Geographic Mapping through the Analysis of Stable Isotopes: Enhancing Post-Mortem Profiles

Mapeamento Geográfico pela Análise de Isótopos Estáveis: Aprimorando Perfis Post-Mortem

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Abstract. The creation of anthropological post-mortem profiles of victims is an essential step in reconstructive human identifications. Geographic origin through the analysis of stable isotopes emerged as valuable reconstructive forensic information, enhancing post-mortem profiles. To perform the analysis of dental stable isotopes, such as Carbon (δ¹³C) and Oxygen (δ¹⁸O), focusing on potential forensic applications. The sample consisted of a randomly selected lower left canine (#33); a lower left second premolar (#35); and a lower right central incisor (#41) collected from patients with unknown age and gender. The teeth were sectioned in the buccal-lingual direction, enabling to remove dentin powder with a rounded drill. The powdered sample was referred for the analysis of δ¹³C and δ¹⁸O through
mass accelerator spectrometry. The obtained data was compared to previous international surveys. The $\delta^{13}C$ values ranged from -9.90 to -11.50 per mil, with standard deviation interval between 0.02 and 0.06 per mil. The $\delta^{18}O$ values ranged from -7.17 to -8.26 per mil, with standard deviation interval between 0.06 and 0.09 per mil. In face of previous surveys, the obtained data followed a similar trend on the concentration of dental $\delta^{13}C$ and $\delta^{18}O$. The analysis of $\delta^{13}C$ and $\delta^{18}O$ was optimally performed, revealing potential application for forensic purposes.

**Keywords:** Carbon; Oxygen; Tooth; Forensic dentistry; Forensic anthropology.

**Resumo.** A elaboração de perfis antropológico post-mortem consiste em uma etapa essencial para identificações humanas reconstrutivas. Assim, a origem geográfica pela análise de isótopos estáveis emerge como uma ferramenta valiosa no contexto forense, acrescentando informações antropológicas post-mortem. Proceder a análise de isótopos estáveis, de Carbono ($\delta^{13}C$) e Oxigênio ($\delta^{18}O$), extraídos de dentes, focando em possíveis aplicações forenses. A amostra foi constituída por um canino inferior esquerdo permanente (#33); um segundo pré-molar inferior esquerdo permanente (#35); e um incisivo central inferior direito permanente (#41). Os dentes foram extraídos por motivos periodontais de pacientes com idade e sexo desconhecidos. Foi realizada secção coronorradicular no sentido bucolingual, permitindo a extração de dentina em pó utilizando brocas esféricas diamantadas. A amostra pulverizada foi encaminhada para análise bioquímica de concentração de Carbono e Oxigênio por espectrometria de aceleração de massa. Os dados obtidos foram comparados a achados previamente relatados na literatura internacional. A concentração de Carbono variou de -9.90 à -11.50 per mil (desvio padrão entre 0.02 e 0.06 per mil), enquanto a concentração de Oxigênio variou entre -7.17 à -8.26 per mil (desvio padrão entre 0.06 e 0.09 per mil). Ambas as concentrações seguiram padrões de similaridade em relação a resultados descritos na literatura. Os resultados obtidos revelam potencial aplicação forense no âmbito odontológico e antropológico, uma vez que apontam tendência à regionalização.

**Palavras-chave:** Carbono; Oxigênio; Dente; Odontologia forense; Antropologia forense.

**1. Introduction**

Lack of ante-mortem (AM) data is one of the major limitations in forensic human dental identification\(^1\). In order to overcome this limitation, anthropologic exams are carried reconstructing a post-mortem (PM) profile of the victims, including predictions of stature, age, gender, and ethnicity\(^2\). Further on, the PM profile will eventually enable potential comparison with data of missing persons.
The analysis of dental and skeletal stable isotopes, such as Carbon ($\delta^{13}C$) and Oxygen ($\delta^{18}O$), is approached as an additional tool into anthropologic exams. Specifically in the palaeoenvironmental routine, stable isotopes indicate animal dietary preferences, ecological niches, air temperature and humidity. Recently, this analysis was performed for forensic purposes, providing clues to geographic origins, consequently enhancing PM anthropologic profiles.

Despite its potential usefulness, stable isotopes are not commonly investigated in the forensic environment of developing countries. Most of the limitations for the research on stable isotopes comprehend the high-cost devices and facilities; technical knowledge; and scarce literature. Based on that, the present study aims to assess the concentration of dental $\delta^{13}C$ and $\delta^{18}O$ in a developing country, reporting our experience and supporting the literature with forensic evidence.

2. Material and Methods
The sample consisted of three randomly selected teeth (A: lower left canine [#33]; B: lower left second premolar [#35]; C: lower right central incisor [#41]) obtained from three different patients from the Southern region of Brazil. The teeth were extracted due to advanced periodontal disease under the patients’ consent. Patients’ demographic data, such as age and gender, were unknown, simulating the routine practice in forensics. The teeth were sectioned in the antero-posterior direction using a cylindrical tungsten carbide drill. Approximately, 4000µg of dentine powder were collected from each tooth using a rounded tungsten carbide drill. The dentine powder was referred to the Laboratory for Analysis of Minerals and Rocks (LAMIR), Brazil. Analysis using an accelerator mass spectrometer (Delta V Advantage, Thermo Fischer Scientific®, USA) was performed aiming the assessment of $\delta^{13}C$ and $\delta^{18}O$. The biochemical process consisted of reacting dentine powder and orthophosphoric acid, under $72^\circ C$, during 72 hours, providing necessary CO$_2$ gas for interpretation. This procedure was repeated four times in order to achieve satisfactory standard deviation values (<0,1 per mil). The obtained data was examined and compared in face of previously reported investigations. Potential forensic applications, as well as detected advantages and limitations, were approached for proper discussion.
3. Results
The accelerator mass spectrometry analysis revealed similar distribution for δ\textsubscript{13}C and δ\textsubscript{18}O values considering the three different teeth (δ\textsubscript{13}C: from -9.90 to -11.50 per mil; δ\textsubscript{18}O: from -7.17 to -8.26 per mil). Reliable standard deviation intervals were achieved (δ\textsubscript{13}C: from 0.02 to 0.06 per mil; δ\textsubscript{18}O: from 0.06 to 0.09 per mil) (Table 1).

Table 1 - δ\textsubscript{13}C and δ\textsubscript{18}O distribution for the three randomly selected teeth.

<table>
<thead>
<tr>
<th>Tooth #</th>
<th>δ\textsubscript{13}C</th>
<th>SD</th>
<th>δ\textsubscript{18}O</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>-11.50</td>
<td>0.02</td>
<td>-7.17</td>
<td>0.06</td>
</tr>
<tr>
<td>33</td>
<td>-09.90</td>
<td>0.06</td>
<td>-8.00</td>
<td>0.07</td>
</tr>
<tr>
<td>41</td>
<td>-08.20</td>
<td>0.06</td>
<td>-8.16</td>
<td>0.09</td>
</tr>
</tbody>
</table>

SD: Standard deviation; Results expressed in "per mil"

4. Discussion
The increasing globalization trend enabled people to live and travel abroad. Consequently, specific identification traits, correlating people to their countries or continents, became necessary in the forensic environment. The analysis of dental Carbon and Oxygen arose as an important forensic tool due to the constant deposition of these isotopes from the atmosphere to foods and water, respectively. Previous studies investigated the potential use of unstable isotopes from dental enamel, such as δ\textsuperscript{14}C, for the improvement of age estimation and prediction of time at death\textsuperscript{5}. On the other hand, stable isotopes, such as δ\textsubscript{13}C, revealed distinct values among countries, indicating its forensic usefulness while predicting geographic origin\textsuperscript{5}. Based on that, the reproducibility and assessment of δ\textsubscript{13}C and δ\textsubscript{18}O in Brazil is justified considering touristic potentials, and ethnical heterogeneity.

The current medical literature is scarce considering the forensic dental applications of stable isotopes. However, Alkass et al.\textsuperscript{4} (2011) optimally supported this branch of forensic anthropology randomly selecting teeth from different countries for the analysis of δ\textsubscript{13}C. In specific, the authors detected different concentrations of stable Carbon isotope for each of the studied countries. It indicates the usefulness of including this analysis as an adjuvant tool in PM profiles of reconstructive identifications. The referred tool potentially enhances the search for a missing person into a list of victims through geographic mapping.
In the present study, the analyzed teeth were extracted from a Southern Brazilian population, resulting in a pattern concentration of $\delta^{13}$C and $\delta^{18}$O compatible with previous reports of the Uruguayan population\(^4\). Oppositely, our results highly differ from a Swedish sample\(^4\). Clearly, these findings may be justified by the geographic position of which the samples were extracted. Specifically the present sample was collected approximately 1200Km close to Uruguay, and 11200Km far from Sweden (Table 2). Despite the optimal results and the apparently advantages, the branch of forensic dental applications of stable isotopes is emergent, deserving detailed attention further on. Additionally, as far as the scientific literature could be consulted up to now, no legal database was found containing comparable results. Other limitations comprehend the lack of properly adapted facilities for the analysis of stable isotopes in developing countries. Consequently, the lack of trained experts also represents a significant limitation. Secondly, the biochemical analysis of stable isotopes consists of a high-cost practice, hampering greater sampling. Once these limitations are overcome in the forensic routine of developing countries, more accurate and improved outcomes will be achieved.

Table 2 - C\(^{13}\) comparison between Sweden, Brazil and Uruguay.

<table>
<thead>
<tr>
<th>Country</th>
<th>Tooth #</th>
<th>$\delta^{13}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweden*</td>
<td>35</td>
<td>-14.63</td>
</tr>
<tr>
<td>Sweden*</td>
<td>34</td>
<td>-14.79</td>
</tr>
<tr>
<td>Sweden*</td>
<td>44</td>
<td>-14.90</td>
</tr>
<tr>
<td>Brazil</td>
<td>35</td>
<td>-11.50</td>
</tr>
<tr>
<td>Brazil</td>
<td>33</td>
<td>-09.90</td>
</tr>
<tr>
<td>Brazil</td>
<td>41</td>
<td>-08.20</td>
</tr>
<tr>
<td>Uruguay*</td>
<td>35</td>
<td>-11.24</td>
</tr>
<tr>
<td>Uruguay*</td>
<td>43</td>
<td>-10.50</td>
</tr>
<tr>
<td>Uruguay*</td>
<td>42</td>
<td>-10.36</td>
</tr>
</tbody>
</table>

Results expressed in “per mil”; *results previously reported by Alkass et al.\(^4\) (2011).

5. Conclusion
The biochemical analysis of $\delta^{13}$C and $\delta^{18}$O represented a potential application for forensic geographic mapping, enhancing the process of reconstructive human identification. However, problem-based surveys must be designed to overcome the limitations highlighted hereby. Further on, validation studies should be carried
reproducing the present investigation in different geographic regions, enabling the creation of geographic databases of stable isotopes from teeth.

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References