ECRONICON
OPEN ACCESS

EC DENTAL SCIENCE
Research Article

The Effect of Age on Calcium, Chlorine, Potassium, Magnesium, Manganese, Sodium, Phosphorus, and Strontium Contents in the Roots of Male Permanent Teeth

Vladimir Zaichick[1]* and Sofia Zaichick[2]

[1]Radionuclide Diagnostics Department, Medical Radiological Research Centre, Russia

[2]Laboratory of Dr. Gabriela Caraveo Piso, Feinberg School of Medicine, Northwestern University, USA

***Corresponding Author:** Vladimir Zaichick, Radionuclide Diagnostics Department, Medical Radiological Research Centre, Russia.

Received: June 07, 2017; **Published:** June 16, 2017

Abstract

Objectives: The aim of the current study was to evaluate quantitatively the effect of age on calcium (Ca), chlorine (Cl), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), and strontium (Sr) contents in the roots of male permanent teeth.

Methods: The Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions were estimated in intact tooth root samples from apparently healthy 46 men, aged from 16 to 58 years. For chemical element mass fractions measurements, instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides was used.

Results: Mean values (M±SEM) for mass fraction (g/kg, dry mass basis) of chemical elements in the roots of male permanent teeth were: Ca 272±8, Cl 1.11±0.12, K 0.911±0.029, Mg 7.99±0.35, Mn 0.00142±0.00015, Na 6.62±0.24, P 148±5, and Sr 0.470±0.036. These mean values for Ca, Cl, Mg, Na, and P mass fractions in the tooth root were within a very wide range of reference data for tooth dentin and close to their median. The means of this work for K and Sr mass fraction were about 3-fold higher and for Mn mass fraction three orders of magnitude lower than the median value of means obtained by other researchers. To estimate the effect of age on the trace element contents we examined two age groups: 16 - 35 and 36 - 58 years. In addition, the Pearson correlation coefficient between age and trace element mass fraction was calculated.

Conclusions: Two ways of statistical data manipulation showed that mass fractions of Ca, Cl, K, Mg, Mn, Na, P, and Sr remain stable in the age range 16-58 years.

Keywords: *Human Tooth Root; Chemical Element Contents; Neutron Activation Analysis*

Abbreviations

INAA-SLR: Instrumental Neutron Activation Analysis with High Resolution Spectrometry of Short-Lived Radionuclides; SRM: Standard Reference Material; CRM: Certified Reference Material; BSS: Biological Synthetic Standards

Introduction

A full assessment of tooth condition requires exact knowledge of the nature and variation of the mineral matrix, including the contents of some apatite and related bulk chemical elements such as Ca, P, Mg, Na, K, and Cl [1,2]. It is known also that apatite phases can apparently be affected by trace element incorporation into teeth with consequent effects on the physicochemical properties [3,4]. This is why deficiency or excess of F, Sr, Mn and some other trace elements is one of the factors which determines the degree of susceptibility to caries and other dental diseases [5,6]. Thus, chemical element analysis of teeth expands the knowledge of etiology of dental diseases and may be used for diagnostic, therapeutic and preventive purposes. Furthermore, teeth have been suggested as monitors for human exposure to elements which accumulate in calcified tissues [7-11]. Accordingly, teeth are used in an occupational medicine and environmental health studies [12-15]. Moreover, elemental analysis of human teeth is often used in paleoanthropology for dietary and environment reconstruction to assess the social and economic status of human groups [16-21].

It should be emphasized that the chemical element contents are different in sound deciduous and permanent teeth and depend on the type of tooth. It should also be noted that the distribution of chemical elements in a tooth is not homogenous. It is therefore evident that for all applications mentioned above it is necessary to establish the normal levels and gender- and age-related changes of chemical elements in a large-scale study of deciduous and permanent teeth take into account type of tooth, part of tooth (crown and root), and tissue of tooth (enamel, dentin, cementum, and pulp).

There is an opinion that roots of permanent teeth are more suitable biological material for the assessment of internal calcified tissue status and the body's environmental exposure history, than crowns [22]. The root includes dentin, cementum, and pulp. Dentin comprises the main portion of the root.

The data on chemical element mass fractions in roots of permanent teeth is apparently extremely limited [22]. There are dozens of studies regarding chemical element content in dentin, using chemical techniques and instrumental methods [1,5,23-38]. However, the majority of these data are based on measurements of processed dentin. In many studies dentin samples are ashed and acid dissolved before analysis. In other cases, dentin samples are treated with solvents (distilled water, ethanol, formalin etc) and then are dried at high temperature for many hours. There is evidence that certain quantities of chemical elements are lost as a result of such treatment [39-41]. Moreover, only few of these studies employed quality control using certified reference materials (CRM) for determination of the chemical element mass fractions.

In our previous studies the peak calcification of human bones, tooth crown, and female tooth root in age about 30 - 35 years was found [42-67]. The primary purpose of this study was to determine reliable values for the calcium (Ca), chlorine (Cl), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), and strontium (Sr) mass fraction in the intact roots of permanent teeth of apparently healthy 16 - 58-year-old men using non-destructive instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR). The second aim was to compare the Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fraction in pre-peak period (group 1, 16 - 35 years old) with those in post-peak period (group 2, 36 - 58 years old) as well as to calculate the Pearson correlation coefficient between age and chemical element contents. The final aim was to estimate the inter-correlations of chemical elements in intact roots of male permanent teeth in the period of life from 16 to 58 years.

All studies were approved by the Forensic Medicine Department of Obninsk City Hospital and the Ethical Committee of the Medical Radiological Research Center.

Materials and Methods

Samples

Non-carious permanent teeth were collected at the Department of Forensic Medicine of the Obninsk Hospital. The molars and premolars were extracted early after death at necropsy (within 24 hours) from 46 men (age range 16 - 58 years). One tooth was obtained from each subject. The typical causes of death in most of these subjects included traffic accident, occupational injury and domestic trauma. All the deceased were citizens of Obninsk (Caucasian, European living habits). None of those who died a sudden death had suffered from any systematic or chronic disorders before. Obninsk is a small city in a non-industrial region 105 km south-west of Moscow.

Sample Preparation

After extraction teeth were immediately frozen at - 18°C until use. The titanium tools were used to cut and to scrub soft tissue and blood off the roots [68]. After separating the root from crown with a titanium knife, samples were freeze dried until constant mass was obtained. Only the roots were used in this study. After drying roots were weighed and sealed in thin polyethylene films washed with acetone and rectified alcohol beforehand. The sealed samples were placed in labeled polyethylene ampoules.

Method and reference materials

A horizontal channel with the pneumatic rabbit system of the WWR-c research nuclear reactor was used to determine Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions by instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR). To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [69]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. Ten standard reference material SRM NIST 1486 (Bone Meal) and certified reference material CRM IAEA H-5 (Animal Bone) sub-samples weighing about 100 mg were treated and analyzed in the same conditions that the teeth samples to estimate the precision and accuracy of results.

The basement of INAA is the irradiation of stable atoms in the sample by neutrons, the transmutation of atoms in radionuclides, and the spectrometry of their self-radiations. Details of nuclear reactions, radionuclides, gamma-energies, methods of analysis and the results of quality control were presented in our previous publications concerning the chemical elements of human bones and teeth [42-55].

Computer programs and statistic

A dedicated computer program of INAA mode optimization was used [70]. Using the Microsoft Office Excel programs, the summary of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for chemical element mass fractions. The difference in the results between two age groups was evaluated by parametric Student's t-test and non-parametric Wilcoxon-Mann-Whitney U-test. For the construction of "age – chemical element mass fraction" diagrams and the estimation of the Pearson correlation coefficient between age and chemical element contents as well as between different chemical elements the Microsoft Office Excel programs were also used.

Results

Table 1 indicates our data for Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions in ten sub-samples of CRM IAEA H-5 (Animal Bone) and SRM NIST 1486 (Bone Meal) and the certified values of this material.

Element	IAEA H5 Animal Bone	This work results	SRM NIST 1486 Bone Meal	This work results
	M ± SD	M ± SD	M ± SD	M ± SD
Ca	212 ± 24[a]	208 ± 4	266 ± 2[a]	267 ± 9
Cl	0.553 ± 193[a]	0.556 ± 0.050	-	-
K	0.637 ± 139[a]	0.620 ± 0.060	0.412 ± 0.004[a]	0.427 ± 0.031
Mg	3.53 ± 0.25[a]	3.62 ± 0.10	4.66 ± 0.17	4.59 ± 0.40
Mn	0.00076 ± 0.00024[b]	0.00085 ± 0.00010	0.00100[b]	0.00102 ± 0.00024
Na	4.77 ± 0.97[a]	4.82 ± 0.13	5.0[b]	5.48 ± 0.17
P	96 ± 44[a]	94.3 ± 2.5	123 ± 2[a]	123 ± 6
Sr	0.104 ± 0.018[a]	0.098 ± 0.008	0.264 ± 7[a]	0.251 ± 7

Table 1: *INAA-SLR data of trace element contents in certified reference material IAEA H-5 (Animal Bone) and SRM NIST 1486 (Bone Meal) compared to certified values (g/kg, dry mass basis).*

M: Arithmetical mean; SD: Standard deviation, a: Certified values; b: Information values

Figure 1 shows individual data sets for the Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fraction in the roots of male permanent teeth between ages 16 - 58 years and their trend lines.

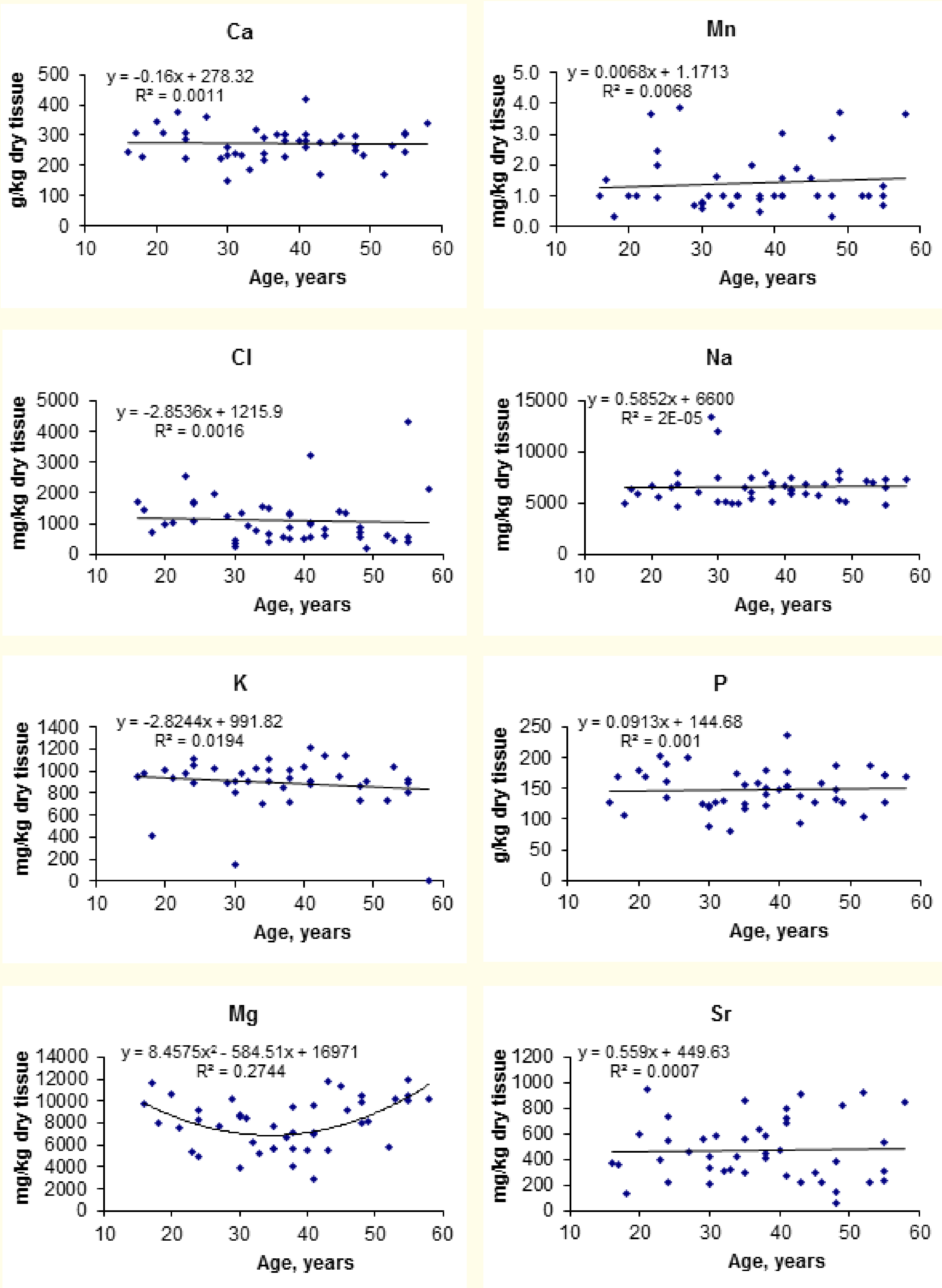

Figure 1: *Individual data sets for the Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fraction in the roots of male permanent teeth between ages 16 - 58 years and their trend lines.*

Table 2 depicts our data for basic statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions (g/kg, dry mass basis) in the intact roots of male permanent teeth.

Element	Mean	SD	SEM	Min	Max	Med.	P0.025	P0.975
Ca	272	54	8.0	150	418	277	170	374
Cl	1.11	0.79	0.12	0.207	4.32	0.903	0.262	3.14
K	0.911	0.187	0.029	0.154	1.21	0.921	0.408	1.14
Mg	7.99	2.32	0.35	2.89	12.0	8.09	3.87	11.8
Mn	0.00142	0.00094	0.00015	0.00034	0.00389	0.00100	0.00035	0.00372
Na	6.62	1.62	0.24	4.73	13.4	6.51	4.79	11.6
P	148	32.9	4.9	79.0	237	149	88.7	202
Sr	0.470	0.236	0.036	0.062	0.946	0.418	0.135	0.920

Table 2: *Basic statistical parameters of Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fraction (g/kg, dry mass basis) in the intact roots of male permanent teeth.*

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

In order to estimate the effect of age on the investigated parameters we used two groups: one with young males, 16 - 35 years, and one with older females, 36 - 58 years. Results are shown in Table 3. In addition, the Pearson correlation coefficient between age and chemical element mass fraction was calculated (Table 4).

Element	Intact roots of male permanent teeth				Ratio
	AG1 6-35 years n = 22	AG2 36-58 years n = 24	t-test *p* ≤	U-test p	AG2 to AG1
Ca	266 ± 13	278 ± 10	0.491	> 0.05	1.05
Cl	1.15 ± 0.13	1.08 ± 0.19	0.761	> 0.05	0.94
K	0.892 ± 0.050	0.929 ± 0.030	0.536	> 0.05	1.04
Mg	7.67 ± 0.47	8.25 ± 0.51	0.405	> 0.05	1.08
Mn	0.00133 ± 0.00021	0.00150 ± 0.00021	0.565	> 0.05	1.13
Na	6.69 ± 0.48	6.56 ± 0.18	0.815	> 0.05	0.98
P	143 ± 8	152 ± 6	0.355	> 0.05	1.06
Sr	0.457 ± 0.046	0.482 ± 0.055	0.726	> 0.05	1.05

Table 3: *Differences between mean values (M±SEM) of chemical element mass fractions (g/kg, dry mass basis) in the intact roots of male permanent teeth of two age groups and ratio of means.*

M: Arithmetic Mean; SEM: Standard Error of Mean; t-test: Student's t-Test; U-test: Wilcoxon-Mann-Whitney U-Test

Element	Ca	Cl	K	Mg	Mn	Na	P	Sr
Age	-0.034	-0.040	0.054	0.179	0.082	0.004	0.031	0.027

Table 4: *Correlations between age and chemical element mass fractions in the intact roots of male permanent teeth (r – coefficient of correlation).*

The data of inter-correlation calculations (values of r – coefficient of correlation) including all chemical elements identified by us are presented in Table 5.

Element	Ca	Cl	K	Mg	Mn	Na	P	Sr
Ca	1.00	0.594[c]	0.126	0.205	0.393[b]	-0.049	0.903[c]	0.137
Cl	0.594[c]	1.00	0.279[a]	0.022	0.260[a]	0.091	0.516[c]	0.058
K	0.126	0.279[a]	1.00	-0.175	0.240	-0.088	0.205	0.209
Mg	0.205	0.022	-0.175	1.00	0.002	-0.018	0.138	-0.142
Mn	0.393[b]	0.260[a]	0.240	0.002	1.00	-0.112	0.368[a]	0.223
Na	-0.049	0.091	-0.088	-0.018	-0.112	1.00	-0.018	-0.032
P	0.903[c]	0.516[c]	0.205	0.138	0.368[a]	-0.018	1.00	0.111
Sr	0.137	0.058	0.209	-0.142	0.223	-0.032	0.111	1.00

Table 5: *Intercorrelations of the chemical element mass fractions in intact roots of male permanent teeth (r – coefficient of correlation).*

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

The comparison of our results with published data for Ca, Cl, K, Mg, Mn, Na, P, and Sr mass fractions in the dentin and roots of intact permanent teeth is shown in Table 6.

Element	Published data [Reference]			This work
	Median of means (*n*)*	Minimum of means M or M±SD, (*n*)**	Maximum of means M or M±SD, (*n*)**	M±SD n=46
Ca	275 (43)	57.7±18.3 (3) [32]	418 (1) [23]	272 ± 54
Cl	0.82 (16)	0.078 (30) [30]	26.7 (1) [28]	1.11 ± 0.79
K	0.333 (8)	0.170 (175) [1]	0.744 (-) [34]	0.911 ± 0.187
Mg	8.50 (35)	0.211 (1) [28]	14.222 (-) [31]	7.99 ± 2.32
Mn	2.85 (24)	0.00019±0.00006 (15) [24]	111 (2) [26]	0.00142 ± 0.00094
Na	5.2(19)	0.54 (25) [29]	58 (1) [27]	6.62 ± 1.62
P	135 (39)	48.9 ± 1.1 (3) [32]	211 (1) [28]	148 ± 33
Sr	0.157(19)	0.064 (10) [25]	1.11 (2) [26]	0.470 ± 0.236

Table 6*: Median, minimum and maximum value of means of chemical element mass fractions (g/kg, dry mass basis) in the intact dentin and roots of human permanent teeth according to data from the literature in comparison with this work results (intact roots of female permanent teeth). M: Arithmetic Mean; SD: Standard Deviation; (n)*: Number of all References; (n)**: Number of Samples*

Discussion

A good agreement of the Ca, Cl, K, Mg, Mn, Na, P, and Sr contents analyzed by INAA-SLR with the certified data of CRM IAEA H-5 (Animal Bone) and SRM NIST 1486 (Bone Meal) (Table 1) demonstrates an acceptable accuracy of the results obtained in the study of chemical element contents in the intact roots of male permanent teeth. presented in Tables 2-6.

The mean values and all selected statistical parameters were calculated for 8 (Ca, Cl, K, Mg, Mn, Na, P, and Sr) chemical elements (Table 2). Our data for 8 chemical element mass fractions in intact roots of permanent male teeth may serve as indicative normal values for non-carious teeth roots of an urban male population of the Russian Central European region, since all subjects were Obninsk citizens and none, who suffered sudden death, had systematic or chronic disorders.

In the intact roots of two age groups, we have not observed a change in mass fraction of Ca, Cl, K, Mg, Mn, Na, P, and Sr with age (Table 3). Moreover, the Pearson correlation coefficient between age and chemical element mass fraction did not show any statistically significant dependence (Table 4). Thus, it was found that the mass fractions of Ca, Cl, K, Mg, Mn, Na, P, and Sr in the intact roots of male permanent teeth remain stable in age from 15 to 55 years.

In the roots of male permanent teeth a statistically significant direct correlation was found, for example, between the mass fraction of Ca and P ($r = 0.903$, $p \le 0.001$), the mass fraction Ca and Cl ($r = 0.594$, $p \le 0.001$), the mass fraction Ca and Mn ($r = 0.393$, $p \le 0.01$), the mass fraction Cl and K ($r = 0.279$, $p \le 0.05$), the mass fraction Cl and Mn ($r = 0.260$, $p \le 0.05$), the mass fraction Cl and P ($r = 0.516$, $p \le 0.001$), and the mass fraction Mn and P ($r = 0.368$, $p \le 0.05$) (Table 5). If some positive correlations between the elements were predictable (e.g., Ca-P), the interpretation of other observed relationships requires further study for a more complete understanding.

For almost lack of reported data of chemical element contents in tooth root we compared our results with published data on tooth dentin and roots [1,5,23-38]. It was acceptable because a tooth root mainly consists of dentin. A number of values for chemical element mass fractions were not expressed on a dry mass basis by the authors of the cited references. However, we calculated these values using published data for water - 10% and ash - 72% contents (on wet mass basis) in dentin of intact permanent teeth [71]. The mean values measured for mass fraction of Ca, Cl, Mg, Na, and P, as shown in Table 6, agree well with medians of the mean values cited by other researchers for tooth dentin. The means of this work for K and Sr mass fraction are about 3-fold higher and for Mn mass fraction three orders of magnitude lower than the median value of means obtained by some other researchers, though they are within the reported range of these mean values. It is known that Sr content in the dentin depends greatly on biogeochemical peculiarities of the residence area [72,73], and this may be the cause for the high Sr content in teeth 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 is respectively large (Tables 2 and 3). This is due to the very wide individual variation of element mass fractions in the intact roots. In particular individual contents of Mn and Sr appear to vary widely between 0.00034 and 0.00389 and between 0.062 and 0.946 g/kg on dry mass basis, respectively. The very wide individual variation of element mass fractions in the intact roots confirms reported data [1,5, 23-38].

Conclusion

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.

Mean values ($M \pm SEM$) for mass fraction (g/kg, dry mass basis) of chemical elements in the roots of male permanent teeth were: Ca 272 ± 8, Cl 1.11 ± 0.12, K 0.911 ± 0.029, Mg 7.99 ± 0.35, Mn 0.00142 ± 0.00015, Na 6.62 ± 0.24, P 148 ± 5, and Sr 0.470 ± 0.036. Two ways of statistical data manipulation; (1) the comparison data for different age groups of 16 - 35 and 36 - 58 years and (2) the Pearson correlation coefficient between age and chemical element mass, showed that mass fractions of Ca, Cl, K, Mg, Mn, Na, P, and Sr remain stable in the age range 16 - 58 years.

This study has several limitations. Firstly, analytical techniques employed in this study measure only 8 chemical elements (Ca, Cl, K, Mg, Mn, Na, P, and Sr) mass fractions. Future studies should be directed toward using other analytical methods which allow extend the list of chemical elements investigated in in the root samples of permanent teeth. Secondly, generalization of our results may be limited to teeth of Russian men. In spite of these limitations, this is the first study that elucidate the relationship between age and chemical elements levels in the intact root of male permanent teeth.

Conflict of Interest

There is no any financial interest or any conflict of interest.

Bibliography

1. Derise NL., *et al*. "Mineral composition of normal human enamel and dentine and the relation of composition to dental caries: I. Macrominerals and comparison of methods of analyses". *Journal of Dental Research* 53.4 (1974): 847-852.
2. Curzon MEJ and Cutress TW. "Trace Elements and Dental Disease". PSG-John Wright, Boston (1983).
3. Koray M., *et al*. "The effects of fluorides and/or trace elements on the solubilities of enamel and cementum". *Journal of Trace Elements in Medicine and Biology* 10 (1996): 255-259.
4. LeGeros RZ., *et al*. "The effect of trace elements on the lattice parameters of human and synthetic apatites". In: Calcified Tissues 1976. Leeds, UK (1977): 362-367.
5. Derise NL and Ritchey SJ. "Mineral composition of normal human enamel and dentine and the relation of composition to dental caries: II. Microminerals". *Journal of Dental Research* 53.4 (1974): 853-858.
6. Davies BE and Anderson RJ. "The epidemiology of dental caries in relation to environmental trace elements". *Experimentia* 43.1 (1987): 87-92.
7. Altshuller LF., *et al*. "Deciduous teeth as an index of body burden of lead". *Journal of Pediatrics* 60 (1962): 224-229.
8. Sharon IM. "The significance of teeth in pollution detection". *Perspectives in Biology and Medicine* 32.1 (1988): 124-131.
9. Zaichick V and Ovchjarenko NN. "In vivo X-ray fluorescent analysis of Ca, Zn, Sr, and Pb in frontal tooth enamel". *Journal of Trace and Microprobe Techniques* 14 (1996): 143-152.
10. Zaichick V., *et al*. "In vivo energy dispersive x-ray fluorescence for measuring the content of essential and toxic trace elements in teeth". *Journal of Applied Radiation and Isotopes* 50.2 (1999): 283-293.
11. Zaichick V. "Neutron activation analysis of Ca, Cl, K, Mg, Mn, Na, P, and Sr contents in the crowns of human permanent teeth". *Journal of Radioanalytical and Nuclear Chemistry* 281.1 (2009): 41-45.
12. Alexander LM., *et al*. "Relative exposure of children to lead from dust and drinking water". *Archives of Environmental Health* 48.6 (1993): 392-400.
13. Baranska-Gadiowska M., *et al*. "Cu content in teeth of children living in the areas contaminated by Zn, Cd, and Pb". *Czasopismo Stomatologiczne* 41 (1988): 406-410.
14. Bercovitz K and Laufer D. "Tooth type as indicator of exposure to lead of adults and children". *Archives of Oral Biology* 35.11 (1990): 895-897.

15. Bloch P., *et al*. "Assessment of lead exposure of children from K-XRF measurements of shed teeth". *Journal of Applied Radiation and Isotopes* 49.5-6 (1998): 703-705.

16. Attramadal A and Jonsen J. "Heavy trace elements in ancient Norwegian teeth". *Acta Odontologica Scandinavica* 36.2 (1978): 97-101.

17. Baluszynska L., *et al*. "Distribution analysis of macro and microelements in the human teeth sequences in aspect of the diet and dynamics of tooth development". In: Ermidou-Pollet S and Pollet S, editors. 3rd International Symposium on Trace elements in human: New Perspectives. Athens, Greece (2001): 364-372.

18. Budd P., *et al*. "Human lead exposure in England from approximately 5500 BP to the 16th century AD". *Science of the Total Environment* 318.1-3 (2004): 45-58.

19. Glen-Haduch E., *et al*. "Cribra orbitalia and teeth element content in human from Neolithic and Early Bronze Age graves in southern Poland". *Journal of Physical Anthropology* 103.2 (1997): 201-207.

20. Grandjean P and Jorgensen PJ. "Retention of lead and cadmium in prehistoric and modern human teeth". *Environmental Research* 53.1 (1990): 6-15.

21. Kuhnlein HV and Calloway DM. "Minerals in human teeth: Differences between preindustrial and contemporary Hopi Indians". *American Journal of Clinical Nutrition* 30.6 (1977): 883-886.

22. Malara P., *et al*. "Do the levels of selected metals differ significantly between the roots of carious and non-carious teeth?" *Science of the Total Environment* 369 (2006): 59-68.

23. Murray MM and Bowes JH. "The composition of enamel, dentine and root in caries and pyorrhea". *British Dental Journal* 61 (1936): 473-477.

24. Söremark R and Samsahl K. "Gamma-ray spectrometric analysis of elements in normal human dentine". *Journal of Dental Research* 41 (1962): 603-606.

25. Lundberg M., *et al*. "Gamma-ray spectrometric analysis of some elements in coronal dentine of unerupted human teeth". *Odontologisk Revy* 16 (1965): 97-100.

26. Hardwick JL and Martin CJ. "A pilot study using mass spectromenry for the estimation of the trace element content of dental tissues". *Helvetica Odontologica Acta* 11.1 (1967): 62-70.

27. Deconninck G. "Analyse des microquantites d'elements traces dans des substences sdides par bombardement d'ions". *Journal of Radioanalytical Chemistry* 17.1 (1973): 29-43.

28. Ahlberg M and Akselsson R. "Proton-induced X-ray emission in the trace analysis of human tooth enamel and dentine". *Applied Radiation and Isotopes* 22 (1976): 279-290.

29. Lakomaa EL and Rytömaa I. "Mineral composition of enamel and dentin of first and permanent teeth in Finland". *Scandinavian Journal of Dental Research* 85.2 (1977): 89-95.

30. Cohen DD., *et al*. "Preliminary investigations of trace element concentrations in human teeth". *Nuclear Instruments and Methods* 188 (1981): 203-209.

31. Knychalska-Karwan Z., *et al*. "Ultrastructural analysis quantitative distribution of macro- and microelements in young teeth hard tissues by the use of an X-ray microanalysis". *Folia Histochemica et Cytobiologica (Krakow)* 19.2 (1981): 87-91.

32. Del Pilar Gutiérrez-Salazara M and Reyes-Gasgaa J. "Microhardness and Chemical Composition of Human Tooth". Materials Research 6.3 (2003): 367-373.

33. Gierat-Kucharzewska B., *et al*. "Concentration of selected elements in the roots and crowns of both primary and permanent teeth with caries disease". *Biological Trace Element Research* 96.1-3 (2003): 159-166.

34. Shishkina EA., *et al*. "Assessment of 90Sr concentration in dental tissue using thin layer beta-particle detectors and verification with numerical calculations". *Radiation Research* 163.4 (2005): 462-467.

35. Nagai K., *et al*. "Morphological and compositional changes in human teeth following 9.6-μm CO2 laser irradiation in vitro". *Journal of Oral Laser Applications* 6.4 (2006): 265-276.

36. Saiki M., *et al*. "Elemental comparison in sound and carious human teeth by instrumental neutron activation analysis". *Journal of Radioanalytical and Nuclear Chemistry* 282 (2009): 29-32.

37. Dilber E., *et al*. "Effect of various laser irradiations on the mineral content of dentin". *European Journal of Dentistry* 7.1 (2013): 74-80.

38. Liu H., *et al*. "Study of P, Ca, Sr, Ba and Pb Levels in Enamel and Dentine of Human Third Molars for Environmental and Archaeological Research". *Advances in Anthropology* 3.2 (2013): 71-77.

39. Zaichick V. "Sampling, sample storage and preparation of biomaterials for INAA in clinical medicine, occupational and environmental health". In: Harmonization of Health-Related Environmental Measurements Using Nuclear and Isotopic Techniques. IAEA, Vienna (1997): 123-133.

40. Zaichick V. "Losses of chemical elements in biological samples under the dry ashing process". *Trace Elements in Medicine* 5 (2004): 17-22.

41. Zaichick V. "Medical elementology as a new scientific discipline". *Journal of Radioanalytical and Nuclear Chemistry* 269.2 (2006): 303-309.

42. Zaichick V., *et al*. "INAA application in the age dynamics assessment of major, minor, and trace elements in the human rib". *Journal of Radioanalytical and Nuclear Chemistry* 244.1 (2000): 189-193.

43. Zaichick V and Tzaphlidou M. "Determination of calcium, phosphorus, and the calcium/phosphorus ratio in cortical bone from the human femoral neck by neutron activation analysis". *Journal of Applied Radiation and Isotopes* 56.6 (2002): 781-786.

44. Tzaphlidou M and Zaichick V. "Calcium, phosphorus, calcium-phosphorus ratio in rib bone of healthy humans". *Biological Trace Element Research* 93.1-3 (2003): 63-74.

45. Zaichick V and Tzaphlidou M. "Calcium and phosphorus concentrations and calcium/phosphorus ratio in trabecular bone from femoral neck of healthy humans as determined by neutron activation analysis". *Journal of Applied Radiation and Isotopes* 58.6 (2003): 623-627.

46. Zaichick V. "INAA application in the age dynamics assessment of Ca, Cl, K, Mg, Mn, Na, P, and Sr contents in the cortical bone of human femoral neck". *Journal of Radioanalytical and Nuclear Chemistry* 259.2 (2004): 351-354.

47. Zaichick V. "NAA of Ca, Cl, K, Mg, Mn, Na, P, and Sr contents in the human cortical and trabecular bone". *Journal of Radioanalytical and Nuclear Chemistry* 269.3 (2006): 653-659.

48. Zaichick V. "INAA application in the assessment of selected elements in cancellous bone of human iliac crest". Journal of Radioanalytical and Nuclear Chemistry 271.3 (2007): 573-576.

49. Zaichick V and Zaichick S. "Instrumental neutron activation analysis of trace element contents in the rib bone of healthy men". *Journal of Radioanalytical and Nuclear Chemistry* 281.1 (2009): 47-52.

50. Zaichick V., *et al*. "The effect of age and gender on Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Sr, V, and Zn contents in rib bone of healthy humans". *Biological Trace Element Research* 129.1-3 (2009): 107-115.

51. Zaichick S and Zaichick V. "The effect of age and gender on 38 chemical element contents in human iliac crest investigated by instrumental neutron activation analysis". *Journal of Trace Elements in Medicine and Biology* 24.1 (2010): 1-6.

52. Zaichick S and Zaichick V. "The effect of age and gender on 38 chemical element contents in human femoral neck investigated by instrumental neutron activation analysis". *Biological Trace Element Research* 137.1 (2010): 1-12.

53. Zaichick S and Zaichick V. "Human bone as a biological material for environmental monitoring". *International Journal of Environment and Health* 4.2-3 (2010): 278-292.

54. Zaichick S and Zaichick V. "Neutron activation analysis of trace element contents in the bone samples of human iliac crest". In: Neutron Spectroscopy, Nuclear Structure, Related Topics. Joint Institute for Nuclear Research, Dubna, Russia (2010): 117-124.

55. Zaichick S., *et al*. "The effect of age and gender on the lithium content in rib bone of healthy humans". *Journal of Trace Elements and Electrolytes* 27.4 (2010): 258-261.

56. Zaichick S., *et al*. "The effect of age and gender on 59 trace element contents in human rib bone investigated by inductively coupled plasma mass spectrometry". *Biological Trace Element Research* 143.1 (2011): 41-57.

57. Zaichick S., *et al*. "Accumulation of rare earth elements in human bone within the lifespan". *Metallomics* 3 (2011): 186-194.

58. Zaichick S and Zaichick V. "Relationship between Ca, Cl, K, Mg, Mn, Na, P, and Sr contents in the human rib bone investigated by neutron activation analysis". In: Neutron Spectroscopy, Nuclear Structure, Related Topics. Joint Institute for Nuclear Research, Dubna, Russia (2012): 349-356.

59. Zaichick V. "Data for the Reference Man: skeleton content of chemical elements". Radiation and Environmental Biophysics 52.1 (2013): 65-85.

60. Zaichick S and Zaichick V. "Relationship between Ca, Cl, K, Mg, Na, P, and Sr contents in the intact cortical bone of human iliac crest investigated by NAA". In: Fundamental Interactions and Neutrons, Neutron Spectroscopy, Nuclear Structure, Related Topics. Joint Institute for Nuclear Research, Dubna, Russia (2013): 295-302.

61. Zaichick V. "Chemical elements of human bone tissue investigated by nuclear analytical and related methods". *Biological Trace Element Research* 153.1-3 (2013): 84-99.

62. Zaichick S and Zaichick V. "Relationship between Ca, Cl, K, Mg, Na, P, and Sr contents in the intact trabecular bone of human iliac crest investigated by neutron activation analysis". In: Neutron Spectroscopy, Nuclear Structure, Related Topics. Joint Institute for Nuclear Research, Dubna, Russia (2014): 322-329.

63. Zaichick V and Zaichick S. “Relationship between Ca, Cl, K, Mg, Mn, Na, P, and Sr contents in the intact trabecular bone of human femoral neck investigated by neutron activation analysis”. In: Fundamental Interactions and Neutrons, Nuclear Structure, Ultracold Neutrons, Related Topics. Joint Institute for Nuclear Research, Dubna, Russia (2015): 334-341.

64. Zaichick V. “Neutron activation analysis of trace element contents in the crowns of human permanent teeth”. In: Neutron Spectroscopy, Nuclear Structure, Related Topics. Joint Institute for Nuclear Research, Dubna, Russia (2009): 288-295.

65. Zaichick V and Zaichick S. “The effect of age and gender on calcium, phosphorus, and calcium-phosphorus ratio in the roots of permanent teeth”. *The Journal of Dentist* 2 (2014): 78-89.

66. Zaichick V and Zaichick S. “The effect of age on Ca, Cl, K, Mg, Mn, Na, P, and Sr contents in the roots of female permanent teeth”. *Journal of Radioanalytical and Nuclear Chemistry* 309.1 (2016): 295-301.

67. Zaichick V and Zaichick S. “The effect of age and gender on calcium, phosphorus, and calcium-phosphorus ratio in the crowns of permanent teeth”. *EC Dental Science* 5 (2016): 1030-1046.

68. Zaichick V and Zaichick S. “Instrumental effect on the contamination of biomedical samples in the course of sampling”. *Journal of Analytical Chemistry* 51 (1996): 1200-1205.

69. Zaichick V. “Application of synthetic reference materials in the Medical Radiological Research Centre”. *Fresenius’ Journal of Analytical Chemistry* 352.1 (1995): 219-223.

70. Korelo AM and Zaichick V. “Software to optimize the multi-elemental INAA of medical and environmental samples”. In: Activation Analysis in Environment Protection. Join Institute on Nuclear Research, Dubna, Russia (1993): 326-332.

71. Burnett GW and Zenewitz JA. “Studies of the composition of teeth. VII. The moisture content of calcified tissues”. *Journal of Dental Research* 37 (1958): 581-589.

72. Nixon GS and Helsby CA. “The relationship between strontium in water supplies and human tooth enamel”. *Archives of Oral Biology* 21.11 (1976): 691-695.

73. Cutress TW. “Teeth, calculus and bone”. In: Curson ME and Cutress TW, editors. Trace Elements and Dental Diseases. PSG-John Wright, Boston (1983): 33-105.

Volume 11 Issue 4 June 2017