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

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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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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

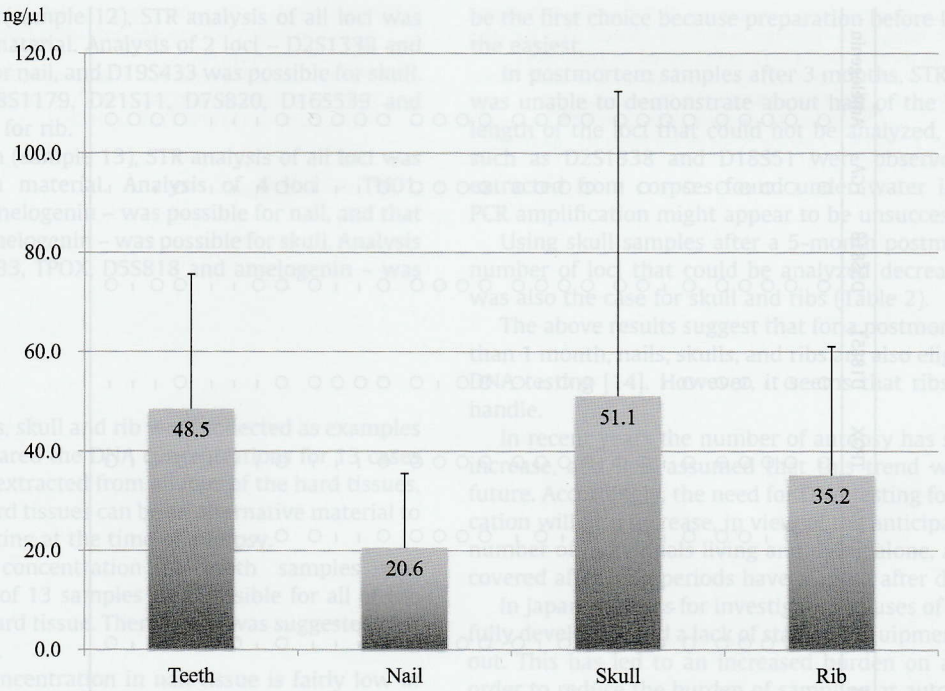

Fig. 1. DNA concentration.

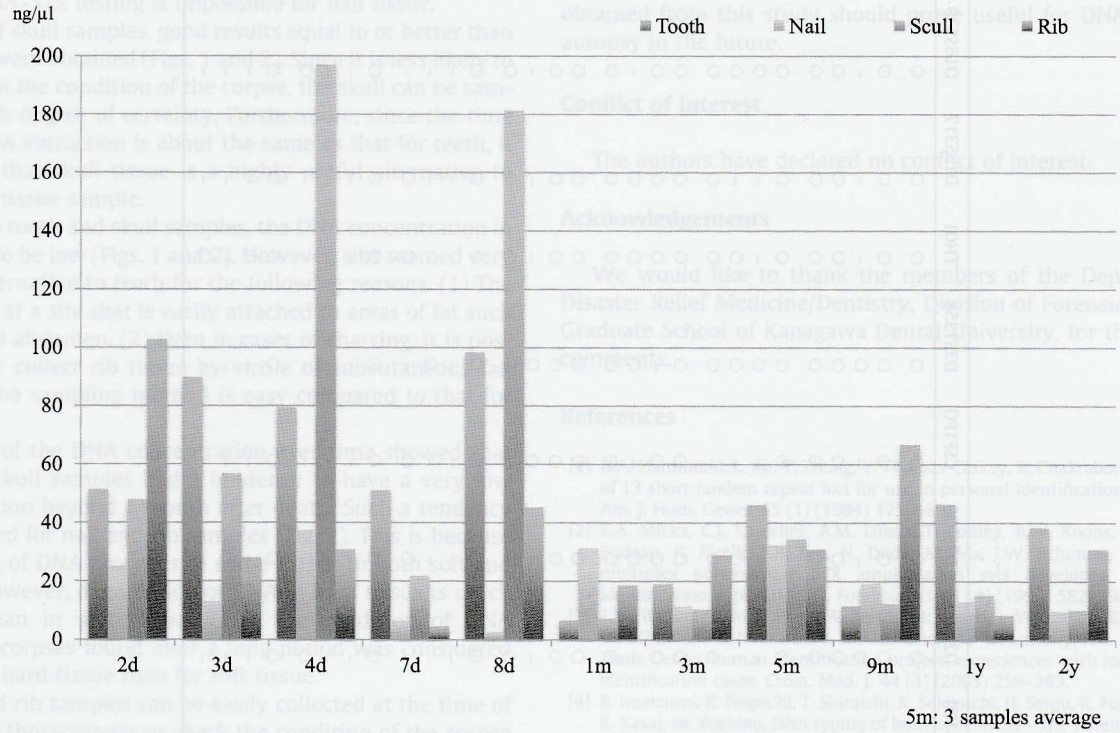

Fig. 2. DNA concentrations in 4 hard tissues. 5 m: 3 samples average.

D13S317, D19S433 and D18S51 – was impossible, and for rib, this was impossible for 3 loci – CSF1PO, D18S51, FGA (sample 8), for nail 2 loci – D3S1358 and vWA – was impossible; for skull, STR analysis of 7 loci – D8S1179, D21S11, CSF1PO, D16S539, D2S1338, D19S433 and D18S51 – was impossible, and for rib, this was impossible for 2 loci – D8S1179, vWA (sample 9), for nail D2S1338 was impossible; for skull, STR analysis of 8 loci – D8S1179, D21S11, D7S820, TH01, D13S317, D16S539, D2S1338, and D18S51 – was impossible, and for rib, this was impossible for 2 loci – D7S820 and D2S1338 (sample 10). Nine months after death (sample 11), STR analysis of all loci was possible for tooth samples. For three loci – D8S1179, D18S51 and amelogenin – STR analysis was possible for nail, and for skull samples this was possible for 3 loci – D3S1358, D18S51 and amelogenin; analysis of 8 loci – D7S820, CSF1PO, TH01, D13S317, vWA, D18S51, D5S818 and amelogenin – was possible for rib samples.

Table 2
DNA typing results.

Sample No.	Hard tissue[a]	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA	Amelogenin
1–6	1–4	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
7	1	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	2	–	○	–	–	○	–	–	–	–	○	–	○	–	○	○	○
	3	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	4	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
8	1	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	2	–	–	–	–	○	–	–	○	–	–	–	–	–	–	–	○
	3	○	–	○	–	○	○	–	○	○	–	○	○	–	○	○	○
	4	○	○	○	–	○	○	○	○	○	○	○	○	–	○	–	○
9	1	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	2	○	○	○	○	–	○	○	○	○	○	–	○	○	○	○	○
	3	–	–	○	–	○	○	○	–	–	–	○	○	–	○	○	○
	4	–	○	○	○	○	○	○	○	○	○	–	○	○	○	○	○
10	1	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	2	○	○	○	○	○	○	○	○	–	○	○	○	○	○	○	○
	3	–	–	–	○	○	–	–	–	–	○	○	○	–	○	○	○
	4	○	○	–	○	○	○	○	○	–	○	○	○	○	○	○	○
11	1	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	2	○	–	–	–	–	–	–	–	–	–	–	–	○	–	–	○
	3	–	–	–	–	○	–	–	–	–	–	–	–	○	–	–	○
	4	–	–	○	○	–	○	○	–	–	–	○	–	○	○	–	○
12	1	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	2	–	–	–	–	–	–	–	–	○	–	–	–	–	○	–	–
	3	–	–	–	–	–	–	–	–	–	○	–	–	–	–	–	–
	4	○	○	○	–	–	–	–	○	–	○	–	–	–	–	–	–
13	1	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	2	–	–	–	–	–	○	–	–	–	○	–	–	–	○	–	○
	3	–	–	○	–	–	–	–	–	–	–	–	–	–	–	–	○
	4	–	–	–	–	–	○	–	–	–	○	–	○	–	○	–	○

–: not conclusive.
[a] 1: tooth, 2: nail, 3: skull, 4: rib.

One year after death (sample 12), STR analysis of all loci was possible only for tooth material. Analysis of 2 loci – D2S1338 and D5S818 – was possible for nail, and D19S433 was possible for skull. Analysis of 5 loci – D8S1179, D21S11, D7S820, D16S539 and D19S433 – was possible for rib.

Two years after death (sample 13), STR analysis of all loci was possible only for tooth material. Analysis of 4 loci – TH01, D19S433, D5S818 and amelogenin – was possible for nail, and that of 2 loci – D7S820 and amelogenin – was possible for skull. Analysis of 5 loci – TH01, D19S433, TPOX, D5S818 and amelogenin – was possible for rib.

4. Discussion

Samples of teeth, nails, skull and rib were collected as examples of hard tissue. We compared the DNA concentrations for 13 cases in which DNA had been extracted from all four of the hard tissues, and verified that such hard tissues can be an alternative material to teeth for use in DNA testing at the time of autopsy.

The average DNA concentration in tooth samples was 48.5 ng/μl. STR analysis of 13 samples was possible for all of the loci using four types of hard tissue. Therefore, it was suggested that tooth DNA is very stable.

Although the DNA concentration in nail tissue is fairly low in comparison with teeth, it was shown that a certain constant concentration can be obtained regardless of the time elapsed after death (Figs. 1 and 2). However, in practice, we have experienced that nuclear DNA–STR testing is impossible for nail tissue.

In the case of skull samples, good results equal to or better than those for teeth were obtained (Figs. 1 and 2). Since it is less likely to be dependent on the condition of the corpse, the skull can be sampled with a high degree of certainty. Furthermore, since the time required for DNA extraction is about the same as that for teeth, it was suggested that skull tissue is a highly useful alternative to teeth as a hard tissue sample.

Compared to tooth and skull samples, the DNA concentration in ribs was found to be low (Figs. 1 and 2). However, ribs seemed very useful as an alternative to teeth for the following reasons. (1) The ribs are located at a site that is easily attached to areas of fat such as the chest and abdomen. (2) Even in cases of charring, it is possible to reliably collect rib tissue by virtue of subcutaneous fat. (3) Moreover, the sampling method is easy compared to that for skull tissue.

Comparison of the DNA concentration over time showed that the tooth and skull samples had a tendency to have a very low DNA concentration beyond 1 month after death. Such a tendency was not observed for nail and rib samples (Fig. 2). This is because the degradation of DNA proceeds to some extent in both soft and hard tissues. However, degradation of DNA in hard tissue is much less marked than in soft tissue. Thus, the condition of DNA extracted from corpses found after a long period was considered to be better for hard tissue than for soft tissue.

The skull and rib samples can be easily collected at the time of craniotomy and thoracotomy to check the condition of the corpse at autopsy. This is one of the strongest arguments for their use as an alternative hard tissue. On the other hand, nails are slightly inferior to skull and rib samples in terms of easy collection and storability. The collection rate for skull and rib was 100%, compared with 92.9% for nails and 88.1% for teeth, suggesting that skull or rib should be selected first as a sample for DNA testing.

The STR results (Table 2) showed that until 1 month after death, any of the four hard tissues could be used as an alternative material for teeth because all of the loci in them could be analyzed. However, from the viewpoint of sampling site and collection method, and size adjustment of the materials, rib might be the first choice because preparation before DNA extraction was the easiest.

In postmortem samples after 3 months, STR analysis using nail was unable to demonstrate about half of the loci. In view of the length of the loci that could not be analyzed, relatively long loci such as D2S1338 and D18S51 were observed. Since the DNA extracted from corpses found under water is fragmented [13], PCR amplification might appear to be unsuccessful.

Using skull samples after a 5-month postmortem interval, the number of loci that could be analyzed decreased gradually. This was also the case for skull and ribs (Table 2).

The above results suggest that for a postmortem interval of less than 1 month, nails, skulls, and ribs are also eligible as samples for DNA testing [14]. However, it seems that ribs are the easiest to handle.

In recent years the number of autopsy has shown a significant increase, and it is assumed that this trend will continue in the future. Accordingly, the need for DNA testing for decedent identification will also increase, in view of the anticipated increase in the number of individuals living and dying alone, and thus being discovered after long periods have elapsed after death.

In Japan, systems for investigating causes of death has not been fully developed, and a lack of staff and equipment has been pointed out. This has led to an increased burden on autopsy doctors. In order to reduce the burden of sampling at autopsy and to achieve a further improvement of rapid identification and DNA testing, there is a need to make sampling sites and sample collection methods at autopsy more stable and reliable. Therefore, the results obtained from this study should prove useful for DNA testing at autopsy in the future.

Conflict of interest

The authors have declared no conflict of interest.

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References

[1] H.A. Hammond, L. Jin, Y. Zhong, Y. Thomas Caskey, R. Chakraborty, Evaluation of 13 short tandem repeat loci for use in personal identification applications, Am. J. Hum. Genet. 55 (1) (1994) 175–189.
[2] K.A. Micka, C.J. Sprecher, A.M. Lins, C.T. Comey, B.W. Koons, C. Crouse, D. Endean, K. Pirelli, S.B. Lee, N. Duda, M. Ma, J.W. Schumm, Validation of multiplex polymorphic STR amplification sets developed for personal identification applications, J. Forensic Sci. 41 (4) (1996) 582–590.
[3] Z.M. Budimlija, M.K. Prinz, A. Zelson-Mundorff, J. Wiersema, E. Bartelink, G. MacKinnon, B.L. Nazzaruolo, S.M. Estacio, M.J. Hennessey, R.C. Shaler, World Trade Center human identification project: experiences with individual body identification cases, Croat. Med. J. 44 (3) (2003) 259–263.
[4] K. Imaizumi, K. Noguchi, T. Shiraishi, K. Sekiguchi, H. Senju, K. Fujii, K. Yoshida, K. Kasai, M. Yoshino, DNA typing of bone specimens – the potential use of the profiler test as a tool for bone identification, Legal. Med. 7 (1) (2005) 31–41.
[5] D.A. Bolnick, H.M. Bonine, J. Mata-Míguez, B.M. Kemp, M.H. Snow, S.A. LeBlanc, Nondestructive sampling of human skeletal remains yields ancient nuclear and mitochondrial DNA, Am. J. Phys. Anthropol. 147 (2) (2012) 293–300.
[6] A. Nakanishi, F. Moriya, Y. Hashimoto, Effects of environmental conditions to which nails are exposed on DNA analysis of them, Legal. Med. 5 (2003) S194–S197.
[7] K. Honda, S. Harihara, C. Wakasugi, S. Sawa, Recovery and identification of skeletal remains by DNA extracted from various hard tissues, Acta Criminologiae et medicinae legalis japonica 60 (6) (1994) 251–259.
[8] L. Pötsch, U. Meyer, S. Rothschild, P.M. Schneider, C. Rittner, Application of DNA techniques for identification using human dental pulp as a source of DNA, Int. J. Legal Med. 105 (3) (1992) 139–143.
[9] D. Higgins, J.J. Austin, Teeth as a source of DNA for forensic identification of human remains: a review, Sci. Justice 53 (4) (2013) 433–441.

[10] Y. Kitagawa, H. Ohira, Y. Yamamuro, I. Yamamoto, N. Kobayashi, Y. Yamada, Personal distinction using DNA from the oral tissue (dental pulp and oral mucosa), J. Kanagawa Odontol. Soc. 43 (1) (2008) 44–48 (in Japanese).
[11] Y. Yamada, Analysis of DNA from tooth and application to forensic dental medicine, Jpn. J. Legal. Med. 43 (1989) 420–423 (in Japanese).
[12] B.C. Manjunath, B.R. Chandrashekar, M. Mahesh, R.M. Vatchala Rani, DNA profiling and forensic dentistry – a review of the recent concepts and trends, J. Forensic Leg. Med. 18 (5) (2011) 191–197.
[13] M. Hashiyada, S. Tkahashi, J. Sakai, K. Usui, M. Funayama, Examination of DNA extraction from a decomposed body using hard tissues, DNA Polym. 18 (2010) 176–178 (in Japanese).
[14] M.P. Evison, D.M. Smillie, A.T. Chamberlain, Extraction of single-copy nuclear DNA from forensic specimens with a variety of postmortem histories, J. Forensic Sci. 42 (6) (1997) 1032–1038.