

STUDY AND ANALYSIS OF LYSOZYME QUANTITY IN BIOLOGICAL FLUIDS (SALIVA, URINE, BLOOD SERUM) OF PATIENTS WITH VARIOUS PATHOLOGICAL CONDITIONS

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The lysozyme is one of the natural protection factors in the organism. It is a matter of an enzyme-protein with a low molecular weight. The lysozyme enhances bacteriolytic activity by attacking the supporting membranes which make up the hard framework of the bacterial cell wall (J. Milleck — 1970). Its content in the various biological fluids of the human organism is by no means equal: the greatest quantity is found in conjunctival secretion (about 2000 mcg/ml), while in the urine only traces are detected (H. Asamer — 1971). It occurs also in the saliva (30-50 mcg/ml), blood serum (5-10 mcg/ml), nasal, genital (E. Maron, B. Bonavida — 1971), bronchial secretion, cerumen and other body fluids (N. L. Petrakis et al — 1971). Lysozyme is likewise contained in some tissues and organs, such as cartilage, stomach, kidneys brain and especially skin (60-120 mcg/ml) where its quantity in the epidermis is three times that in the dermis (H. Ogawa — 1971). Differences exist also in terms of its activity — in the egg white it is ten times weaker than in the human organism (F. Elliott et al — 1966). Insofar as human organism is concerned its activity is the highest in the umbilical cord of the fetus just prior to birth, and in the blood of newborns but, subsequently, it shows a gradual age-related fall with the lowest values being recorded in the blood serum of healthy adults (Z. M. Mihaylova and G. A. Mikeeva — 1967).

Literature data on the problem show that a certain interdependence exists between lysozyme quantity and a number of pathological conditions. A rise in its amount is recorded in the urine and blood serum of patients with a variety of leukosis forms (E. Yordanova et al — 1973), in conjunctival secretion of conjunctivitis patients (K. McEwen and S. Rimura — 1955), in the urine of patients with a variety of renal derangements (Prcckop, Davidson — 1964), with rather considerable values being reached by the lysozyme in patients with decreased glomerular filtration (H. Jensen — 1973), as well as in those with creatinine clearance below 30 ml/min (E. F. Nielsen et al — 1973). By the elevated lysozyme level a great number of authors (P. K. C. Chun et al — 1972) have made an attempt to diagnose rejection and inflammation processes in transplanted kidneys, as well as concomitant development of heavy infections (J. F. Harrison et al — 1972), or affection of the transplant by pyelonephritis and glomerulonephritis (J. F. Sennenkamp et al — 1972). Moreover, K. Keeler (1969) reports on the so-called renal dependent factor in the absence of which lysozyme level in the urine shows a 50 per cent increase.

Notwithstanding the above data we could hardly reach the conclusion that the problem about the diagnosis and prognosis of pathological conditions on the basis of the method outlined is solved. Hence, it is the purpose of the present work to study lysozyme quantities in the different biological fluids of patients from the clinics of stomatology, obstetrics and gynecology, and nephrology in the Faculty Hospital — Varna, and to analyze the obtained results in compliance with the clinical picture and paraclinical indicators in individual cases on the basis of the listed parameters' normal values in healthy subjects.

Material and Method

The study was conducted in three stages: I — investigation of 225 healthy individuals (controls) and 402 stomatological patients (194 with gingivitis and 208 with caries) from whom saliva was taken and was diluted with distilled water at 1:5 ratio; II — investigation of 67 healthy individuals and 112 patients with nephrological problems (30 with glomerulonephritis, 35 — pyelonephritis, 27 — nephrolithiasis and 20 — cystopyelitis) from whom urine was obtained; III — investigation of 36 healthy non-pregnant women, 59 healthy pregnant women and 28 pregnant women with habitual abortions or stillbirths in a previous pregnancy. In all instance blood was obtained from the veins and the blood serum was separated.

Quantitative lysozyme determination in single biological fluids was carried out after the method of F. Elliott et al (1966), as modified by Zucker et al (1970). To 100 ml 1 per cent agar (Difco) in 0.067 M sodium-phosphate buffer (pH=6.2), thawed and cooled to 60° C, are added 60 mg *Micrococcus lysodeikticus* from 24-hour agar culture. After mixing, a certain amount is decanted in a Petri dish (at 4 mm thickness of the layer). Using a syringe needle (diameter of the lumen 4 mm) wells are made in the agar and filled up with the material under study (saliva at 1:5 dilution, urine and blood serum). Following 24-hour stay at room temperature, the diameters of the cleared zones (lesion zones) are measured using plotting paper. To express the obtained results in mcg/ml lysozyme a semilogarithmic curve was made use of, constructed on the basis of data from standard egg lysozyme dilutions.

Results and Discussion

The results of quantitative lysozyme determination in the saliva of patients studied in the first stage are presented in Table 1. From the data submitted it becomes evident that the lysozyme amount in the saliva of healthy individuals (30 mcg/ml) is considerably lower than in patients with caries and gingivitis (75 and 125 mcg/ml resp.). Expressed in percentages the values augment from 100 per cent for the healthy subjects to 250 and 416 per cent for the affected with caries and gingivitis respectively. The higher lysozyme content in the saliva of the latter group is most probably due to the peculiar state of the organism brought about by the pathological process, and to the simultaneous mobilization of natural resistance factors. The processes just mentioned are more pronounced in gingivitis patients because of the heavier and more significant inflammatory element.

Table 1

	Number	Mean lysozyme quantity
Healthy	225	30 mcg/ml
Patients	Caries	75 mcg/ml
	Gingivitis	125 mcg/ml

Table 2

	Number	Mean lysozyme quantity
Healthy	67	1,44mcg/ml
Patients	Cystopyelitis	8,77 mcg/ml
	Nephrolithiasis	10,72 mcg/ml
	Pyelonephritis	11,99 mcg/ml
	Glomerulonephritis	17,55 mcg/ml

Table 3

	Number	Mean lysozyme quantity
Healthy non-pregnant women	36	6,40 mcg/ml
Healthy pregnant women	59	17,08 mcg/ml
Pregnant women with habitual abortions or stillbirth in a preceding pregnancy	28	22,58 mcg/ml

Table 2 illustrates the data from lysozyme amount determinations in the urine of the subjects under study in stage II. The average quantity for healthy persons is 1.44 mcg/ml, and for renal patients — 12.252 mcg/ml, i. e. an 8.51 times increase is present. The various groups of nephrologic patients displayed different mean lysozyme values in the urine, namely: glomerulonephritis — 17.55 mcg/ml (the greatest amount of all groups studied in this particular stage), pyelonephritis — 11.99 mcg/ml, nephrolithiasis — 10.72 mcg/ml and cystopyelitis — 8.77 mcg/ml. In per cents the latter quantities augment from 100 per cent for the healthy, to 609 per cent for cystopyelitis patients, 744 per cent — for nephrolithiasis, 832 per cent — pyelonephritis, and 1218 per cent — for patients with glomerulonephritis. Analysis of the lysozyme quantity, cli-

nical symptomatics and paraclinical indicators, performed in each patient, showed that in all instances with a high lysozyme level in the urine there is a parallel rise in creatinine and urea in the blood serum (up to 15 resp. 300 mg % in one of them), of protein in the urine (up to 2.6 g % in the same case), as well as a reduction of the concentration capacity of the kidneys (up to 1010 sp. w. according to Fohlhard in the already cited patient). The rise of creatinine and urea in the blood serum, and of protein in the urine, and the reduced concentration capacity of kidneys coincides with the lysozyme rating in the urine of the various groups of patients (the lowest in cystopyelitis, and the highest in glomerulonephritis). All data submitted point to an enhanced natural resistance against renal affections. It is quite likely that a correlation exists between the increase in lysozyme quantity in the urine of patients and the reduction of their glomerular filtration, as well as a reverse correlation between lysozyme and creatinine clearance (the former increases parallel to the reduction of the latter). Lysozyme excretion in rather heavy, close to uremia cases is higher than that in medium heavy and slight renaltubular lesions. The possibility that two routes exist for the transfer of proteins with low molecular weight into the proximal tubules is also considered.

The study in stage III, illustrated in table 3, proves that the mean lysolyme quantity in the blood serum of healthy individuals amounts to 6.40 mcg/ml. It grows up to 17.08 mcg/ml in healthy pregnant women, and up to 22.58 mcg/ml in pregnant women with a past history of habitual abortion or stillbirth in a previous pregnancy. Expressed in per cents this rise ranges from 100 per cent for healthy non-pregnant women to 267 per cent for healthy pregnant women, and up to 353 per cent for the women in the other group. Such a difference in the lysozyme level between non-pregnant and healthy pregnant women is most probably due to the specific state of the maternal organism during the development of fetus, a state which demands a substantially heavier strain on behalf of a number of organs and systems, natural resistance inclusive. The still greater amount of lysozyme found in the blood serum of pregnant women with a past history of habitual abortion or stillbirth in a previous pregnancy is explained by the possibility of enhancing natural protection against the heavy pathological changes in the maternal organism (anatomical, gynecological, related to blood group incompatibility, genetic and the like) which have caused rejection of the fetus, and which, during subsequent pregnancy, once again trigger the natural resistance factors in connection with the peculiar state of the mother and of the developing fetus.

The results of our study demonstrate the indisputable role played by lysozyme in the protection of the organism against pathological variations. Whether or not the lysozyme has a diagnostical or prognostical value as a paraclinical indicator remains to be seen in the forthcoming researches along this line.

Conclusions

1. The mean quantity of lysozyme in the saliva, urine and blood serum of healthy subjects, according to the present study, amounts to 30.0 mcg/ml, 1.44 mcg/ml and 6.40 mcg/ml respectively.
2. The saliva from patients with caries and gingivitis shows a much higher content of lysozyme as compared to healthy persons, while in caries patients lysozyme displays lower values compared to gingivitis cases.

3. The quantity of lysozyme in the urine of patients with renal diseases is much higher than in healthy subjects:

a) the highest lysozyme level in the urine is established in glomerulonephritis cases, next ranking pyelonephritis, nephrolithiasis and cystopyelitis, that is, in rather heavy renal derangements the lysozyme quantity is greater than in slighter and medium heavy lesions:

b) an inverse correlation exists between lysozyme level in the urine and creatinine clearance.

4. The amount of lysozyme in the blood serum of healthy pregnant women is substantially higher than in healthy non-pregnant women. A still greater increase is recorded in pregnant women with habitual abortions or stillbirth from a preceding pregnancy.

5. The quantity of lysozyme in biological fluids of healthy and ill persons varies within a relatively wide range, depending on the individual state of the organism.

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ИССЛЕДОВАНИЕ И АНАЛИЗ КОЛИЧЕСТВА ЛИЗОЗИМА В БИОЛОГИЧЕСКИХ ЖИДКОСТЯХ (СЛЮНА, МОЧА, СЫВОРОТКА КРОВИ) БОЛЬНЫХ С РАЗЛИЧНОЙ ПАТОЛОГИЕЙ

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РЕЗЮМЕ

Исследованы последовательно в три этапа количества лизозима в биологических жидкостях у 328 здоровых (контроль) и 601 больных людей с различной патологией.

Установлено повышение уровня лизозима в слюне больных кариесом (в среднем 75 мкг/мл) и гингивитом (в среднем 125 мкг/мл), в сравнении с уровнем лизозима у здоровых людей (в среднем 30,0 мкг/мл).

Повышение количества лизозима отмечают и в моче при заболеваниях почек (при гломерулонефрите — в среднем 17,55 мкг/мл; при пиелонефрите — в среднем 11,99 мкг/мл; при нефролитиазе — в среднем 10,72 мкг/мл и при цистопиелите — в среднем 8,77 мкг/мл) по сравнению со здоровыми людьми (в среднем 1,44 мкг/мл).

При исследовании количества лизозима в сыворотке крови беременных женщин с хроническими абортными или при мертворожденном плоде еще раз установили повышенные показатели (в среднем 22,58 мкг/мл), в сравнении с показателями у здоровых беременных женщин (в среднем — 17,08 мкг/мл). У обеих групп количество лизозима значительно выше, чем у здоровых небеременных женщин (в среднем — 6,4 мкг/мл).

Проведен анализ полученных результатов согласно клинической картине и параклиническим показателям в каждом отдельном случае.