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Dedicated to Professor Ionel Haiduc,
President of The Romanian Academy at his 70th anniversary

METALLOGRAMS OF SIALOCONCREMENTS WITH METABOLIC AND NUTRITIONAL ASPECTS NOTE I. ALKALINE AND ALKALINE-EARTH METALS

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ABSTRACT. Investigations concerning the biogenesis of various concrements are of interest for biochemistry and pathobiochemistry (by the induced homeostatic disturbances); pathophysiology (by their composition and mechanism of formation, i.e. precipitative and / or co-precipitative processes) and morphopathology (due to their localization).

From an anatomic point of view, lithiasis presents numerous localizations, the most important ones being the urolithiasis, cholelithiasis and the sialolithiasis.

Our investigations were performed on a number of 23 salivary calculi (extracted surgically) and the metallogram of the alkaline and alkaline-earth metals was obtained. For this purpose by atomic absorption spectroscopy (AAS) the concentrations of some alkaline (Na, K), alkaline-earth metals (Ca, Mg) were determined. The obtained results revealed that the highest quantities of metals have been in the case of alkaline-earth metals (in the relation Ca>Mg) and alkaline metals (in the relation Na>K) – values expressed in mg/g calculus.

Keywords: salivary calculi, alkaline and alkaline-earth metals

Introduction

Saliva is a complex fluid produced by a number of specialized glands which discharge into the oral cavity of the glands of mammalian vertebrates. Most of the saliva is produced by the major salivary glands (parotid,

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submandibular, and sublingual), but a small contribution is made by the numerous small labial, buccal, and palatal glands which line the [1- 4].

Saliva is a viscous, colorless and opalescent fluid having a pH value around 6.2 which, in the presence of air, may become more acid [5]. Under microscope, the saliva shows nucleated squamous cells from the oral lining, disintegrating leucocytes and gland cells, as well as a large variety of microorganisms. Saliva is the watery fluid containing 98% water and many important substances, including electrolytes (cationic such as Na, K, Ca, Mg, Cu and anionic: P, Cl, I, F) , mucus, antibacterial compounds and various enzymes [6]. In the presence of carbon dioxide or by bacterial action salivary constituents can precipitate and their deposition takes place on the teeth as tartar or as calculi in the salivary ducts [7]. At the level of salivary glands, especially of salivary ducts, crystals with the specificity of lithiasis and sometimes even concrements can be produced. These are known as salivary lithiasis (sialolithiasis). Biogenesis of salivary calculus similar to renal and biliary calculi [8, 9] starts from the heterogeneous nucleation principles. In nucleation an important role is played by the organic compounds which, generally, define the type of sialolithiasis, and the inorganic compounds which are especially metallic ions. In the first phase crystallization nuclei are formed which often are called “primers” or “starters” of the lithogenic process [10]. Literature data in the domain discuss about microliths [11, 12]. Microliths are visible only at the microscope and are considered to simply be evolutive/intermediary forms between starters and calculus.

Materials and methods

Analytical investigations were made on surgically obtained salivary calculi. The sialoconcrements were mainly extracted from the level of submandibular glands and their ducts. Salivary calculi were collected from the Clinic of maxilo-facial surgery in a period of 10 years. Sizes of concrements were 3-11 mm. After the obtainment, calculi were washed repeatedly with distilled water, desiccated and thereafter powdered.

The purpose of the investigations was to measure the concentrations of the main alkaline (Na, K), alkaline-earth (Ca, Mg) metals. In our investigations we have determined biometals from the group of alkaline metals Na, K, alkaline-earth metals Ca, Mg and trace elements Zn, Cu, Mn. Also we have determined some elements with toxic potential Pb, Cd, Al, Cr [13]. In the present “report” are presented the results referring only to the alkaline and alkaline-earth metals.

Analytical determinations were made by atomic absorption spectroscopy (AAS) method. It was used successively a Pye-Unicam - Series 1900 apparatus and an Analyst-100 apparatus produced by Perkin Elmer.

Obtained data were statistically evaluated, determining the mean value (X) and the standard deviation (SD).

Results and discussions

Saliva, like other body fluids, is a dilute aqueous fluid containing both electrolytes and protein with an osmolality less than or equal to that of plasma [14]. Also present in saliva is a certain amount of cell debris arising from the epithelial cells of the mouth together with food residues [15]. The osmolality is principally determined by the type of gland and by secretory activity, whose degree is affected by many factors including sex, age, nutritional or emotional state, season of the year [16, 17], darkness and a variety of diseases and many pharmacological agents.

The total volume of saliva produced each day in adults is 500 to 1500 ml [18]. Mixed saliva consists mainly of the secretions of submandibular (65%), parotid (23%), and sublingual (4%) glands, the remaining 8% being provided by the minor numerous glands [15]. These proportions are a function of the type, intensity and duration of stimulation. The important stimulus for secretion is the presentation and ingestion of food; the quantity and quality of the secretion vary with the nature of nutrition.

Systematic studies on the composition of sialolithiasis with specific analytical investigations for that period were related in 1938 by Lock and Murray [19].

Between the organic components that are found more frequently in sialolithiasis one can mention the presence of phosphates, carbonates, cholesterol, urates. In order to evidence these substances various chemical and physico-chemical methods are used. For practical considerations, the infrared spectroscopy was more often utilized.

In general, saliva contains the usual electrolytes of the body fluids, the principal ions being sodium, potassium, chloride and bicarbonate. The acinar cells forming the secretory endpiece of the salivary gland actively pump sodium ions from the blood into the lumen of the endpiece. The resulting osmotic pressure difference between the blood and the fluid in the endpiece causes water to flow from the blood, through the tight junctions between the acinar cells, and into the lumen of the endpiece. Thus, the primary secretion (as it leaves the endpiece) is thought to be almost isotonic with plasma [20].

The initial fluid moves down the ductal system of the salivary gland and, an energy-dependent transport process reabsorbs sodium and chloride. Potassium, bicarbonate and lithium ions are actively secreted into saliva. However, the ductal membranes are relatively impervious to water, so the resulting saliva becomes increasingly hypotonic as it moves down the ductal system [21]. Data referring to the composition of saliva are compiled from literature after Altman and Dittmer [22, 23] – see Table 1.

Table 1.
Chemical composition of saliva - inorganic substances

Component		UM	Saliva (sample)	Average	Range
Cations	Ca total	mg / dL	Mixed Submaxillary Parotid	5.80 8.80 3.50	5.2 – 9.7 4.4 – 13.1 2.1 – 6.7
	Co	µg / dL	Mixed	2.44	0 – 12.53
	Cu	µg / dL	Mixed	31.70	5.0 – 76.0
	Mg	mg / dL	Mixed	0.50	0.15 – 0.93
	K	mg / dL	Mixed	80.3	56 - 148
	Na	mg / dL	Mixed	23.2	8 – 56
Anions	Cl	mEq / L	Mixed	15.5	8.4 – 17.7
	F	mEq / L	Mixed		0 – 0.005
	I	µg / dL	Parotid Submaxillary	6.46 3.65	
	P total	mg / dL	Mixed	20.40	
	P anorg	mg / dL	Mixed	14.90	7.4 – 21.1
	P org	mg / dL	Mixed	5.50	
	Bicarbonate	mEq / L	Mixed	6.44	3.48 – 10.70

Salivary glands lithiasis (sialolithiasis) is the most common disease of the major salivary glands after mumps and accounts for approximately 30% of all salivary disorders and about 0.01-1.0% of the population is held to be affected, with a higher incidence in males aged between 30 and 60 years.

Metallograms of sialoconcrements may offer important data for the biochemistry, physiology and physiopathology of lithogenic processes and can be used as a clinical guideline in dentistry [7, 24].

In the present study the concentrations of alkaline (Na, K), alkaline-earth (Ca, Mg) were determined. The obtained results are given in table 2.

Table 2.
Concentration of the main alkaline and alkaline-earth metals

Speci-fication	UM	Nr. of calculi	Concentration X ± SD	Range of concentration
Na	mg/g	23	4 843.21 ± 1 071.16	3 170 – 8 750
K		23	518.74 ± 88.31	320 – 710
Ca		23	227 293.00 ± 50 193.12	154 250 – 221 400
Mg		23	1 185.93 ± 329.84	920 – 2 150

Metabolism of calcium and magnesium is conditioned by the quantity of micronutrients (minerals and vitamins – especially vitamins D) in the consumed food and influenced by thyro-parathyroid hormones. In this context we mention that parathyroid hormone increases blood calcium level while calcitonine (thyrocalcitonine) lowers blood-calcium level

The most common localization is the submandibular gland where 92% of calculi are found, the duct being more frequently affected than the parenchyma. The parotid gland is affected in 6% of cases. The sublingual gland is affected in 2% of cases and other minor salivary glands in another 2%.

Sialoliths are concrements detectable only microscopically in the ductal system of the salivary glands. They contain calcium and phosphorus with formation of hydroxylapatite crystals as well as organic secretory material in granulated form and necrotic cellular residues.

The chemical composition of the sialoliths varies from one gland to another. Calculi generally consist of a mixture of different calcium phosphates (mainly hydroxy-apatite and carbonate-apatite) together with an organic matrix [25-28]. Submandibular gland stones tend to have higher concentrations of calcium, which explains why 85% of submandibular stones are visible on x-ray, whereas only 15% of parotid duct stones are visible.

Despite the relative frequency of sialolithiasis, current research has not led to definitive conclusions about the exact cause of sialolith formation. Several theories have been proposed to explain the development of sialoliths.

One early theory suggested that sialoliths resulted from disturbances in the secretion and precipitation of the components of the saliva as a result of inflammatory processes within the salivary gland [2, 12]. A later theory proposed that specific changes in the structure of the organic molecules in saliva allowed the formation of a supportive frame for calcium crystals [12, 29]. By contrast, Rauch [12, 30], was of the view that the primary precipitation was of minerals, upon which the organic substances later accumulated. Another theory explains the formation of sialoliths as a metabolic disturbance, caused by the alkalinity of bicarbonate and the precipitation of calcium phosphates [12,31].

Due to the fact that sialoliths appear mainly in a single gland, especially the submandibular gland, the local factors (morpho-anatomic such as: salivary duct stenosis, salivary duct diverticuli, etc.) for development of sialoliths are etiologically significant.

Disturbed salivary secretion and a change in the composition of saliva (high supersaturation, crystallization inhibitor deficit, etc.), which is called dyschylia, can lead to an increase in salivary viscosity and to a mucous obstruction in the terminal ducts of the salivary gland [11, 12, 32].

Studies on animal models showed that microliths are generated due to autophagocytosis of organelles that are rich in calcium. The accumulation of organic substances, especially of glycoproteins with higher calcium affinity, and mineralization of the organic matrix in the ductal system, is the most important phase of lithogenesis [12]. Mineralization is supported by accumulation of calcium and an increase in pH, which then decreases the solubility of the calcium phosphates in saliva

Usually treatment of salivary lithiasis means surgical intervention and, in the last decade, lithotripsie procedures based on treatment with ultrasounds.

Nowadays, the first choice treatment of salivary gland stones is shock wave lithotripsy, because it is considered a non-invasive and successful treatment with low risk [33].

Sialolithogenesis is related to the biochemical homeostasis variations which must be correlated with the nutrients intake and also with metabolic disturbances generated by the presence of infections, the appearance of antibodies and salivary ducts anatomic anomalies. These kinds of situations facilitate the stagnation of secretions and, in time, the initiation of heterogeneous nucleation process.

Conclusions

1. Metallograms of sialoconcrements evidenced the decrease of concentration in the successive series for alkaline and alkaline-earth metal bioelements:

$$Ca > Na > Mg > K$$

2. Data referring to metal composition of sialoconcrements are of importance for physiopathology and is a veritable guideline for the dentistry clinic for the prophylaxy and metaphylaxy of sialolithiasis. We note that our results differ slightly from other analyses published in the field (e.g., Ref. [34], reporting a somewhat larger concentration for Ca), which opens up a discussion on the origin and nature of such differences (with instrumental methods as well as patient-related factors bound to be investigated). Such aspects constitute the focus of further research from our part.

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