Crystallization of Oral Fluid Components in Patients with Type 1 Diabetes Mellitus

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We systematized and described morphological criteria characterizing crystalline aggregates in mixed saliva from patients with type 1 diabetes mellitus.

Key Words: *oral fluid; crystallization; type 1 diabetes mellitus*

Micromorphological signs of dried biological fluids were extensively studied over the past 10 years [4,5]. These characteristics can include diagnostic criteria of diseases. Study of mucin-containing biological fluids (*e.g.*, tear, saliva, and nasal secretion) is of considerable importance in this respect. Published data show that various diseases, including cancer, are accompanied by quantitative and qualitative changes in the expression of mucin genes [8]. The use of crystallography in clinical practice is limited due to the fact that specific condition often develops against the background of this or that somatic pathology, *e.g.* diabetes mellitus (DM) [1].

Here we studied microcrystallization of the oral fluid (OF) in patients with type 1 DM (DM-1).

MATERIALS AND METHODS

Mixed saliva (OF) was collected during free discharge from the oral cavity (baseline secretion). The control group included 20 conventionally healthy volunteers (20-27 years). The main group included 30 patients (13 men and 17 women); 50% patients had moderate DM. The diagnosis of DM-1 was made according to WHO criteria [6]. In women saliva was collected during the lutein phase of the menstrual cycle.

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A drop of OF (0.1 ml) was placed on the surface of Petri dish and substrate for drying biological fluid. The samples were completely dried in a horizontal position at 18-25°C. The method for evaluating structural characteristics of saliva samples was described elsewhere [2].

The data were processed by means of cluster analysis.

RESULTS

In healthy volunteers crystallization of OF considerably varied. We revealed 16 types of backbone crystals (crystallographic terminology). In physics this form of crystals is designated as dendrite. The images of grown crystals (graphic files) were standardized by rotation in such a manner that they looked like a shrub or tree. The method of unification allows us to describe quantitatively 6 signs. It should be emphasized that up to 10 additional signs can be characterized qualitatively [3].

Microcrystals were not detected in 30% saliva samples from patients with DM-1. The image was textured, but not homogenous. Microcrystals were revealed in saliva samples from other patients. Some crystals had abnormal qualitative characteristics. The body had modified processes and apexes. Bulk crystals were found in 74% samples (Fig. 1, *a*). Unilateral first-order processes were formed in 61% samples from DM-1 patients (Fig. 1, *b*). Splitting of the apex was often seen (Fig. 1, *c*). The formation of processes

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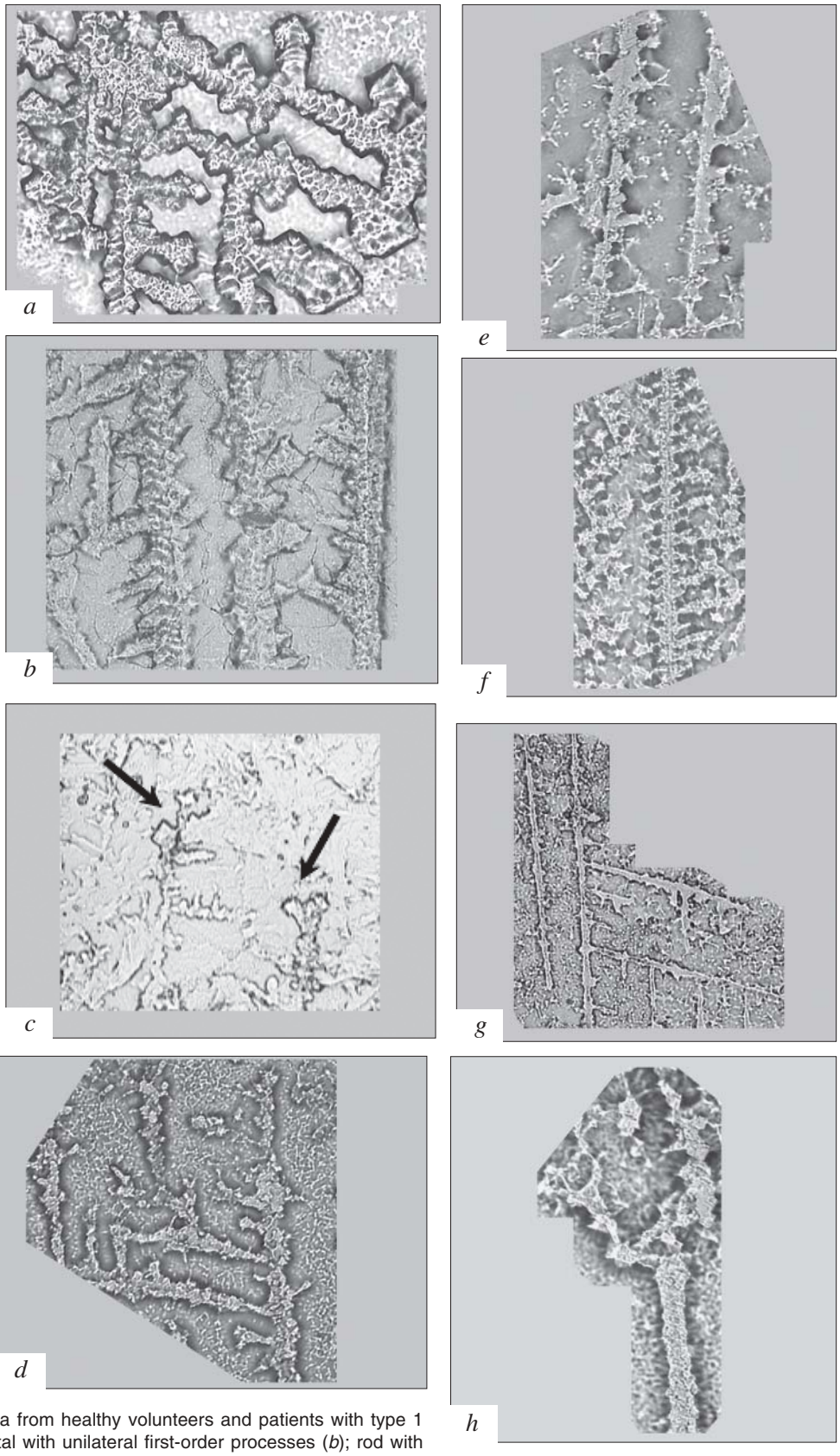

Fig. 1. Dendrite crystals in mixed saliva from healthy volunteers and patients with type 1 diabetes mellitus: bulk crystal (*a*); crystal with unilateral first-order processes (*b*); rod with split apex (arrows, *c*); crystals with long branching processes (*d*); non-branching rods with short deformed processes (*e*); crystals resembling a coral branch (*f*); crystals with single branching process (*g*); absence of processes, naked crystals (*h*).

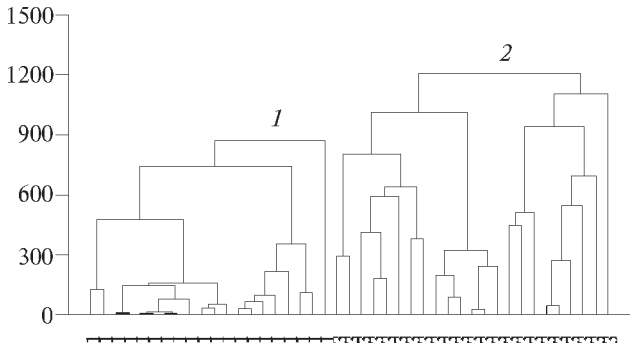

Fig. 2. Cluster analysis. Dendrogram was constructed by the method of wards for 2 clusters. Normal (*1*) and diabetes mellitus (*2*). Abscissa: combined observations (according to the analysis). Ordinate: distance for each step of the agglomerative hierarchic algorithm in clustering.

was impaired. In 48% samples, crystals had long branching processes (Fig. 1, *d*). We also visualized short deformed processes (Fig. 1, *e*). The growth of microcrystals in 39% samples resembled a coral branch (Fig. 1, *f*).

Crystals with a single branching process were found in 26% samples from DM-1 patients (Fig. 1, *g*). Naked crystals without processes were rarely seen (22% samples, Fig. 1, *h*).

Our results show that the growth of microcrystals in mixed saliva remained qualitatively unchanged in patients with insulin-dependent DM.

Statistical treatment of the results also included multivariate analysis. Processing of the results for the control (20 volunteers, 16 parameters) and main groups (30 patients, 31 parameters) involved cluster analysis. We took into account 5 additional parameters: sex, age, duration of the disease, presence of periodontitis, and vertical dimension. The method of joining suggests construction of a dendrogram or agglomerative tree. Cluster analysis was performed by the method of wards (selection of clusters with approximately equal number of members). The group served as a point label. After clustering the patients and healthy volunteers were divided into clusters (Fig. 2). Our results are consistent with published data that synthetic processes in the salivary glands change little in patients with DM [7,9,10].

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