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Determining comparative elemental profile using handheld X-ray fluorescence in humans, elephants, dogs, and dolphins: Preliminary study for species identification

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ABSTRACT

Species identification is a crucial step in forensic anthropological studies. The aim of this study was to determine elemental profiles in bones from four mammal species, to be used for species discrimination. Human, elephant, dog, and dolphin bones were scanned by X-ray fluorescence (XRF); the differences in elemental profiles between species were determined using discriminant analysis. Dogs had the greatest number of elements (23), followed by humans (22) and elephants (20). Dolphins had the lowest number of elements (16). The accuracy rate of species identification in humans, elephants, dogs, and dolphins was 98.7%, 100%, 94.9%, and 92.3%, respectively. We conclude that element profiles of bones based on XRF analyses can serve as a tool for determining species.

1. Introduction

Knowledge of the elemental composition of bones is essential for better insight into bone metabolism and for various applications, particularly in forensic science [1–3]. Our research group hypothesized that elements in connective tissue (such as bones and teeth) can be used as a tool for species identification or sex identification. We recently conducted an elemental analysis of Asian elephant (*Elephas maximus*) teeth, both deciduous (first molar, second molar, and tusk) and permanent (molar and tusk). Further, we compared the elemental composition of permanent teeth among 15 species: Asian elephant, dog (*Canis lupus familiaris*), hyena (*Hyaena hyaena*), pig (*Sus scrofa domesticus*), white-tailed deer (*Odocoileus virginianus*), goat (*Capra aegagrus hircus*), sheep (*Ovis aries*), cow (*Bos taurus*), horse (*Equus ferus caballus*), cat (*Felis catus*), tiger (*Panthera tigris*), Malayan tapir (*Tapirus indicus*), monkey (*Macaca assamensis*), spinner dolphin (*Stenella longirostris*), and crocodile (*Crocodylus siamensis*) [4]. Discriminant analysis of the results demonstrated that the combination of Ca/P + Ca/Zn + Ca/Pb + Ca/Fe + Ca/Sr + Zn/Fe could generate two equations that successfully classified six species (dog, pig, goat, tapir, monkey, and elephant) out of a total of 15 at 100% specificity. In another report, our team presented the elemental differences between male and female human bones [2]. Based on the results of such studies on the cranium, three elements (S, Ca, and Pb) were at significantly higher levels in males, and five elements (Si, Mn, Fe, Zn, and Ag) plus light elements (atomic number <12) were at higher levels in females. In the humerus and os coxae, nine elements were at significantly elevated levels in males, whereas one element was at a higher level in females. The accuracy rate for sex estimation was 60%, 63%, and 61% for the cranium, humerus, and os coxae, respectively, and 67% when data for all three bones were combined. Our group is not the first to explore the use of elemental analysis in forensic science, as many

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researchers have also attempted the same. For example, in a previous study, Christensen et al. applied elemental analysis and found it to be a valid and effective means of determining the osseous or dental origin of unknown material [3].

X-ray fluorescence (XRF) is used for routine, relatively nondestructive chemical analyses of rocks, minerals, sediments, and fluids. It can provide important information on the elemental composition of various biological sample types such as bones [1–3,5], teeth [3,4], and antlers [6]. However, most reports on the application of XRF in human forensic science have focused on metal contamination in human remains [7–9]. Some studies have shown that this technique can distinguish bone and dental tissue from non-bone or non-apatite material [3,10–12]. To broaden the application of XRF in forensic anthropology, especially for species identification, we studied the use of elemental analysis for differentiating four mammalian species, including long-living (humans and elephant), short-living (dog), and marine (dolphin) species. Moreover, we also applied the XRF technique for species identification using elemental levels in bones.

2. Materials and methods

2.1. Samples

Adult animal bones (Asian elephants, dogs, and dolphins) were obtained from the Animal Anatomy Museum, Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand. Adult human bone and teeth samples were obtained from the Excellence Center in Osteology Research and Training Center, Chiang Mai University. All samples were dry bones maintained at room temperature. None of the samples exhibited pathological lesions or disease conditions. The use of human bones was approved by the Human Ethics Committee, Faculty of Medicine, Chiang Mai University, Thailand, in 2015. We did not require ethical approval for using animal bones from the Animal Anatomy Museum, according to the guidelines of the Animal Ethics Committee, Faculty of Veterinary Medicine, Chiang Mai University.

Bones from six female human (*Homo sapiens*) skeletons were scanned, including the humerus, radius, ulna, rib, femur, tibia, fibula, and metatarsus. We also scanned bones from two female Asian elephants (*E. maximus*), including the humerus, radius, ulna, rib, femur, tibia, and fibula; 10 female dogs (*C. lupus familiaris*), including the cranium, mandible, cervical vertebrae, scapula, humerus, radius, ulna, metacarpus, femur, tibia, and fibula; and two dolphins (spinner, *S. longirostris*, of unknown sex), including the cranium, mandible, rib, cervical vertebrae, coccygeal vertebrae, scapula, humerus, radius, ulna, and metacarpus.

2.2. XRF measurement

Bone elemental analyses were conducted using a Handheld XRF Analyzer (DELTA Premium; Olympus, Waltham, MA, USA), which includes a silicon drift detector that can identify elements ranging from magnesium (12 Mg) through bismuth (83 Bi) on the periodic table. The collimator size was set to 0.3 mm as the diameter of the scanned area, and operating voltages of 10 and 40 kV were used as the source of incident radiation. Six different locations on each bone were scanned. To verify results, before scanning, the machine was calibrated with a standard reference material, and software was used to monitor quality control [13–15]. In our experiment, we used a measurement time of 2 min. Calibrations were performed before the first and after the last use of the portable XRF for sample analysis each day.

2.3. Statistical analysis

XRF measurements followed by Kruskal–Wallis and Mann–Whitney tests were used to determine differences in the composition of 25 elements, including the Ca to P ratio, in bones obtained from four different species: humans, elephant, dog, and dolphin. A p-value <0.01 was considered to be a significant difference. An element that showed significant difference in three species was used as a denominator for creating a ratio data set. Then, the acquired ratio data set was used to evaluate the feasibility of using the elemental composition of animal bones for species classification, by stepwise discriminant analysis. The accuracy rates of species classification were tested by a "leave-one-out" method to evaluate whether the elemental profile in bones could be used to identify animal species [16–18]. In cross-validation, each case was classified by the functions derived from all cases other than that case. The accuracy rate was calculated as the number of correctly predicted samples/total samples $\times$ 100; the error rate was calculated as the number of incorrectly predicted samples/total samples $\times$ 100.

3. Results

3.1. Distribution of elements across different bone species

Light elements could be found in all four animals (Table 1); other elements differed among species. The highest number of elements (23) was found in dogs (Mg, Al, Si, P, S, Cl, K, Ca, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, Zr, Mo, Ag, Cd, Sn, Sb, and Pb), followed by humans (22 elements: Al, Si, P, S, K, Ca, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, Zr, Mo, Ag, Cd, Sn, Sb, W, and Pb) and elephants (20 elements: Al, Si, P, S, K, Ca, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, Zr, Ag, Cd, Sn, Sb, and Pb). Dolphins had the lowest number of elements (16; Si, P, S, K, Ca, Ti, V, Cr, Mn, Fe, Zn, Zr, Ag, Cd, Sn, and Sb).

Ca and P were found in the highest proportions. We also concluded that Mg and Cl were likely to be specific to dog bones, whereas W was present only in human bones. In dolphins, the presence of Ni, Cu, and Pb could not be determined. When comparing the different proportions of elements in bones across species, two elements, Cu and Mo, showed no significant difference ($p > 0.05$), whereas 20 out of 25 displayed a significant difference of elemental proportions. Six elements (Si, P, Fe, Zr, Ag, and Cd) were found to be of varying proportions in three species. Moreover, as shown by the ratios of Ca/P presented in Fig. 1, humans (2.36 ± 0.34) had a significantly lower ratio than elephants (2.74 ± 0.38), dogs (2.695 ± 0.613), and dolphins (2.675 ± 0.438) did.

3.2. Species classification by discriminant analysis

The ratio data set was analyzed by stepwise discriminant analysis for species classification. Thus, the number of ratios used to create functions for classification was 26 (Table 2). Fig. 2 displays the classification of each species based on these elemental ratios. The classification result of cross-validation showed that elephants could be correctly predicted in 100% of cases, followed by humans, dogs, and dolphins with 98.7%, 94.9%, and 92.3% accuracy rates, respectively (Table 3). In the prediction of dog species, any species can be indicated, exhibiting a high variation for classification.

4. Discussion

The use of elemental analysis in forensic science has been increasingly applied in practice. This technique has been proven to distinguish bone or dental tissue from non-apatite material.