Patterns of Homeostatic Alterations during Delayed Aftereffects of Radiation: Detection using Laser Correlation Spectroscopy

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Identification of the subfraction composition is performed using the method of laser correlation spectroscopy, which makes it possible to estimate the contribution of 1-10^4-nm particles in the total effect of quasi-elastic light scattering. Special classification software is used to analyze statistically the complex multicomponent spectra, bringing to light reliable shifts differentiating the pathological processes.

Key Words: *laser correlation spectroscopy; risk groups; ecological anomalies; medical monitoring*

Laser correlation spectroscopy (LCS), also known as Doppler spectroscopy, optical shift spectroscopy, quasi-elastic light scattering, etc., is widely used in biology, chemistry, and physics as a rapid and precise method of estimating the hydrodynamic size of particles of various nature in solutions. Meanwhile, by means of mathematical methods of regularization of the fluctuation spectra of the photocurrent, has become possible to estimate the subfraction composition of the light-scattering particles in a solution depending on their size. Thus, in principle it is now possible to a analyze multicomponent liquid media, including such complex ones as blood serum or plasma [3]. The ratios of molecular components vary greatly above all, depending on the pathognomonic nature of individual pathological processes in the organism, as has been shown by several investigators [2].

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In view of this, it was of interest to study the variability of plasma subfraction composition by examining diverse pathologies in a human population that had been exposed to chronic radiation [1]. On the one hand, a multiparameter characterization of homeostatic shifts should contribute to a better understanding of the nature of postradiation complications. On the other hand, new techniques for multicomponent classification of recorded disorders open up the possibility for a new express method of diagnosing postradiation disorders, involving new hardware and software yielding objective results during population analysis that can be used in monitoring studies of ecologically abnormal regions.

MATERIALS AND METHODS

The studies were carried out using a laser correlometer adapted for the analysis of minimized volumes of plasma and/or serum (as little as 0.1 ml). The device was manufactured at the Research-Manufacturing Enterprise *Progress* at the Russian Academy of Medical Sciences. The principles of data processing and the construction classification software were described earlier [2].

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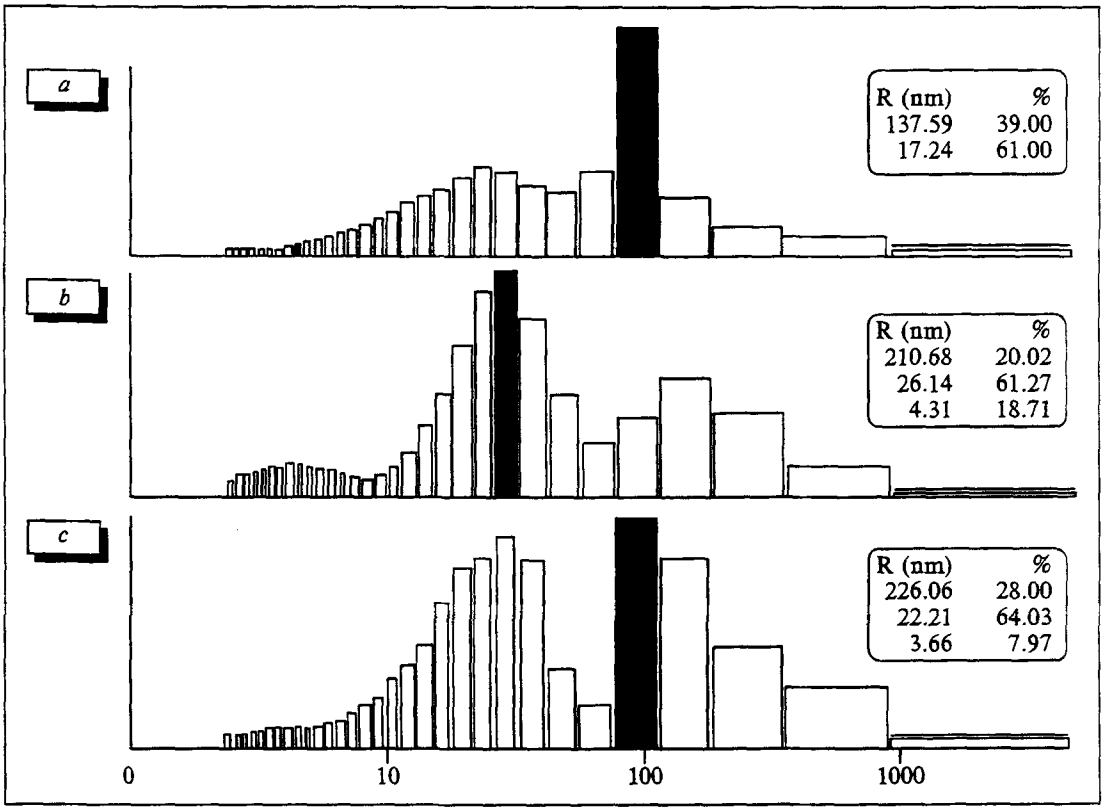

Fig. 1. Averaged spectrum of plasma subfraction composition in patients with different pathologies. *a*) 1st group without clinical symptoms at time of examination; *b*) 2nd group with chronic inflammatory diseases; c) 3rd group with degenerative–dystrophic diseases. Abscissa: hydrodynamic radius of each identified fraction, nm. Ordinate: relative contribution of each identified fraction to total intensity of light scattering. Each histogram presents the mean of modes and their percent contribution to the total intensity of light scattering of samples.

In the present work LCS was used to examine groups of patients who had been exposed to radiation due to radioactive contamination of the Techa River and a reference group. Irradiated patients were divided into 6 groups: 1) individuals who had no clinical symptoms at the time of examination (reporter group); 2) patients with chronic inflammatory diseases (chronic bronchitis, chronic cholecystitis, pneumonia, chronic pyelonephritis); 3) patients with degenerative-dystrophic syndromes (osteochondrosis, primary osteoarthrosis deformans); 4) patients with autoimmune syndromes (systemic lupus erythematosus, autoimmune thyroiditis, autoimmune thrombocytopenia, periarteritis nodosa, rheumatoid arthritis); 5) patients with obligate precancerous disorders (chronic atrophic gastritis, polyposis of sigmoid colon and rectum, fibroadenomatosis, multiple gastric polyposis, etc.); 6) patients with malignant diseases.

The reference group consisted of people who lived in the same regions but had not been exposed to radiation.

Blood was taken from the finger using a standard blood pipette prewetted with an anticoagulant (Na citrate). The contents of the pipette were carefully transferred to an Eppendorf-type 0.7 ml test tube containing 3 volumes of saline per volume of blood and stored for 15-20 min, after which the tubes were centrifuged for 5 min at 2.5×10^3 rpm, and 100 μl of supernatant from each tube were transferred to a clean tube with a hermetically sealed plastic cap. All procedures were performed at room temperature. The diluted specimens of plasma were stored at -20°C and were thawed just before use.

Prior to analysis the specimens were incubated for 15-20 min at room temperature, and then 0.3-0.4 ml of saline was added to each tube, and the samples were thoroughly mixed and centrifuged for 15 min at 2.5×10^3 rpm. The supernatant was placed in the measuring cuvette of the laser spectrometer. Thus, the object studied represented native plasma diluted 12-15 times with saline.

RESULTS

It is known that many delayed radiation-induced aftereffects do not differ from analogous diseases of spontaneous origin in either their clinical or morphological parameters. In the interests of further

discrimination of risk groups according to different postradiation complications, it was useful to differentiate the revealed shifts in terms of the severity of particular postradiation disorders.

The averaged spectrum of plasma taken from patients with predominantly degenerative-dystrophic processes is presented in Fig. 1. Even simple visual analysis reveals obvious differences in the plasma subfraction composition of this group as compared to that in the group exposed to radiation but free of clinical symptoms. First, we should note the multicomponent nature of the spectrum differences. For example, whereas in the reference group the main contribution to light scattering (up to 80%) is assigned to the subfractions consisting of 30-60-nm particles, in the group with chronic degenerative-dystrophic processes the main role (up to 60%) belongs to the small-size component (particle size less than 30 nm). It is worth noting that in accordance with the severity of individual clinical features the recorded spectra differ to some degree depending on the pathognomonicity of the degenerative-dystrophic and inflammatory processes (Fig. 1). Multicomponent differences are seen between the total spectra of patients with predominantly osteochondrosis pathology and of patients with predominantly chronic bronchitis and gastritis. The quantitative differences between these groups can be established from the data of multiparameter classifiers. As can be seen from the classification tables (Fig. 2), only 4 out of 30 observations (14%) coincided with the group free of clinical symptoms. The same number of spectra revealed no reliable analogy with any of the groups that were compared (in the classification table such results are entered under "else"). In view of the marked heterogeneity of clinical symptoms in groups 2 and 3, it seemed important to assess the degree of variability in the subfraction composition of the plasma when comparing specific pathological types in these groups. For this purpose, only cases with severe bronchial symptoms (subgroup 2a) were chosen from group 2, and cases with mainly osteochondrosis pathology (subgroup 3a) were chosen from group 3. As can be seen in Fig. 2, the differential sensitivity of the method was not increased as a result of such assortment; in fact, in certain cases the degree of differences even sloped down slightly (to 60%). This confirms the hypothesis that the main homeostatic differences are governed by the symptomatics of the pathologies compared (degenerative-dystrophic or inflammatory) and to a lesser degree are related to the localization of the pathology. It is worth noting a specific feature of the "classifier" software used for statistical evaluation, namely owing to the specially

a

		3	1
else	10%	13%	6%
3	53%	73%	33%
1	37%	14%	61%

		3	1
else	10%	4	1
3	53%	22	6
1	37%	4	14

b

		3	1
else	10%	6%	11%
3	32%	59%	5%
1	58%	35%	84%

		3	1
else	10%	0	0
3	32%	4	0
1	58%	2	11

Fig. 2. Statistical data of multicomponent classification for comparative analysis of plasma subfraction composition in patients from 1st and 3rd groups (*a*) and subgroups 2a and 3a (*b*). Results are presented as absolute numbers of coincidences and noncoincidences in each pair compared and as percents of expected level of coincidence for the same pairs compared. In composite tables the group "else" is formed on the basis of spectra statistically different from pairs of groups analyzed. The column preceding the groups analyzed gives the statistical probabilities of *apriori* ratios of contribution of each group according to the number of spectra analyzed.

organized statistics of *apriori* expectation (% in the first column), it counterbalances somewhat the raised degree of reliability that results from the limited number of cases compared and extrapolates the expectation probability to a more representative number of observations. For example, if formally all 11 observations coincide only with the group proper, i.e., within a given group, then the reliability should approximate 100%, whereas if only 2 of 6 observations fall into another reference group, the reliability should be about 67%. However, the classifier, by extrapolating the low number of expected results to certain *apriori* statistical weights of frequency, estimated the significance as 84% and 59%, respectively.

Thus, these results form the basis for an objective differential diagnosis of the homeostasis status using LCS, as a function of the nature of possible delayed aftereffects of radiation on the human organism.

The above thesis is confirmed by other observations we performed of individuals with prevailing symptoms of precancerous and autoimmune diseases. Visual analysis of the plasma subfraction composition (Fig. 3) reveals multiparameter differences in both the intensity (contribution to light scattering) and dimensions of certain spectrum modes. Generally speaking, the histograms of precancerous patients are distinguished by a pronounced monomodality with a prevalence of 40-80-nm particles. Autoimmune disorders are characterized by a fraction of some 100-120 nm, which is close to the size of the known characteristics of circulating immune complexes that are

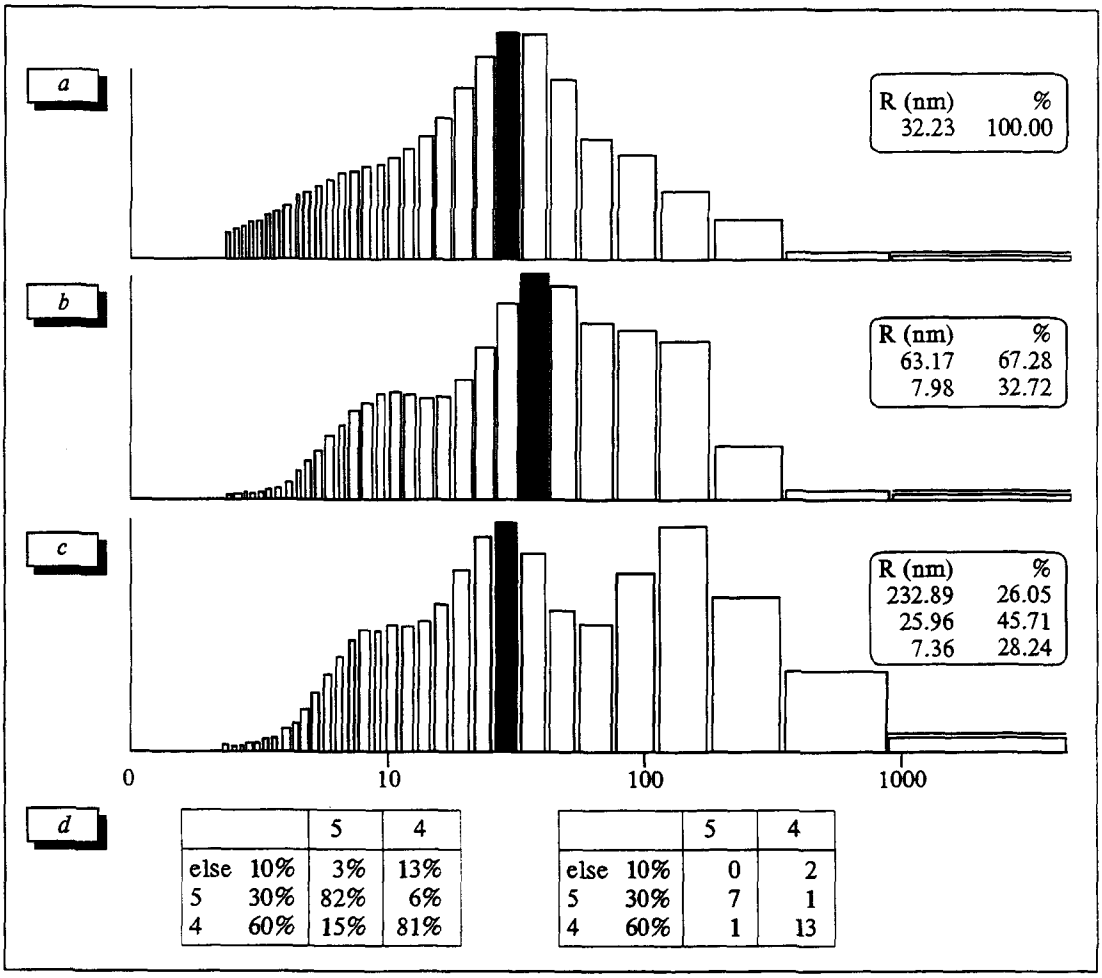

Fig. 3. Total spectra of plasma subfraction composition in patients with obligate forms of premalignancy (5th group, *a*), with autoimmune diseases (4th group, *b*), and with malignancies (*c*); *d*) results of multicomponent classification of groups of patients with autoimmune diseases and obligate premalignancies. All designations for histograms and tables are the same as in Figs. 1 and 2.

often identified in such diseases in numerous clinical and laboratory studies.

In full accordance with the differences discussed are the results of multicomponent classification (Fig. 3), demonstrating a significant distinction in the pattern of homeostatic shifts in the diseases compared (up to 80%).

At this stage of our study differentiation of malignancies cannot be done with the same certainty as that for the diseases discussed above. The problem has to do with the limited number of observations (5) with strictly verified malignancies. A possible solution lies in the fact that the total spectrum of the limited information on many components shows significant differences of differential value vis-a-vis all the variants (Fig. 3). In no case did we find such a pronounced fraction of high-molecular-weight complexes (up to 200-500 nm) against the background of the contribution of 60-80-nm particles (in this fraction are the ribo- and deoxyribonucleoproteins reflecting the pattern of changed differentiation of cells in some malignancies).

From the data presented here the conclusion can be drawn that the differential sensitivity of the method for elucidating multicomponent differences in the homeostasis system is sufficiently informative for a dynamic study of different pathologies and is also useful for screening risk groups for different pathologies by means of standard questionnaires, medical examinations, and certain differentially significant clinical-laboratory tests.

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