

Detection of Microbial Biomarkers by The Method of Chromatography Mass Spectrometry as A Factor of Urinary Stone Formation in Patients with Urolithiasis

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Short Communication

Pathogenetically, urolithiasis is regarded by most urologists as a multifactorial, dynamically progressive disease, with a number of complex physicochemical processes occurring both in the body as a whole and at the level of the urinary system. Among the endogenous causes of the formation of urinary stones, there are urinary tract infections, foci of infection at another location (tonsillitis, furunculosis, osteomyelitis, salpingo-oophoritis), metabolic diseases (gout, hyperparathyroidism), deficiency or hyperactivity of a number of enzymes, severe injuries or diseases associated with prolonged immobilization of the patient, diseases of the digestive tract, liver and biliary tract, hereditary predisposition to urolithiasis [1]. The relationship between the chemical composition of stones and urine pH was revealed: acidic urine (pH <5.5) is determined more often in patients with stones formed by uric acid, and a pH value of about 8 indicates an infectious stone. Until recent years, it was believed that the urine of a healthy person is sterile, since microorganisms are not detected in the process of standard research methods [2].

However, studies in recent years have shown that urine contains non-cultivated microbiota, i.e., she is not sterile. So, normally, in the urine of an adult woman, bacteria are found that are not detected during standard urine culture for microbiota. These microorganisms, identical in all women, were called "non-culturable bacteria" [3]. The resulting material was subjected to NASBA molecular analysis (ribosomal RNA detection), light microscopy, and bacteriological culture. The human microbiota is represented by various microorganisms with a population of about 10¹⁵ and a total biomass of 2.5–8.0 kg. Of particular interest today are nanobacteria (nanoparticles), which have round or oval

mineral structures ranging in size from 30 to 200 nm. They are represented by giant pseudomolecules having a complex internal structure, in many cases a core and a shell, often external functional groups [4]. It has been established that nanobacteria are inanimate crystallized nanoparticles of minerals and organic molecules, and many scientists admit that they may play an important role in human pathology.

From a scientific standpoint, the question of the role of nanoparticles in the pathogenesis of urolithiasis remains open, requires further research, due to the fact that they are found in the air, in water, even in humans. Calcifying nanoparticles have been found in calcified arteries and heart valves [5]. Under our supervision there were 273 patients with urolithiasis. There were 167 men (61.1%), 106 women (38.9%). The age of the surveyed ranged from 19 to 83 years and averaged 46.6±15.7 years. Newly diagnosed urolithiasis was observed in 142 (52.1%) patients, recurrent urolithiasis occurred in 131 (47.9%) patients. The data of the anamnesis of the disease and the clinical picture made it possible to diagnose infectious and inflammatory diseases of the urinary tract in 147 (53.8%) of 273 patients with urolithiasis, with a thorough clinical and laboratory examination using standard methods in 226 (82.8%) and using gas chromatography - mass spectrometry performed in a licensed specialized laboratory - chronic pyelonephritis occurred in all patients. The study of microbial markers of 57 microorganisms in urinary stones by gas chromatography - mass spectrometry.

The study of nanoparticles in urinary stones by electron microscopy. bacteriological examination of urinary stones, - electron emission microscopy of stones to detect nanoparticles. -

for the detection of ureaplasma infection in patients suffering from urogenital pathology, PCR and the corresponding test systems of CJSC Implementation of Systems in Medicine (Russia) containing highly specific ligands for a certain DNA of *Ureaplasma urealyticum* and *Mycoplasma hominis* were used. Bacteriological examination of urine was carried out in St. Petersburg State Budgetary Institution of Healthcare "City Polyclinic No. 75". Urinalysis is aimed at isolating the causative agent of the disease and at quantifying the degree of bacteriuria. For the study, an average portion of freely released urine was taken, taken in an amount of 3–5 ml in a sterile dish after a thorough toilet of the external genital organs. The material was taken before the start of antibiotic therapy. For the qualitative and quantitative determination of microorganisms in urine, the method of sector crops (Gould's method) was used, which allows not only to determine the degree of bacteriuria, but also to isolate the pathogen in a pure culture.

When interpreting the obtained data, we took into account the complex tests: the degree of bacteriuria, the type of isolated cultures, their repetition discharge during the course of the disease, the presence in the urine of a monoculture or association of microorganisms. The degree of bacteriuria makes it possible to differentiate the infectious process in the urinary tract from urine contamination with normal microflora. To this end, we used the following criteria: - degree of bacteriuria not exceeding 10³ microbial cells in 1 ml of urine, indicates the absence of an inflammatory process and is usually the result of urine contamination; - the degree of bacteriuria, equal to 10⁴ microbial cells in 1 ml of urine, regarded as a dubious result, the study repeated; - the degree of bacteriuria is higher than 10⁴ microbial cells in 1 ml of urine indicated the presence of an inflammatory process in the urinary tract. The microbiota of urinary stones and urine was studied at the Center for Dysbiosis (St. Petersburg, B. Sampsonievsky pr., 60 lit. A, tel/fax +7(812)336-93-95) certified by Roszdravnadzor (Permission FS 2010/038 dated 24.02.2010) by gas chromatography - mass spectrometry. The method of mass spectrometry of microbial markers was first described in 1987, and since 1991 it has been used in our country for quantitative analysis of the generic or species composition of the microbiota [6].

The method is based on the detection by a highly sensitive and selective method of gas chromatography - mass spectrometry of markers from among their cellular lipids, aldehydes, alcohols and sterols in the analyzed sample, allowing you to simultaneously measure more than a hundred microbial markers directly in blood, urine, biopsy specimens, punctuates, sputum, and other biological fluids and tissues without prior inoculation on nutrient media or the use of test biochemical materials. At the same time, an automatic analysis algorithm is used using standard programs that allow determining the concentration of more than 50 microorganisms in the material two hours after it enters the laboratory. The method has found application in clinical practice [7]. The gas chromatography mass spectrometry method makes it possible to detect the components of a microbial cell of a wide

range of microorganisms of a person's own and foreign microbiota. The method is easy to standardize, automated, which makes laboratory diagnostics simple, and allows simultaneous detection of dozens of markers of microorganisms during the analysis of one sample. The course of the analysis consists in the direct extraction by means of a chemical procedure of higher fatty acids from the sample to be investigated, their separation on a chromatograph in a high-resolution capillary column and analysis of the composition in dynamic mode on a mass spectrometer.

The chromatograph is connected in a single device with a mass spectrometer and equipped with a computer with appropriate programs for automatic analysis and data processing; the analysis process itself takes 30 min and taking into account the time of sample preparation and data calculation, about 3 hours. Its result is the determination of the concentration of microbial markers and the subsequent reconstruction of the composition of the microbial community. In general, the method of gas chromatography - mass spectrometry is characterized by: - a wide diagnostic spectrum: determination of markers of dozens of microorganisms simultaneously in one analysis; - universality: determination of different groups of microorganisms: bacteria, fungi, viruses; - express - the time of one analysis is not more than 3 hours; - high sensitivity: 0.01 ng/ml marker; - selectivity: determination of a microorganism to a species - in the presence of a species marker; - independence from the equipment of the microbiological laboratory and the possibility of direct analysis of clinical samples without seeding and growing; - cost-effectiveness - the method does not require biological and biochemical test materials, culture media, enzymes, primers.

Conclusion

The use of gas chromatography mass spectrometry in clinical practice in patients with urolithiasis proves that the presence of calculi in the urinary tract in 100% is combined with the presence of a high content of microbial bodies that affect the stability of the urine colloidal system (Tamm-Horsfall protein), which should be considered as starting factor in urinary crystallogenesis. This fact should be the basis for the metaphylaxis of urinary stone formation.

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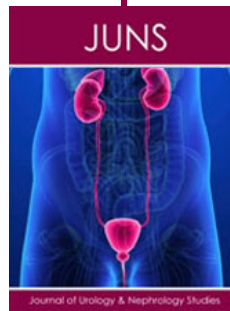
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