Forensic Science International 232 (2013) 237.e1–237.e5

Contents lists available at ScienceDirect

Forensic Science International

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

Forensic Anthropology Population Data

A trial of the utilization of stable isotope analysis for the estimation of the geographic origins of unidentified cadavers

Stuart J. McLean [b], Hiroshi Ikegaya [a,b,*], Pekka J. Saukko [c], Huang Yung Zheng [d], Tomoko Akutsu [a], Daisuke Miyamori [b], Noboru Ishikawa [b], Koichi Sakurada [a]

[a] First Department of Forensic Science, Third Biology Section, National Research Institute of Police Science, 277-0882 Japan
[b] Department of Forensic Medicine, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, 602-8566 Japan
[c] Department of Forensic Medicine, University of Turku, 20520 Finland
[d] Department of Urology, Fujian Medical University Union Hospital, 350001 China

ARTICLE INFO

Article history:
Received 5 May 2011
Received in revised form 14 March 2013
Accepted 22 July 2013
Available online 2 August 2013

Keywords:
Stable isotope analysis
Geographic origin
Identification

ABSTRACT

The number of unidentified cadavers is increasing worldwide and the effective methods which reveal their geographic origin are not well known. In this study, we analyzed the urine stable isotope ratio of hydrogen and oxygen collected from three locations: Chiba (Japan), Fuzhou (China), and Denpasar (Indonesia) from healthy volunteers. In addition, analysis of the effect of drinking bottled water on stable isotope ratios found in urine, and the comparison of the stable isotope ratios of urine and saliva, were conducted. Statistically significant differences in δ^2H and $\delta^{18}O$ values from the three locations were found. In this pilot study, urine $\delta^{18}O$ values became increasingly similar to those of bottled drinking water during an eight-day period of drinking only bottled water. In a separate pilot study significant differences in $\delta^{18}O$, $\delta^{13}C$, and $\delta^{15}N$ values from urine and saliva were found, but not in δ^2H values. In all three studies, although the number of samples was limited, the results suggest that with further research, stable isotope analysis from urine samples might be used to identify the origins of unidentified corpses, assist in determining the length of time an individual has been in a given area and distinguish between body fluids.

1. Introduction

Recently, due in part to increasing globalization, the number of unidentified cadavers is increasing in Europe and Japan [1,2]. Especially, in the case of large natural disasters and acts of terrorism, the determination of a cadaver's geographic origin is extremely important to facilitate later DNA identification. However, forensic science requires effective ways in addition to ordinal anthropological methods [3] to determine a human's geographic origin. In 2002, it was shown that genotypes of the JC virus within urine or renal tissue closely relate to a human's geographic origin [4]. In addition, JC virus genotyping has been effective in cases where soft tissue remained on unidentified cadavers [5,6]. However, the JC virus is not always detectable. Therefore, detecting other virus genotypes was also used to speculate on the geographic origin of other cadavers and to speculate much more precisely on the geographical origin of cadavers by analyzing the distribution of various viruses [7–9]. However, alternative techniques of establishing the geographical origin of cadavers are also necessary.

While belonging to archaeometry, geochemistry and hydrology; stable isotope analysis is an increasingly important technique in determining the origin of unidentified corpses. Hydrogen (H), sulphur (S), carbon (C), oxygen (O) and nitrogen (N) are present in nature in variable ratios. From the soil, water and air, through the food chain, and into plants and animals, local isotopic signatures are present in flora and fauna. As a result, there is a large body of literature documenting the use of stable isotope analysis through the analysis of biogenetic tissue in determining the authenticity and origin of beverages, food and food ingredients [10–20]. For instance, in Japan the stable isotope of strontium is used to establish where rice was produced [21], distinguishing among imports from Australia, China, or California.

Isotope ratios of light elements in human tissue and body fluids bear information about the preferred food and nutrition of organisms ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$), and the geographic and climatic conditions (δ^2H, $\delta^{18}O$) of the habitat as a result of the food chain passing along isotopic signatures of the environment [22–25].

Recently, within the field of forensic science, stable isotope analysis has been used to determine the origins of drugs [26], banknotes [27], gunshot [28], humans [29–31] and a cadaver [32].

* Corresponding author at: Department of Forensic Medicine, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, 456 Kajiicho, Kamigyo, Kyoto 602-8566 Japan. Tel.: +81 75 251 5343; fax: +81 75 251 5343.
E-mail addresses: ikegaya-tky@umin.ac.jp, ikegaya@koto.kpu-m.ac.jp (H. Ikegaya).

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http://dx.doi.org/10.1016/j.forsciint.2013.07.019

In 2007 the utilization of stable isotope analysis in assisting authorities in the identification of an unknown male body found on an expressway in Germany, following different criminalistic and forensic methods (e.g. tooth status, fingerprint or DNA analysis) failing to identify the person in question [32], showed the potential value of stable isotope analysis to forensic science.

This study aims to be the first study to consider whether stable isotope analysis from urine samples, because of the ease with which samples can be collected, may be utilized to estimate the origin of unidentified cadavers. In addition, this study aims to be the first study to demonstrate the use of O stable isotopes in determining the origins of humans. A pilot study was conducted with samples collected from Chiba (Japan), Fuzhou (China), and Denpasar (Indonesia). The stable isotope ratios of hydrogen (H) and oxygen (O) of the three locations were analyzed and compared. In addition, the impact of drinking nothing but foreign mineral water on a human's stable isotope ratios from urine samples was investigated. Then finally, the stable isotope ratios of urine and saliva were compared to evaluate the feasibility of body fluid determination through stable isotope analysis.

2. Materials and methods

2.1. Urine and saliva samples

Two milliliters of urine were collected from six individuals living in each of the three study locations: Chiba (Japan), Fuzhou (China), and Denpasar (Indonesia). Two milliliters of saliva were also collected from four of the urine donors living in Chiba. All samples were sealed and stored at −80 °C until analysis. All samples were collected under the informed consent of the ethical committee of the National Institute of Police Science, Japan.

2.2. Urine stable isotope analysis of samples taken from a subject drinking foreign mineral water

A male living in Chiba drank two liters of European mineral water for eight days, during which time urine samples were collected. During this period, he did not drink any other water or other liquids. Two milliliters of urine were collected on the 1st, 3rd, 5th and 8th days. All samples were immediately sealed and stored at −80 °C until analysis.

3. Stable isotope analysis

3.1. Sample preparation

Prior to analysis samples were centrifuged at 1500 × G to settle out any sediment to ensure that only the liquid component of the samples was analyzed.

3.2. Deuterium analysis

Deuterium analysis of the samples was performed in duplicate using the equilibration technique. In brief, sample aliquots were pipetted into Exetainer tubes and an insert vial containing 5% platinum on alumina added. The tubes were sealed and then filled with pure hydrogen. Samples were left to allow complete equilibration of the water with the hydrogen gas. Analysis was undertaken using continuous-flow isotope ratio mass spectrometry using a Europa Scientific ANCA-GSL and GEO 20–20 IRMS.

3.3. A brief outline of the calibration method

The samples were measured against three reference standards, the first standard being IA-R054 with $\delta^2H_{V\text{-}SMOW}$ = + 4.93‰, the second being IA-R052 with $\delta^2H_{V\text{-}SMOW}$ = −157.12‰ and the third being IA-R053 with δ^2H_{VSMOW} = −61.97‰. All three standards are traceable to the primary reference standards V-SMOW2 (Vienna-Standard Mean Ocean Water) and V-SLAP2 (Vienna-Standard Light Antarctic Precipitation) distributed by the International Atomic Energy Agency (IAEA).

The IA-R054 standard was used as the reference to which the samples and other standards were measured. The IA-R052 standard was used for calibration of the δ^2H values and the IA-R053 standard was used as a check of this calibration.

4. Oxygen-18 analysis

After deuterium analysis, the vials were flushed with pure CO_2 and left to allow complete equilibration of the water with the CO_2 gas. Reference waters (including a quality control standard) were prepared in the same manner. The samples and references were then analyzed using continuous-flow isotope ratio mass spectrometry using a Europa Scientific ANCA-G and 20–20 IRMS.

4.1. A brief outline of the calibration method

The samples were measured against three reference standards, the first standard being IA-R054 with $\delta^{18}O_{V\text{-}SMOW}$ = + 0.56‰, the second being IA-R052 with $\delta^{18}O_{V\text{-}SMOW}$ = −19.64‰ and the third being IA-R053 with $\delta^{18}O_{V\text{-}SMOW}$ = −10.18‰. All three standards are traceable to the primary reference standards V-SMOW2 (Vienna-Standard Mean Ocean Water) and V-SLAP2 (Vienna-Standard Light Antarctic Precipitation) distributed by the IAEA.

The IA-R054 standard was used as the reference to which the samples and other standards were measured. The IA-R052 standard was used for calibration of the $\delta^{18}O$ values and the IA-R053 standard was used as a check of this calibration.

4.2. Nitrogen-15 and carbon-13 analysis

The technique used for this analysis was EA-IRMS (elemental analyzer isotope ratio mass spectrometry). In this technique, samples and reference materials are pipetted and dried into tin capsules, sealed, and then loaded into an automatic sampler on a Europa Scientific Roboprep-CN sample preparation module. From there they were dropped in a furnace held at 1000 °C and combusted in the presence of oxygen. The tin capsules flash combust, raising the combustion temperature of the samples to 1700 °C. The combusted gases were swept in a helium stream over a combustion catalyst (Cr_2O_3), copper oxide wires (to oxidize hydrocarbons), and silver wool to remove sulphur and halides. The resultant gases (N_2, NO_x, H_2O, O_2, and CO_2) are sucked through a reduction stage of pure copper wires held at 600 °C. This removes any oxygen and converts NO_x species to N_2. A magnesium perchlorate chemical trap removes any water. Nitrogen and carbon dioxide are separated using a packed column of gas chromatograph held at an isothermal temperature of 65 °C. The resultant chromatographic peaks enter the ion source of the Europa Scientific 20–20 IRMS, where they are ionized and accelerated. Gas species of different mass are separated in a magnetic field and then simultaneously measured on a Faraday cup universal collector array. For N_2, masses 28, 29, and 30 were monitored and for CO_2, masses 44, 45, and 46 are monitored.

Both references and samples were converted to gases and analyzed in the same manner. The analysis proceeded in a batch process, whereby a reference was analyzed followed by a number of samples and then another reference.

The reference material used during analysis of all samples was a mixture of IA-R045 (Iso-Analytical working reference standard ammonium sulphate with a $\delta^{15}N$ value of −4.71‰ vs. Air) and IA-R005 (Iso-Analytical working reference standard beet sugar with a $\delta^{13}C$ value of −26.03‰ vs. V-PDB). IA-R045 is traceable to IAEA-N-1 (ammonium sulphate, $\delta^{15}N$ = 0.40‰ vs. Air). IA-R005 is traceable to IAEA-CH-6 (sugar, $\delta^{13}C$ = −10.43‰ vs. V-PDB). The reference mixture was prepared so that it had a similar C:N ratio to the urine samples.