

RELATIONSHIP BETWEEN Ca, Cl, K, Mg, Mn, Na, P, AND Sr CONTENTS IN THE INTACT CROWNS OF FEMALE TEETH INVESTIGATED BY NEUTRON ACTIVATION ANALYSIS

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Introduction

To use chemical element composition as estimation of bone and teeth health in stomatological, geographical, environmental and occupational medicine, paleoanthropology, and other directions, it is necessary to know normal levels and age- and gender-related changes of chemical element contents and their ratios.^[1-22]

In our previous studies it was shown that samples are contaminated by trace elements from stainless steel tools during the sample preparation.^[23] There is evidence that some chemical elements are lost if tooth samples are treated with solvents in order to remove the organic matrix, and are then ashed and acid digested.^[24, 25]

This work had three aims. The first was to determine the Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions in the intact crowns of female teeth by instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) and to calculate some statistical parameters of Cl/Ca, K/Ca, Mg/Ca, Mn/Ca, Na/Ca, P/Ca, Sr/Ca, Ca/P, Cl/P, K/P, Mg/P, Mn/P, Na/P, Sr/P, Ca/Mg, Cl/Mg, Mn/Mg, Na/Mg, P/Mg, Sr/Mg, Ca/Cl, K/Cl, Mg/Cl, Mn/Cl, Na/Cl, P/Cl, Sr/Cl, Ca/K, Cl/K, Mg/K, Mn/K, Na/K, P/K, Sr/K, Ca/Na, Cl/Na, K/Na, Mg/Na, Mn/Na, P/Na, and Sr/Na mass fraction ratios. The second aim was to evaluate the effect of age on mean values of ratios of chemical element mass fractions. The third aim was to estimate the inter-correlations between Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions in the intact crowns of female teeth.

All studies were approved by the Ethical Committee of the Medical Radiological Research Center, Obninsk.

Experimental

Tooth root samples were obtained at postmortems from intact cadavers (38 female, 16–55 years old) within 24 h of death. Samples were freeze dried until constant mass was obtained. All subjects died mainly due to trauma. A titanium tool was used to cut and to scrub samples as well as to cut teeth crowns. The samples weighing about 100 mg for INAA-SLR were sealed separately in thin polyethylene film washed beforehand with acetone and rectified alcohol. The sealed samples were placed in labeled polyethylene ampoules.

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol–formaldehyde resins and chemically pure compounds were used.^[26] Ten certified reference materials (CRM) IAEA H-5 (Animal Bone) and standard reference materials (SRM) NIST 1486 (Bone Meal) sub-samples weighing about

50–100 mg were analyzed in the same conditions as teeth samples to estimate the precision and accuracy of results.

The mass fractions of Ca, Cl, K, Mg, Mn, Na, P, and Sr were determined by INAA-SLR using a horizontal channel equipped with the pneumatic rabbit system of the WWR-c research nuclear reactor. The information of used nuclear reactions, radionuclides, gamma-energies, neutron flux, spectrometer and other details of the analysis including the quality control of results were reported by us before.^[6, 26]

A dedicated computer program of NAA mode optimization was used.^[27] Using the Microsoft Office Excel software, the summary of statistics were calculated for different ratios of chemical element mass fractions. The reliability of difference in the results between two age groups of females was evaluated by Student's *t*-test. The Pearson's correlation analysis was used to identify relationships between elements.

Results and discussion

Table 1 represents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of 42 different ratios of Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions in the intact crowns of female teeth.

The obtained values for Ca/P ratio, as shown in Table 1, agree well with median of means cited by other researchers for the teeth enamel.^[28,29] No published data referring to ratios of Ca/P or other chemical element mass fractions in the intact crowns of female teeth was found.

To estimate the effect of age on the chemical element ratios in the intact crowns of female teeth we examined two age groups: one comprised a younger group with ages from 15 to 35 years and the other comprised older people with ages ranging from 36 to 55 years (Table 2). In the intact crowns of female teeth it was found a statistically significant age-related decrease of the Sr/Ca, Sr/P, Sr/Mg, Sr/Na, and Cl/Na ratios accompanied an increase of the K/Cl, Na/Cl, and P/Cl ratios.

No published data referring to age-related differences of ratios of chemical element mass fractions in the intact crowns of female teeth were found.

Table 3 depicts the inter-correlation calculations including all chemical elements identified by us. For example, the positive inter-correlations of P mass fractions with Ca ($p < 0.001$) and Cl ($p < 0.01$), as well as Mn mass fractions with K ($p < 0.05$) and Sr ($p < 0.01$) mass fractions were found in female teeth crowns. If some correlations between the elements were predictable (e.g., Ca–P), the interpretation of other observed relationships requires further study for a more complete understanding.

Conclusions

INAA-SLR is a satisfactory analytical tool for the non-destructive precise determination of mass fraction of 8 chemical elements (Ca, Cl, K, Mg, Mn, Na, P, and Sr) in the crown samples of permanent teeth. It was found the statistically significant changes of some chemical element mass fraction ratios in the intact crowns of female teeth with age. All the deceased were citizens of Obninsk, a small city of non-industrial region near Moscow. None of those who died a sudden death had suffered from any systematic or chronic disorders before. Thus, our data for element mass fraction ratios in the intact crowns of female teeth may serve as indicative normal values for residents of the Central European region of Russia.

Table 1. Some statistical parameters of 42 different ratios of Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions in the intact crowns of female (16-55 years)

Ratio	M	SD	SEM	Min	Max	Med	P0.025	P0.975
(Cl/Ca) $\times 10^3$	8.41	4.05	0.72	2.37	17.3	8.03	2.71	17.0
(K/Ca) $\times 10^3$	2.84	2.67	0.47	0.351	15.2	2.27	0.788	10.0
(Mg/Ca) $\times 10^2$	1.28	0.65	0.11	0.127	2.80	1.16	0.510	2.77
(Mn/Ca) $\times 10^6$	7.68	5.98	1.07	0.273	30.4	7.96	1.04	19.8
(Na/Ca) $\times 10^2$	1.84	0.35	0.06	1.08	2.68	1.75	1.32	2.55
P/Ca	0.527	0.075	0.013	0.377	0.714	0.522	0.402	0.764
(Sr/Ca) $\times 10^3$	0.974	0.589	0.103	0.342	3.11	0.819	0.356	2.17
Ca/P	1.93	0.28	0.05	1.40	2.65	1.91	1.42	2.49
(Cl/P) $\times 10^2$	1.57	0.72	0.13	0.581	3.11	1.44	0.599	3.09
(K/P) $\times 10^2$	0.502	0.387	0.069	0.0719	2.13	0.432	0.0996	1.73
(Mg/P) $\times 10^2$	2.48	1.32	0.23	0.239	6.17	2.23	0.947	5.43
(Mn/P) $\times 10^6$	14.2	10.9	1.93	0.615	56.1	15.5	1.28	35.4
(Na/P) $\times 10^2$	3.48	0.85	0.15	1.43	5.55	3.34	2.40	5.46
(Sr/P) $\times 10^3$	1.88	1.14	0.20	0.650	5.73	1.53	0.709	4.69
Ca/Mg	111	127	22	35.7	790	86.3	36.1	290
Cl/Mg	0.942	1.04	0.18	0.121	5.95	0.717	0.154	3.27
K/Mg	0.316	0.427	0.074	0.0348	1.87	0.182	0.0455	1.71
(Mn/Mg) $\times 10^4$	8.5	12.5	2.3	0.183	70.8	5.88	0.687	32.7
Na/Mg	1.89	1.72	0.30	0.656	10.9	1.51	0.749	4.37
P/Mg	59.0	66.6	11.4	16.2	416	45.0	18.5	149
Sr/Mg	0.098	0.076	0.013	0.0137	0.383	0.080	0.0234	0.253
Ca/Cl	156	93	17	58.0	421	135	58.9	370
K/Cl	0.378	0.312	0.055	0.0829	1.48	0.287	0.104	1.14
Mg/Cl	2.14	1.00	0.35	0.168	8.26	1.30	0.341	6.62
(Mn/Cl) $\times 10^4$	10.4	8.46	1.52	0.100	34.3	10.4	0.905	31.0
Na/Cl	2.86	1.83	0.32	0.952	7.43	2.14	0.996	6.87
P/Cl	79.1	30.5	6.9	32.1	172	60.4	32.4	167
Sr/Cl	0.149	0.120	0.021	0.0374	0.510	0.126	0.0408	0.505
Ca/K	561	484	86	65.7	2847	441	106	1487
Cl/K	4.29	2.83	0.50	0.675	12.1	3.49	0.893	9.72
Mg/K	7.34	6.14	1.07	0.535	28.7	5.50	0.586	20.8
(Mn/K) $\times 10^4$	34.5	19.8	3.5	9.79	87.1	33.9	9.95	78.4
Na/K	10.3	9.2	1.6	1.30	56.1	7.78	2.23	26.3
P/K	305	255	44	46.9	1391	231	58.4	1029
Sr/K	0.558	0.542	0.094	0.0438	2.76	0.376	0.0566	2.05
Ca/Na	56.2	11.2	1.9	37.3	92.9	57.3	39.3	76.4
Cl/Na	0.498	0.276	0.048	0.134	1.05	0.468	0.146	1.00
K/Na	0.150	0.131	0.023	0.0178	0.767	0.129	0.046	0.479
Mg/Na	0.709	0.317	0.054	0.0916	1.52	0.664	0.293	1.34
(Mn/Na) $\times 10^4$	4.20	3.41	0.60	0.165	17.4	3.89	0.548	12.1
P/Na	30.6	9.0	1.5	18.0	69.9	30.0	18.3	43.8
Sr/Na $\times 10$	0.566	0.359	0.062	0.181	1.78	0.437	0.204	1.46

M arithmetic mean, *SD* standard deviation, *SEM* standard error of mean, *Min* minimum value, *Max* maximum value, *Med* median, *P0.025* percentile with 0.025 level, *P0.975* percentile with 0.975 level

Table 2. Effect of age on mean values (M±SEM) of ratios of chemical element in the intact crowns of female teeth (Student's *t*-test)

Ratio	Age			Ratio M ₂ /M ₁
	15-35 year (M ₁)	36-55 year (M ₂)	<i>p</i> ≤	
(Cl/Ca)×10 ³	9.79±1.06	7.33±0.92	0.09	0.75
(K/Ca) ×10 ³	2.29±0.22	3.27±0.82	0.26	1.43
(Mg/Ca) ×10 ²	1.22±0.11	1.35±0.18	0.54	1.11
(Mn/Ca) ×10 ⁶	9.13±1.98	6.48±1.06	0.25	0.71
(Na/Ca) ×10 ²	1.74±0.07	1.92±0.09	0.12	1.10
P/Ca	0.519±0.013	0.534±0.021	0.56	1.03
(Sr/Ca) ×10 ³	1.28±0.20	0.750±0.075	0.022	0.59
Ca/P	1.95±0.06	1.92±0.07	0.83	0.99
(Cl/P) ×10 ²	1.82±0.17	1.37±0.17	0.07	0.75
(K/P) ×10 ²	0.415±0.041	0.575±0.121	0.22	1.39
(Mg/P) ×10 ²	2.31±0.21	2.61±0.37	0.49	1.13
(Mn/P) ×10 ⁶	16.3±3.6	12.4±1.8	0.33	0.76
(Na/P) ×10 ²	3.28±0.24	3.65±0.17	0.23	1.11
(Sr/P) ×10 ³	2.43±0.35	1.44±0.16	0.018	0.59
Ca/Mg	90.3±7.7	127±38	0.36	1.40
Cl/Mg	0.889±0.106	0.986±0.323	0.78	1.11
K/Mg	0.201±0.031	0.412±0.131	0.13.	2.05
(Mn/Mg) ×10 ⁴	7.47±1.56	9.40±3.97	0.66	1.26
Na/Mg	1.51±0.13	2.19±0.51	0.22	1.45
P/Mg	48.3±4.4	67.5±20.2	0.36	1.40
Sr/Mg	0.115±0.017	0.084±0.019	0.23	0.73
Ca/Cl	123±17	182±25	0.06	1.48
K/Cl	0.260±0.043	0.484±0.091	0.036	1.86
Mg/Cl	1.58±0.36	2.60±0.55	0.13	1.65
(Mn/Cl) ×10 ⁴	9.79±2.16	11.0±2.2	0.70	1.12
Na/Cl	2.16±0.35	3.45±0.47	0.036	1.60
P/Cl	63.2±7.0	92.4±10.3	0.026	1.46
Sr/Cl	0.160±0.033	0.139±0.028	0.63	0.86
Ca/K	507±59	603±146	0.55	1.19
Cl/K	5,23±0.75	3.46±0.62	0.08	0.66
Mg/K	6.69±0.95	7.89±1.81	0.57	1.18
(Mn/K) ×10 ⁴	37.3±5.9	31.9±4.1	0.46	0.86
Na/K	9.16±1.08	11.2±2.8	0.50	1.22
P/K	304±53	306±70	0.98	1.01
Sr/K	0.661±0.112	0.472±0.146	0.31	0.71
Ca/Na	58.6±2.2	54.4±3.0	0.26	0.93
Cl/Na	0.610±0.070	0.404±0.059	0.032	0.66
K/Na	0.130±0.013	0.166±0.040	0.41	1.28
Mg/Na	0.726±0.058	0.696±0.088	0.78	0.96
(Mn/Na) ×10 ⁴	5.05±1.13	3.45±0.51	0.21	0.68
P/Na	33.2±3.0	28.5±1.3	0.16	0.86
Sr/Na	0.785±0.110	0.393±0.035	0.004	0.50

M arithmetic mean, *SEM* standard error of mean, *bold* statistically significant

Table 3. Intercorrelations of the chemical element mass fractions in the intact crowns of female teeth (r – coefficient of correlation)

Element	Ca	Cl	K	Mg	Mn	Na	P	Sr
Ca	1.00	0.34	-0.04	-0.13	0.03	0.24	0.68 ^c	-0.10
Cl	0.34	1.00	0.15	-0.29	0.19	-0.20	0.46 ^b	0.04
K	-0.04	0.15	1.00	-0.22	0.39 ^a	0.21	0.26	-0.09
Mg	-0.13	-0.29	-0.22	1.00	-0.16	0.27	-0.17	0.02
Mn	0.03	0.19	0.39 ^a	-0.16	1.00	0.19	0.21	0.50 ^b
Na	0.24	-0.20	0.21	0.27	0.19	1.00	0.18	-0.18
P	0.68 ^c	0.46 ^b	0.26	-0.17	0.21	0.18	1.00	0.01
Sr	-0.10	0.04	-0.09	0.02	0.50 ^b	-0.18	0.01	1.00

Statistically significant values: ^a $p \leq 0.05$, ^b $p \leq 0.01$, ^c $p \leq 0.001$.

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