Forensic Science International 271 (2017) 33–42

Contents lists available at ScienceDirect

Forensic Science International

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

Variation in elemental composition of human teeth and its application for feasible species identification

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ARTICLE INFO

Article history:
Received 5 September 2016
Received in revised form 4 November 2016
Accepted 11 December 2016
Available online 21 December 2016

Keywords:
Animal
Classification
Element
Forensic
Human
Teeth

ABSTRACT

Identifying human remains is a primary task in forensic science. In this study, we propose a possible new technique, handheld X-ray fluorescence (HHXRF), for determining whether a suspected tooth is an authentic human tooth. A total of 444 teeth obtained from 111 human skulls (male = 62, female = 49) aged between 30–67 years (51.81 ± 8.37 years) were used as subjects. The teeth were scanned by HHXRF to acquire their elemental profile. Differences in elemental composition were analyzed for different tooth positions (numbers 1–32), between crown and root, and between sexes (male and female); also, the proportion of elements in relation to different human ages was examined. Teeth from 20 different animal species, serving as non-human teeth samples, were used to distinguish between human and non-human teeth through a stepwise discriminant analysis. Our results revealed that different tooth positions, different regions (crown and root) of a tooth, and different sexes demonstrated disparities in the proportion of several elements. The accuracy rate of predicting sex based on the elemental profile of human teeth was 65.5%. Likewise, a dissimilar distribution of elements between human and non-human teeth was observed, leading to a high degree of correctness of 83.2% for distinguishing them. In conclusion, elemental analysis by HHXRF could serve as a promising candidate tool for identifying human teeth in forensic science, but is ineffective for sex determination.

1. Introduction

Studies of the elemental analysis of a variety of body tissues (i.e. internal organs, body fluid, hair, bone or teeth) have been widely applied for many purposes, such as biology [1,2], physiology [3,4] or contamination by environmental pollutants [5,6]. Recently, the use of an elemental profile, or 'fingerprint', has been of increasing interest in forensic science, and there have been an increasing number of investigations: for example, distinguishing between osseous and non-osseous materials using the Ca/P ratio and the amount of other elements [7–9], estimating the origin of elephant tusks, determining the sex of human bones, etc. However, more academic data is needed to support the concept of the use of elemental analysis in this field.

Indeed, many techniques have been applied for studying the elemental composition of materials, such as inductively coupled plasma mass spectrometry (ICP-MS) [10], atomic absorption spectroscopy (ASS) [11] and X-ray fluorescence (XRF) [9]. Nonetheless, these techniques have different advantages and limitations. In this study, handheld XRF (HHXRF) was employed as a tool for the acquisition of elemental data, because this technique does not necessitate destruction of the subject materials, which were the property of museums which did not allow their destruction. In addition, several previous studies have disclosed that HHXRF can be applied on a range of biological tissues, such as horns/antlers [12,13], bones [12,14], teeth [12,15] and tusks [16].

Our research team previously demonstrated that measuring the elemental composition in bone and teeth can be a promising method for species identification, such as determining whether a tusk's origin was from an Asian elephant or African elephant, with a 94% accuracy rate [16], and for differentiation of various species

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http://dx.doi.org/10.1016/j.forsciint.2016.12.017
0379-0738/

among humans, elephants, dogs and dolphins, with high accuracy rate [17]. Moreover, elemental content in teeth has been used to distinguish between humans and other species (deer, dog, Asian elephant, horse, monkey, dolphin and crocodile) with an 80% accuracy rate [12]. The lower accuracy rate in the latter study might have been caused by the limited number of samples from each species, particularly humans (five samples). However, these studies have provided evidence for validation of the concept that elements in teeth (and bone as well) can be applied as a tool for species identification.

Here, human (*Homo sapiens*) teeth were used as subjects for detecting their elemental profile by HHXRF. The elemental distribution of human teeth was examined for differences between teeth (tooth numbers 1st–32nd), parts of the tooth (crown and root), sex (male and female), and age variation. In order to distinguish between human teeth and teeth from other species, an assortment of common animals (such as dog, pig, horse and cattle, and other wild animals such as deer, tiger, lion, hyena, camel and kangaroo) was used for establishing a discrimination equation.

2. Materials and methods

2.1. Samples

Human teeth samples (in dry bones) were obtained from cadavers donated to the Department of Anatomy, Faculty of Medicine, Chiang Mai University, Thailand. Human teeth used in this study were taken from a total of 111 human skulls (male = 62, female = 49) aged 30–67 years (51.81 ± 8.37). Animal teeth samples were obtained from different institutions, including: (1) Animal Anatomy Museum, Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand; (2) Phuket Marine Biological Center, Phuket, Thailand; (3) Tiger Kingdom–Chiang Mai, Mae Rim district, Chiang Mai, Thailand; and (4) Mae Sa Elephant Camp, Mae Rim district, Chiang Mai, Thailand.

A total of 173 animals were used in this study, including African elephant (*Loxodonta africana*, $n = 20$), Asian elephant (*Elephas maximus*, $n = 20$), water buffalo (*Bubalus bubalis*, $n = 1$), Arabian camel (*Camelus dromedarius*, $n = 1$), cattle (*Bos taurus*, $n = 4$), deer (*Cervidae* spp., $n = 17$), dog (*Canis lupus familiaris*, $n = 25$), spinner dolphin (*Stenella longirostris*, $n = 10$), dugong (*Dugong dugon*, $n = 43$), horse (*Equus ferus caballus*, $n = 8$), striped hyena (*Hyaena hyaena*, $n = 1$), red kangaroo (*Macropus rufus*, $n = 1$), lion (*Panthera leo*, $n = 1$), monkey (Assam macaque; *Macaca assamensis*, $n = 3$), pig (*Sus scrofa domesticus*, $n = 8$), sheep (*Ovis aries*, $n = 1$), Asian tapir (*Tapirus indicus*, $n = 1$), Bengal tiger (*Panthera tigris tigris*, $n = 6$), waterbuck (*Kobus ellipsiprymnus*, $n = 1$) and sperm whale (*Physeter macrocephalus*, $n = 1$).

To use human teeth samples, consent was waived by the Human Ethics Committee, Faculty of Medicine, Chiang Mai University, Thailand; the samples were also anonymized in our study. The use of animal bones did not require approval by the Animal Ethics Committee, Faculty of Veterinary Medicine, Chiang Mai University. All teeth samples were dry, and maintained at room temperature. They were immediately cleaned upon death, but were not otherwise manipulated (burned or buried). None of the samples exhibited pathological lesions or disease conditions.

2.2. X-ray fluorescence

Teeth elemental analyses were conducted using a handheld X-ray fluorescence (HHXRF) analyzer (DELTA Premium; Olympus, Tokyo, Japan) with a silicon drift detector, which was able to detect magnesium (Mg^{12}) through bismuth (Bi^{83}) on the periodic table. The collimator size was set at 0.3 mm for the analysis area (diameter), and used the standard mining plus mode. Calibrations were performed before the first use for sample analysis each day. Light elements (LE) were those with an atomic number lower than Mg (H^1–Na^{11}), which could not be differentiated as separate elements. For each scan (2 min each), the XRF unit was secured in a stand and the sample was placed directly adjacent to the puncture-resistant window of the machine to limit the distance between the detector and the sample. Each element was expressed as a percentage. The XRF method was noninvasive, and samples were not manipulated or destroyed during the scanning process.

2.3. Study design

Measurements of the difference in distribution of elements under certain conditions were performed. First, we looked at the difference between tooth type, from tooth number 1–32 (study 1), and then we investigated the difference between crown and root (study 2). We also investigated the accumulation of elements according to sex (study 3) and the correlation of element proportion and age (study 4). Finally, we used the elements in teeth for species identification (study 5). Moreover, the ratio between Ca and P was calculated in all studies because it is used as a representative of calcium hydroxyapatite in teeth and bone [2,7].

2.3.1. Study 1: elemental analysis of 32 teeth

One hypothesis of this study was that there is a difference in elemental accumulation across 32 types of teeth, according to position. A total of 20 skulls (male = 10, female = 10), aged between 36–51 years (45.3 ± 4.7), were used. Permanent teeth were assigned numbers using the 'universal numbering system', in which the number 1 represents the upper right third molar; the numbering system then follows around the upper arch to the upper left third molar (16), descending to the lower left third molar (17) and following around the lower arch to the lower right third molar (32). Incomplete teeth (fractured or cracked) or teeth with implants (Fig. 1) were excluded. The enamel at the buccal surface of each tooth was scanned, using HHXRF, with the number of scans of each tooth depending on tooth size. Elements were presented as percentages and were compared between 32 teeth using one-way ANOVA followed by Mann–Whitney *U* test.

2.3.2. Study 2: elemental analysis of crown and root of teeth

Another hypothesis of this study was that there is a dissimilarity in elemental composition between the crown and root of teeth. This study did not categorize enamel and dentin parts of teeth, as has been done in other reports, because a different measurement technique was employed. Performing XRF on the crown will measure both enamel and dentin, because the enamel thickness averages 0.675–1.450 mm [18], while radio beams from HHXRF penetrate up to 3 mm in depth. A total of 30 human teeth (randomized for sex and age) taken from different skulls were subjected to HHXRF. Two parts of each tooth were scanned: the crown and root. Elements were presented as percentages, and comparisons between the two groups were performed using Mann–Whitney *U* test for nonparametric data analysis.

2.3.3. Study 3: elemental analysis between sexes in humans

A further hypothesis of this study was that there is a disparity in elemental composition between male and female teeth. A total of 46 male teeth, age range 30–67 years (52.2 ± 8.8), and 62 female teeth, age range 35–65 years (51.1 ± 8.3), were scanned. Four teeth from each skull were used as subjects, and each tooth was scanned once due to size limitations. Elemental composition was presented as percentages, and comparisons between the two groups were performed using Mann–Whitney *U* test for nonparametric data analysis. The data of elemental composition for male and female