Relationship between Ca, Cl, K, Mg, Mn, Na, P, and Sr Contents in the Intact Roots of Female Teeth Investigated by Neutron Activation Analysis

V. Zaichick[1], S. Zaichick[1,2]

[1] *Medical Radiological Research Centre, Korolyev St., 4, Obninsk, 249036, Russia, e-mail: vezai@obninsk.com*

[2] *Current address: University of Illinois College of Medicine Chicago, IL 60612, USA*

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 roots 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 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 roots 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 roots. 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 roots 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 roots of female teeth was found.

To estimate the effect of age on the chemical element ratios in the intact roots 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 roots of female teeth it was found a statistically significant age-related decrease of the Mg/Na ratio accompanied an increase of the Mg/Ca, Sr/Ca, Sr/P, Sr/Mg, Sr/Cl, Sr/K, and Sr/Na ratios.

No published data referring to age-related differences of ratios of chemical element mass fractions in the intact roots 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 Ca mass fractions with P ($p<0.001$) and Mg ($p<0.01$) mass fractions were found in female teeth roots. If some correlations between the elements were predictable (e.g., Ca–P, Ca–Mg), 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 root samples of permanent teeth. It was found the statistically significant changes of some chemical element mass fraction ratios in the intact roots 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 roots 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 roots of female teeth

Ratio	M	SD	SEM	Min	Max	Med	P0.025	P0.975
(Cl/Ca)×10^3	2.71	1.59	0.30	0.880	7.77	1.89	1.27	6.38
(K/Ca) ×10^3	3.12	1.16	0.23	0.305	5.13	3.32	0.804	4.62
(Mg/Ca) ×10^2	2.90	0.60	0.11	1.14	3.85	2.97	1.65	3.85
(Mn/Ca) ×10^6	3.47	2.22	0.43	0.517	9.31	3.37	1.89	5.64
(Na/Ca) ×10^2	2.67	1.07	0.20	1.79	6.69	2.33	1.89	5.64
P/Ca	0.538	0.069	0.013	0.370	0.655	0.540	0.383	0.642
(Sr/Ca) ×10^3	1.52	0.74	0.13	0.499	2.96	1.30	0.565	2.94
Ca/P	1.89	0.28	0.05	1.53	2.70	1.85	1.56	2.61
(Cl/P) ×10^2	0.518	0.270	0.050	0.150	1.25	0.383	0.217	1.07
(K/P) ×10^2	0.587	0.211	0.041	0.053	0.910	0.637	0.133	0.883
(Mg/P) ×10^2	5.49	1.22	0.22	2.54	8.47	5.55	3.47	7.91
(Mn/P) ×10^6	6.23	3.88	0.73	0.789	17.6	5.87	1.06	15.6
(Na/P) ×10^2	5.03	1.85	0.34	2.73	10.5	4.36	3.08	9.31
(Sr/P) ×10^3	2.87	1.45	0.26	0.891	6.26	2.30	1.09	5.80
Ca/Mg	36.5	11.5	2.14	26.0	87.3	33.7	26.0	63.8
Cl/Mg	0.097	0.053	0.010	0.035	0.255	0.078	0.038	0.246
K/Mg	0.107	0.036	0.007	0.013	0.171	0.113	0.035	0.158
(Mn/Mg) ×10^4	1.21	0.85	0.16	0.171	3.17	0.959	0.195	3.15
Na/Mg	0.975	0.482	0.088	0.505	2.22	0.789	0.540	2.20
P/Mg	19.3	5.18	0.95	11.8	39.4	18.0	12.7	29.8
Sr/Mg	0.055	0.037	0.007	0.020	0.224	0.045	0.022	0.127
Ca/Cl	468	211	40	129	1136	529	160	834
K/Cl	1.42	0.89	0.17	0.053	4.09	1.14	0.256	3.10
Mg/Cl	13.0	6.0	1.1	3.92	28.8	12.8	4.07	26.6
(Mn/Cl) ×10^4	15.0	11.8	2.3	1.76	47.2	12.8	2.42	40.2
Na/Cl	13.1	11.0	2.0	2.87	58.9	12.3	3.35	41.4
P/Cl	246	124	23	79.9	665	261	94.6	485
Sr/Cl	0.698	0.528	0.093	0.158	2.21	0.499	0.168	1.85
Ca/K	475	602	118	195	3281	301	218	1796
Cl/K	1.64	3.54	0.70	0.244	18.7	0.877	0.341	8.69
Mg/K	12.5	13.6	2.6	5.83	77.6	8.82	6.36	41.2
(Mn/K) ×10^4	17.3	25.9	5.2	2.02	135	9.19	2.62	75.5
Na/K	12.0	16.2	3.1	4.93	89.9	7.34	5.05	46.4
P/K	256	344	66	110	1892	157	113	1029
Sr/K	0.623	0.595	0.109	0.171	2.96	0.488	0.183	2.49
Ca/Na	41.0	9.6	1.8	15.0	55.9	42.9	18.0	53.0
Cl/Na	0.117	0.076	0.014	0.017	0.349	0.081	0.026	0.302
K/Na	0.129	0.054	0.010	0.011	0.203	0.136	0.032	0.198
Mg/Na	1.20	0.40	0.07	0.450	1.98	1.27	0.455	1.86
(Mn/Na) ×10^4	1.31	0.89	0.17	0.257	4.42	1.40	0.273	3.50
P/Na	22.0	6.3	1.2	9.52	36.7	23.0	10.8	32.6
Sr/Na	0.603	0.293	0.052	0.167	1.26	0.553	0.242	1.15

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 roots of female teeth (Student's *t*-test)

Ratio	Age			Ratio
	15-35 year (M_1)	36-55 year (M_2)	$p\leq$	M_2/M_1
$(Cl/Ca)\times10^3$	3.13±0.61	2.48±0.32	0.36	0.79
$(K/Ca)\times10^3$	3.29±0.32	3.01±0.32	0.54	0.91
$(Mg/Ca)\times10^2$	3.19±0.16	2.75±0.14	**0.048**	0.86
$(Mn/Ca)\times10^6$	3.78±0.81	3.29±0.50	0.61	0.87
$(Na/Ca)\times10^2$	2.56±0.46	2.72±0.19	0.75	1.06
P/Ca	0.559±0.022	0.527±0.016	0.24	0.94
$(Sr/Ca)\times10^3$	1.18±0.15	1.72±0.18	**0.023**	1.46
Ca/P	1.81±0.07	1.94±0.07	0.24	1.07
$(Cl/P)\times10^2$	0.588±0.094	0.475±0.056	0.32	0.81
$(K/P)\times10^2$	0.617±0.063	0.566±0.054	0.54	0.92
$(Mg/P)\times10^2$	5.86±0.34	5.27±0.29	0.20	0.90
$(Mn/P)\times10^6$	6.16±1.20	6.27±0.95	0.94	1.02
$(Na/P)\times10^2$	4.62±0.63	5.27±0.39	0.40	1.14
$(Sr/P)\times10^3$	2.17±0.26	3.30±0.35	**0.014**	1.52
Ca/Mg	32.0±1.6	38.9±3.1	0.056	1.22
Cl/Mg	0.101±0.017	0.094±0.012	0.76	0.93
K/Mg	0.105±0.010	0.110±0.010	0.72.	1.05
$(Mn/Mg)\times10^4$	1.10±0.26	1.28±0.21	0.59	1.16
Na/Mg	0.820±0.140	1.065±0.110	0.18	1.30
P/Mg	17.7±1.1	20.2±1.3	0.17	1.14
Sr/Mg	0.0371±0.0040	0.0649±0.0100	**0.014**	1.75
Ca/Cl	406±55	503±53	0.22	1.24
K/Cl	1.26±0.20	1.53±0.27	0.42	1.21
Mg/Cl	12.2±0.20	13.5±1.6	0.53	1.11
$(Mn/Cl)\times10^4$	13.3±3.9	16.2±2.8	0.55	1.22
Na/Cl	10.4±2.6	14.7±2.9	0.28	1.41
P/Cl	218±33	263±31	0.33	1.21
Sr/Cl	0.476±0.090	0.831±0.132	**0.034**	1.75
Ca/K	354±61	551±188	0.33	1.56
Cl/K	1.05±0.19	2.07±1.20	0.41	1.97
Mg/K	10.7±1.3	13.8±4.4	0.51	1.29
$(Mn/K)\times10^4$	10.5±2.1	22.7±9.0	0.21	2.16
Na/K	8.21±1.16	14.6±5.2	0.24	1.78
P/K	197±39	297±109	0.40	1.51
Sr/K	0.371±0.040	0.791±0.170	**0.026**	2.13
Ca/Na	44.6±3.5	39.1±1.9	0.19	0.88
Cl/Na	0.144±0.027	0.100±0.015	0.18	0.69
K/Na	0.141±0.015	0.121±0.014	0.32	0.86
Mg/Na	1.41±0.12	1.09±0.08	**0.041**	0.77
$(Mn/Na)\times10^4$	1.37±0.24	1.28±0.23	0.80	0.93
P/Na	24.3±2.1	20.7±1.3	0.16	0.85
Sr/Na	0.482±0.043	0.675±0.075	**0.034**	1.40

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

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

Element	Ca	Cl	K	Mg	Mn	Na	P	Sr
Ca	**1.00**	0.0316	0.177	0.375[b]	-0.0391	0.0504	0.598[c]	0.0160
Cl	0.0316	**1.00**	-0.0704	0.222	0.171	-0.321[a]	0.0938	-0.129
K	0.177	-0.0704	**1.00**	0.521[c]	-0.0105	0.0895	0.0706	0.101
Mg	0.375[b]	0.222	0.521[c]	**1.00**	0.0148	-0.239	0.408[b]	0.0457
Mn	-0.0391	0.171	-0.0105	0.0148	**1.00**	0.183	0.162	0.237
Na	0.0504	-0.321[a]	0.0895	-0.239	0.183	**1.00**	-0.0685	0.261
P	0.598[c]	0.0938	0.0706	0.408[b]	0.162	-0.0685	**1.00**	-0.356[a]
Sr	0.0160	-0.129	0.101	0.0457	0.237	0.261	-0.356[a]	**1.00**

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

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