Study on Chemical Composition of Urinary and Salivary Gland Stones in Relationship with Laboratory Parameters and Lifestyle Habits of Patients with Lithiasis

ENIKO NEMES NAGY[1], MARIANA CORNELIA TILINCA[1]*, ALINA IACOB[1], ALINA ORMENISAN[1], ZITA FAZAKAS[1], HORIA MIHAIL BARBU[2], MELINDA KOLCSAR[1], ADRIAN CORNEL MAIER[6], ARPAD OLIVER VIDA[1], MARTHA ORSOLYA[1]

[1]University of Medicine and Pharmacy, 38 Gh. Marinescu Str., 540139, Targu Mures, Romania

[2]Titu Maiorescu University of Bucharest, Romania, Faculty of Dental Medicine, 67A Gh. Petrascu Str., 031593, Bucharest, Romania

[3]Dr. Aristide Serfioti Military Emergency Hospital, 199 Traian Str., 800008, Galati, Romania

Lithiasis is a disease with increasing incidence, may occur in the urinary tract, salivary glands and bile ducts. Genetic susceptibility, diet, low fluid intake, endocrine disorders, infections and other factors can influence the development of the disease and its recurrences. The aim of the study was to reveal the relationship between chemical composition of the stones, lifestyle and dietary habits and the results of laboratory analysis in patients with urinary tract and salivary gland lithiasis. The data of 258 patients with urinary tract and salivary gland lithiasis collected between September 2009 – May 2016 were studied. The subjects were from the Urology and Oral Surgery Hospital, and from the Marmed and Procardia medical units in Tirgu Mures. We evaluated the lifestyle habits using a questionnaire, microscopical examination, size measurement and chemical analysis of the stones were made and we processed the results of urinary strip analysis and sediment. In a subgroup of patients (48 samples) calciuria, phosphaturia, serum uric acid, calcium, phosphate, alkaline phosphatase and parathormone (PTH) levels were determined; in case of pathological values calcitonin measurement and endocrinology consultation were performed. The most frequent composition was the combination of calcium oxalate and phosphate in case of both urinary and salivary gland stones, in several uroliths we detected uric acid/xanthine crystals, rarely cystine and carbonate were found. The incidence of hematuria and leukocyturia exceeded 70%. Excessive calcium intake was observed in the diet of the several patients having calcium oxalate stones. In the subgroup tested for biochemical and hormonal analyses we found increased serum PTH concentration in 20.83% of the patients. Some of the subjects had pathological serum calcium, uric acid and alkaline phosphatase results, or the elimination of minerals in the urine was out of the normal range. We found pathological laboratory results in several patients suffering from lithiasis. Proper diet and medical treatment in many cases could prevent the recurrences of stone formation, but first a complex investigation of the patients is necessary to adapt the treatment plan to individual requirements.

Keywords: lithiasis, diet, chemical composition of stones, parathormone

Lithiasis occurs most frequent in the urinary tract, but it also can appear in the salivary glands and bile ducts. It has increasing incidence worldwide, affecting around 5% of the population, especially males between 30-50 years of age. Recurrencies of lithiasis are quite often, several factors contribute to it such as genetic background, imbalanced diet, low fluid intake, medication, infections and endocrine disorders (including hyperparathyroidism).

Prevention of new episodes are currently the goal of the treatment in patients with lithiasis, evaluation of lifestyle habits and complex laboratory investigations are necessary to apply the proper strategy in case of each individual. Determination of the stones' chemical composition is also necessary, providing important information regarding the possibilities of secondary prevention. Gallstones consist mainly of cholesterol, along with biliary pigments and calcium salts [1], while uroliths contain mainly calcium oxalate cristals, often mixed with calcium phosphate, uric acid or other components. Sedentary lifestyle and overweight, decreased water intake contribute to the higher incidence of calcium oxalate uroliths over the past decade. Acid urine enhances uric acid crystallization, while alkaline urine determines the physical condition for the formation of calcium phosphate and struvite (ammonium magnesium phosphate) stones [2].

Salivary gland stones are most common in the submandibulary gland, sialoliths often occur concomitant with nephroliths [3]. Calcium oxalate, calcium phosphate, carbonate and uric acid can be found in the composition of sialoliths [4].

Experimental part

The first aim of this study was to evaluate the lifestyle habits of patients suffering from urinary tract and salivary gland lithiasis in relationship with the chemical composition of their stones.

A second goal was to investigate a subgroup of these patients, performing biochemical and hormonal measurements besides routine test such as urinary strip analysis and sediment, to reveal a possible connection between laboratory test results and their condition.

Our research team used a complex questionnaire for evaluation of lifestyle habits. This included personal data, hight and weight, associated diseases, information regarding lithiasis in the family history, data about different food types (meat, eggs, fruits, vegetables, diary products, bread, fish roes, peas, beans) and drinks ingested (mineral water, fruit juice, tea, coffee, cocoa) in a semiquantitative approach. Body mass index was calculated using the formula: BMI = weight (kg)/height (m^2). Normal weight

* email: mariana.tilinca@umftgm.ro,

was considered in case of BMI between 18.5-24.9 kg/m^2, overweight at values between 25-29.9 kg/m^2, and obesity for BMI of 30.0 kg/m^2 and above.

Each stone underwent microscopic examination (Elerom photomicroscope, 3,4X) for studying the aspect of the cystals in their composition. Chemical analysis of uroliths and sialoliths was made using small amounts of stone powder and colour reactions revealed the presence of oxalate, phosphate, uric acid/xanthine, cystine and carbonate. The presence of oxalate was tested by the reaction with 1‰ $KMnO_4$ in the presence of 5% H_2SO_4, discoloration was observed after heating. Formation of ammonium phosphomolybdate revealed the presence of phosphate by the reaction with HNO_3 (d = 1.42 g/mL) and 5% ammonium molybdate at high temperature. Urate cristals were identified using the murexid reaction (HNO_3, 25% NH_3 and 10% NaOH and heating are necessary). Formation of black PbS showed the presence of cysteine reacting with NaOH and $Pb(CH_3COO)_2$ solution. Carbonate caused CO_2 gas release after 2N HCl was added [5].

Testing the presence of pathological compounds in the urine was made by reflectometry (HandUReader and Uryxxon analyzers), the sediment obtained by centrifugation underwent photomicroscopic evaluation (20x, 40x).

Biochemical tests in serum and urine (calciuria, phosphaturia) were performed by photometric procedures. The Konelab20XTi analyzer was used for measurement of serum uric acid, calcium, phosphate concentration and alkaline phosphatase activity.

Serum parathormone (PTH) levels were tested by ELISA method, in a subgroup of patients (those having pathological values) also calcitonin was determined by chemiluminiscence.

The results obtained have been compared to the following normal ranges: 2.15-2.57 mmol/L for calcemia, 2.50-4.90 mg/dL for phosphatemia, 3-6 mg/dL for uric acid in female patients (up to 7 mg/dL in male individuals); 11-67 pg/mL for serum PTH concentration, calcitonin normal under 18.2 pg/mL; 42-353 mg/day for calciuria, 0.4-1.3 g/day for phosphaturia.

Results and discussions

The average age of the studied patients was 51.41 years +/- 13.50 (SD), 48.06% being female subjects.

Calcium oxalate was present in the composition of the large majority of uroliths (96.15%), while the reaction for phosphate was positive in 67.45% of the urinary stones. These two components were found in all sialoliths analyzed (n=12). Uric acid/xanthine crystals were present in 16.70% of the uroliths, while cystine and carbonate represented 1.93 and 0.77%, respectively. The composition of urinary stones was mixed in 74.05% of the cases. The size of the stones was quite different, ranged from 1x1 mm urolith to 43 mm long sialolith extracted from the parotid gland.

Based on evaluation of diet in patients with lithiasis we could observe that 80.23% of the patients developing calcium oxalate stones have higher than normal calcium intake, mostly due to excess of dairy products, and 19.40% of these patients consume large amounts of coffee, which enhances calciuria. Excess of carbohydrates and animal proteins could also be observed in a few patients. Large amounts of food containing nucleic acids were in some cases in relationship with the presence of uric acid crystals in the composition of the stones. 92.0% of the patients presenting urinary stones with mixed composition (calcium oxalate and phosphate) consumed large amounts of fruits and vegetables, containing oxalate and vitamin C. We found one case having currently sialolithiasis and previously urinary stone, mentioned in the questionnaire. The large majority of patients with lithiasis (uro-and sialoliths) consume daily too small amounts of liquids (under 2 L/24 h or even less than 1.5 L/day).

Based on the data regarding height and bodyweight included in the questionnaire we calculated BMI of the patients. The mean value was 27.55 kg/m^2 +- 3.46, minimum value 22.1, maximum 33.7 kg/m^2. The distribution of the patients in different categories based on their BMI value can be observed in figure 1.

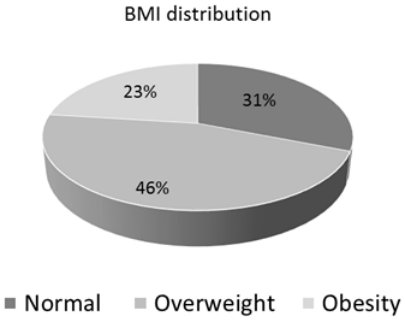

Figure 1. Distribution of patients based on their BMI value

The incidence of hematuria and leukocyturia was 79.06% and 71.70%, respectively; proteinuria occurred in 32.17% of the cases, nitrite test was positive in 15.50% of the patients. The urine of 24.03% of the patients contained crystals. Urinary infection was present in 36.82% of the patients having urolithiasis, the most frequent microorganism being E. coli.

The mean value of PTH was 39.02 pg/mL +/- 29.40 (normal range: 11-67 pg/mL), minimum value 8.29 pg/mL, maximum 131.24 pg/mL in a female patient who underwent surgery of the thyroid gland. Her calcemia was at the lowest limit of the normal range, she has recently had her first manifestation of urolithiasis with acute renal colic. The symptoms stopped after spontaneous elimination of the stone by the urine. 20.83% of the studied patients had increased PTH serum concentration, but only in one case this condition was associated with high serum calcium value. All the other cases, with increased PTH values, but normal serum calcium and phosphate concentration underwent endocrinology consultation with ultrasound. One male patient had increased value of phosphaturia associated to high PTH level. No pathological modifications of the parathyroid gland could be observed in these patients, but scintigraphy would be necessary to completely rule out a possible hyperplasia. Mean value of calcitonin was 6.51 pg/mL +/- 4.59 (SD). Average serum calcium was 2.27 mmol/L +/- 0.24 (SD), minimum value 1.75 mmol/L, maximum 3.04 mmol/L.

Serum uric acid concentration was elevated in case of one female patient (she had traces of uric acid in the composition of her sialolith) and a result very close to the highest normal value has been obtained in a male individual. Mean value of serum uric acid concentration was 4.13 mg/dL, minimum value 1.98 mg/dL, maximum 8.02 mg/dL.

Serum alkaline phosphatase activity was high in two male patients with urolithiasis, and female patient with sialolithiasis had a slightly low value. Average alkaline phosphatase activity was 189 IU/L +/- 88.46 (SD), minimum value 94 IU/L, maximum 362 IU/L.

Calciuria was normal in every studied patient with one exception, mean value was 178.95 mg/day +/- 95.83 (SD), minimum value being 40 mg/day, maximum 322 mg/day.

Phosphaturia was increased in 12.50% of the patients and lower than normal in 16.67% of the cases. Average phosphaturia was 0.73 g/day +/- 0.43 (SD), minimum value 0.16 g/day and maximum 1.63 g/day. The male patient with urolithiasis having the highest value of phosphaturia also presented increased serum PTH concentration.

The relationship between imbalanced diet and occurrence of lithiasis has been confirmed by our study. According to long term studies, the association of calcium-rich diet and stone formation can be observed especially in male patients under 60 years of age. A large amount of vitamin C intake seems to increase the risk of symptomatic nephrolithiasis [6].

Hormonal disorders, especially hyperparathyroidism represents high risk for development of stones, increased PTH concentration occasionally in combination with other pathological laboratory results could be observed in over 20% of our patients. A study on a group of patients suffering from primary hyperthyroidism concluded that those who exhibited lithiasis showed higher values of calcium, alkaline phosphatase, PTH, osteocalcin, and chlorine/phosphate, calciuria and phosphaturia indexes compared to those patients who did not develop stones. Student's t test revealed significant differences in calcium levels, intact PTH and osteocalcin ($p<0.05$) [7].

The precise measurement of urine volume influences the results of phosphaturia and calciuria. The lowest calciuria has been obtained in a female patient with urolithiasis having nephrostomy after kidney surgery, so the accurate measurement of her 24 h urine volume encountered difficulties. For the rest of the patients the exact 24 h urine volume could be recorded. Modifications in urinary *p*H can be caused by genetic variants or mutations in transport pathways, by or metabolic diseases or lifestyle habits such as different diets. Inappropriately acidic or alkaline urine affects the solubility of various metabolites and salts enhancing urolithiasis [8].

The urinary strip analysis reveals several pathological compounds in patients with urolithiasis. Examination of urine sediment for the presence of crystals cannot replace the information provided by the chemical analysis of stones. The easily available semiquantitative determination of the components offers sufficient data for clinicians and nutrition specialists to propose changes in the diet of the patients. Quantitative measurements of the components are very expensive and require sophisticated equipment such as computerized tomography or crystallography [9], [10].

High frequency of urinary tract infections, especially in female subjects, is an important factor strongly associated with urolithiasis, our results confirm date in the literature. Uropathogen bacteria synthesize adhesion factors, tissue-damaging toxins, enzymes thus influencing the *p*H of the urine, and represent a severe public health problem [11]. A limitation of our study is the absence of mineral concentration measurements in the saliva of the patients presenting sialolithiasis and the lack of microbiological cultivation from the affected salivary glands, which could have revealed the contribution of local factors to the development of sialolithiasis.

Based on the calculated BMI results the large majority of our patients (69%) can be included in the category of overweight or type 1 obesity (BMI between 30-35 kg/m^2), which underlines the role of imbalanced diet, sedentarism and weight gain in the development of lithiasis. According to data described in medical literature overweight and obesity are strongly associated with an increased risk of stone formation in both genders due to an elevated urinary excretion of promoters of calcium oxalate stone formation. Overweight and obese men are more predisposed to stone formation compared to overweight women [12].

Conclusions

The data of our study revealed pathological laboratory results in several patients suffering from lithiasis, most frequently elevated serum PTH, high phosphaturia, increased serum alkaline phosphatase activity. Low fluid intake, imbalanced food consumption, infections and overweight also represent important risk factors for stone development.

Proper diet, larger fluid ingestion and medical treatment (antibiotics, management of hormonal and metabolic disorders) in many cases could prevent the relapses of the disease, but a precondition is a complex investigation of the patients to elaborate an individualized treatment plan.

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