Legal Medicine 17 (2015) 547–552

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Comparison of hard tissues that are useful for DNA analysis in forensic autopsy

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ARTICLE INFO

Article history:
Received 20 April 2015
Received in revised form 30 June 2015
Accepted 2 July 2015
Available online 3 July 2015

Keywords:
Forensic autopsy
Hard tissues
DNA typing

ABSTRACT

Forensic analysis of DNA from hard tissues can be important when investigating a variety of cases resulting from mass disaster or criminal cases. This study was conducted to evaluate the most suitable tissues, method and sample size for processing of hard tissues prior to DNA isolation. We also evaluated the elapsed time after death in relation to the quantity of DNA extracted. Samples of hard tissues (37 teeth, 42 skull, 42 rib, and 39 nails) from 42 individuals aged between 50 and 83 years were used. The samples were taken from remains following forensic autopsy (from 2 days to 2 years after death). To evaluate the integrity of the nuclear DNA isolated, the percentage of allele calls for short tandem repeat profiles were compared between the hard tissues. DNA typing results indicated that until 1 month after death, any of the four hard tissue samples could be used as an alternative to teeth, allowing analysis of all of the loci. However, in terms of the sampling site, collection method and sample size adjustment, the rib appeared to be the best choice in view of the ease of specimen preparation. Our data suggest that the rib could be an alternative hard tissue sample for DNA analysis of human remains.

1. Introduction

Forensic autopsy is an important process for personal identification and investigation of cause of death. In recent years in Japan, the number of corpses handled by police has been increasing steadily, being 169,047 in 2013 and thus 1.24 times the number handled in 2004. This has resulted in an increase in the number of autopsies, having increased 1.68-fold from 4969 in 2004 to 8356 in 2013. Against this background, legislation to promote the investigation of causes of death has been introduced.

DNA testing at autopsy is a highly effective approach for personal identification [1–3]. DNA testing is used when antemortem records are missing and corpses are in a poor state of preservation. DNA identification becomes essential at times of mass disasters. When putrefaction has occurred after a corpse has remained undiscovered for a long period, DNA testing using soft tissue becomes difficult. However, hard tissues such as tooth and bone tend to remain better preserved, even after long periods have elapsed. In comparison with soft tissues, hard tissues are resistant to autolysis and putrefaction resulting from environmental exposure. For this reason, bones, teeth and nails may be the only source of DNA in many forensic cases [4–7]. Therefore, DNA extraction from hard tissue is employed when investigating a variety of cases involving crimes and disasters.

A number of reports have indicated that DNA testing using teeth is very useful and practical [8–12]. However, we often encounter situations in which teeth cannot be used for DNA testing, for example in aged individuals who are edentulous or young infants in whom tooth eruption has not yet occurred. There are many situations in which DNA testing using teeth cannot be performed, such as burnout of teeth in charred corpses, mixing of DNA due to root canal treatment, or paucity of DNA in the remaining root. In particular, tooth desorption or loss is frequently observed in corpses that have remained undiscovered for long periods or in corpses of elderly individuals, or infants less than 6 months old in which tooth eruption has not yet occurred. Thus, cases in which tooth samples cannot be collected for DNA analysis are not uncommon.

Here we examined methods for collection and extraction of DNA from hard tissues that can be used an alternative to teeth when DNA testing using teeth is not possible.

2. Materials and methods

Cleaning of samples, DNA extraction and amplification were conducted following the generally accepted safety and DNA typing guidelines.

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http://dx.doi.org/10.1016/j.legalmed.2015.07.001
1344-6223/

2.1. Samples

Forty-two samples with different histories and states of degradation were used (teeth, nails, skull and rib samples from 30 males and 12 females aged 50–83 years). Considering the uniformity of samples and the amount of DNA, samples were collected from prespecified portions. That is, vital tooth material from the upper right canine lacking any caries or treatment was collected. Similarly, nails from the right thumb, the skull just above the scaly suture, and the right second rib close to its connection with the rib costal cartilage were collected.

This study was carried out with the approval of Kanagawa Dental University Research Ethics Examination Committee.

2.2. DNA extraction

2.2.1. DNA extraction from teeth

The surface of the tooth samples was washed with sterilized water to eliminate potential contamination and dirt. After drying, the samples were fixed to an aluminum block (BUEHLER Ltd., USA), cut into three thin slices (1-mm vertical sections, each approximately 620 mg) and washed with sterilized water. The samples were then decalcified by incubating with 35 ml of 0.5 M EDTA solution, pH 7.5, at 56 °C for 3 days. The DNAs were prepared by the proteinase K-SDS method. Briefly, the decalcified slices were incubated at 56 °C for 3 h with 400 μl of a cell lysis buffer composed of 10 mM Tris–HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA, 2% SDS and 2 mg of proteinase K. The digest was mixed with an equal volume of TE saturated phenol, vortexed for 10 s, then centrifuged at 15,000 rpm for 5 min. The aqueous phase was extracted using equal volumes of phenol/chloroform/isoamyl alcohol (25:24:1). The mixture was spun through a QIAamp DNA Mini Spin column (QIAGEN, Chicago, USA). The columns were centrifuged for 1 min at 15,000 rpm and then cell lysis buffer was added. The membrane of the QIAamp mini column was washed with 500 μl AW1 and AW2 buffer following centrifugation at 15,000 rpm for 1 min. The DNA was eluted in 50 μl of AE buffer.

2.2.2. DNA extraction from skull and rib

Prior to DNA extraction, approximately 120 mg of skull, and approximately 70 mg of rib was calculated as the appropriate amount. Each skull and rib sample was decalcified by incubation with 35 ml of 0.5 M EDTA solution, pH 7.5, at 56 °C for 3 days. The DNAs were prepared by the proteinase K-SDS method. Briefly, the decalcified samples were incubated at 56 °C for 3 h with 400 μl of a cell lysis buffer composed of 10 mM Tris–HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA, 2% SDS and 2 mg of proteinase K. The digest was mixed with an equal volume of TE saturated phenol, vortexed for 10 s, then centrifuged at 15,000 rpm for 5 min. The aqueous phase was extracted using equal volumes of phenol/chloroform/isoamyl alcohol (25:24:1), and then the DNA was extracted according to the instructions of the QIAamp DNA Mini Kit (QIAGEN, Chicago, USA). The final volume of eluted DNA was 50 μl.

2.2.3. DNA extraction from nail

Nail samples were wiped with alcohol and dried. Approximately 50 mg was cut off with a surgical blade. DNAs were extracted according to the instructions of the QIAamp DNA Micro Kit. The final volume of eluted DNA was 25 μl.

2.3. DNA concentration and quantification

DNA concentrations were measured using a Nanodrop-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Samples were quantified using the 260/280 ratio via Nanodrop-2000.

2.4. STR typing and analysis

All DNA extracts were amplified in duplicate using the AmpFlSTR Identifiler plus PCR Amplification Kit (Applied Biosystems, Foster City, CA, USA) in order to confirm the genetic profile of the samples. The kit was used in accordance with the manufacturer's protocol with a 9700 Thermal Cycler (Applied Biosystems). From every sample, 10 ng of the DNA extract was subjected to PCR as a template. In all amplification reactions a positive control and negative PCR controls were used, as well as reagent blanks from extractions. The STR fragments were separated by capillary electrophoresis using a 310 Genetic Analyzer (Applied Biosystems), POP4 polymer and the collection software 310 Data Collection ver. 3.1.0. The samples were genotyped using GenemapperlD-X ver.1.4 (Applied Biosystems).

3. Results

Samples of hard tissue, i.e. teeth, nails, skull, and rib, were obtained from 42 forensic autopsy cases (male 30, female 12; time elapsed from death: 2 days to 2 years). Skull and rib samples were obtained from all cases, but tooth samples were obtained from 37 cases and nail samples from 39.

In this study, we examined 13 out of 42 cases for which DNA was extracted from all four hard tissues. Sample profiles are listed in Table 1.

The average DNA concentration of each sample per microliter was: tooth 48.5 ng, nail 20.6 ng, skull 51.1 ng, and rib 35.2 ng (Fig. 1). We also examined the DNA concentration with time for each sample of hard tissue (Fig. 2). Tooth DNA concentration became very low after 1 month. Although the nail DNA concentration was low, it showed a constant value regardless of time elapsed after death. The skull DNA concentration became very low after 1 month, as was the case for tooth DNA. The rib DNA concentration was similar to that for tooth material.

STR analyses were performed on DNA extracted from each hard tissue (Table 2). Until 1 month after death (samples 1–6), STR analysis of all loci was possible using all four hard tissues (Table 2). Three months after death (sample 7), STR analysis of all loci was possible for tooth, skull and rib, but for nail STR analysis of 9 loci – D8S1179, D7S820, CSF1PO, TH01, D13S317, D16S539, D2S1338, vWA and D18S51 – was impossible. Five months after death (sample 8, 9, 10), STR analysis of all loci was possible for tooth, but for nail 13 loci – D8S1179, D21S11, D7S820, CSF1PO, TH01, D13S317, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA – was impossible; for skull, STR analysis of 5 loci – D21S11, CSF1PO,

Table 1
Sample profiles.

Sample No.	Sex	Age	Elapsed time after death	Cause of death
1	F	79	2 days	Cardiac tamponade
2	F	83	3 days	Burned
3	F	69	3 days	Drowing
4	M	63	7 days	Intercranial bleeding
5	M	51	8 days	Subdural bleeding
6	M	71	1 m	Cerebellar bleeding
7	M	51	3 m	Drowing
8	M	62	5 m	Unknown
9	M	67	5 m	Unknown
10	F	80	5 m	Unknown
11	M	70	9 m	Unknown
12	M	75	1 year	Unknown
13	M	50	2 year	Unknown