Crystallization of Oral Fluid Components in Patients with Type 2 Diabetes Mellitus

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Crystal aggregations of oral fluid from normal subjects and patients with type 2 diabetes mellitus were examined. Morphological signs characterizing crystal aggregations of salivary pools from patients with type 2 diabetes mellitus are described and classified.

Key Words: *oral fluid; crystallization; type 2 diabetes mellitus*

Structural changes in the crystallized oral fluid (salivary pools) can serve as diagnostic signs of various pathologies [2,3]. The presence of numerous concomitant somatic diseases notably impedes wide use of this method in the clinical practice. One of these diseases is type 2 diabetes mellitus. The number of diabetics amounts to 8 million in Russia; the number of subjects with the prediabetes stage is virtually the same [1].

The aim of our study was to describe variants of microcrystallization of oral fluid in type 2 diabetes mellitus.

MATERIALS AND METHODS

The object of the study was mixed saliva (oral fluid, OF) collected during its free flowing from the mouth (basal saliva). Control group consisted of 20 normal subjects aged 20-27 years, experimental group (type 2 diabetes mellitus) consisted of 36 patients (6 men and 30 women). The disease was diagnosed in accordance with WHO criteria [5]; 55% patients presented with severe diabetes mellitus. In women saliva was collected during the luteal phase of the menstrual cycle. A droplet of OF was placed in a Petri dish with a cover and dried on free surface. Droplets of OF (0.1 ml) were applied on the underlayer surface and dried at 18-25°C in a strictly horizontal position until complete drying [2]. The structure of the salivary samples was examined using a Leica DM-LS light microscope fitted with a Sony SSC-DC30P videocamera. The image was transferred on the monitor. First the entire surface of the dried droplet was scanned at low magnification, then individual fragments of the surface with different morphology were examined at higher magnification. The fragments of crystallograms were recorded in a PC as graphic files with the following parameters: 362×280 pixels with 256 gray scale shades. The files were saved as scanned images with 300 dpi resolution in the BMP format. A total of 400 videofiles were examined. Graphic images were processed (rotation on the plane, brightness and contrast modification, size marking) in the Adobe Photoshop 6.0 and PhotoDraw 2000 software. Biometrical results were transferred into the electron table Excel 2002 from Microsoft Office XP software. The data were analyzed using Statgraphics Plus 5.0 (Manugistic Inc. USA) software. The zero hypothesis was evaluated using Student's t test at $p<0.05$.

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RESULTS

Crystallization of oral fluid in normal subjects was different. We distinguished up to 16 variants of skeletal crystal signs (according to crystallographic terminology); in physics these crystals are called dendrites.

Images of grown crystals (graphic files) were standardized by rotation on the plane, so that they looked like bushes or trees. This unification permits stable quantitative description of 6 signs. Ten more signs can be described qualitatively (yes/no). For more con-

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venient statistical processing expert name of the sign was presented as a three-letter code.

The most rare (10% cases) were "splitted terminals" (CFS); the most rare crystal shapes were "flat" (FCT) and cross-shaped (CRE).

The saliva from patients with type 2 diabetes mellitus crystallized differently. Microcrystals did not form in 18% cases, though the image was not homogeneous, but texture-like. In other cases microcrystals formed, some of them differing qualitatively from the normal. Crystal bodies with modified processes and apexes formed (Fig. 1). Normally the body of the crystal and its processes are clearly seen (Fig. 1, *a*), while in disease one branching process (CBM) originates from the crystal body in 72% cases (Fig. 1, *b*).

Normally clearly seen first-order processes originating at a small angle (Fig. 1, *c*) are forming, while in diabetes the processes are short and wide (Fig. 1, *d*, **1**, **2**), which can end by a blurred texture-like structure (CBD, 56%).

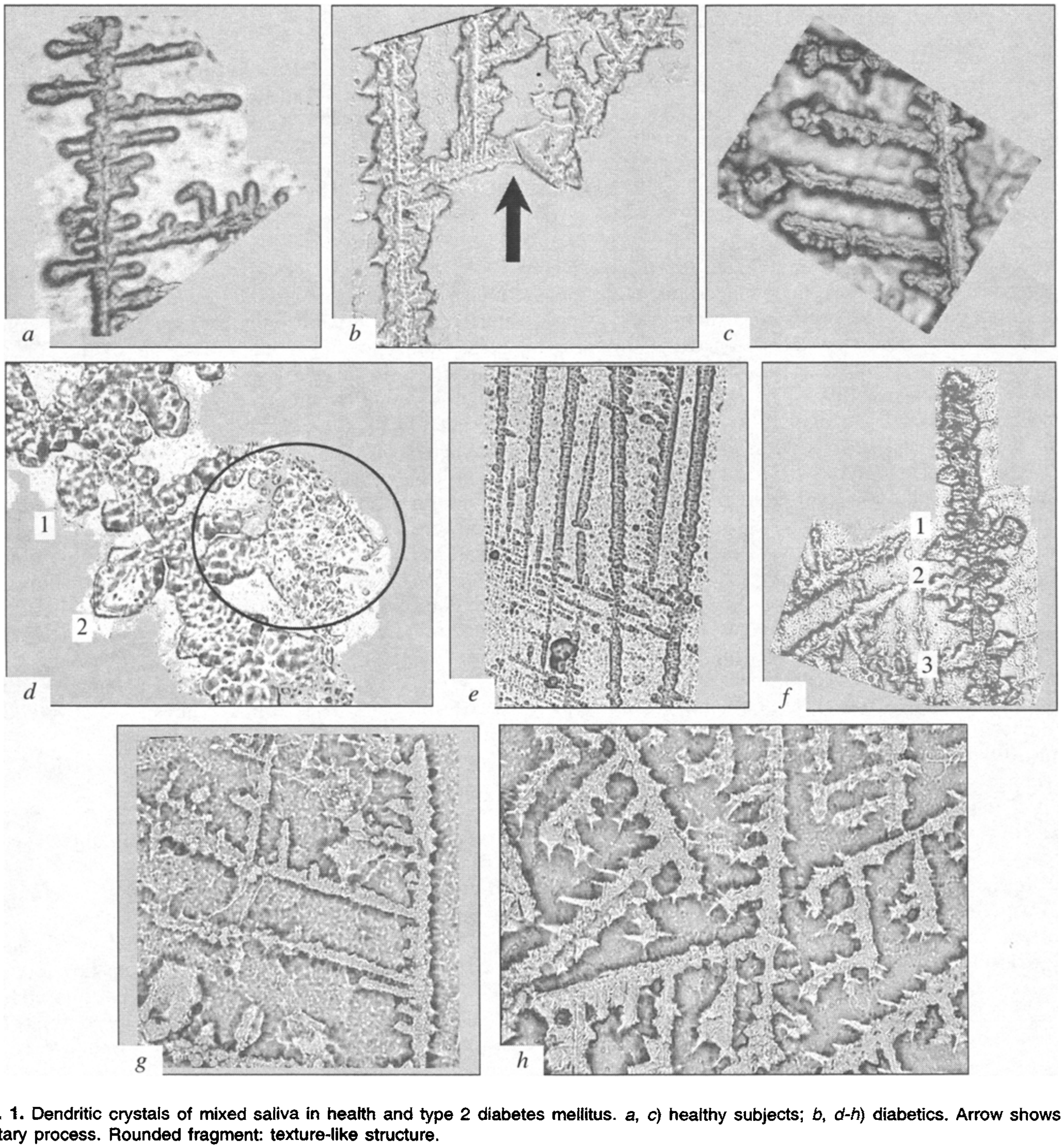

Fig. 1. Dendritic crystals of mixed saliva in health and type 2 diabetes mellitus. *a, c*) healthy subjects; *b, d-h*) diabetics. Arrow shows a solitary process. Rounded fragment: texture-like structure.

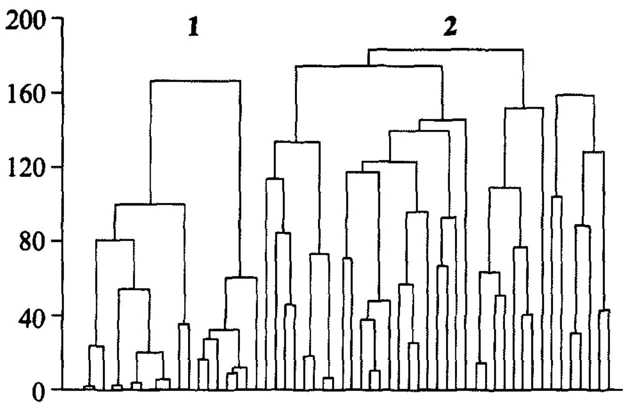

Fig. 2. Cluster analysis. Dendrogram (Wards method for 2 clusters). Abscissa: cases complied in accordance with analysis; *1*) normal subjects; *2*) diabetics. Ordinate: distance for each step of agglomerative hierarchical clusterization algorithm.

Needle-shaped crystals (CAN) were observed in 17% diabetics (Fig. 1, *e*); sometimes the processes had peak-shaped apexes (Fig. 1, *f*, **1**, **2**). "Coral branch" crystals (CCB) were observed in 39% cases (Fig. 1, *h*). Crystals with unilateral growth of microprocesses (CUM) were observed in 72% diabetics (Fig. 1, *g*). Hence, salivary microcrystal growth is modified during the development of non-insulin-dependent diabetes mellitus.

Multidimensional statistical analysis was carried out using the data from 20 normal subjects (16 parameters) and 36 patients (31 parameters) and 5 additional parameters: sex, age, disease duration, presence of periodontitis, occlusion height, which were introduced in the Statgraphics electron table and subjected to cluster analysis. The joining method (tree clustering) was used, consisting in dendrogram or "joining tree" plotting. Cluster analysis was carried out by the Wards method for the analysis of clusters with approximately equal numbers of members. The study group was chosen as the key label. Clusterization resulted in complete separation of patients (Fig. 2, **2**) and normal subjects (Fig. 2, **1**) into clusters. This indicates the possibility of detecting crystals of normal subjects and patients by multidimensional statistical analysis. These changes in the crystal morphology were detected for the first time: it was previously considered that synthetic processes in the salivary glands were little changed in diabetes mellitus [4,5,7].

Hence, we for the first time classified and described morphological signs characterizing crystal aggregations of salivary pools from patients with type 2 diabetes mellitus.

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