NEUTRON ACTIVATION ANALYSIS OF Ca, Cl, Mg, Mn, Na, P, AND Sr CONTENTS IN THE ENAMEL OF PRIMARY TEETH

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

[1] *Northwestern University, Chicago, IL, 60611, USA*

[2]*Medical Radiological Research Centre of RAMS,*
Koroleva str., 4, Obninsk, 249020, Russia,
e-mail: vezai@obninsk.com

Introduction

The apatite phases of teeth can apparently be affected by trace element incorporation into teeth with effects on the physicochemical properties[1-4]. It is the reason why deficiency or excess of F, Fe, Mg, Mn, Sr, Zn and some other trace elements is one of the factors which determines the degree of susceptibility of caries and other dental diseases.[5-7] So, chemical element analysis of teeth expands the knowledge of etiology of dental diseases and may be apply for diagnostic, therapeutic and preventive purposes.

Furthermore, chemical element intake plays an important role in human health. Deficiency and excess (toxicity) of F, Fe, Mg, Mn, Sr, Zn and some other trace elements, resulting from exposure to both the natural and man made environment, can lead to a wide variety of clinical effects. The monitoring of trace element status via tissue sampling has important implications for the identification and correction of such effects. Teeth are reported to be suitable indicators of trace element exposure for a wide range of elements concentrated in calcified tissues.[8-11] Geographical variation in the trace element content of teeth has been demonstrated too. [12-15] In accordance with it teeth are used in an occupational medicine, environmental health studies and geographical medicine.[10, 14, 16-18]

Several other studies demonstrate that it is possible to detect nutritional deficiencies through the chemical composition of teeth and that such deficiencies can have an important effect on the critical growth period of the dentition.[19-20] Differences in the availability of foods and food choice, often based on social and cultural practices, may be important factors in determining trace element intake in man and complicate the modelling of intake.[14]

Moreover, a chemical element analysis of human teeth are often used in paleoanthropology for dietary and environment reconstruction, assessment of the social and economic status of human groups.[21]

Therefore, for efficient application of teeth analysis in all above-mentioned directions it is necessary to know the normal levels and age- and gender-related changes of teeth trace elements in large scale. Primary teeth are particularly suitable because they are easily to obtained since they naturally exfoliate eruption of permanent teeth.

There are several reviews and articles regarding the trace element analysis of teeth, using chemical analysis techniques and instrumental methods.[1,2,5,22-25] However, the majority of these data are based upon non intact teeth. In most cases, teeth are washed in water, and then maintained in a NaCl, physiological and other solution for conservation. In some cases teeth are treated with solvents in order to remove organic materials and exterior layer. If an

investigator chooses to use destructive methods, teeth are ashed and acid digested. There is evidence that some chemical elements are lost during these preparations and that the relationship between some trace elements is affected. [26,27]

In the present study the Ca, Cl, Mg, Mn, Na, P, and Sr mass fraction in enamel of primary teeth were analyzed with three objectives. The first objective was to use intact enamel. The second objective was to compare element contents in enamel of sound primary teeth received in normal exfoliation time with enamel of teeth extracted by reason of chronic periodontitis. The third objective was to receive the indicative normal values of element contents in enamel of sound primary teeth for residents of the Russian Central European region. The Ethic Committee of the Medical Radiological Research Centre of Russian Academy of Medical Sciences approved this research.

Experimental

Twenty primary teeth (incisors, canines and molars) from 20 children (7 girls and 13 boys, age range: 5-16 years), living in Obninsk were collected by Municipal Dental Clinic. Obninsk is a small city situated in a rural region approximately 100 km south-west of Moscow. Twelve teeth were naturally exfoliated and eight were extracted by reason of chronic periodontitis. All teeth were caries free or without visual defects.

The crowns contacted with the stainless steel surgical instruments were cleaned by the alcohol moist gauze tampons. A tool made of titanium was used to cut and to scrub soft tissue and blood off the roots. The enamel was removed from each of the 20 teeth using a diamond dental drill. To prevent the resulting enamel dust from becoming air-bone and lost, the whole mechanical grinding procedure was undertaken inside a transparent plastic bag. The pooled enamel dust was collected on a plastic sheet at first and than transferred to clean plastic containers. After freeze drying enamel samples were weighed and sealed in thin polyethylene films previously washed with acetone and rectified alcohol. The sealed samples were then placed in labeled polyethylene ampoules. The weights of enamel samples ranged from 20 to 50 mg.

A horizontal channel in the pneumatic rabbit system of the WWR-c research nuclear reactor was used to determine Ca, Cl, Mg, Mn, Na, P, and Sr by instrumental neutron activation analysis (INAA) using short-lived radionuclides. Samples of the standard reference material, NIST SRM1486 bone meal and certified reference material IAEA H-5 animal bone, were analyzed under the same conditions as enamel samples to estimate the precision and accuracy of results. Details of nuclear reactions, radionuclides, gamma-energies, methods of analysis and the results of quality control were presented in our earlier publications concerning the chemical elements of human bones and crowns of teeth.[28,29]

Irradiation, expose and measurement times as well as the sample-detector distance were regarded optimum in terms of providing feasibility of simultaneous measurement for the maximum number of elements with acceptable statistical error for each of them. A dedicated computer program of INAA mode optimization was used for preliminary estimation of these parameters.[30]

Results

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 Ca, Cl, Mg, Mn, Na, P, and Sr contents and Ca/P ratio in intact enamel of sound primary teeth received in normal exfoliation time.

Table 1. Some statistical parameters of chemical element contents and Ca/P ratio in intact enamel of sound primary teeth received in normal exfoliation time (on dry weight basis)

Physiological exfoliation	Age years	Ca g/kg	Cl mg/kg	Mg mg/kg	Mn mg/kg	Na mg/kg	P g/kg	Sr mg/kg	Ca/P
n	12	12	12	12	12	12	12	12	12
M	10.0	395	2318	4104	2.72	5074	203	330	1.98
SD	3.0	46	1278	1583	0.79	354	39	187	0.24
SEM	0,9	13	369	457	0.23	102	11	54	0.07
min	6	326	1030	1320	1.71	4590	139	68	1.62
max	16	457	5660	6920	4.36	5790	276	565	2.51
Med	10	407	1980	4150	2.58	5095	194	323	1.94
Per0.025	6.0	328	1091	1548	1.71	4620	145	73.2	1.66
Per0.975	15.2	454	5129	6532	4.23	5691	267	563	2.44

Abbreviations: n – number of samples, M –arithmetical mean, SD – standard deviation, SEM – standard error of mean, min – minimal value, max – maximal value, P0.025 – percentile with 0.025 level, P0.975 – percentile with 0.975 level.

Table 2 gives some statistical parameters of 7 chemical element contents and Ca/P ratio in intact enamel of primary teeth extracted by reason of chronic periodontitis such as arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels.

Table 2. Some statistical parameters of chemical element contents and Ca/P ratio in intact enamel of primary teeth extracted by reason of chronic periodontitis (on dry weight basis)

Chronic periodontitis	Age years	Ca g/kg	Cl mg/kg	Mg mg/kg	Mn mg/kg	Na mg/kg	P g/kg	Sr mg/kg	Ca/P
n	8	8	8	8	8	8	8	8	8
M	6.75	396	2110	3332	3.15	4850	201	293	1.98
SD	1.67	23	458	1026	1.39	264	22	202	0.15
SEM	0.59	8.3	162	363	0.48	93	7.8	72	0.05
min	5	365	1560	1840	1.87	4510	183	56	1.79
max	9	439	2700	4720	5.92	5160	243	658	2.22
Med	7	394	2120	3160	2.76	4870	192	263	1.99
Per0.025	5	367	1574	1968	1.92	4526	183	70	1.80
Per0.975	8.83	434	2700	4694	5.63	5160	240	618	2.20

Abbreviations: n – number of samples, M –arithmetical mean, SD – standard deviation, SEM – standard error of mean, min – minimal value, max – maximal value, P0.025 – percentile with 0.025 level, P0.975 – percentile with 0.975 level.

The comparison of mean ((M± SEM) values of chemical element contents and Ca/P ratio in enamel of sound primary teeth received in normal exfoliation time and extracted by reason of chronic periodontitis is shown in Table 3.

Table 3. Comparison of mean ((M± SEM) values of chemical element contents and Ca/P ratio in enamel of sound primary teeth received in normal exfoliation time and extracted by reason of chronic periodontitis.

Chemical element	Unit (on dry weight basis)	Physiological exfoliation 10.0±0.9 years n=12	Chronic periodontitis 6.8±0.6 years n=8	*p* (t-test)
Ca	g/kg	395±13	396±8	N.S.
Cl	mg/kg	2318±369	2110±162	N.S.
Mg	mg/kg	4104±457	3332±363	N.S.
Mn	mg/kg	2.72±0.23	3.15±0.48	N.S.
Na	mg/kg	5074±102	4850±93	N.S.
P	g/kg	203±11	201±8	N.S.
Sr	mg/kg	330±54	293±72	N.S.
Ca/P	-	1.98±0.07	1.98±0.05	N.S.

Abbreviations: n – number of samples, M –arithmetical mean, SEM – standard error of mean, N.S. - not significant.

No statistically significant difference in all investigated parameters in enamel of sound primary teeth received in normal exfoliation time and extracted by reason of chronic periodontitis has allowed us to combine both groups of teeth. Table 4 depicts our data for 7 chemical element contents and Ca/P ratio in intact enamel of primary teeth (exfoliated and extracted teeth taken together).

Table 4. Some statistical parameters of chemical element contents and Ca/P ratio in intact enamel of primary teeth (exfoliated and extracted teeth taken together) from children of age range 5-16 years (on dry weight basis)

Enamel	Age	Ca	Cl	Mg	Mn	Na	P	Sr	Ca/P
of primary teeth	years	g/kg	mg/kg	mg/kg	mg/kg	mg/kg	g/kg	mg/kg	
n	20	20	20	20	20	20	20	20	20
M	8.70	396	2235	3795	2.89	4985	202	315	1.98
SD	2.99	38	1017	1410	1.04	333	32	189	0.20
SEM	0.67	8.5	227	315	0.23	74	7.3	42	0.05
min	5	326	1030	1320	1.71	4510	139	56	1.62
max	16	457	5660	6920	5.92	5790	276	658	2.51
Med	8	401	2120	3910	2.58	5005	192	323	1.99
Per0.025	5	329	1135	1567	1.71	4548	156	61.7	1.68
Per0.975	14.6	453	4743	6250	5.18	5619	260	614	2.39

Abbreviations: n – number of samples, M –arithmetical mean, SD – standard deviation, SEM – standard error of mean, min – minimal value, max – maximal value, P0.025 – percentile with 0.025 level, P0.975 – percentile with 0.975 level.

To estimate the effect of age on Ca, Cl, Mg, Mn, Na, P, and Sr contents and Ca/P ratio we examined two age groups: one (1) comprised a younger group with ages from 5 to 10 years and the other (2) comprised children and adolescents with ages ranging from 11 to 16 years (Table 5).

Table 5. Effect of age on mean ((M± SEM) values of chemical element contents and Ca/P ratio in intact enamel of primary teeth

Chemical element	Unit (on dry weight basis)	Group 1 from 5 to 10 years Mean 6.9±0.4 years n=13	Group 2 from 11 to 16 years Mean 12.0±0.8 years n=7	*p* (t-test)
Ca	g/kg	400±10	389±17	N.S.
Cl	mg/kg	2046±127	2584±614	N.S.
Mg	mg/kg	3951±374	3507±599	N.S.
Mn	mg/kg	2.95±0.32	2.79±0.32	N.S.
Na	mg/kg	4931±99	5084±104	N.S.
P	g/kg	210±9	188±12	N.S.
Sr	mg/kg	286±60	368±46	N.S.
Ca/P	-	1.92±0.04	2.09±0.09	N.S.

Abbreviations: n – number of samples, M –arithmetical mean, SEM – standard error of mean, N.S. - Not significant.

We used the entire dataset for both girls and boys taken separately, seeking to detect the presence of gender-related differences (see Table 6).

Table 6. Effect of gender on mean ((M± SEM) values of chemical element contents and Ca/P ratio in intact enamel of primary teeth

Chemical element	Unit (on dry weight basis)	Boys Mean 8.1±0.7 years n=13	Girls Mean 9.9±1.4 years n=7	*p* (t-test)
Ca	g/kg	399±10	389±16	N.S.
Cl	mg/kg	2112±183	2463±575	N.S.
Mg	mg/kg	3798±319	3791±723	N.S.
Mn	mg/kg	3.07±0.35	2.56±0.09	N.S.
Na	mg/kg	4892±70	5157±155	N.S.
P	g/kg	211±9	187±11	N.S.
Sr	mg/kg	330±56	287±65	N.S.
Ca/P	-	1.91±0.04	2.11±0.10	N.S.

Abbreviations: n – number of samples, M –arithmetical mean, SEM – standard error of mean, N.S. - Not significant.

Finally, Table 7 presents the comparison of our and reference data of Ca, Cl, Mg, Mn, Na, P, and Sr contents and Ca/P ratio in primary teeth.

Table 7. Median, minimum and maximum value of means of chemical element contents and Ca/P ratio in enamel of sound primary teeth according to data from the literature in comparison with our results (on dry weight basis)

Element	Unit	Published data [Reference]			This work results
		Median of means [n]*	Minimum of means M or M±SD, (n)**	Maximum of means M or M±SD, (n)**	M±SD n=20
Ca	g/kg	365 [7]	316±44 (105) [31]	397 (39) [32]	396±38
Cl	mg/kg	2700 [3]	<1370 (16) [35]	3600 (93) [36]	2240±1020
Mg	mg/kg	2600 [8]	0.08±0.01 (105) [37]	3100 (105) [19]	3800±1400
Mn	mg/kg	1.49 [9]	0.5±0.2 (13) [38]	58 (10) [39]	2.89±0.23
Na	mg/kg	6300 [6]	~3000 (16) [35]	8400 (39) [32]	4990±330
P	g/kg	158 [6]	116±21 (-) [33]	192 (39) [32]	202±32
Sr	mg/kg	78 [12]	24.2±9.5 (13) [38]	161 (114) [40]	315±189
Ca/P	-	2.06 [3]	2.01±0.15 (8) [34]	2.21 (39) [32]	1.98±0.20

Abbreviations: M - arithmetic mean, SD – standard deviation, [n]* – number of all references, (n)** - number of samples

Discussion

As was shown in our previous reports[28,29] the mean contents for each element in ten sub-samples of the IAEA H-5 animal bone and NIST SRM1486 bone meal certified reference materials were in a good agreement with certified values indicating an acceptable accuracy of the results obtained in the study of tooth enamel.

No statistically significant differences were found between element contents in enamel of sound primary teeth received in normal exfoliation time and extracted by reason of chronic periodontitis (Table 3). Age- and gender-related comparison did not show any statistically significant differences in Ca, Cl, Mg, Mn, Na, P, and Sr contents and Ca/P ratio in tooth enamel (Table 5 and 6).

The mean values measured for Ca, Cl, Mg, Mn, Na, P, and Sr contents and Ca/P ratio, as shown in Table 7, agree well with ranges and medians of the mean values cited by other researchers for enamel of primary teeth.[1,2,5,12,19,31-40] The Sr means are about 4-fold higher than the median value of means obtained by some other researchers. It is known that Sr content in the enamel depends greatly on biogeochemical peculiarities of the residence area, and this may be the cause for the high Sr content in tooth enamel of citizens living in the Russian Central European region.

The standard deviation and standard error of the mean obtained for all elements including Ca and P are respectively large (Tables 1, 2, and 4). This is due to the very wide individual variation of element mass fractions in the intact human enamel of primary teeth.[31,33,34,38]

Conclusions

INAA using short-lived radionuclides is an efficient technique for the determination of many important chemical elements in tooth enamel. The method is simple, fast, multielemental, and non-destructive.

No statistically significant differences were found between element contents in enamel of sound primary teeth received in normal exfoliation time and extracted by reason of chronic periodontitis. Age- and gender-related comparison did not show any statistically significant differences in Ca, Cl, Mg, Mn, Na, P, and Sr contents and Ca/P ratio in tooth enamel.

Our data for Ca, Cl, Mg, Mn, Na, P, and Sr mass fractions in enamel of sound primary teeth may serve as indicative normal values for residents of the Russian Central European region.

References

1. Derise N.L., Ritchey S.J., Furr, A. J. Dent. Res., 1974, **53**, 847-852.
2. Curzon M.E.J., Cutress T.W. (eds.): Trace Elements and Dental Disease. PSG-John Wright, Boston, 1983.
3. Koray M., Őner-Iyidogan Y., Soyman M., Gűrdől F. J. Trace Elements Med. Biol., 1996, **10**, 255-259.
4. LeGeros R.Z., Miravite, M.A., Quirolgico, G.B., Curzon M.E.J. In: Calcified Tissues 1976. Leeds, 1977, p. 362-367.
5. Derise N.L., Ritchey S.J. J. Dent. Res., 1974, **53**, 853-858.
6. Davies B.E., Anderson R.J. Experimentia 1987, **43**, 87-92.
7. Alvarez J.O., Eguren J.C., Caceda J., Navia J.M. J Dent Res 1990, **69**, 1564-1566.
8. Altshuller L.F., Halak D.B., Landing B.H., Kehoe A.R. J. Pediatr., 1962, **60**, 224-229.
9. Sharon I.M. Perspect. Biol. Med., 1988, **32**, 124-131.
10. Alexander L.M., Heaven A., Delves H.T., Moreton J., Trenouth M.J. Arch. Environ. Health., 1993, **48**, 392-400.
11. Al-Mahroos F., Al-Saleh F.S. Ann Trop Paediatr 1997, **17**, 147-154.
12. Curzon M.E.J., Losee F.L., MacAlister A.D. N.Z. Dent. J., 1975, **71**, 80-83.
13. Carvalho M. L., Casaca C., Marques J. P., Pinheiro T., Cunha A. S. X-ray spectrometry, 2001, **30**, 3, 190-193.
14. Brown C. J., Chenery S. R. N., Smith B., Mason C., Tomkins A., Roberts G. J., Sserunjogi L., Tiberindwa J. V. Archives of Oral Biology, 2004, **49**, 705-717.
15. Salah H., Arab N. Journal of Nuclear and Radiochemical Sciences, 2007, **8**, 1, 31-34.
16. Baranska-Gadiowska M., Kaminski M., Owcarczak K., Kabza Z. Czas Stomatol., 1988, **41**, 406-410.
17. Bercovitz K., Laufe D. Archs. Oral. Biol., 1990, **35**, 895-897.
18. Bloch P., Shapiro I.M., Soule L., Close A., Revich B. Appl. Radiat. Isot., 1998, **49**, 703-705.
19. Lakomaa E.L., Rytömaa I. Scand. J. Dent. Res., 1977, **85**, 89-95.
20. Nakamoto T., Mallak H.M., Miller S.A. J. Dent. Res., 1979, **58**, 1115-1122.
21. Budd P., Montgomery J., Evans J., Trickett M. Sci. Total Environ.,2004, **318**, 45-58.
22. Cutress T.W. In: Curson, M.E., Cutress, T.W. (eds.) Trace Elements and Dental Diseases, p.33-105. PSG-John Wright, Boston, 1983.

23. Molleson T. In: Grupe G., Herrmann B. (eds). Trace Elements in Environmental History, p. 67-82. Springer-Verlag, Berlin, 1988.
24. Nixon G.S. Caries Res., 1969, **3**, 60-74.
25. Priest N.D., Van de Vyver F.L. (eds.): Trace Metals and Fluoride in Bones and Teeth. CRC Press, Boca Raton, Floride, 1990.
26. Grynpas M.D., Pritzker K.P., Hancock R.G.V. Biol. Trace Elem. Res., 1987, **13**, 333-344.
27. Zaichick V. Trace Elements in Medicine, 2004, **5**, 3, 17-22. (in Russian)
28. Zaichick V. J. Radioanal. Nucl. Chem., 2006, **269**, 653-659.
29. Zaichick V. J. Radioanal. Nucl. Chem., 2007, **271**, 573-576.
30. Korelo A.M., Zaichick V.Ye. In: Activation Analysis in Environment Protection, Dubna, JINR, 1993, pp.326-332.
31. Helle A., Haavikko K. Proc. Finn. Dent. Soc., 1977, **73**, 87-98.
32. Naujoks R., Schade H., Zelinka F. Caries Res., 1967, **1**, 2, 137-143.
33. Vernois V., Deschamps N., Boulay A., Goldberg M. In: Metals in Bone (Ed.: Priest N.D.). MTP Press, Lancaster, U.K., 1985, pp. 407-415.
34. De Sant'Anna G.R., Dos Santos E.A.P., Soares L.E.S., Do Espirito Santo A.M., Martin A.A., Duarte D.A., Pacheco-Soares C., Brugnera A. Photomedicine and Laser Surgery, 2009, **27**, 5, 771-782.
35. Norén J.G., Lodding A., Odelius H., Linde A. Caries Res., 1983, **17**, 6, 496-502.
36. Haataja J., Pohto P., Kleemola-Kujala E., Hausson L. Proc. Finn. Dent. Soc., 1972, **68**, 67-72.
37. Shashikiran N.D., Subba Reddy V.V., Hiremath M.C. Indian Journal of Dental Research, 2007, **18**, 4, 157-162.
38. Vernois V., Deschamps N., Revel G. J. Trace Elem. Electrolytes health Dis., 1989, **3**, 2, 67-70.
39. Owczarczak K. Czas. Stomat., 1985, **38**, 11-12, 752-757.
40. Nixon G.S., Helsby C.A. Arch. Oral Biol., 1976, **21**, 691-695.