Electronic Supplementary Material (ESI) for Analytical Methods

Supporting Information (SI)

Elemental analysis of burnt human bone for classifying sex and age at death by logistic regression

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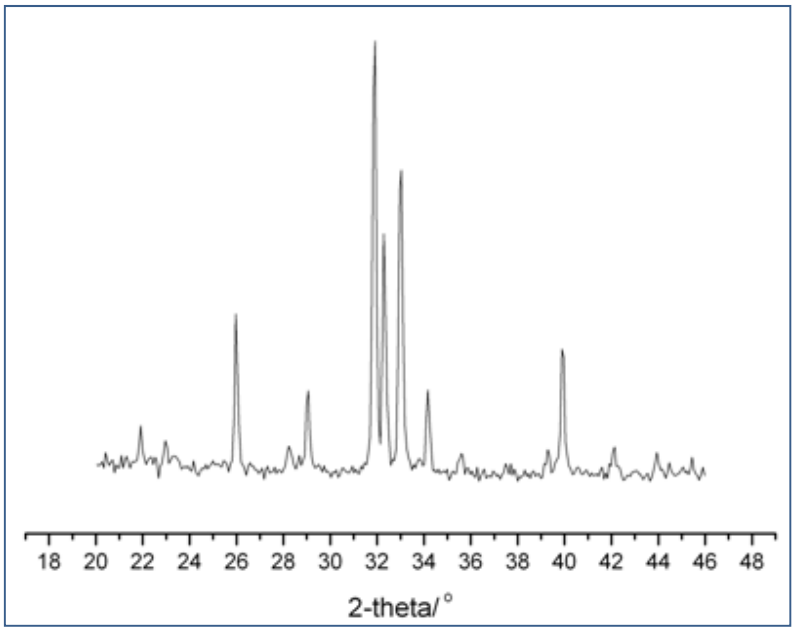

Figure S1 Powder X-ray diffraction pattern of the majority of the burnt bone samples (from 75 individuals) showing a typical feature of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$.

Table S2. Significant elemental concentration ratios for the bone fragments from Individual I (23 year old male) and Individual II (80 year old female), the average values of the ratios and the estimated parameters used to classify the sex of the deceased by binary regression analysis.

Individual I	**Elemental concentration ratios**						
	[Ca]/[K]	**[Mg]/[Al]**	**[S]/[Al]**	**[P]/[Mg]**	**[K]/[Mg]**	**[P]/[K]**	**[P]/[S]**
i	228.58	265.39	217.39	25.05	0.25	101.45	30.59
ii	135.92	58.95	48.36	21.58	0.35	61.1	26.31
iii	164.03	61.84	29.28	22.55	0.32	71.05	47.67
iv	39.55	75.78	5.19	18.13	1.06	17.09	31.24
v	98.36	22.85	13.78	22.79	0.52	44.12	37.78
Average [a]	133.29	83.59	62.8	22.02	0.5	58.96	34.72
Individual II	**[Ca]/[K]**	**[Mg]/[Al]**	**[S]/[Al]**	**[P]/[Mg]**	**[K]/[Mg]**	**[P]/[K]**	**[P]/[S]**
i	155.19	23.08	13.17	26.84	0.4	67.68	47.11
ii	145.73	42.33	28.86	25.83	0.42	61.5	37.96
iii	237.96	91.24	58.94	28.05	0.28	99.15	43.51
iv	98.46	16.95	9.74	21.52	0.53	40.81	37.48
v	138	15.18	6.43	22.84	0.39	59.03	53.93
Average [a]	155.07	37.76	23.43	25.02	0.4	65.63	44
Estimates(β_i) [b]	-0.06	0.03	-0.04	0.32	13.45	0.18	-0.18

[a] The mean values of elemental concentration ratios calculated for each individual;
[b] Elemental concentration ratio coefficients obtained by binary logistic regression calculated with respect to sex.

ICP-OES was employed to measure the content of Ca, P, Mg, S, K and Al in the burnt bone samples from Individual I (23 year old male) and Individual II (80 year old female). Following the developed protocol discussed in the main article, classifying sex is required prior to classify age of the deceased. Binary logistic analysis was performed using the chemical data (seven elemental concentration ratios) from these 10 bone fragments to obtain the estimates (β_i) or the elemental concentration ratio coefficients quantified with respect to sex, for which β_0 = -7.88. When applying the elemental concentration ratios and their coefficients in Eq (1), the fitted logistic equation can be written as follows.

$$P[E] = \frac{e^{-7.88-0.06\left(\frac{[Ca]}{[K]}\right)+0.03\left(\frac{[Mg]}{[Al]}\right)+\cdots-0.18\left(\frac{[P]}{[S]}\right)}}{1+e^{-7.88-0.06\left(\frac{[Ca]}{[K]}\right)+0.03\left(\frac{[Mg]}{[Al]}\right)+\cdots-0.18\left(\frac{[P]}{[S]}\right)}}$$

The values of $P[E]$, were employed to classify sex of the deceased using two constraints; $P[E] < 0.5$ for female and $P[E] > 0.5$ for male.

Next, prediction of age at death will be performed by using multinomial regression equation, Eq (4). Six elemental concentration ratios and mean values of the ratios for each individual are given in Table S3 where the ratio coefficients were calculated with respect to three age groups (20-30, 31-60 and ≥ 61 years old).

Table S3. Significant elemental concentration ratios of each bone fragment from Individual I (23 year old male) and Individual II (80 year old female) and the average values of the ratios.

Individual I	Elemental concentration ratios					
	[Ca]/[Al]	[Ca]/[S]	[Mg]/[Al]	[P]/[Al]	[K]/[Al]	[S]/[Al]
i	14943.22	68.90	265.39	6650.53	65.83	217.39
ii	2829.10	58.52	58.95	1272.87	20.83	48.36
iii	3213.62	110.04	61.84	1394.21	19.65	29.28
iv	375.58	72.25	75.78	1374.20	9.47	5.19
v	1161.23	84.22	22.85	520.67	11.80	13.78
Average [a]	4504.55	78.79	83.59	2000.05	25.52	62.80

Individual II	[Ca]/[Al]	[Ca]/[S]	[Mg]/[Al]	[P]/[Al]	[K]/[Al]	[S]/[Al]
i	1420.23	108.01	23.08	619.30	9.16	13.17
ii	2591.48	89.96	42.33	1093.93	17.78	28.86
iii	6163.85	104.41	91.24	2558.28	25.81	58.94
iv	880.44	90.40	16.95	364.89	8.94	9.74
v	810.14	91.81	15.18	346.59	5.87	6.43
Average [a]	2373.23	96.92	37.76	996.60	13.51	23.43

[a] The mean values of elemental concentration ratios calculated for each individual.

Table S4. Estimates calculated for three age groups; 20-30, 31-60 and ≥ 61 years old. These estimated parameters were further used to classify the age at death of the deceased by multinomial regression analysis.

Group	Estimates (β_i) [a]								
	β_0	[Ca]/[Al]	[Ca]/[S]	[Mg]/[Al]	[P]/[Al]	[K]/[Al]	[S]/[Al]	Female	Male
$g_1(20-30)$	-36.03	-0.014	0.262	-0.212	0.03	0.461	0.413	4.502	0
$g_2(31-60)$	-34.985	-0.014	0.266	-0.23	0.029	0.465	0.43	3.672	0
$g_2(\geq 61) = 0$ (set as reference)									

[a] Elemental concentration ratio coefficients obtained by binary logistic regression calculated with respect to age group.

When applying the elemental concentration ratios and their coefficients in Eq (5)-(6), to obtain the fitted logistic equations, Eq. (4) giving g_i and the probability for the Individual I (predicted as female from binary regression analysis) of each age group as follows.

$$g_1(20-30) = -36.03 - 0.014\left[\frac{\mathbf{[Ca]}}{\mathbf{[Al]}}\right] + \cdots 0.413\left[\frac{\mathbf{[S]}}{\mathbf{[Al]}}\right] + 4.502$$

$$g_2(31-60) = -34.985 - 0.014\left[\frac{[\mathbf{Ca}]}{[\mathbf{Al}]}\right] + \cdots 0.43\left[\frac{[\mathbf{S}]}{[\mathbf{Al}]}\right] + 3.672$$

$$P[20-30] = \frac{e^{0.23}}{e^{0.23} + e^{0.1} + e^{0}}$$

$$P[31-60] = \frac{e^{0.1}}{e^{0.23} + e^{0.1} + e^{0}}$$

$$P[\geq 61] == \frac{e^{0}}{e^{0.23} + e^{0.1} + e^{0}}$$

Similarly, the probability for the Individual II of each age group can be quantified using the chemical information of the individual and the estimate vales for male. Following the developed protocol, the group with maximum value of *P[E]* will represent the predicted result.