AUTOMATED MORPHOMETRIC INVESTIGATION OF THE KIDNEY EOCENE THE "DIAMORPH" COMPUTERIZED SYSTEM

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A further improvement in the quality of morphologic diagnosis of kidney diseases can be achieved through objectivization of morphologic investigations [1] by the wider use of quantitative methods [6]. Quantification of a pathological process also enables correlation analysis to be undertaken, with the detection of parallel trends in morphology and function [5]. Computerized systems enabling automation of image analysis, highly accurate analysis of a large number of morphometric parameters, accumulation of data, and statistical analysis of the results, are being actively developed at the present time and introduced into the investigative side of medical practice [2-4, 7, 9].

The aim of this investigation is to assess the possibility of using computer systems of image analysis in order to obtain new informative morphometric parameters reflecting interaction between structure and function in the kidney.

METHODS

The investigation was carried out with the aid of "Diamorph" computerized morphometric complex (CMC), developed at the Research Institute of Physicochemical Medicine, Ministry of Health of the RSFSR, in conjunction with the Leningrad Institute of Fine Mechanics and Optics and the Leningrad Optico-Mechanical Combine, and consisting of a specialized image analysis system. The "Diamorph" CMC is based on an IBM PC/AT personal computer. The CMC possesses advanced software, oriented for working with biological objects, and also an improved "Kontrast" light microscope (based on the MBI-15 instrument). It enables up to 100 morphometric and densitometric parameters in cytological objects and up to 30 such parameters on histological objects to be measured and calculated. By means of a television camera and special converter, images from a light microscope of "Kontrast" type (Research Institute of Physicochemical Medicine, Leningrad; Leningrad Institute of Fine Mechanics and Optics, USSR), or "Universal" (Opton, Germany) type, or drawings or copies made directly from preparations, to be led into the computer. The test material consisted of biopsy specimens from the kidneys of nine patients with insulin-dependent diabetes (IDD) aged from 16 to 51 years (four men and five women). Group 1 (control) consisted of five patients with a periodic form of the disease, the study of kidney biopsy material from whom revealed no pathological changes, group 2 consisted of four patients with a manifest form of diabetes for not more than 1 year (1.5-12 months), and group 3 consisted of five patients in whom the duration of the disease exceeded 1 year (1.5-26 years). Clinically in all patients of groups 2 and 3 the glomerular filtration rate was increased from 144 to 285 ml/min × 1.73 m^2. In addition, in the patients of group 3 microalbuminuria of between 30 and 300 mg/day was found. Semithin sections (thickness 1 μ) from the kidney biopsy material, processed by the standard method for electron-microscopy, were used. The semithin sections were stained with methylene blue—azure II—fuchsine (Fig. 1). During automated investigation with the "Diamorph" CMC, at least three glomeruli in each biopsy specimen were subjected to morphometry. The following basic parameters

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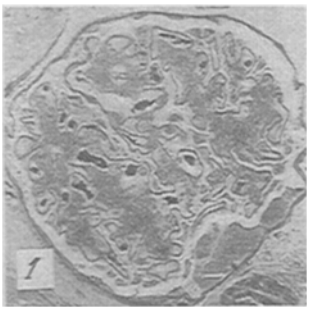

Fig. 1

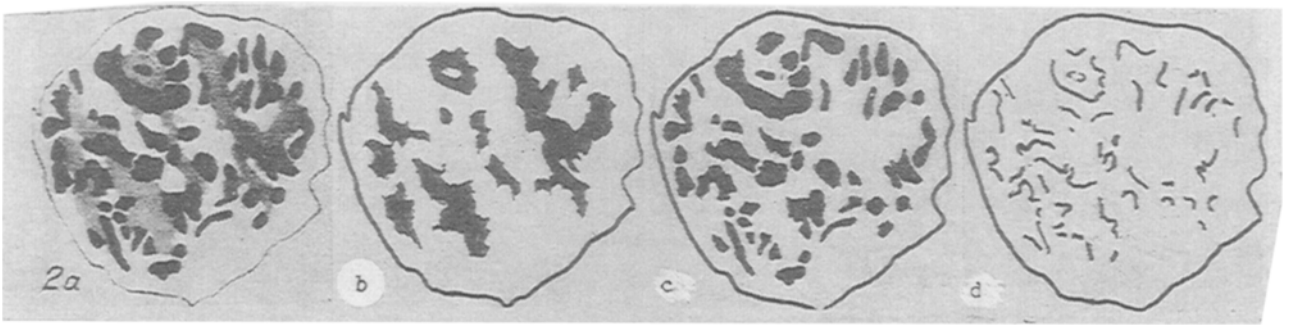

Fig. 2

Fig. 1. Diabetic glomerulosclerosis. Staining with methylene blue—azure II—fuchsine. Semithin section, 360 ×.

Fig. 2. Image of preparation after computer analysis (a) and results of computer-assisted dissection (discrimination) (b-d). Pale regions — mesangium; dark — glomerular capillaries; b)mesangium; c) capillaries of glomerulus; d) computer image of "capillary—mesangium" contacts.

of traditional morphometry were determined: the area of the renal corpuscle and the perimeter of Bowman's capsule, the area and perimeter of the mesangium and capillaries of the glomeruli. These traditional parameters are easily modified by standard programs of the majority of computerized image analysis systems [4]. A morphometric study was made of the "capillary—mesangium" zone of contact, for which purpose a special program was devised from the program modules of the "Diamorph" CMC. Computer-assisted dissection of the glomeruli into mesangial and capillary parts was carried out by methods of mathematical morphology (Fig. 2b, c), after which the boundaries of their mutual contact were determined and outlines of the mesangial part of the capillary (paramesangial region) were distinguished, i.e., the boundary of "capillary—mesangium" contact (Fig. 2d) was characterized, and the average extent of these contacts (LKM) was measured. The mean fraction of the filtration surface of the glomerular capillaries (FS) also was calculated by the equation:

$$FS = \frac{(PK - LKM) \times 100\%}{PK}$$

where PC denotes the mean perimeter of a capillary. The "Statgraphics" standard program package was used for statistical analysis of the results. To calculate Kullback's information modulus and other criteria of difference, programs of statistical analysis of results, constituting a part of the software for the "Diamorph" CMC, were used.

RESULTS

Light-optical study of renal biopsy material from patients with IDD of groups 2 and 3 revealed only minor changes: minimal segmental enlargement of the mesangium, cloudy-swelling or hyaline-droplet degeneration of the

nephrocytes of the convoluted tubules. The morphometric investigation showed that analysis of the parameters traditionally used [8] — relative area of the mesangium (AMC), relative area of the capillaries of a glomerulus (ACC) — enabled group 3 to be reliably ($p < 0.01$) distinguished from group 1. However, group 2 did not differ from group 1.

By the use of the suggested parameter and its derivatives, it was possible to distinguish all groups of patients, including groups 2 and 1, those most closely resembling each other at the light-optical level, reliably ($p < 0.01$).

Determination of Kullback's information modulus confirmed the greater dependence of the suggested parameters LKM and FS (15.0 and 11.0, respectively) than the parameters AMC and ACC (1.5 and 0.7, respectively).

The suggested morphometric parameter LKM can be used not only for the differential diagnosis of various diseases of the kidneys, especially when used in conjunction with other parameters, but also to assess changes in structural—functional correlation of the glomerular capillaries and mesangium at different stages of the pathological process.

Using morphologic investigation of the renal glomeruli in diabetic nephropathy as the example, it is shown that CMC can be used in the morphological diagnosis of glomerulopathies. With the aid of CMC it is possible to look for new quantitative parameters for the differential diagnosis of pathological states, including their early (preclinical) stages, to speed up the process of morphometry and to make quantitative criteria more accurate and informative.

LITERATURE CITED

1. G. G. Avtandilov, *Klin. Med.*, No. 1, 8 (1987).
2. G. G. Avtandilov, *Medical Morphometry* [in Russian], Moscow (1990).
3. A. V. Zhukotskii, S. I. El'yutina, and É. M. Kogan, in: Abstracts of Conference of Specialists of Comecon Countries, Suzdal' (1989), pp. 21-22.
4. P. Fraser (ed.), *Microcomputers in Physiology*, Oxford (1988).
5. M. A. Pal'tsev, *Arkh. Patol.*, No. 1, 62 (1987).
6. A. I. Strukov and L. V. Kakturskii, *Arkh. Patol.*, No. 6, 3 (1979).
7. V. V. Shlyapnikov, N. T. Raikhlin, N. N. Vasil'eva, et al., *Arkh. Patol.*, No. 7, 58 (1988).
8. R. Osterby, H.-H. Parving, E. Hommel, et al., *Diabetes*, **39**, 1057 (1990).
9. A. V. Zhukotskii (A. V. Zhukotsky), N. N. Butusova, Kh. M. Umudov, et al., *Biomat. Artific. Cells Artific. Organs*, **15**, 137 (1987).