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TECHNICAL NOTE

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Reliability in Age Determination by Pulp/Tooth Ratio in Upper Canines in Skeletal Remains

ABSTRACT: Estimation of age of skeletal remains is one of the most complex questions for anthropologists. The most common macroscopic methods are based on dental wear and histological evaluation of bone remodeling. These methods are often qualitative, require great technical expertise, and have proved inexact in the estimation of ages over 50 years. Certain dental methods investigate the apposition of secondary dentine, in the study of tooth cross-sections, and X-rays to study width, height, and pulp area. The primary author previously proposed a method of estimating the age of a living person based on the pulp/tooth ratio (PTR) method in the upper canines. The aim of the present study is to verify whether the PTR method can also be used to estimate the age at death of skeletal remains. This paper investigates the study of historical samples of known age as a means to validate the proposed method.

KEYWORDS: forensic science, age estimation by teeth, secondary dentine, pulp/tooth area, Aragonese princes

Age estimation is a continuing challenge for anthropologists and dentists. Some difficulties exist in anthropological methods' wide range of results and inaccuracy in the study of subjects over 50 years of age, when using skeletal remains. Methods differ according to whether the skeleton is adult or nonadult. Age estimation in a nonadult is based on three criteria: dentition, the presence and fusion of the diaphyses, and the length of long bones not yet fused with the epiphysis. Age estimation of adults over 50 is more complex. Current methods are based on macroscopic wear and histological evaluation of bone remodeling. The most common macroscopic methods involve teeth (1–3), cranial suture (4,5), or pubis (6,7), auricular surface of the ilium (8), and the sternal rib (9). Although there are many dental methods, some are very complex, destructive, and are therefore not normally used in anthropology (10, 11). Dental wear is the method most widely used by anthropologists. The apposition of secondary dentine (12,13) is also often taken into account, because the pulp is surrounded not only by hard tissue such as enamel but also by dentine, which changes during an individual's life. The pulp contains a fine, fibrillar, specialized connective tissue, with odontoblasts bordering on the dentine surface. The apposition of secondary dentine by the odontoblasts occurs during the life of a healthy tooth. Various methods are used to study the pulp, including tooth cross-sections and X-rays. Radiological methods are used to study tooth width, height, and pulp area. Cameriere et al. (14) analyzed an age estimation method based on pulp/tooth ratio (PTR) method in the upper canine. This internal examination eliminates certain environmental factors such as weather, fire, water, etc., which can affect the human remains. The aim of this study is to verify the age estimation method reported in (14) and investigate its use to estimate the age of known skeletal remains.

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Materials and Methods

Samples of skeletons came from three sites. The first was a cemetery dating back to the early Middle Ages, on a hill near Poggio Imperiale, in the province of Siena, Italy. The cemetery occupied a flat area of about 40 × 25 m. The skeletons were only of adults as children were probably buried separately. Some skeletons were in a fragile condition. Skulls were fragmentary and incomplete, the sterna was often missing, and vertebrae, ribs, and limbs were fragmentary (this last phrase is confusing). The maxillae and mandibles were often incomplete and required long, careful laboratory restoration to reconstruct. The study sample from this site was composed of 33 skeletons of the best-preserved remains.

The second site was a cemetery dating from the 11th and 12th centuries A.D., near the church of Sts. Miniato and Romolo in the castle of Monte Croce, at Pontessieve, Italy. Ten skeletons having complete and connected skulls were analyzed from this site.

The anthropological methods used in both sites to assess an age interval within which a subject died were cranial suture (4,5), dental wear (1–3), sternal rib (9), auricular surface of the ilium (8), and symphysis surface (6,7). This interval, called anthropological age (AA), is used as an estimate of the actual age at death of the subject.

The third site was the Basilica of San Domenico Maggiore, which dates back to the early 14th century, and is one of the largest and most important churches in Naples. The sample size was

nine. The humanist Giovanni Pontano and the philosophers Tommaso Campanella and Giordano Bruno studied here. St. Thomas Aquinas taught in the annexed monastery of the Dominicans. The impressive Sacristy of San Domenico Maggiore, built into a gateway close to the vault, contains 38 wooden sarcophagi (Fig. 1) containing the bodies of 10 Aragonese princes and Neapolitan nobles, who died in the 15th and 16th centuries (15). In particular, they include the Aragonese kings Alfonso I (who died in 1458), Ferrante I (1494), and Ferrante II (1496), Queen Giovanna IV (1518) and Isabella of Aragona, Duchess of Milan (1525), who employed Leonard da Vinci at her court (16).

Most bodies were embalmed, which is not surprising, considering the high social class to which the individuals buried in San Domenico belonged. Some well-preserved bodies showed no apparent signs of embalming and their natural mummification can probably be attributed to the very dry microclimatic conditions of the Basilica. The age at death is known for these historically significant personalities. This enabled us to test the PTR method in the ancient samples without using the anthropological methods.

In all three sites, the ages of skeletons were estimated by evaluation of secondary dentine of the upper canines (12). Radiographs were digitized by a scanner, and images were then recorded in a computer file. Radiograph images of canines (RIC) were processed using computer-aided drafting (CAD) (AutoCAD2000, Install Shield 3.0, 1997). Twenty points from each tooth edge and 10 points for pulp edge were identified, and were used to evaluate tooth and pulp areas. Age estimation applied the equation:

$$\text{Age} = 84.31 - 473.86\,\text{RA} \qquad (1)$$

where RA is the pulp/tooth area. This method, previously used successfully with modern subjects (12), was tested in this study for the first time on a group of historical subjects.

Statistical Analysis

As regards the remains in the first two sites, Poggio Imperiale and Pontessieve (Italy), the age at death was estimated by both RA and anthropological methods (AA methods). The two methods were defined as being in agreement if the estimate of the age at death obtained using Eq. (1) belonged to the interval of the ages at death estimated on the same skeleton using anthropological methods. Also computed were the standard deviations (SDs) of the estimates of the ages at death obtained with the PTR method and the interval defined by $IC_{68} = (Age_{estim} - SD,\ Age_{estim} + SD)$ where Age_{estim} and SD are, respectively, the estimate of the age of skeletal remains including its SD.

The age at death and its SD for the remains in the third site were estimated for each skeleton using the PTR method. Also evaluated was the error of the estimate of the age at death of each subject as the difference between its actual and estimated ages by means of Eq. (1). Lastly, the extent of the mean error was estimated by using its 95% confidence interval (IC_{95}).

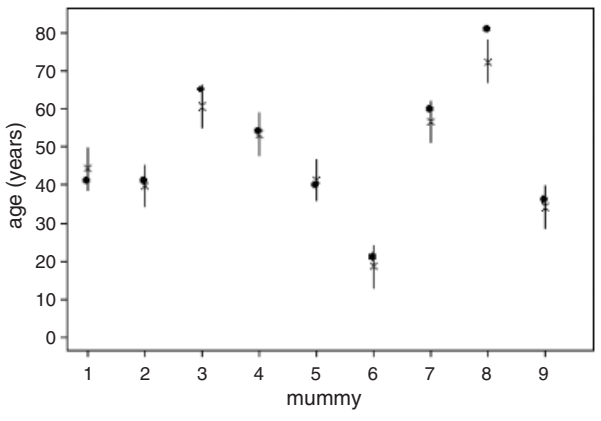

FIG. 1—*Plots of ages at death of the nine mummies in the Basilica of San Domenico Maggiore (black dots) overlaid with an indicator of ages estimated with the pulp/tooth ratio method. Each estimate (cross) is reported with ± 1 SD (vertical segments).*

Results

The AA or AA and age estimated with the PTR method were concordant in 84% of the skeletal remains from the two cemeteries (Table 1).

The IC_{68} intervals intersected the corresponding intervals of the AA in nearly all the cases (95%).

As regards the remains in the third site, Table 2 shows the actual ages of the mummies in the Basilica of San Domenico

TABLE 1—*Age at death of the skeletons in two cemeteries estimated using AA and PTR methods.*

AA	RA	SD_{RA}
18–22	21.29	5.52
18–24	21.95	5.52
18–24	22.01	5.52
22–25	26.4	5.49
25–30	27.45	5.49
28–40	28.39	5.48
20–35	28.43	5.48
25–33	31.24	5.47
30–40	31.43	5.47
28–30	32.19	5.47
35–40	35.5	5.46
30–36	35.92	5.46
34–42	37.86	5.46
30–32	38.82	5.46
35–45	41.05	5.46
40–45	41.66	5.46
30–40	42.64	5.46
35–45	43.56	5.46
36–45	44.51	5.46
30–50	46.4	5.47
40–50	49.5	5.48
>50	50.67	5.48
38–50	49.7	5.48
>50	60.66	5.54
25–30	22.71	5.51
25–40	38.82	5.46
31–50	40.24	5.46
25–35	25.08	5.50
25–40	35.98	5.46
33–50	37.87	5.46
24–35	30.29	5.48
24–30	27.92	5.48
25–40	34.55	5.46
25–40	40.71	5.46
22–26	24.13	5.50
20–24	22.15	5.51
24–35	29.82	5.48
33–50	37.87	5.46
30–40	52.56	5.49
22–27	26.03	5.49
18–22	21.76	5.52
19–25	19.45	5.53
25–30	27.35	5.49

AA, anthropological age; PTR, pulp/tooth ratio; RA, pulp/tooth area, SD, standard deviation.

TABLE 2—*Actual and estimated ages with their standard deviations of the mummies in the Basilica of San Domenico Maggiore.*

Age	RA	SD_{RA}	Error
41	44.23	5.46	− 3.23
41	39.83	5.46	1.17
65	60.57	5.53	4.43
54	53.36	5.49	0.64
40	41.3	5.46	− 1.3
21	18.68	5.54	2.32
60	56.63	5.51	3.37
81	72.4	5.64	8.6
36	34.21	5.47	1.79
43	48.34	5.47	− 5.34

RA, pulp/tooth area; SD, standard deviation.

Maggiore, the age estimates (SD) yielded by the apposition of secondary dentine (PTR method), and the error of estimates.

The first mummy to be examined was that of Francesco Ferdinando of Avalos, Marquis of Vasto and Pescara (1530–1571). The body lay in a large sarcophagus in the wall of the left apse, facing the entrance. Age as estimated by apposition of secondary dentine was 44.23 years (with an error of − 3.23 years).

Antonio of Aragon lay in the first large sarcophagus in the wall of the left apse, in an orthogonal position, near the sarcophagus of King Alfonso I of Aragon. The mummy was in good condition, with little postmortem damage. The age at death was 41 years and the age as evaluated was 39.83.

Isabella of Aragon was Duchess of Milan, the wife of Duke Gian Galeazzo Sforza. She escaped from imprisonment in Milan and took refuge in Bari, where she died in 1524. Her sarcophagus is in the right wall, near that of Queen Giovanna IV of Aragon and her age was estimated at 53.36. Her recorded age at death was 54.

The fourth mummy was that of Giovanna IV of Aragon. Her sarcophagus was near that of Isabella of Aragon in the upper apse. Estimated age was 41.30 years, and recorded age at death was 40 years.

The fifth large sarcophagus of in the upper row of the left apse was that of Luigi Carafa, Prince of Stigliano. The natural mummy was in average condition. Estimated age was 56.63, and recorded age at death was 60 years.

Ippolito Guevara, the sixth mummy, died in 1593, aged 21. Using apposition of secondary dentine, his age was estimated to be 18.68 years.

The seventh mummy was that of Maria of Aragon, Marquise of Vasto, lying in a large sarcophagus in the right apse, in front of that of Isabella of Aragon. It was an artificial mummy in good condition, aged 60.57 years according to apposition of secondary dentine. The Marquise died in 1568, aged 65.

The eighth mummy was that of Giacomo Francesco of Aragon, who was born in 1699 and died in 1780, aged 81 years. The body lay in a large sarcophagus in the wall of the left apse. In this case, age was estimated to be 72.40 years. It is important to highlight here the fact that previous studies (14) have shown that measurement of apposition of secondary dentine provides valid results up to 72 years of age. Therefore, the age of this mummy was considered to be an outlier.

The ninth mummy was that of Ferdinando Francesco of Avalos (1489–1525), Marquis of Pescara. Under the sarcophagus is a frame with the following inscription: "FRANCISCUS FERDINANDUS D'AVALOS D'AQUINO MARCHIO PISCARIAE CESAREAE MAIESTATIS VICARIUS GENERALIS ITALIAE—OBIIT ANNO DOMINI 1525". His estimated age was 34.21 years (with an error of 1.79 years).

The case of Flavio Orsini is different, because his year of birth is not known for a certainty but it was probably in 1538. The year of his death was certainly 1581, so that he was 43 when he died. The age as estimated by the PTR method was 48.34 years.

Figure 1 summarizes the actual and estimated ages of the mummies in the Basilica of San Domenico Maggiore.

The mean error of the estimates of the age at death, leaving out Giacomo Francesco of Argon (eighth mummy), was 0.26 years with a 95% confidence interval $IC_{95} = (-2.45, 2.97)$. This indicates that the absolute error of the estimates of the age at death of the mummies in this site was even lower than the estimated SD obtained with the PTR method (about 5 years).

Discussion

The methods for age estimation most often used by anthropologists are macroscopic. They are often qualitative, require great technical expertise, and have proved inexact in the estimation of ages over 50 years. Age estimation by apposition of secondary dentine has often been used (12,13). This quantitative method, more controllable scientifically, is less dependent on technical ability. The PTR of the upper canine is a method applied in modern studies, but until now it has never been tested in historical samples of known age.

This study shows that the PTR method, as reported in (14), is not only a useful technique to assess the chronological age of living persons, but it is also a reliable tool in the determination of age at death in skeletal remains. In particular, as shown by the estimates of the ages of the mummies in the third site, when a mummy is well preserved, the absolute error of the estimate of its age at death may be even more faithful than the estimate of the age of a living person.

Specifically, age estimation was very precise in eight mummies aged less than 72 years. The method can be used to provide age estimation of old subjects, who died over 50, with great reliability. Therefore, for simplicity and reliability, this method can also be proposed for age estimation of historical subjects.

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