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Microelemental Composition of Saliva Stones

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Abstract

A microelemental composition has been studied for the collection of saliva stone samples taken from patients living in the Novosibirsk, Vladivostok and Omsk. The analysis of the microelemental composition of the samples has demonstrated that the elements under determination are distributed in an extremely non-uniform manner throughout saliva stone groups for the patients living in different regions. So, saliva stones from the Vladivostok collection are characterized by a higher content of selenium, iodine, vanadium, chromium and manganese; however, they less often contain indium and cadmium as compared to the samples from the Novosibirsk and Omsk. There is more titanium, copper and zirconium contained in saliva stones from the Omsk collection comparing to the samples from the Novosibirsk and Vladivostok, whereas a number of the samples demonstrate an almost complete absence of selenium and iodine, which could be caused by a specific character of the region.

Key words: saliva stones, X-ray fluorescence elemental analysis, microelemental composition, regional ecology

INTRODUCTION

It is known that solid and soft tissues, as well as the blood of humans contain microelements playing an important role in the functioning of an organism [1, 2]. The level of microelemental content increased with respect to the normal one for an organism of a human being could be caused both by naturally occurring factors, and by a man-caused environmental pollution. Nowadays the extent of the man-caused pollution have reached a considerable level, which promotes the growth of disease incidence and pathologies, including pathogenic mineralization almost within all the tissues and organs including salivary glands in humans.

The pathogenic organomineral formations in the maxillofacial sphere (saliva stones) are found out in patients already since the age of 12 [3]. They are known to be formed from saliva more frequently within the submandibular glands and their canals [4]. In the ducts one can observe the formation of cylindrically shaped oblong stones, whereas in the glands the stones formed are round, cylindrical or shapeless, *i. e.* the shape of stones depends on the location and on the fact whether they are single or multiple. The size of the organomineral formations varies within the range from several millimetres to several centimetres. As a rule, saliva stones represent rhythmically deposited layers of organic and inorganic nature around a central nucleus [5] presented either by leukocyte clusters, or by alien bodies (a slice of a seed shell, fish bone, *etc.*) [6]. According to [6], saliva stones use to arise due to the abnormality in the outflow of saliva or due to deranged metabolism with respect to any of its elements (more often due to calcium metabolic disturbance) and a considerable participation of microorganisms. The authors of the review [7] have concluded that the formation of this pathogenic mineralization is connected with inflammatory processes in salivary glands, plastic surgical operations and improper nutrition. In addition, the occurrence of pathogenic organomineral formations in a human organism could be promoted by environmental conditions, including the condition of patients' living environment [8–10].

The goal of the present work consisted in the investigation and comparative analysis of the microelemental composition for the sample collection saliva stones taken from patients living in different regions, in order to reveal interrelations between the data obtained and the condition of living environment.

EXPERIMENTAL

As the objects of our investigation we used 19 samples of saliva stones taken from patients living in the Vladivostok, 12 samples taken from patients living in the Novosibirsk and 11 samples taken from patients of living in the Omsk. The mineral (phase) composition of pathogenous minerals was established earlier with the use of XFA [11, 12]. According to the XFA data, the basic mineral component in all the samples of saliva stones from the Omsk collection is presented by hydroxyapatite. Among five samples the three are composed by hydroxyapatite and an organic component, one of the samples alongside with hydroxyapatite contains whitlockite, another sample contains brushite [12]. According to IR spectroscopy, mineral components are prevailing in the composition of saliva stones.

All the samples of saliva stones were analyzed at the energy dispersive X-ray fluorescent elemental analysis VEPP-3 station on the basis of the Siberian Synchrotron Radiation Centre of the INP, SB RAS (Novosibirsk). X-ray fluorescence spectra of the samples under investigation were excited by a beam of 25 keV polarized monochromated radiation. In order to prepare samples, a powder was compacted to obtain pellets 30 mg in weight and 5 mm diameter, with superface density 0.15 g/cm^2. Two excitation energy levels were employed: 22 keV for the quantitative determination of Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, Ga, As, Se, Br, Rb, Sr, Y, Zr, Nb, Mo, Pb, Th, U as well as 42 keV for the determination of Ag, Cd, In, Sn, Sb, Te, I, Cs, Ba, La and Ce.

The calculation of elemental content was carried out by means of an external standard technique. Reliably certified standards of rocks such as ST-1a (trap), SA-1 (aleurolite), SG-2 (granite), SL-1 (limestone) were used as reference. The correctness of employing similar standards is confirmed by the investigation of the phosphorite sample proposed as International standard BCR-32 [13, 14], as well as by the results obtained from atomic emission spectral analysis with inductively coupled plasma (AES-ICP). Normalizing coefficients for the calculation of the content for the elements those were not certified by the aforementioned standards have been obtained *via* the interpolation of corresponding data for neighboring groups of chemical elements. The detection limits for the elements under loading the spectrometric path with the frequency of 10 kHz and measurement time of 1000 s ranged within $(0.1-2.0) \cdot 10^{-4}$ %. The processing of X-ray fluorescence spectra was performed by means of special software. For the quantitative calculation we used an external standard technique. The measurements were carried out in triplets. The determination error for the elements amounted to 2–5 rel. %.

RESULTS AND DISCUSSION

The analysis of microelemental composition demonstrates that the distribution of the elements under investigation throughout the groups of saliva stones exhibits non-uniform character for samples of different collections. For each series of samples we performed examining the character of variants distribution, rejection of crude errors, calculation and comparison of confidence intervals of the experimental data obtained (Table 1).

For a number of elements we observed a considerable scatter of the content even within the samples from the same collection. For example, the content of selenium in the samples of the Vladivostok collection varies from 3663 to 40.8 μg/g (except for one sample where it amounts to only 1.11 μg/g), the content of iodine varies from 5466 to 3.08 μg/g. In the samples of the Novosibirsk collection the content of selenium varies within the range of 190–1.7μg/g, the content iodine ranges within 261–0.5 μg/g. A high content of selenium (1318 μg/g) has been revealed in the only sample of the Novosibirsk collection. The data obtained allow us to assume that the microele-

TABLE 1

Average values for the content of elements in saliva stone samples under investigation taken from patients living in different regions, mass %

Element	Region		
	Omsk	Vladivostok	Novosibirsk
K	0.316±0.079	0.419±0.049	0.247±0.014
Ti	$(5.81\pm1.19)\cdot10^{-2}$	–	–
V	$(2.89\pm0.31)\cdot10^{-2}$	$(1.42\pm0.38)\cdot10^{-3}$	$(4.86\pm1.84)\cdot10^{-4}$
Cr	$(7.3\pm1.4)\cdot10^{-3}$	$(1.32\pm0.33)\cdot10^{-3}$	$(4.56\pm2.15)\cdot10^{-4}$
Mn	–	$(8.03\pm2.68)\cdot10^{-4}$	$(2.74\pm0.72)\cdot10^{-4}$
Fe	$(6.7\pm2.4)\cdot10^{-3}$	$(7.59\pm1.37)\cdot10^{-3}$	$(9.45\pm4.23)\cdot10^{-3}$
Ni	$(4.7\pm2.2)\cdot10^{-4}$	$(3.86\pm2.29)\cdot10^{-4}$	$(4.87\pm0.46)\cdot10^{-4}$
Cu	$(1.3\pm0.8)\cdot10^{-3}$	$(1.10\pm0.47)\cdot10^{-3}$	$(5.35\pm0.57)\cdot10^{-4}$
Zn	$(2.97\pm0.75)\cdot10^{-3}$	$(3.50\pm0.97)\cdot10^{-3}$	$(2.98\pm1.71)\cdot10^{-3}$
Sr	$(3.6\pm1.7)\cdot10^{-3}$	$(3.84\pm0.66)\cdot10^{-3}$	$(3.97\pm0.78)\cdot10^{-3}$
Zr	$(1.6\pm0.2)\cdot10^{-4}$	–	–
Ba	$(6.9\pm3.4)\cdot10^{-4}$	$(6.77\pm2.94)\cdot10^{-4}$	$(3.47\pm2.27)\cdot10^{-4}$
La	$(6.3\pm0.9)\cdot10^{-4}$	–	–
I	$(5.9\pm3.7)\cdot10^{-3}$	$(5.88\pm3.82)\cdot10^{-2}$	$(5.52\pm4.86)\cdot10^{-3}$
Ga	$(2.8\pm0.4)\cdot10^{-4}$	$(1.26\pm0.25)\cdot10^{-4}$	$(3.76\pm0.28)\cdot10^{-4}$
As	$(2.1\pm0.9)\cdot10^{-4}$	–	–
Se	$(2.8\pm1.1)\cdot10^{-4}$	$(6.88\pm4.67)\cdot10^{-2}$	$(1.41\pm2.30)\cdot10^{-2}$
Br	$(3.9\pm2.8)\cdot10^{-3}$	$(3.35\pm2.11)\cdot10^{-3}$	$(8.84\pm5.58)\cdot10^{-4}$
Ge	–	$(1.81\pm0.34)\cdot10^{-4}$	$(3.35\pm0.34)\cdot10^{-4}$

mental composition of biominerals formed in salivary glands as in the systems closed enough is determined by the living environment of a patient as well as by the features of metabolic processes in his organism.

The interrelation between microelemental composition of saliva stones and the region where the patients are living is confirmed by the results of discriminant analysis (Statistica 6.0, StatSoft). The scatter plot of canonical discriminant analysis (Figure 1) clearly demonstrates the collections of saliva stones taken from patients living in different regions to be distinctly separate. Similar laws have been found out earlier for the collections of nephroliths, gallstones and odontoliths [15].

Separate elements are distributed throughout the groups of samples from different collections in an extremely non-uniform manner (Fig. 2). For example, whereas chromium, copper, nickel, titanium and vanadium prevail in the composition samples from the Omsk collection, manganese, selenium and iodine are inherent in the composition of samples from

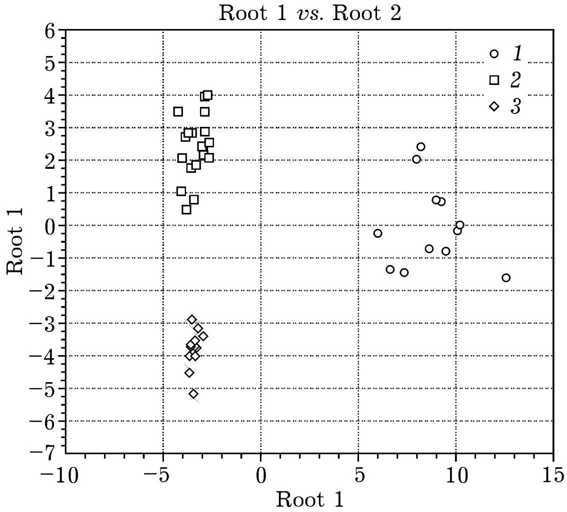

Fig. 1. Scatter plot for the group distribution of saliva stones taken from patients living in different regions: *1* – Omsk, *2* – Vladivostok, *3* – Novosibirsk.

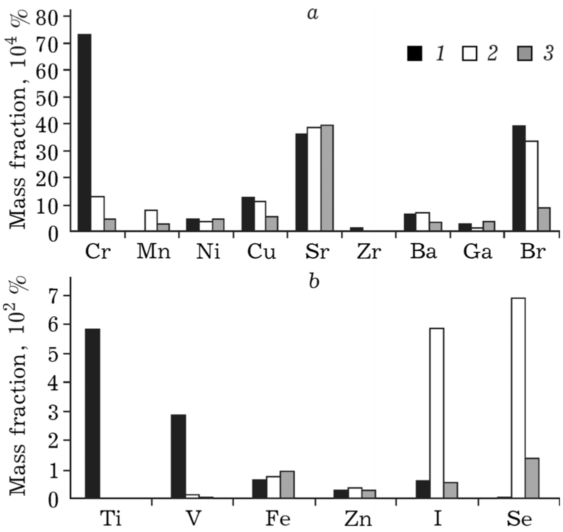

Fig. 2. Regional features of microelemental distribution in the saliva stones taken from patients living in different regions: Omsk (*1*), Vladivostok (*2*), and Novosibirsk (*3*).

Vladivostok. The samples of the Novosibirsk collection are characterized by an increased content of iron and strontium as compared to other collections. To all appearance, the elemental composition of various biological substrates including saliva stones reflects a total inflow of pollutants into a human organism. So, according to the data received from the Ob-Irtysh Interregional Territorial Department for hydrometeorology and environmental monitoring, the Irtysh River water is to a considerable extent polluted with zinc (14 MPC), phenols (20 MPC), iron (24 MPC), copper (23 MPC), mineral oil (23 MPC), compounds of manganese (44 MPC). The concentration of manganese and copper in the Om River sometimes amounted up to 134 and 138 MPC, respectively [15].

The amount of pollutants ingress into the atmosphere of the Novosibirsk of is equal to almost 300 thousand t/year, whose main sources are presented by motor transport, heat power system enterprises, municipal boiler-houses and chimneys of private sector. In the course of unannounced ecological investigations, more than 2000 different violations of the environmental regulations have been revealed.

It should be noted that the non-uniform distribution of chemical elements is observed within the one sample, too. So, in the fragmentary elemental analysis of three stones from the Novosibirsk and Omsk collections we have revealed that at the centre of one stone the content of iodine amounted to 903 μg/g, whereas on its surface it is equal 5.05 μg/g. The same tendency concerning the content of iodine is observed for another sample, too: 32.9 μg/g iodine is revealed at the centre of the stone and 0.7 μg/g on its surface. For the same stones the content of selenium within the central parts is higher as compared to its content at the surface (Fig. 3). The content of strontium determined for these two samples, on the contrary, is lower in the central part (2.63 and 27.1 μg/g) as compared to the surface (40.6 and 45.7 μg/g, respectively).

The non-uniform distribution of elements within one stone irrespective of the living region of a patient indicates a considerable change in the conditions of the medium with respect to the crystallization of the compounds composing the stones [11]. Thus at a certain stage of growing this pathogenic formation there is an accumulation of the elements excessively entering a human organism either from the exterior environment, or as the result of metabolic processes derangement in an organism. The level-by-level distribution of chemical elements, to all appearance, could be caused by incorporating the microelements into the structure of hydroxyapatite due to an isomorphic substitution or their interaction with the organic components of the stone.

This assumption is confirmed also by the results of saliva stone mineral composition analysis performed using an IR spectroscopy method. It has been established that in the central part of the stone the content of organic substances is higher as compared to their content in the surface layer (Fig. 4), which is indicated by much more intense absorption bands corresponding to C–H bonds of organic components (2850, 2930 cm^{-1}), as well as by wider absorption bands corresponding to inorganic components of saliva stones (1030, 950 cm^{-1})

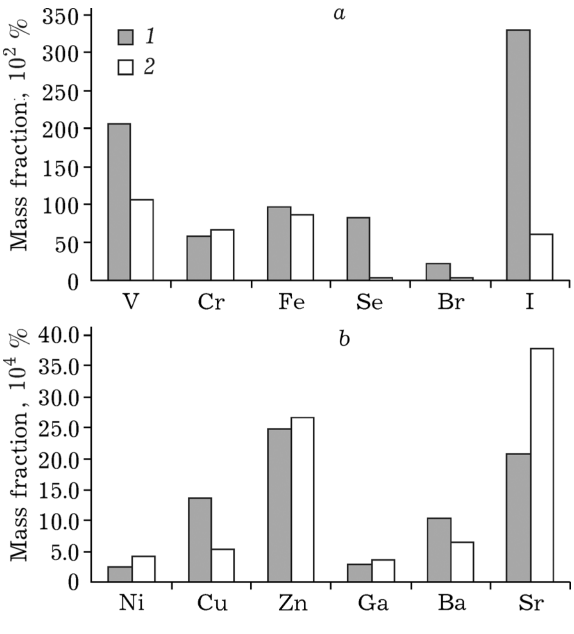

Fig 3. Fragmentary distribution of chemical elements over the parts of saliva stones: *1* – central part, *2* – surface.

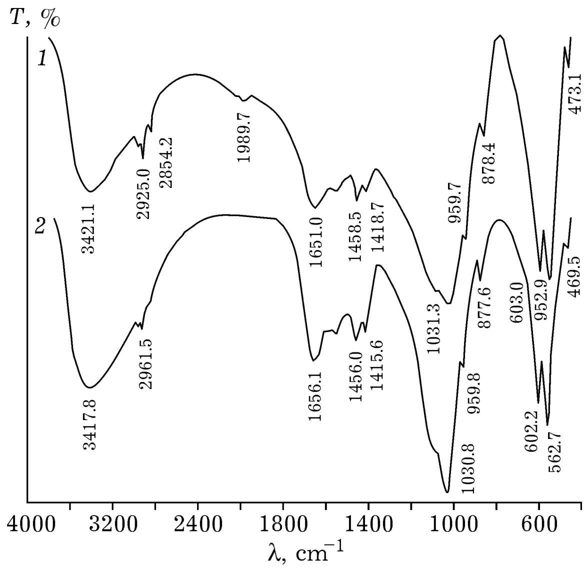

Fig. 4. IR spectrum of a saliva stone: *1* – central part, *2* – surface.

Basing on the data obtained one could hypothesize one of probable mechanisms for the formation of saliva stones in a human organism. It is known, that the saliva represents a structured biological liquid whose volume is distributed between micelles, i.e. colloidal formations [16]. Their nuclei consist of the molecules of calcium phosphate and are surrounded with water-protein envelops. At the first stage the organic matrix of a saliva stone is formed as the result of the structure abnormality of excretory ducts, changes in the saliva composition and properties, as well as of phosphorus-calcium metabolic disorder [17]. In the composition of saliva stone proteins there are such amino acids prevailing as glutamic acid, glycine, serine, *etc*. The accumulation of iodine, selenium and copper in the central part of stones could be connected with their high affinity with respect to proteins or to separate amino acids composing the centre of the stone. For a number of chemical elements one can observe reliably high correlation coefficients (for example, the values of stepwise stability constants of nickel chelate complexes with glycine are $K_1 = 1.4 \cdot 10^6$, $K_2 = 8.9 \cdot 10^4$). To all appearance, under the excess content of microelements in the saliva there is the formation of chelate complex compounds with amino acids contained within protein, as well as the destruction of the shielding envelops of colloidal micelles. Under an increased content of metal cations the micelles can lose their stability being subject to coagulation, which results in disturbing the mineralizing and structural properties of the saliva as well as to the formation of saliva stones in the mouth cavity of a human.

The basic protein of the saliva is presented by glycoprotein mucin, exhibiting strong affinity with respect to calcium ions. In this connection at the following stage of the stone formation there is a mineral phase formation to occur on the basis of the organic matrix. The mineral layers of saliva stones presented mainly by hydroxyapatite in the process of formation and growth could isomorphically incorporate a number of microelements into the structure, for example, strontium, which is confirmed by close values of ionic radii for these elements (0.106 and 0.127 nm for Ca^{2+} и Sr^{2+}, respectively).

The saliva represents the main source of mineral substances, whereas its composition and properties could vary to a considerable extent even within 1 day, which fact determines the difference in the microelemental composition within one sample. Nevertheless, the characteristic set of chemical elements is determined, most likely, by patients' living environment.

CONCLUSION

The microelemental composition of the saliva stones under investigation with respect to a number of chemical elements exhibits certain regional features. The samples of the Vladivostok collection are richer in iodine, selenium, chromium and vanadium as compared to the samples of the Novosibirsk collection, however indium and cadmium are less often found out therein. The fragmentary analysis of the three samples indicates the mineral and elemental composition to be different in the central part and on the surface of these stones. Basing on the results obtained one can conclude that during the formation of pathogenous organomineral lumps in the maxillofacial sphere the conditions in the medium where the crystallization of the compounds composing them are varying to a considerable extent. It has been established that the elemental composition of saliva stones depends on patient's living environment.

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