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Morphological Indicators of Dehydrated Saliva Microcrystals as an Indicator of One's Prenosological Level

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Abstract

Based on morphological and functional indicators of the body, the prenosological health level of 43 practically healthy students at a medical university was evaluated. The morphological parameters of the microcrystals of their oral fluid in a solid phase, obtained by microdrop dehydration, were also studied. It was found that there was a statistically significant dependence of the value of this indicator on the health levels of the examined individuals.

The values of indicators of the morphological structure of microcrystals of a dehydrated sample of oral fluid, which are recommended as diagnostic criteria for the prenosological condition of a healthy person, are established.

Keywords: Prenosological conditions, Health levels, Oral fluid, Morphological parameters of the facies of dehydrated oral fluid, Prenosological diagnosis, Diagnostic criteria

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Introduction

The actualization of the concept of a health protection system aimed at maintaining the health of a healthy person attaches particular importance to the problem of assessing the level of an individual's health. Thus, at the present stage of development of society, the problem of diagnosing the level of health of a healthy person is particularly acute. However, in most cases, modern medicine reveals the presence of a disease and does not diagnose one's level of health. Doctors do not receive information about a decline in a healthy person's condition, which leads to a delay in preventive measures. If there is a well-developed and generally accepted nomenclature (classification) of diseases, then, until recently health had no corresponding classification. Currently, the term "health" is often interpreted as the absence of disease. The concept of health, developed in space medicine and preventive medicine [1-3], considers the transition from health to disease as a gradual reduction in the adaptive capacity of the body and the development of prenosological conditions that occurs as a result of a decrease in the functional reserve of its regulatory systems. Thus, the manifestation of the disease as a result of the failure of adaptation is preceded by prenosological and premorbid conditions. In practically healthy people, it is these conditions that are the object of prenosological control.

Note that the concept of a prenosological state in assessing human health is actually based on the laws of ancient medicine, laid out more than a thousand years ago by the famous physician and philosopher Abu Ali Ibn Sina - Avicenna, who identified six human health conditions [3]:

1. The body is the healthiest.
2. The body is healthy but not the healthiest.
3. The body is neither healthy nor sick.
4. A body that easily declines in health.
5. The body is sick but not the sickest.
6. The body is the sickest.

Of these conditions, only the last two relate to disease. Between the two extreme levels of health (according to Avicenna), we distinguished five transition states with different degrees of tension of regulatory systems: normal, moderate, pronounced, acutely pronounced and overstrain. The transition from health to disease occurs through the overstrain and breakdown of adaptation mechanisms. The sooner it is possible to foresee such an outcome, the more likely it is that one will be able to maintain his or her health. Thus, the problem boils down to learning to determine the degree of tension of the body's regulatory systems, that is, to evaluate its prenosological level and, consequently, manage one's health. In this regard, the implementation in practice of the WHO strategy, which involves monitoring the body's functional reserves; prenosological diagnosis at the early stages of the adaptation process; and timely preventive correction of the functional state are considered the best methodology for protecting one's health. Currently, with the active formation of the science of health, prenosological diagnosis has become the main part of preventive medicine, as it provides an assessment of the level of health in various functional states of the body of a healthy, not in an ill person [2,4,5]. The fundamental importance of the functional diagnosis of prenosological conditions is due mainly to the presence of functionally reversible, and not morphological, abnormalities in the state of individual wellness in a practically healthy person, which can easily be restored. In other words, by prenosological diagnostics, it is necessary to understand the assessment of the functional state of the body and its adaptive capabilities in a period when there are still no obvious signs of disease and to recognize conditions that are borderline between normal and pathological.

The practice of prenosological studies shows that for the recognition and assessment of the functional states of the body on the verge of transitioning from normal to pathological, taking into account the absence of pronounced pathological signs and the disorganized nature of the location of the objects to be examined, it is necessary to use the most modern methods and technologies. In this regard, the development of new methods of prenosological diagnosis remains an urgent task of preventive medicine [4-6]. First, it should be noted that the collection of information in prenosological studies should be focused on remoteness, non-invasiveness, minimal time requirements and high information content. In this case, the most accessible and frequently used method is the study of the composition of biological fluids (BFs), which carries integral information about the person's levels of anabolism and catabolism, hormonal status, and the functional state of various organs and systems. The liquids of a living organism are the basis in which all its activities take place; therefore, they reflect the state of the organism. It is known that deviation of body parameters from the norm outside the limits of adaptation always leads to the occurrence of processes in the body at the cellular, organ or system level and, accordingly, a change in the parameters and properties of biological fluids. These changes are especially pronounced in the morphology of their solid phase and, accordingly, are indicators of a person's state of health, both of its individual elements, and in the whole organism. The phase transition of the BFs to a solid state makes it possible to fix the molecular interactions in this complex system and make these interactions easily accessible to a researcher. In this regard, one of the new diagnostic and prognostic technologies in medicine has become the functional morphology of human biological fluids [7-10]. It is based on the identification of the morphological picture of crystalline and non-crystalline structures in the facies of dehydrated BFs. In general, this technology allows us to assess the levels of functional states of the body, including in the prenosological period. The morphology of biological fluids as a fundamentally new scientific direction in the field of clinical diagnostics is now developing at an exceptionally fast pace. There is an substantial increase in the volume of new scientific knowledge on identifying pathology markers, the quality of previously obtained data is being clarified, and diagnostic methods are being actively introduced into laboratory and clinical practice. Simplicity, novelty of the approach to obtaining information, and reliability are the advantages of this method; these advantages have made this approach attractive for clinical diagnosis. It should be noted that recently, the medical community has shown increasing interest in oral fluid (OF) as a material for biocrystalloscopic examination, which is considered an integral test that provides generalized information on the composition and properties of this biofluid [11-14]. In these works, the authors established quantitative parameters for the visual assessment of the facies of OF microcrystals in healthy adults. However, the literature on the morphological texture of OF microcrystals, depending on various donosological levels of the body's health and the identification of morphological markers for a donosological diagnosis in healthy humans, is practically absent in the literature.

In this context, the present study aimed to establish a relationship between the morphology of microcrystals in the solid-phase OF of the pancreas and the health conditions of healthy people and to assess whether the possibility of their use in prenosological diagnosis is relevant. Based on this, we formulated the purposes of our research: to study and identify the features of the relationship between the level of physical health of practically healthy people with indicators of the morphology of the microcrystals of the solid phase of their OF; to assess the sensitivity and specificity of the morphological structure of saliva microcrystals to prenosological levels of health and changes in these levels; and to develop a non-invasive, informative method of prenosological assessment of the condition of healthy individuals.

Materials and Methods

The study involved 43 (19 boys and 24 girls) 1- to 3-year students of the Fergana branch of the Tashkent Medical Academy who were not involved in sports and were aged 19-25 years. As a rule, prenosological studies are carried out among practically healthy people. In this regard, among the group of persons proposed for inclusion in this study, a survey was conducted on specially prepared questionnaires, and based an analysis of the results; the preliminary selection of the possible subjects was based on whether the individuals were practically healthy.

The study was carried out in 3 stages—in the first stage, on the basis of the actual morphological and functional indicators of the body of the subjects, the level of health and the adaptive potential and functional reserves of the subjects' bodies were evaluated according to the methods of Apanasenko GL [14] Baevsky RM [5] and Kournikova I [15], respectively. The results of these studies allowed us to classify the examined contingent of individuals according to the prenosological levels of the health indicators of their bodies.

The proposed rating system consists of a number of simple indicators that are ranked, and each rank is assigned a corresponding score. The overall health score is determined by the total score and allows all practically healthy people to be distributed into 5 health levels. For these purposes, at rest, the following parameters were measured: vital capacity of the lungs (VCL), heart rate (HR), blood pressure (BP), weight, standing growth, and brush dynamometry. Then, a standard functional test with squats (Martine-Kushelevsky) was performed. Based on the data acquisition, the following metrics were calculated: body mass index (body weight, kg/height, m2), life index (VCL, ml/ body weight, kg), power index (brush strength, kg/body weight, kg) x100%, and Robinson index (HR пок, hit/min) x (BP sys.)/100. With a stress test, the pulse recovery time to the initial level was determined. The level of physical health was determined as low, below average, average, above average, and high based on the total score, in accordance with the recommended criteria [14]. At the second stage of the study, microcrystals of dehydrated pancreatic cancer samples were examined. Note that the principle of the crystallographic method is based on the concept of the morphology of biological fluids. This concept suggests that structural engineering in the process of dehydration reflects not only the physicochemical composition but also the functional state, as well as the vital properties of the body. The subject of the study was the OF of the subjects obtained by collecting it in sterile tubes. Samples were separated into sediment and supernatant components by centrifugation at 3000 rpm for 5 minutes at room temperature. This study used the supernatant component. A supernatant droplet was transformed to the solid phase according to a previously reported procedure [7] by drying them under standard conditions. To do this, using a semiautomatic dispenser with a variable volume, 20 µl of oral fluid was applied to a fat-free glass slide located in a strictly horizontal arrangement and dehydrated by air-drying the sample at 24-25 °C and a relative humidity of 60-65% for 24 hours. The obtained facies of the oral fluid were visualized using a light microscope in transmitted light, and a computer atlas was created using the built-in digital camera for their subsequent morphological analyses. The third stage of the study is associated with morphological analysis and assessment of facies microcrystallization. In this case, each of the samples of the facies of the OF, using a marker, was divided into 4 equal quadrants; in each quadrant, a type of microcrystallization was established. There are several approaches to the classification of microcrystal types. When classifying the morphological types of RG facies, we took into account the indices of the method of visual morphometry [13], the severity of individual zones of the facies, the presence of crystalline and amorphous formations, the degree of their destruction and uniform distribution over the texture of the sample, as well as the severity of the edge zone, due to the protein content in samples of RJ. Four morphological types [16] of oral microcrystals were isolated: I type—a clear pattern of interconnected large crystal-prismatic structures of a tree-like (fern-like) form, evenly spaced along the main layer of the drop, and in which there are single organic inclusions around the perimeter; II type—in the central part, individual crystal-prismatic structures of a tree-like (fern-like) form are determined, some of the crystals are not interconnected, and a moderate amount of organic inclusions is located on the periphery; III type—a large number of randomly placed irregularly shaped structures, as well as a significant amount of organic inclusions adhering to crystals, are visible throughout the area; and IV type—single small crystals of irregular shape are detected in the field of view along the entire perimeter, without a clear orientation with signs of disaggregation or the complete absence of crystals (**Figure 1**).

Quantitative indicators of microcrystallization of the oral fluid are calculated by the method of Porodenko-Chudakova IO [17], with the modification the reported Domenyuk DA [16]. In this methodology, the type of microcrystallization (I, II, III, or IV) was determined in each quadrant of the OF facies with the subsequent calculation of its index (M) according to the formula:

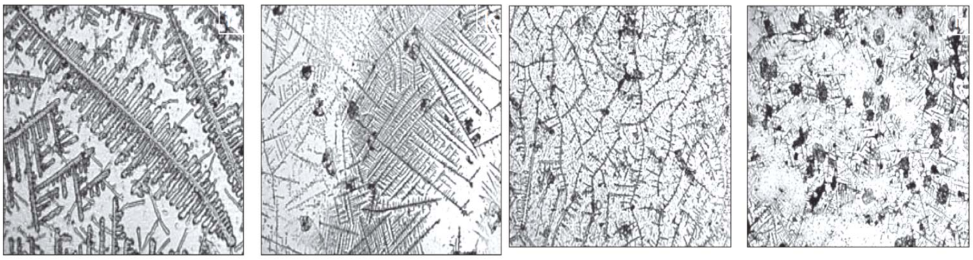
$$M = \frac{1NI + 2NII + 3NIII + 4NIV}{NI + NII + NIII + NIV}$$

Where NI, NII, NIII, and NIV represent the number of quadrants with microcrystallization of the OF of types I, II, III, and IV, respectively; 1, 2, 3, 4 are the weighting factors for microcrystallization types I, II, III, and IV. Note that this technique of morphological texture analysis of OF microcrystals allows us to identify 35 combinations of facies structural engineering, with 13 quantitative indicators varying in the range from 1 to 4, in discrete steps of 0.25. This makes it possible to almost double the sensitivity of this method to morphological changes in facies microcrystals compared with the existing methods [18], in which only 7 morphological types of facies can be classified.

Research results and discussion

Based on the morphological and functional data, the individual body indicators of the included individuals were measured, and in accordance with the known criteria [14], individuals were classified according to the corresponding health levels, the results of which are presented in the **Table 1**.

As seen from the data presented, 67.4% of the examined participants had average and near average health levels and 32.6% had low health levels level. At the same time, as shown by the assessment of their adaptive indicators, 76.8% of the participants had satisfactory indicators, and 23.2% did not, signifying stress on the body's adaptation mechanisms. At the same time, the proportion of persons with intense adaptation among young men was almost 2 times that of girls. The analysis of individual adaptation indicators of the examined participants and the corresponding health levels showed that all persons with a tense adaptation mechanism had a low level of health, with satisfactory adaptation—average and below average; with a good level of adaptation, their health was rated as average and above average. In this study, the morphological texture of 172 quadrants in 43 samples of the OF was analysed. As a result, type I microcrystallization was identified in only 2 quadrants, which was 1.2% of all samples; type II was identified in 39 quadrants (22.7%); type III was identified in 101 quadrants (58.7%); and type IV was identified in 30 quadrants, which was 17.4% of all samples. The parameters of OF microcrystallization calculated according to the above formula indicated that their values in the studied OF samples varied from 1 to 4, with certain distribution trends, with a dominant interval depending on the health levels of the body. The results of the analysis of the distribution of the obtained indicator values, depending on the prenosological levels of health of the examined individuals, are presented in the **Table 2.**



а- 1 type б -2 type в -3 type г – 4 type

Figure 1 Type of crystals.

Table 1: The distribution of the examined individuals by health level.

Levels health by Apanasenko	The Quantity of Examined					
	Total		Including			
			Young Men		Girls	
	N	%	N	%boys	N	%girls
High	Not found					
Above average	1	2,3	-	-	1	4,2
Medium	19	44,2	11	57,9	8	33,3
Below average	9	20,9	2	10,5	7	29,2
Low	14	32,6	6	31,6	8	33,3
Total	43	100	19	100	24	100

Table 2: The results of the analysis of the distribution of the obtained indicator values, depending on the prenosological levels of health of the examined individuals.

Health levels	Amount persons and their proportion in % of the total number of examined individuals		Of these. according to micro-crystallization index M in intervals (in arbitrary units and in % of the total number of persons with given health levels)										M_{mid} by health level
	N	%	1.0-1.25		1.5-1.75		2.0-3.0		3.25-3.5		3.75-4.0		
			N	%	N	%	N	%	N	%	N	%	
High	-	-	-	-	-	-	-	-	-	-	-	-	1.25±0.25 forecast
Above average	1	2.3	-	-	1	100	-	-	-	-	-	-	1.75±0.25
Medium	19	44.2	-	-	-	-	14	73.7	5	26.3	-	-	2.75±0.75
Below average	9	20.9	-	-	-	-	4	44.4	4	44.4	1	11.2	3.25±0.5
Low	14	32.6					5	35.7	6	42.8	3	21.4	3.5±0.5
Total	43	100	-	-	1	2.3	23	53.5	15	34.9	4	9.3	-

In the examined contingent, people with a high level of health, as well as people with unsatisfactory and poor adaptation, were not identified. The latter is because patients and persons with severe chronic pathology were not included in the group of examined individuals. As for the lack of people with a high level of health, this is inherent in modern youth who are not actively involved in sports, have low physical activity and have high levels of psycho-emotional stress; this population includes the bulk of students of medical schools, from which our study group was formed. Note that with a high level of health, when the crystallizing abilities of saliva are maximized, facies of the first type are formed, and with unsatisfactory and disruption of adaptation, its crystallizing abilities sharply decrease, and the formation of amorphous structures (facies type 4) increases; that is, a change in the morphology of microcrystals saliva from 1 to 4 characterizes the transition from the prenosological state of the body to a premorbid state and, in some cases, even to a pathological state.

As already noted, among the individuals examined by us, people with high levels of health and people with unsatisfactory and disruptive adaptation were not identified. At the same time, in the samples of OF facies examined by us, we also did not establish facies having microcrystallization type 1 in all quadrants, which logically indicates the possibility of using the microcrystallization index equal to M=1 as probable indicators of a high level of health and M=4 as an indicator of a low prenosological level of health. Thus, as a result of the analysis of the data array that we obtained, we can formulate the following conclusions:

1. For the first time, the dependence of quantitative indicators of the visual morphometry of microcrystals of a dehydrated cancerous sample of practically healthy young people on prenosological levels of their body's health was established. At the same time, the value of the indicator of microcrystallization of OF equal to -1 corresponds to the maximum crystallization ability of the saliva, which occurs at a high level of health of the body; with a deterioration in health, there is a discrete increase in the maximum crystallization ability at a low level of health, up to a value of 4.

2. The morphology indicators of microcrystals of oral fluid can serve as a sensitive and objective indicator of the levels of the functional state of the body of a healthy person and can be considered a fairly simple, economical, non-invasive and informative way to test the body's health level between healthy and disease states, that is, as a method of prenosological diagnosis.

3. Generally, quantitative information on the intrinsic crystallogenic ability and morphological parameters of OF microcrystals formed after dehydration can be used as reference criteria for assessing the levels of prenosological states of the body of practically healthy people.

Conclusion

A morphological analysis of the structure of dehydrated microdrops of oral fluid can be recommended as a biophysical test for diagnosing the prenosological state of an organism's health, and the measurements of the morphological structure of facies microcrystals are the criteria for distinguishing an organism's health levels.

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