ONCOLOGY

Use of Saliva Crystallogenic Properties for Early Diagnostics of Prostate Cancer

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Multivariate analysis was used to examine the morphology of mixed saliva crystals in 55 healthy men and the patients with suspected malignant prostate gland diseases. The established changes in the shape of dendritic crystals in the saliva from prostate-problematic patients were corroborated by the cluster and discriminatory analyse.

Key Words: *saliva; crystallization; malignant prostate pathology*

The development of the early diagnostic methods for detection of malignant prostate gland (PG) diseases remains actual. Evaluation of microcrystallization potency of biological fluids is a modern method of diagnosis of oncological diseases.

Crystallography was used for the analysis of blood in patients with PG hypertrophy and carcinoma. The crystalogenic properties of PG secretion are known to change during andropause. The key role in the tumor and metastatic growth is played by dysregulation of mucin expression [10]. Neoplastic transformation is related to dysregulation of the synthesis of the membrane mucins of the basic protein epitope and to changes in mucin glycosylation [9]. Methodically, saliva is a suitable biological fluid enriched with mucins [4]. We previously developed the method salivary microcrystals classification [2].

Our present aim was to examine the crystallographic properties of the saliva in the norm and during malignant PG pathology.

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MATERIALS AND METHODS

The study was carried out on healthy men (n=25) aged 20-27 years and patients with suspected malignant tumor in PG (n=30) aged 45-85 years. Mixed saliva (oral fluid) was obtained by its free flow from the oral cavity (the baseline saliva). The crystallograms were obtained as described previously [1]. The specimens of oral fluid were placed onto a horizontal surface as a 0.1-ml drop and dried at 18-25°C. The structure of saliva specimens was examined under a Leica DLMS-LS light microscope equipped with a Nikon DM v.581-80 photo camera. The photos were made under the white light provided by incandescent lamp with the matrix exposition mode (1024×768). The video grabbing was performed by a FlyVideo'98 Capture driver v.1.0.0.0 video board. The image was transferred to a display.

Initially, the entire surface of the dried saliva drop was scanned at a low magnification, thereafter the individual surface areas with various morphology were zoomed at greater magnification. The selected crystallogram fragments were saved into a graphic file as a raster image with RGB resolution of 24 bits in BMP format. The off-line correction was performed with Adobe Photoshop 7.0 software. The total number of 110 video files were examined.

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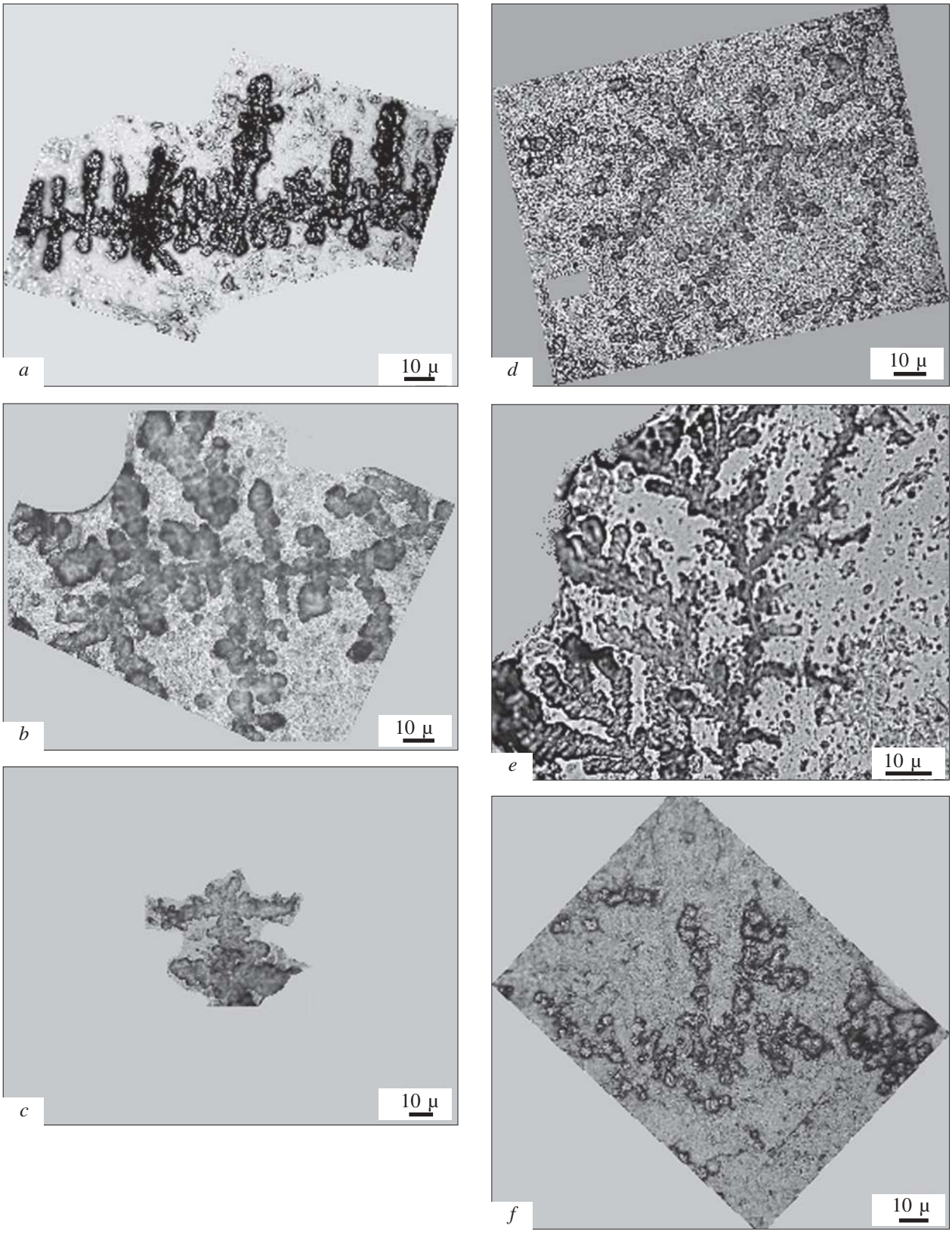

Fig. 1. Qualitative morphological signs of the dendritic crystals grown from the mixed saliva, which are revealed in patients with PG tumor. *a*) a mimosa-like process; *b, d*) mosaic structure of the crystals and a mimosa-like process; c) the symmetrically deformed microprocesses; *e*) unilateral long deformed processes; f) mosaic structure of the crystals.

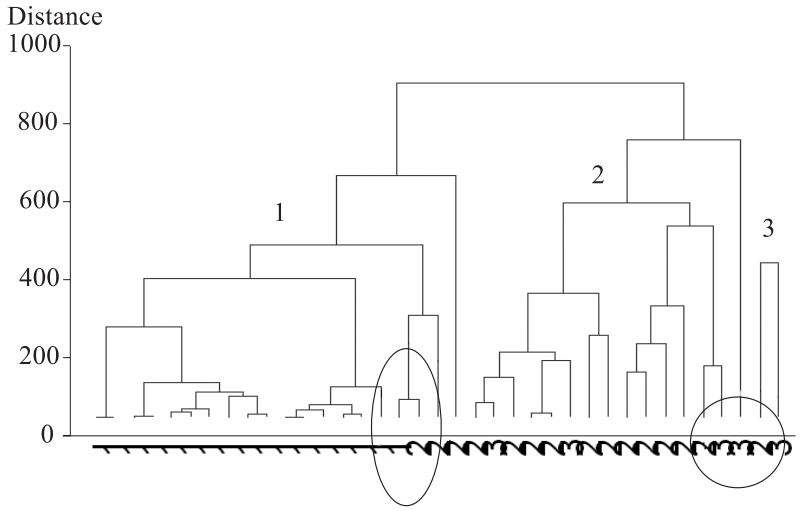

Fig. 2. Morphological separation of crystallograms from healthy men and patients with PG pathology into 3 clusters. *1*) normal; *2*) benign PG hyperplasia; *3*) PG cancer. Ordinate: distance for each step of agglomeration hierarchical clusterization algorithm. Ovals show data for two clusters.

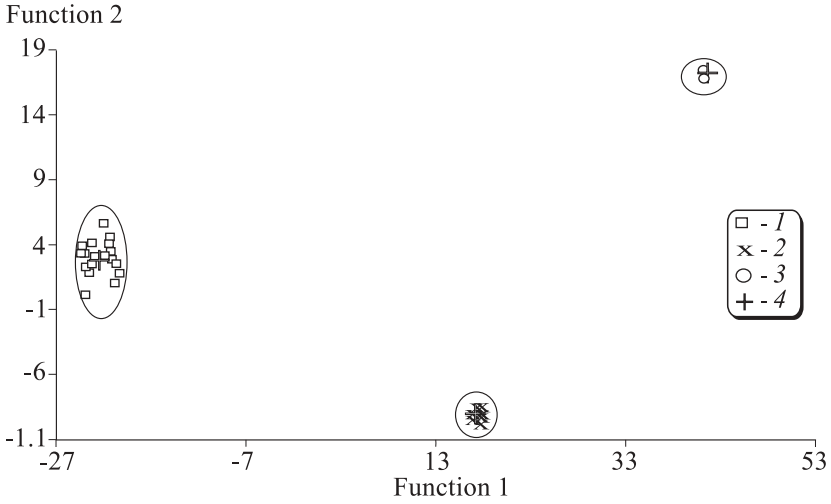

Fig. 3. A two-dimensional scatter diagram of morphological signs of crystallograms on the plane of the first two discriminatory functions. *1*) normal; *2*) benign PG hyperplasia; *3*) PG cancer; *4*) cluster center.

The data were processed statistically with a STATGRAPHICS Plus 5.0 software using multivariate cluster and discriminatory analyses [5].

RESULTS

Normally, crystallization of the oral fluid in healthy people is not strictly identical. Up to 16 signs of skeletal (dendritic) crystals can be distinguished. Only the smaller part of these features (signs) can be described quantitatively, while the greater part of signs can be characterized only quantitatively by the "present" or "absent" attributes [2].

In the experimental group, the number of variants of the dendritic crystals increased to 32. We previously described the appearance of novel species of dendritic crystals in the mixed saliva in other types of pathology [3]. Of principal importance is the appearance of four variants of microcrystals, which are specific of PG only. They were *1*) mimosa-like process (33%); *2*) symmetrical location of deformed microprocesses (33%); *3*) mosaic structure (67%); and *4*) the unilateral long deformed processes (20%).

The cluster analysis (Joining method of construction of a dendrogram or the neighbor-joining tree) showed that the entire data file could be subdivided into clusters corresponding to healthy individuals, patients with benign tumor, and patients with PG cancer. However, these clusters were not separated by clear-cut boundaries.

Another tool, the discriminatory analysis (construction of the scatter diagram on the plane of the first two discriminatory functions) yielded a clear

separation of microcrystals corresponding to healthy individuals and both tumor groups.

The attempts to use microcrystallization of biological fluids to diagnosticate cancer were frequently made in the past [7], but they were not further developed. This can be explained by a number of reasons. First, in most studies the formation of the crystals in biological fluids was carried out under the action of external factors, usually copper sulfate. How and why this salt would modify the character of crystallization under the pathological conditions remain unclear. Second, the majority of researchers analyzed the data within the paradigm of univariate statistics, which says that one type of crystals (for example, a confocal beam of crystals emerging from a common center in the presence of $CuCl_2$) corresponds to normal, while another type of crystals indicates different functional states or some pathologies [6]. At the same time, crystallization on glass or other matrices results from complicated physical processes, which yield not strictly identical crystals [8]. The observed polymorphism results from polydispersity of the specimen in mass and configuration of the macromolecules and from the presence of the ordered macromolecular complexes (mucins) in the substrate [7]. Therefore, we used multivariate statistics, which simultaneously assesses many signs instead of a single one. This approach yielded the positive unequivocal results.

Thus, this paper is the first report of systematized and described morphological signs, which characterize the crystalline aggregates formed from the mixed saliva of the patients with tumor growth in PG.

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