Medical University, Shenyang, People’s Republic of China
³Department of Forensic Genetics and Biology, School of Forensic Medicine, China Medical University, Shenyang, People’s Republic of China

Abstract

Human teeth have become a prominent source of DNA for human forensic identification as their biological structure is highly resistant to extreme conditions. Previous forensic identification was mainly dependent on the pulp and the other hard tissues of intact teeth. However, there is high likelihood that only carious teeth can be available for forensic analysis. This study aimed to validate the use of the carious part of the teeth for forensic identification and to compare two DNA extraction methods—the operative technique with the cervical cut technique for human identification using STR typing. The reliability of STR markers in carious part of the teeth was evaluated in 120 carious teeth (60 dental pulp and 60 dentinal carious tissues, respectively) with considerable coverage of gender type and age range to avoid false exclusions. The study was performed on genuine data set where samples have been extracted by proficient dentist during the treatment operation and collected for further analysis. Complete DNA was extracted and the corresponding human identification profile was obtained using the GoldenEye™DNA ID system 20A kit. The operative technique showed a conservative approach to the sampling of carious tissues and allowed safe access to collect carious tissues, whereas the cervical cut technique permitted access to the root canals and complete sampling of the pulp tissues. The findings indicated that there was no significant association between the cervical cut and operative cut techniques (p = 0.165). In addition, there was no statistically significant association between the various teeth types and the obtained profiles observed. The operative technique, by drilling holes on the defected surface of

Corresponding author:
Yi Liu, School and Hospital of Stomatology, China Medical University, Liaoning Provincial Key Laboratory of Oral Diseases, Shenyang 110002, China.
Email: liuyi@cmu.edu.cn
carious human teeth and gentle hand excavation of carious tissues, was indicated to be very efficient, preserving, time-saving, and cost-effective in the recovery of human DNA from carious teeth. The result gives new insights that the carious tissues of human carious teeth might be as valid as the healthy teeth for forensic human identification.

Keywords
Forensic odontology, STR typing, caries, degraded human tissues

Introduction
The latest advantages in DNA technology have revolutionized the field of forensic sciences. Nowadays, the importance of forensic DNA profiling has increased tremendously due to the identification issues in instances of terrorist attacks and mass murders. Furthermore, it is critical to determine the exact identity results to establish paternity and maternity cases, property disputes and burial of victims of disasters. This becomes highly significant as the world faces many crises like acts of terrorism, earthquakes, tsunamis, and airplane crashes. A comparison between fingerprint records and different medical ante and post-mortem records is often difficult and not practicable due to the lack of sufficient ante-mortem records. DNA can be extracted from soft tissues as well as from hard tissues like bones and teeth. In accidents soft tissues may not remain in good conditions, therefore hard tissues are usually preferred. Among bones teeth are the hardest tissues in human body; it may survive in extreme temperatures and in adverse environmental conditions.

Teeth are considered as an important source of DNA due to their composition which provides protection from different microorganisms and environmental factors responsible for decay. While pulp is recognized as the main source of DNA in healthy fresh teeth, many factors can reduce its value such as age, dental disease, and postmortem disintegration.

Dental caries is one of the most common oral diseases globally; approximately 2.43 billion people, 36% of the world’s population, have carious lesions in permanent teeth. The prevalence is increasing day by day with changing dietary habits and lifestyles. It is widely accepted that the consumption of sugar-rich foods increases the incidence of caries. Caries affects tooth structure as it destroys enamel and dentin and also the pulp also undergoes necrosis depending upon the extent of the carious lesion.

Dental caries status in China shows typical characteristics found in developing countries. According to the national third epidemiological report of 2005, the prevalence of dental caries is high in children of 5–6 years age (70.2%), adults of 35–44 (60.6%) years, and of 65–74 years age (75.5%).

In recent years different techniques for DNA extraction from teeth have been used, that are sectioning of teeth horizontally or vertically (cervical cut), crushing or cryogenic grinding, and conventional access cavity preparation. Crushing or grinding is not recommended as the tooth is ground and is not available for any further evaluations. The access cavity preparation techniques or operation techniques: Occlusal perforation (Perforation of the occlusal surface), cervical
perforation (through the tooth neck) are simple, relatively low cost and preserve the tooth. The cervical cut method, a longitudinal cut through the teeth using a very thin disc or saw, facilitates access to the cells of the dental pulp. The cervical cut is less conservative but allows direct access to the pulp cavity of the tooth. Cervical cut and use of endodontic files to access the pulp cavity were considered the most optimum conservative method for DNA recovery by Hervella et al.\textsuperscript{11}

Short tandem repeats (STRs) and Single nucleotide polymorphism (SNPs) are different types of DNA t markers which have revolutionized the field of genetic identification. Among the 3 million or so DNA bases that do not code for proteins are regions with multiple copies of short repeating sequences of these bases, which make up the DNA backbone. These sequences repeat a variable number of times in different individuals. Such regions are called “short tandem repeats,” and they are the basis of STR analysis which are important in forensic analysis.

DNA may be extracted from various tissues present in the tooth, such as from pulp tissues, dentine and cementum.\textsuperscript{12} Teeth are frequently subjected to decontamination processes preceding sampling to remove exogenous DNA, environmental contaminants, and micro-organisms.\textsuperscript{13} Decontamination includes one or both of the following processes: removal of the outside covering layer of the tooth by sanding or grinding;\textsuperscript{13} washing or soaking in bleach.\textsuperscript{14}

The caries are known to destroy enamel and dentin and also the pulp may also undergo necrosis due to caries. As the pulp tissues are major source of DNA in forensic evaluation, implications of caries need to be evaluated.

The aim of this study was to establish a DNA extraction technique from human teeth, which is valid for carious teeth, cost-effective, time-saving, and conserves the tooth integrity and to validate STR profiling from carious teeth.

**Materials and methods**

**Sample demographics**

The nature of study is based on clinical data set. The study was performed on genuine data set where samples have been extracted by proficient dentist during the treatment operation and collected for further analysis. It was not based on literature data set but from actual data set.

The rationale for sample size selection is highlighted as follows. The study was evaluated in 120 carious teeth with considerable coverage of gender type and age range to avoid false exclusions. The number of samples was chosen to adequately represent the case study as per the common researches published in this domain. Sample number for our study lies on the high band in comparison to other highly referenced/cited researches on the same topic indicating 20 to 120 samples used.\textsuperscript{11,15}

All participants included in the current study were from the same geographical area and the same dentist performed dental extractions.

Total 37 females and 23 males participated in the operative cut technique group, whereas 42 females/18 males participated in the cervical cut technique group. The
age range was of participants was 15–68 in the operative cut group, whereas the age range was 17–64 in the cervical cut group.

**Teeth collection**

In this study, 120 carious human teeth were collected during 2016–2018 in the First Affiliated Hospital of China Medical University (Shenyang, China). Each patient declared verbal consent that was approved by the ethical review committee of China Medical University, Shenyang, Liaoning Province, P.R. China. The inclusion criteria were the presence of carious pathology diagnosed either radiographically or visually, whereas the exclusion criteria were root canal treated teeth, internal root resorption, periapical pathology, and severe open root apex because they have minimal bacterial interaction. The carious teeth were extracted under strict sterilized conditions by professional dental surgeons and stored into sterilized gauze. Next, the teeth were decontaminated by gentle bleaching and complete soft tissue curettage and then preserved at $-20^\circ C$ until further process. The collection of carious lesion samples was performed by a skillful dentist, as per International caries detection and assessment system (ICDAS) classification, radiographic images and whether soft or hard carious lesion was present.

Whenever the sample showed presence of superficial debris/plaque, these contaminants were removed by reduced transport fluid (RTF; Syed & Loesche, 1972) twice.

Consequently, comparative study was performed between two different DNA extraction procedures on carious teeth which are listed below (a,b):

(a) Operative technique: (40 molars, 11 premolars, and 9 incisors). We used sterile diamond bur cut around hard carious lesion and hand excavated soft carious dentine with sterile spoon excavators of different sizes. The collected hard and soft dentine caries fragments were preserved in microcentrifuge tubes and analyzed separately (Figures 1 and 2). Crushing was needed for a few samples where hard carious enamel was collected.

(b) Cervical cut: (45 molars, 7 premolars, 8 incisors) A cut at the neck level of the carious tooth using tungsten carbide surgical bur facilitates direct access to the pulp. Pulpal tissue samples were collected using endodontic K-files of different sizes from the pulp chamber, root canals, and the dental apex of the root (Figure 3). In both groups, 6–20 g of samples was collected in microcentrifuge tubes.

**Statistical analysis**

The median and interquartile ranges (Percentile 25 and 75) of data chi-square test was applied to compare the obtained genetic profile proportion between the operative cut and cervical cut to access the pulp cavity and root canals. DNA purity and concentration between both groups was compared using the $U$ of Mann-Whitney test.
Statistical analysis was performed using IBM SPSS Statistics V.20 (Cytel software Co., Cambridge, USA). The $p$-value of $\leq 0.05$ was considered statistically significant.

**DNA extraction from carious material**

DNA from hard and soft carious materials and dental pulp tissues was extracted by the commercial QIAamp Mini Kit (Qiagen, Hilden, Germany) following the manufacturer instructions. The concentration of DNA was quantified by absorption at 260 nm using an ultraviolet spectrophotometer (UV-2800AH, UNICO).

**PCR amplification and STR typing**

PCR co-amplification of one sex locus and 19 autosomal STRs, including 13 combined DNA index system (CODIS) STRs and 6 other loci were performed in a
fluorescence-based multiplex reaction using the Goldeneye 20A systems (D5S818, FGA, D3S1358, TH01, D13S317, D16S539, D8S1179, D21S11, D7S820, CSF1PO, vWA, TPOX, D18S51, Penta E, Penta D, D2S1338, D19S433, D12S391, and D6S1043). From 1 to 2 ng of the target DNA was amplified according to the manufacturer's recommended protocol. Thermal cycling was conducted under the following conditions: 95°C for 5 min; 30 cycles of 94°C for 30 s, 60°C for 60 s, 70°C for 60 s; and a final extension of 60°C for 30 min. All loci were amplified in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA).

Analysis of the results was obtained in four different possibilities: (1) Full profile (where all 19 STR loci markers and amelogenin gene were present), (2) Partial profile (More than 10 STR loci could be identified and amelogenin gene), (3) Low profile (less than 10 STR loci could be identified and amelogenin gene), (4) No profile (No STR loci could be identified, whether amelogenin gene could be identified or not).

**Results**

The operative technique showed a conservative approach to the sampling of carious tissues and allowed safe access to collect carious tissues, whereas cervical cut technique permitted access to the root canals and complete sampling of pulp tissues. The process of DNA extraction was successful in both groups, purity (A260/280) values were symmetric, yet nucleic acid concentrations showed wide variations (Figure 4). Descriptive data for both groups are presented in Tables 1 and 2.

Of the 120 samples, 118 (98%) samples obtained genetic profiles that could be used in forensic identification.

---

**Figure 3.** Scheme of both sampling methods evaluated: cervical cut and operative cut techniques for DNA recovery from carious teeth.
There was no significant association between the cervical cut and operative cut techniques ($p = 0.165$). However, the cervical cut method obtained higher complete STR genetic profiles with less variation in the distribution of complete (51), partial

| Tooth type     | Classification | DNA (ng/μl) | Obtained profile |
|---------------|---------------|-------------|------------------|
|               |               | Median  | IQR | Full | Partial | Low | No-profile |
| Incisors and  | 4 (5)         | 1.95    | 0.5 | 4    | 1        |     |            |
| Canines (9)   | 5/6 (4)       | 2       | 2   |      |          |     |            |
| Premolar (11) | 4 (3)         | 1.91    | 0.22| 2    | 1        | 1   | 1          |
|               | 5 (4)         |          |     | 3    |          | 1   |            |
|               | 6 (4)         |          |     | 2    |          | 2   |            |
| Molar (40)    | 4 (24)        | 1.91    | 0.33| 22   | 2        | 1   |            |
|               | 5 (11)        |          |     | 7    | 3        | 1   | 2          |
|               | 6 (5)         |          |     | 2    | 1        | 2   | 1          |
| Total (60)    | 1.91          | 0.22–0.5|     | 43   | 11       | 4   | 2          |

*0: sound, 1: seen only after prolonged air drying or restricted to first visual change in enamel (confines of a pit or fissure) 2: distinct visual change in enamel, 3: localized enamel breakdown (without clinical visual signs of dentinal involvement), 4: underlying dark shadow from dentin, 5: distinct cavity with visible dentin, 6: extensive distinct cavity with visible dentin.

*Interquartile range.
The obtained profiles from operative cut were: 43 complete, 11 partial, 4 low, and 2 no profile results. There was no statistically significant association between various teeth types and the obtained profiles observed. Purity (A260/280) values and concentrations of DNA were obtained from both groups.

Discussion

According to the World Health Organization (WHO) Oral Health Data Bank, dental caries is the most prevalent bacterial pathology globally and its prevalence is increasing daily. The aim of this study was to validate the use of carious enamel and dentine lesions to produce full short tandem repeats (STR) profiles in decayed dental tissues and establish the most suitable sampling method for carious teeth.

The carious lesions in both techniques comprised of both enamel and dentine caries (Tables 1 and 2). It is difficult to locate DNA from hard structure of enamel. Hence the samples in this study were carious enamel and some mild dentinal caries.

The limitation of this study was the use of freshly extracted teeth that are not exposed to postmortem, environmental and thermal changes. Despite this limitation, the study suggests that carious teeth may be used for human identification. However, further studies using forensic samples should be performed.

Higgins and Austin proved that it is preferable to use the dental pulp of healthy teeth rather than carious ones to obtain human DNA for corpse identification in cases where classic identification methods are of no significance as in cases of mass disasters. With the evolution in the sensitivity and specificity of genomic

| Tooth type | Classification | DNA (ng/μl) | Obtained profile |
|------------|----------------|-------------|------------------|
|            |                | Median      | IQR^b            | Full | Partial | Low  | No-profile |
| Incisors and Canines (8) | 4 (3) | 1.96 | 0.22 | 3 |
| Premolar (7) | 4 (3) | 1.85 | 0.66 | 2 | 1 |
| Molar (45) | 4 (26) | 1.97 | 0.22 | 24 | 2 |
| | 5 (13) | | 11 | 2 |
| | 6 (6) | | 4 | 2 |
| Total (60) | | 1.96 | 0.22–0.66 | 51 | 6 | 3 |

^a: sound, 1: seen only after prolonged air drying or restricted to first visual change in enamel (confines of a pit or fissure) 2: distinct visual change in enamel, 3: localized enamel breakdown (without clinical visual signs of dentinal involvement), 4: underlying dark shadow from dentin, 5: distinct cavity with visible dentin, 6: extensive distinct cavity with visible dentin.

^b Inter-quartile range.
DNA extraction and amplification procedures, we hypothesized that various tissues of carious teeth could be potential sources for obtaining human DNA. We also hope that it will benefit identification process in instances of mass disasters.

The major possible sources of dental human nuclear DNA are the odontoblasts and its odontoblastic processes which usually end at the Dentino-enamel junction or become embedded in the enamel as it forms or the reparative dentine in carious teeth.17 Collection of the carious dental tissues using spoon excavators facilitated obtaining non-damaged genetic material from such places.

Several nondestructive methods for tooth sampling have been compared for DNA extraction, as the procedure used is a major factor that decisively affects the recovery of DNA profile.18,19 Most of the previous studies focused on nuclear DNA extraction from pulpal tissues. However, cells within pulpal tissues become nonviable within a short period of time. Contrary to expectations, even after pulpal tissues are completely destroyed, DNA is present in the hard tissues as the teeth of extended postmortem periods have demonstrated the presence of nuclear DNA.20

The purity (A260/280) and concentrations of DNA obtained are limiting factors in determining the efficiency of obtained genetic profiles. For purity values in the range of 1.8–2.0 are considered to be adequate, while values less than 1.7 considered having protein or membrane contamination, whereas more than two are considered as RNA or minerals involvement.21,22 Most of our samples showed adequate purity with large variation in DNA concentration.

Full profile results were obtained from most of our samples. However, 10–15 samples were needed to re-amplification process two to three times when the first instance of amplification did not yield any genetic profiles.

The cervical cut technique and operative cut technique together yielded genetic profile in 98% of cases that could be used in forensic identification. Both techniques allowed successful extraction of DNA from the carious teeth. Thus our findings in the present study are consistent with the findings of Alia-García et al.15, which stated that carious teeth could be used for forensic identification purposes as in DNA extraction to the same extent as healthy ones.

**Conclusion**

It was concluded that the Operative technique by drilling holes on the defected surface of carious human teeth and gentle hand excavation of carious tissues would be an efficient, preservative, time-saving, and cost-effective in the recovery of human DNA from carious teeth.

In addition, soft and hard carious material could be used as a source for forensic identification purposes.

**Author contributions**

MF and YL contributed to the design of the study, data collection. PH, AA, did data analysis and interpretation. MF prepared the manuscript. AA modified the manuscript. All authors read and approved the final manuscript.
Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The work was supported by Liaoning Province key research and development plan project (2020JH2/10300038) and Shenyang Science and Technology Project (20-205-4-099).

Ethical approval
The study has been approved by the Ethical Review Committee of China Medical University, Shenyang, Liaoning Province, P.R. China. Ethics and health compliance committee of China Medical University with Registration number (1006701211).

Informed consent
Informed consent was obtained from all individual participants included in the study.

ORCID iD
Mohammed Shbair https://orcid.org/0000-0003-4416-2880

Availability of data and material
The full dataset supporting the conclusions of this article can be obtained upon a reasonable request to the corresponding author liuyi@cmu.edu.cn.

References
1. Nuzzolese E and Di Vella G. Future project concerning mass disaster management: a forensic odontology prospectus. Int Dent J 2010; 57: 261–266.
2. Sarode SC, Zarkar GA and Kulkarni MA. Role of forensic odontology in the world’s major mass disasters: facts and figures. Dent Update 2009; 36: 430–432, 435–436.
3. Edson SM, Ross JP, Coble MD, et al. Naming the dead-Confronting the realities of rapid identification of degraded skeletal remains. Forensic Sci Rev 2004; 16: 63–90.
4. Nelson K and Melton T. Forensic mitochondrial DNA analysis of 116 casework skeletal samples. J Forensic Sci 2007; 52: 557–561.
5. Malaver PC and Yunis JJ. Different dental tissues as a source of DNA for human identification in forensic cases. Croat Med J 2003; 44: 306–309.
6. Trivedi R, Chattopadhyay P and Kashyap VK. A new improved method for extraction of DNA from teeth for the analysis of hypervariable loci. Am J Forensic Med Pathol 2002; 23: 191–196.
7. Vos T, Flaxman AD, Naghavi M, et al. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380: 2163–2196.
8. Kämppi A, Tanner T, Päkkilä J, et al. Geographical distribution of dental caries prevalence and associated factors in young adults in Finland. *Caries Res* 2013; 47: 346–354.

9. Liu L, Zhang Y, Wu W, et al. Prevalence and correlates of dental caries in an elderly population in Northeast China. *PLoS One* 2013; 8(11): e78723.

10. Mohandesan E, Prost S and Hofreiter M. Case study: using a nondestructive DNA extraction method to generate mtDNA sequences from historical chimpanzee specimens. *Methods Mol Biol* 2012; 840: 101–110.

11. Hervella M, Iñiguez MG, Izagirre N, et al. Nondestructive methods for recovery of biological material from human teeth for DNA extraction. *J Forensic Sci* 2015; 60: 136–141.

12. Divakar KP. Forensic odontology: the new dimension in dental analysis. *Int J Biomed Sci* 2017; 13: 1–5.

13. Kemp BM and Smith DG. Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. *Forensic Sci Int* 2005; 154: 53–61.

14. Sweet D and Hildebrand D. Recovery of DNA from human teeth by cryogenic grinding. *J Forensic Sci* 1998; 43: 1199–202.

15. Alia-García E, Parra-Pecharromán D, Sánchez-Díaz A, et al. Forensic identification in teeth with caries. *Forensic Sci Int* 2015; 257: 236–241.

16. Higgins D and Austin JJ. Teeth as a source of DNA for forensic identification of human remains: a review. *Sci Justice* 2013; 53: 433–441.

17. Smith BC, Fisher DL, Weedn VW, et al. A systematic approach to the sampling of dental DNA. *J Forensic Sci* 1993; 38: 1194–1209.

18. Rubio L, Martínez LJ, Martínez E, et al. Study of short- and long-term storage of teeth and its influence on DNA. *J Forensic Sci* 2009; 54: 1411–1143.

19. Alonso A, Andelinović S, Martín P, et al. DNA typing from skeletal remains: evaluation of multiplex and megaplex STR systems on DNA isolated from bone and teeth samples. *Croat Med J* 2001; 42: 260–266.

20. Higgins D, Kaidonis J, Austin J, et al. Dentine and cementum as sources of nuclear DNA for use in human identification. *Aust J Forensic Sci* 2011; 43: 287–295.

21. Anthonappa RP1, King NM and Rabie AB. Evaluation of the long-term storage stability of saliva as a source of human DNA. *Clin Oral Investig* 2013; 17: 1719–1725.

22. Sosa C, Baeta M, Núñez C, et al. Nuclear DNA typing from ancient teeth. *Am J Forensic Med Pathol* 2012; 33: 211–214.

Author biographies

Mohammed Shbair was born in AbuDhabi, UAE. he was fascinated with forensic medicine, and this interest led to some early exposure to reading most stories related to forensic medicine. previous articles focused on Sella turcica and cephalometric x rays to evaluate patient’s age, sex and racial discrimination.

Atif Adnan practiced forensic medicine in Anthropology department of human anatomy school of basic medicine he got his PhD from China Medical University and He had more than 20 publications as co author and corresponding author. Skillful in SPSS, data analysis and next generation sequencing.
Pang Hao is the dean of the department of forensic genetics and biology in China Medical University for more than 25 years. He published lot of papers and books related to forensic medicine in both Chinese and English. He dedicated all his life for forensics medicine and he is well known in China for solving many complicated paternity and maternity cases.

Yi Liu is the head dean of orthodontic department in China Medical University for more than two years. With more than 16 publications as corresponding author in international SCI journals, more than 50 publications in local Chinese journals. Great skills and strategies to treat more than 2000 orthodontic patients successfully. Many patients visit her from all around China for consultation. She’s considered as Godfather for orthodontics in Liaoning province.