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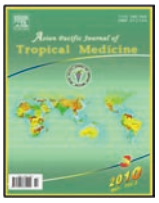

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Crystallodiagnostics of some animals' helmintosis

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ABSTRACT

Objective: To investigate the teziocrystalloscopic features of urine which were collected from both healthy rodents and those with parasitosis. **Methods:** Physical-chemical characteristics of urine samples that were collected from 15 mice and 20 rats were estimated by their ability to crystallize. Crystallization test results were interpreted with integrated identity table which includes 5 main classes of the crystalline and amorphous formations. Additional quantitative and semiquantitative evaluation indicators were also used. **Results:** Presence of *Trichinella spiralis* in animal organism transformed results of the biofluid free crystallogenesis. The main components of rats' urine facia were single-crystalline rectangles which were made of cholesterol and its derivatives. The biofluid microsamples of healthy rats and rats with echinococcosis had both common and difference. **Conclusions:** Urine teziocrystalloscopic analysis is an informative method for diagnosis of helminthiasis in animals.

1. Introduction

Present development of medicine and biology is characterized by wide public attention to the crystallographic methods of testing. They are based on the analysis of the biological fluids crystallization phenomenon from the aspect of diagnostic significance of the dehydration (facia) results [1-3].

In Russia the most dynamic crystallographic researches have been doing in last thirty years. Development of the approaches for the biological fluids dried samples analyses are being carried out in several scientific centers [4-6]. Metabolism data accessing methods were changed into classical crystalloscopy based on the analysis of the biological substratum direct crystallization and used for marker structures detection for the specific pathologic process or disease. Teziography which analyses the crystallization results in the dynamic system "biological fluid-basic substance" is also based on the facia qualitative study [5,7, 8].

Works made by E.G. Rapis initiated free crystallization analysis which was carried out for the first time on human being and animals' vitreous body [3]. The female scientist's activity created generations of mathematically and physicochemically grounded crystallization theory based on numerous clinicoexperimental researches. However, this theory covers only the protein structures [3].

Nowadays, the crystalloscopic analysis is wide spread in medical field, whereas there are only few works devoted to study the animals' biological fluids crystallization by comparing with that of human being [3]. In these cases the description concerns solely the characteristics abstraction of the facia which is appropriate to the dehydrated samples of the human liquid fluids.

The purposeful analysis of free (it is crystalloscopy) and initiated (it is teziography) crystallogenesis of the animals biofluids hasn't ever been carried out. That is why such a research has great importance in the tezigraphic and crystalloscopic diagnosis patterns of animals' functional and pathological states.

From our point of view, to access data of the animals´organism metabolism and homeostasis mathematical methods should be used to visualize the state's shifts for crystallodiagnostics objectives.

That is why the aim of our work is the teziocrystalloscopy pattern analysis of urine collected from both healthy small rodents and sick ones with parasitosis.

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2. Materials and methods

The urine samples were taken from both healthy animals and sick ones with helminthiasis including trichinosis, echinococcosis.

The physical-chemical characteristics of 15 mice and 20 rats' urine samples were estimated by their ability to crystallize since classic urine crystalloscopy was considered as the optimal crystallographic method. It can show the free (own) biofluids crystallogenesis and indicate the metabolic reorganization of its compound [9-13].

0.3 mL biological material samples (urine) were put on the fatness, cleanse and dried object-plate [11-13]. The micro-preparation drying is done in warm blast. The samples association is inadmissible.

The microsamples estimation was made using the microscopes at overall magnification ×56. The crystal structures were counted in three fields of view; the mean value was calculated and rounded to integer.

Interpretation of the crystallization test results was made using the integrated identity table which includes 5 main classes of the crystalline and amorphous formations. Most of them have decoded chemical structures[13]. Besides, additional quantitative and semiquantitative evaluation indicators including picture porosity, distribution density homogeneity of the crystalline and amorphous formations, and intensity of the rim zone and facia destruction degree were also used because such a representation is more convenient for further information processing.

Statistic processing of the data was accomplished by the Microsoft Excel XP spreadsheet using the built-in functions.

3. Results

Crystalloscopic pictures of both healthy and sick animals were compared. According to data in Table 1, Table 2 as well as Figure 1 and 2, we saw clear variations of the crystalloscopic pictures between the animal species as well as between their conventional norm and pathology (helminthiasis).

Presence of *Trichinella spiralis* in animal organism transformed results of the biofluid free crystallogenesis. The main components of rats' urine facia were single-crystalline rectangles which were made of cholesterol and its derivatives.

The biofluid microsamples of healthy rats and rats with echinococcosis had both common and difference (Table 2). The morphological approach we used as additional evaluating criteria specified the data of visual morphometry (Figure 1 and 2).

Table 1

Urine crystalloscopic characteristic of the healthy mice and those with trichinosis .

Structures		Healthy mouse **	Mouse with *T. spiralis* **
Single crystals	Rectangles	0	2
	Prisms	1	0–1
	Pyramids	1	2–3
	Octahedrons	0	0
Dendritic structures	Linear	2	0
	Rectangles	0	2
	Moss, onion, comet	0	0
	Crosses	0	0
	Horsetail	0	0
	Rosettes	0	0
Amorphous formations	size	small	small
	quantity	mean	few

The mean value (rounded to integer) of the structures count in the field of view was shown; interaction mode of the large crystals and amorphous formations: "*" – edging; "+" – chains presence.

Table 2

Urine crystalloscopic characteristic of the healthy rats and those with echinococcosis (the mean value (rounded to integer) of the structures count in the field of view is showed).

Structures		Healthy rat **	Rat with *echinococcus* **
Single crystals	Rectangles	∞	∞
	Prisms	0	1
	Pyramids	1	2–3
	Octahedrons	0	till 10
Dendritic structures	Linear	2	4
	Rectangles	3	2
	Moss, onion, comet	0	0
	Crosses	2	0–1
	Horsetail	0	1–2
	Rosettes	0	0
Amorphous formations	size	small	small
	quantity	mean	many

The mean value (rounded to integer) of the structures count in the field of view was shown; interaction mode of the large crystals and amorphous formations: "*" – edging; "+" – chains presence.

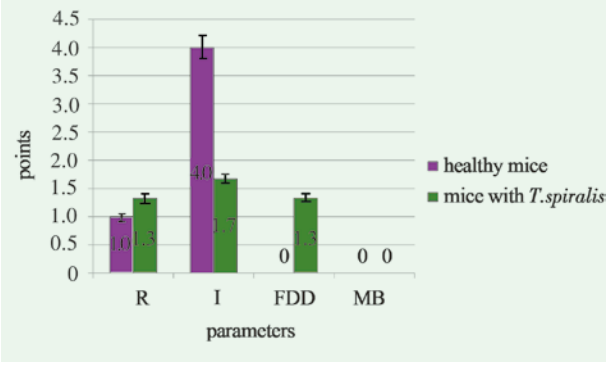

Figure 1. Additional evaluating criteria of the free crystallogenesis results of the healthy and sick mice.

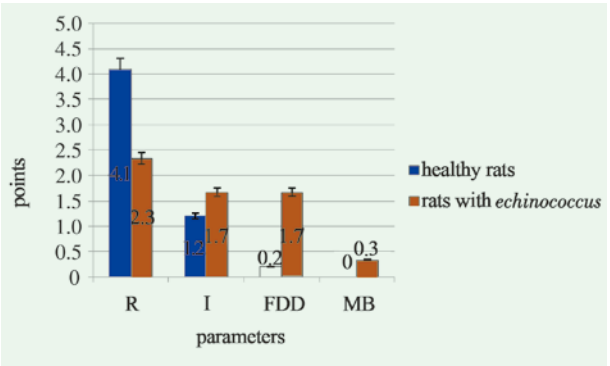

Figure 2. Additional evaluating criteria of the free crystallogenesis results of the healthy and sick rats.

4. Discussion

We estimated the crystallization ability of the mice urine and formed the pattern typical for healthy mice. Crystalloscopic picture of healthy mice was characterized by crystallogenesis of single-crystalline component type including prisms and pyramids figures which were magnesium and calcium phosphates, respectively; dendritic component was only represented by the few linear polycrystalline structures. Amorphous picture of the healthy mice urine was represented by the small formations (calcium carbonate) which were clearly distinguished from the large crystals. As a special structure, chains were found in the crystallogram. They were also variably registered in the urine crystallization of the healthy and sick people [10].

The classic crystalloscopic pattern of the mice urine is similar to the character criteria values of this human biofluid [10,14,15]. The drying results of urine collected form mice with *Trichinella spiralis* were different from the microsamples of healthy animals. Particularly, maximum variations were registered at the single-crystalline component analysis. At the free crystallization of the biofluid rectangles were clearly seen. Chemically they were cholesterol and its derivatives were absent in the facias of the healthy mice, but presented in few healthy people.

Quantitative differences among animals with trichinosis were marked by the occurrence of the "pyramids" figures which indicated higher renal excretion of phosphate-anions. The number of the amorphous structures decreased considerably in the field of view for healthy mice, while their size and type of interaction with the large crystals remained as the same. This may be a compensatory mechanism because calcium ions also took part in genesis of the amorphous structures.

Sick animals as well as healthy ones had a weakly pronounced dendritic component which is presented not by the linear structures but the lamellar rectangles, a chemical structure which has not decoded yet. Human with no somatic or mental pathology had a dendritic picture with the greater number of the figures types [11,16]. Crystallogram of the healthy mice urine differed considerably from human at all characteristics. Presence of *Trichinella spiralis* in animal organism transformed results of the biofluid free crystallogenesis.

We also analyzed the urine crystalloscopic picture of the healthy rats and those with echinococcosis, the biofluid crystallization differed considerably from what were typical for human and mouse. This was clearly defined in single crystals and dendritic structures[9-11]. The main components of rats' urine facia were single-crystalline rectangles which were made of cholesterol and its derivatives. It covered one third of the field of view. Besides, single pyramids were also founded. Dendritic composition of the urine microsamples was much more various than those of mice but comparable to those of man. Particularly "lamellar rectangles" and "linear dendrites" figures were typical for these samples despite the fact that their proportions and "concentration" (the mean value in the field of view) are the differentiative indices. This confirmed significance of the mathematical formulation of the crystallization results, but may be indicative of greater similarity between rats and human urine than mice urine.

The "cross" dendritic figure with unknown chemical composition was specific enough for the biofluid crystallograms.

The amorphous component of the urine facias (made according to the classic crystalloscopy methods) was almost similar to that of mice, but was different from that of human. The formed pattern of the urine dried samples of a healthy rat was original enough and differed from morphology of dehydrated biofluid of both human and mice qualitatively and quantitatively.

The biofluid microsamples of healthy rats and rats with echinococcosis had both common and difference. The identifying parameters of the urine microsample were predominance of the single rectangles which covered one third of the field of view; and absence of the "moss", "onion" and "comet" figures that are typical for dehydrated urine of human. These parameters should be regarded as specific features of urine facias of healthy and sick rats.

From the aspect of physiological and diagnostic significance, clearly defined rise of the "range" and quantity of the crystalline and amorphous structures of dried biofluid of rats with echinococcosis was interesting. This phenomenon called crystallizability and was considered as the main quantitative index of the biological fluid crystallogenesis. It may be explained by increase of the mineral substances renal excretion in sick animals.

All the known types of the single crystals were found in quantity in the urine samples of the sick rats. The dendritic component included different polycrystalline morphotypes. Linear crystals with the fragments divergence angle at 180°, lamellar rectangles and large "horsetail" figures which were not found in the urine facias of healthy human and rats were the most common shapes for sick rats. These peculiarities may be associated with echinococcus presented in the animal organism. The amorphous picture had only higher quantitative indices in comparison with that of the healthy rats.

Under traditional morphometric analysis of the free crystallogenesis results of the small rodents, clear patterns were registered. They had special crystalloscopic structures and defined shifts connected with helminthiasis in animals (indicated elements and their combinations, and quantitative proportions in the facia).

Besides, the morphological approach we used as additional evaluating criteria specified the data of visual morphometry. We studied steadiness of distribution density of the facias crystalline and amorphous elements (R), intensity of porosity (I), clearness of the rim zone formation (MB) by the six-point scale (from 0 to 5). Facias destruction degree (FDD) was calculated by four-degree scale (from 0 to III). All these criteria served to evaluate multivariately the integral index of the free crystallogenesis qualitative aspect, its regularity.

The dried urine microsamples of the healthy mice and those with *Trichinella spiralis* had low and unauthentically distinguished steadiness of structures distribution. This showed the biofluid crystallization disorder in health and sick animals.

The most sizeable and significant differences ($P<0.05$) were found between the picture of porosity intensity and its destruction degree. It's revealed that the first of the above indices decreased 2.35 times ($P<0.05$). This showd indirectly reduction of seats (areas) of the crystalline hydrates contraction and probable concentration changes of the crystalloscopically nonvisualized aggregations in the baseline urine. But the supposition about transformations of the biofluid protein structure was denied by the absence of the rim zone (according to its clearness, Rz) in the control and test samples.

Thus the imaginary contradiction (between R and I parameters data) about system chaotization (increase of entropy) and its stabilization (entropy drop) was not well-grounded. The data may be interpreted as a generalization of the destruction tendencies localized in norm (biosystem pursuit to increase its own entropy). This was completely confirmed by the FDD analysis (the sick animals had reliably higher FDD level than healthy ones, $P<0.05$).

Tendencies ascertained for the urine facias of the healthy and sick mice were shown for the dehydrated samples of the rats' biofluid, including both animals with and without echinococcus. But they had special features of the helminth response.

Particularly common features were authentic decrease of the structural elements equitability on the facia at sick rats in comparison with healthy ones ($P<0.05$). It was accompanied with increase of the facia destruction degree from its absence to moderate disruption of the picture elements (Figure 2) at animals in comparison with healthy rats ($P<0.05$). This showed clear chaotization of the results of the rats' urine free crystallogenesis.

The potential difference in these effect realization mechanisms should be mentioned. Mice had destruction of the mineral genesis proper, and the protein constituent of the biological fluid may took part in the crystallization at rats. This was confirmed by little increase of the rim zone clearness in the rats' urine facias. It testified indirectly to presence of the protein constituent in the biofluid [3,6,17] at the unauthentic higher level of the parameter I ($P>0.05$) which was identifiable with the number of the crystallization initiation centers in the dehydrated sample.

The analysis of the urine dried microsamples enabled to determine the clear patterns of helminthiasis. The characteristics analysis of free crystallogenesis of both healthy rodents and sick ones with helminthiasis shows adaptive mechanisms of the biofluid composition homeostasis. They come out as the qualitative and quantitative morphology reorganizations of the biofluid dried samples and are attended by the facia chaotization. This has diagnostic sense and is realized in different ways subjective to the animal species and their uropoiesis and urination systems as well as neuro-immune-endocrine mechanisms of regulation.

Besides we ascertained that classic crystalloscopy is a sensitive indicator of the composition and physical-chemical characteristics of biological fluids (urine and other liquid biological substrata) which show the metabolic state of the animal organism.

Conflict of interest statement

We declare that we have no conflict of interest.

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