Crystallization of Components Oral Fluid in Diabetics in Case of Absence of Crystal Structures

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Crystallization of mixed salivary pools was studied in patients with types 1 and 2 diabetes mellitus. The formation of microcrystals in two types of diabetes varied and could be differentiated by multidimensional analysis. If salivary crystallization was absent, the material could be evaluated by the texture analysis methods.

Key Words: *mixed saliva microcrystallization; types 1 and 2 diabetes mellitus; texture analysis*

We studied morphological features of crystals forming after drying of oral fluid from patients with diabetes mellitus of two types [4,5]. The saliva of diabetics does not always form crystals after drying (in 18% cases in type 2 and in 30% cases in type 1 diabetes).

We analyzed and summarized the data and evaluated the cases when dried saliva did not form crystals.

MATERIALS AND METHODS

Mixed saliva (oral fluid, OF) was collected under conditions of spontaneous flow from the oral cavity (basal saliva). Control group consisted of 20 normal subjects aging 20-27 years. Diabetes mellitus was diagnosed in accordance with WHO criteria [4]. Experimental group consisted of 96 diabetics: type 1, 20 men and 21 women, and type 2, 10 men and 45 women. 55% patients presented with severe diabetes. In women the saliva was collected during the lutein phase of the cycle. A droplet of OF was put onto the surface of a Petri dish with a cover (laboratory plastic TU 64-2-19-79) and dried on free surface; 0.1 ml droplet of OF was put onto the underlayer surface (on which the biological liquid dried) and dried at 18-25°C in a strictly horizontal position [1]. The structure of the saliva samples was studied under a Leica DM-LS optical microscope with a Sony SSC-DC30P analog videocam. The image was captured with an Asus 3 DP-V264 GT/PRO videoplate. The resultant image was

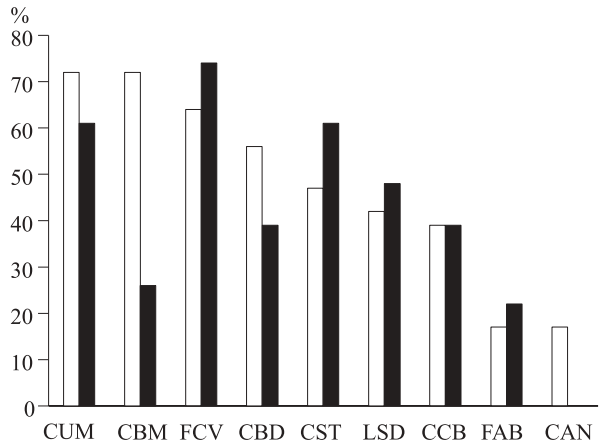

Fig. 1. Comparative frequency of different crystal types in different types of diabetes mellitus. Ordinate: frequency of crystals in types 1 and 2 diabetes. Abscissa: types of crystals: CUM: crystal with unilateral processes; CBM: stems with a single ramifying process; FCV: crystal of volumic form; CBD: stem without ramification, with a short deformed microprocess; CST: stem with cleaved deformed apex; LSD: crystal with a long process, ramifying at its end; CCB: coral branch crystal; FAB: "naked crystal", no ramification at all; CAN: needle-shaped crystal. Light bars: type 1 diabetes; dark bars: type 2 diabetes.

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TABLE 1. Texture Analysis of Dry Saliva without Apparent Signs of Crystallization

Group	Mean absolute value	Entropy	Contrast	Energy	Maximum probability	Correlation	Homo-geneity
Type 1 diabetes (n=10)	217.1±34.7	3.54±1.32	52 020±5588	0.75±0.05	0.80±0.06	0.99±0.36	0.810±0.063
Type 2 diabetes (n=13)	238.3±33.1	3.58±1.08	58 817±5799	0.93±0.04*	0.94±0.06	0.99±0.35	0.95±0.065

Note. Minimum value of the parameter is "0", -0.990 for correlation. Maximum values±standard error are shown; n is number of measurements. *p=0.005 according to Student's test.

transferred onto the monitor. At first the entire surface of the dried droplet was scanned at low magnification, and then individual areas of the surface with different morphology were examined at greater magnification. The selected areas of the crystallogram were recorded as graphic files with the following parameters: 362´280 pixels with 256 brightness shades (gray scale). The files were saved as scanning images with 300 dpi resolution in the BMP format. A total of 400 videofiles were examined. The data were analyzed using Statgraphics Plus 5.0 statistic software. Texture analysis of images by means of Hesperus software (Am. Lab. USA 3.0 beta) was used. The zero hypothesis was evaluated using Student's t test. The results were considered reliable at $p \leq 0.05$.

RESULTS

New modifications of microcrystals appear in types 1 and 2 diabetes [3-5]. Some microcrystal types (CUM, FCV, LSD, and CCB) [3] appeared at about the same frequency in diabetes of different types (Fig. 1). Other (CBM and CAN) [3] were more incident in type 2 diabetes, other types were more incident in type 1 condition.

In order to detect the differences in crystal structures forming in diabetes of different type, we compared the data using multidimensional (discriminant) analysis, which permits comparison of all qualitative and quantitative signs in general.

Microcrystals of expert descriptions in health and diabetes of two types differ in space and form individual groups, not crossing with other classes (Fig. 2). The set of microcrystal variants formed by mixed saliva, described in our study, indicates that they are characteristic of the studied diabetes types, despite their partial identity.

Many saliva pools did not form crystals (up to 30% in type 1 diabetes), and hence, microcrystals could not be studied. The microscopic picture of the dried saliva was not homogeneous, but textured. We therefore applied texture analysis. The notion of texture has many definitions, depending on the sphere of application [1,2]. We used Hesperus software, intended for

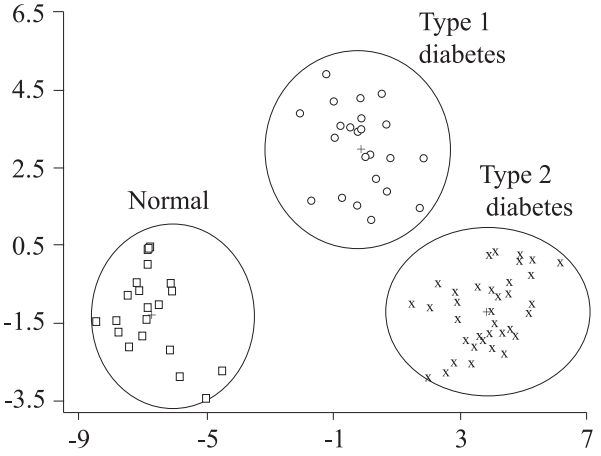

Fig. 2. Discriminant analysis. Two-dimensional diagram of dissemination of morphological signs of diabetics' crystallograms on the plane for two first discriminant functions. Abscissa: observations classified in accordance with analysis. □: normal; x: type 2 diabetes; o: type 1 diabetes; +: cluster center. Ordinate: distance for each step of the agglomerative hierarchical clusterization algorithm work.

imaging and processing two-dimensional data of any nature (Table 1). Salivary textures virtually did not differ from each other by six of seven parameters, differing only by the "energy" parameter (24% higher in type 2 diabetes; t=-3.163, p<0.005).

Hence, the totality of microcrystal variants formed in different types of diabetes mellitus is different and can be discriminated by multidimensional analysis.

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