A STUDY OF LYSOZYME (MURAMIDASE) ACTIVITY IN THE SERUM AND URINE OF PATIENTS WITH BLOOD DISEASES

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In the past few years, researches in the lysozyme have greatly extended. It is well known that the lysozyme is contained in the serum, in the urine — in insignificant quantities, as well as in neutrophil granulocytes and monocytes of the peripheral blood of healthy persons. Among bone marrow elements, lysozyme has been established in promyelocytes, and in the rather mature white blood cells (1, 5).

Changes in this particular enzyme in the serum and urine of leukosis patients have been subjected to a comparatively more detailed study. In the literature the opinions are unanimous in terms of the lysozyme increase in patients with monocytoid type of acute leukosis and chronic myelogenic leukosis where it is considered that changes in lysozyme have both diagnostic and prognostic value (2, 3, 4, 5, 7, 9). There are no literature reports in this country concerning lysozyme studies in malignant hemopathies.

The purpose of the present work is to try to find changes in muramidase in the serum and urine of patients with various systemic blood affections, as well as to determine their dependence on the type and stage of disease, and the effect of the treatment performed.

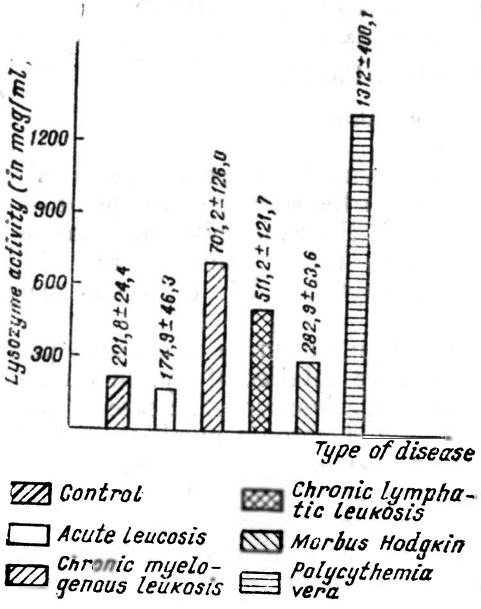
Material and methods

The study is conducted on a series of 76 clinically affected cases of which 43 with leukosis diseases (acute leukosis, chronic myelogenic leukosis and chronic lymphatic leukosis), 20 with malignant lymphoblastoma, and 13 with other hemopathies (polycythemia vera, reticulosis). Forty seven practically healthy individuals were studied for control purposes.

Quantitative lysozyme determination in the serum and urine was made after the method of Osserman and Lawlor, as modified by Zucker (8, 10). To about 100 ml 1 per cent agar (Difco) in 0.067 M sodium phosphate buffer (pH 6.2), thawed and cooled to 60° C, 60 mg Micrococcus lysodeicticus from a 24-hour agar culture was added. Following mixture, a determined quantity was decanted in petri dishes at 4 mm thickness of the layer. Using a needle with 3 mm diameter, small wells were formed in the agar, and filled with the material under study by means of a Pasteur's pipette. The batches were kept at room temperature for a duration of 24 hours, and thereafter the diameter of the zones lysed by the enzyme was measured. Readings of the lysozyme in mcg/ml were made after the semilogarithmic curve, built on the basis of data from standard dilutions of egg lysozyme because of the lack of a human one. Lysozyme activity recordings in mcg/ml egg lysozyme have been described in the literature. (9).

Results and discussion

The patients under study are 20 to 70 years old, including 50 males and 26 females. The subjects in the control group are of both sexes and of comparable ages.



Diag. 1.

Summed up data from the study on lysozyme activity are presented in Diagram 1. It shows that the mean lysozyme values in the serum of the control group subjects amounts to 221.8±24.4 mcg/ml (single values ranging from 50 to 500), and in the urine — 2.33±1.02 mcg/ml (single values ranging from 0 to 50).

The group of leukosis diseases includes 13 patients with acute leukosis, of which seven with lymphocytoid morphological type, five with mixed type, and only one with myeloblast type. The mean value of serum lysozyme in all the patients of this group amounts to 174.9±46.3 mcg/ml which points to the fact that the enzyme activity remains inaltered as compared to that of the control group ($P<0.05$). In the urine as well no changes in the activity of the lysozyme were established in this particular group ($P< 0.05$).

In patients with acute leukosis, the specific data about lysozyme activity in the serum and urine show no increase; on the contrary, in three of them it is below the norm (20 mcg/ml). The latter had low leukocyte values. Essential variations of lysozyme activity were neither found in the various stages of the disease, namely before, during and after the treatment. It is evident from the dynamic follow up study of seven patients that only in two of them where monocytoid—myeloblast transformation took place, the lysozyme activity in the serum and urine was reliably increased (P 0.05).

The lack of a rise in the lysozyme activity among the patients with acute leukosis may be explained by the fact in that them it is a matter of mainly lymphocytoid morphological type in which, as already pointed out and according to literature data, no changes in the enzyme take place.

The mean value of a serum lysozyme activity in the 16patiens with myelogenic leukosis under study is substantially higher (701.2±126.0 mcg/ml) in comparison with the control group ($P>0.05$). The latter increase is conditioned by the high lysozyme values in four patients (two in the stage of exacerbation, and two free of exacerbation phenomena). In the remainder (12 cases) it was within normal limits.

A considerable lysozymuria (reaching up to 2 000 mcg/ml) was recorded in two cases of the series, with pronounced renal lesions, most likely due to the leukosis process.

In this group no correlation was established between changes in lysozyme and number of leukocytes, duration and type of cytostatic therapy indertaken.

Among the patients with chronic lymphatic leukosis (14), the mean value of serum lysozyme activity is likewise considerably elevated (511.2±121.7 mcg/ml) (P 0.05). In four of the patients the activity of serum muramidase was very high — exceeding 1 000 mcg/ml, whereas in the other ten it was unaltered. Lysozymuria was established in there patients, and in two it was accompanied by parallel intensification of the enzyme activity in the serum. Moreover, we failed to establish a one-way correlation between the changes in activity of serum lysozyme in the beginning, in remissions and in recurrence of the disease, on the one hand, and leukocyte count dynamics, on the other. Perillie and co-authors claim a reduction of its content (9).

In the group of malignent lymphoblastoma (15 patients with morbus Hodgkin, and 5 with lympho- and reticulosarcoma), the mean value of lysozyme activity in the serum is 282.9±63.6 mcg/ml, and shows no variations from the norm. Two patients were with strongly reduced lysozyme activity in the serum, reaching 10 mcg/ml. There were two cases in the series with elevated enzyme activity — a woman in IV B clinical stage of morbus Hodgkin with heavy renal lesion, and a man with secondary inflammatory process subsequent to biopsy. No literature reports were found with a reference to lysozyme studies in malignent lymphoblastomas.

Of the group with other hematologic diseases, it is worth noting the data about the activity of the serum lysozyme in the six cases with polycythemia vera where the mean value is the highest — 1 312.0±400.1 mcg/ml. After analysis of the specific values of the enzyme, it was established that the latter increase concerns three cases in the initial stage. In the remainder (3 cases) which were in the stage of post-treatment remission, the enzyme activity was within normal limits. The fact that in these patiens activity enhancement is by no means associated with lysozymuria is impressive. Malmquist (6) reports a moderate rise of the serum lysozyme in all patients with polycythemia vera.

No variations in lysozyme activity were detected among the patients with myeloma and reticulosis, included in this group.

The questions concerning clarification of the mechanism of changes in muramidase activity have not been fully explained in the literature thus far. Presumably, it is a matter of changes conditioned by a variety of causes, namely: nephrotoxic effect of the enzyme, leukemic lesion of the kidneys, enhanced production in the leukemic cells or intense cellular decomposition (7) 8, 9).

Notwithstanding the fact that our lysozyme study is caried out on a comparatively limited number of patients with various nosological entities the following preliminary inferences could be reached:

1. Lysozyme activity in the serum is increased in acute leukosis patients with monocytoid-myeloblast transformation of the affection, and in some patients with chronic myelogenic and chronic leukosis, and polycythemia vera.
2. There is no definite correlation between changes in muramidase activity and peripheral blood indicators, more particularly, the leukocyte count, and the type and duration of cytostatic treatment performed. A rather definite correlation between these changes and renal lesions, conditioned by the systemic blood disease, was established in most of the patients.

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ИССЛЕДОВАНИЕ АКТИВНОСТИ ЛИЗОЗИМА (МУРАМИДАЗЫ) В СЫВОРОТКЕ КРОВИ И МОЧЕ БОЛЬНЫХ С ЗАБОЛЕВАНИЯМИ КРОВИ

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РЕЗЮМЕ

Обсуждаются результаты исследования лизозимной активности сыворотки и мочи у 76 больных с системными заболеваниями крови. Устанавливается повышенная активность энзима у некаторых болъных с хроническими миелолейкозом и лимфолейкозом и истинной полицитемией. Отсутствует определенная зависимость между изменениями в активности лизозима и числом лейкоцитов, стадией заболевания, видом и продолжительностью цитостатического лечения.