NOTES ON SOME SERUM PROTEIN CHANGES IN VIRAL HEPATITIS — BIOCHEMICAL ASPECTS

D. Hadzhilarska, P. Kokosharov, M. Radkov

Serum protein changes in viral hepatitis have been studied by various authors. Attempts have been made to assess a number of laboratory tests in the different periods of the disease, failing to discover changes in terms of increased, respectively, decreased values (1, 2, 4, 5, 7, 8, 9, 10, 12, 13, 16, 19).

Our attention was focused on the changes in free serum amino acids, protein fractions, total protein, immunoglobulins, and on the colloid stability tests — thymol turbidity and Weltmann — in the first day of the patients' admission to the clinic, that is after the beginning of the icteric period.

Material and methods

Eighty blood sera not containing, and 46 containing Australia antigen (hepetitis-associated antigen — HAA) were used in the study, at a control group of ten clinically healthy individuals without a past history of viral hepatitis, responding negatively against the Australia antigen.

Study of the Australia antigen was carried out using electrophoretic immunoprecipitation according to Pesendorfer and assoc, (18) free amino acids — according to Pashina (6), serum protein fractions through paper electrophoresis after Todorov (15), immunoglobulins after Mancini(17), total protein, thymol turbidity test and Weltmann — according to routinely used methods. The data were elaborated statistically afted the method of variation analysis — Sepetliev (11).

Results and discussion

Among the studied in blood sera 14 free amino acids, not containing Australia antigen, seven show elevated values, namely: cystine, lysine, histidine, aspariginic acid, glycine, leucine ($P<0.001$) and serine ($P<0.01$) — Table 1.

The other amino acids, regardless of the rise, do not show statistically reliable differences in comparison with control sera. In Australia-antigen containing blood sera, the following amino acids are increased: cystine, lysine, histidine, aspariginic acid, serine, glycine, valine, leucine ($P<0.001$), and arginine ($P<0.02$), whereas phenylalanine is lowered ($P<0.01$).

In the same sera, the listed below amino acids are increased relative to those not containing Australia antigen: lysine, histidine, aspariginic acid, alanine, valine ($P<0.001$), cystine and leucine ($P<0.01$), whereas tyrosine and phenylalanine show a reduction, $P<0.001$ and $P<0.05$ respectively.

Nowadays, the standpoint that viral hepatitis is an infectious disease with viral etiology has been universally adopted. In the opinion of Tihonenko (1966),

Table 1

Naturally Occurring Amino Acids in Blood Sera of Viral Hepatitis Patients, in mg %

Naturally occurring amino acids	Patients with viral hepatitis		Clinically healthy subjects
	+HAA	−HAA	
Cystine	2.90±0.15	2.40±0.10	1.28±0.15
Lysine	3.00±0.14	1.91±0.08	0.93±0.13
Histidine	3.37±0.12	2.57±0.13	1.11±0.12
Arginine	3.71±0.08	3.29±0.32	2.87±0.41
Aspariginic acid	8.09±0.14	5.94±0.15	4.03±0.48
Serine	2.35±0.15	2.07±0.23	1.27±0.12
Glycine	4.49±0.17	4.50±0.07	2.86±0.34
Glutamine	3.37±0.29	3.04±0.21	3.31±0.47
Alanine	3.73±0.12	3.01±0.18	3.59±0.29
Tyrosine	2.28±0.08	3.60±0.09	3.05±0.55
Phenylalanine	1.80±0 05	2.20±0.18	2.80±0 32
Methionine	2.62±0.28	2.21±0.07	1.95±0.48
Valine	2.09±0.10	1.32±0.19	1.11±0.10
Leucine	2 01±0.10	1.64±0.08	1.21±0.03

viral proteins are built up by the basic amino acids — arginine. lysine and histidine, and because of that their elevated values are worthy of notice. Certainly, these amino acids are essential for man, but an eventual synthesis under the effect of an introduced viral information is by no means ruled out.

Total protein is within normal limits at reduced albumins ($P<0.001$) and increased gammaglobulins, especially in Australia antigen containing sera ($P<0.001$) — Table 2.

Table 2

Serum Protein Fractions in the Blood Sera of Viral Hepatitis Patients, in %

Serum protein fractions	Viral hepatitis patient		Clinically healthy subjects
	+HAA	−HAA	
Albumins	44.7±0.78	44.9±1.3	54.2±2.4
Alpha$_1$-globulins	7.1±0.24	6.4±0.2	6.8±0 8
Alpha$_2$-globulins	6.1±0.21	9.1±0.3	8.2±0 7
Beta-globulins	13.2±0.63	15.6±0.4	11.7±0.9
Gamma-globulins	28.9±0.58	24.00±0.6	19.1±0.9
Total protein	7.4±0.13	7.1±0.3	7.9±0.5

The albumin-globulin coefficient is lowered. These changes are essential for the elevated thymol turbidity test values ($P<0.001$). Of the immunoglobulins (Table 4), IgG ($P<0.001$ for sera not containing, and $P<0.01$ for those containing Australia antigen) and IgA ($P<0.1$, resp. $P<0.001$) show an appreciable increase. IgM is reduced in either types of sera, with statistical reliability in those containing Australia antigen ($P<0.02$). The elevated values of globu-

lins, respectively IgG, and the low IgM values along with the other changes indicate that upon admission to the clinic, the patients with viral hepatitis are already in an advanced stage of the infectious process, and it is completely justified to pose the question whether or not the occurrence of the icteric period should be considered as the peak period of the disease.

Table 3

Quantitative Values of the Thymol and Weltmann Tests in Viral Hepatitic

Tests	Viral hepatitis patients		Clinically healthy subjects
	+HAA	−HAA	
Thimol	69.4±4.0 FU	57.7±4.2 FU	33.9±4.4 FU
Weltmann	7.7±0.4 T. t.	7.6±0.4 T. t.	6.3±0.3 T. t.

Table 4

Serum Immunoglobulins in the Blood Sera of Patients with Viral Hepatitis, in mg %

Immunoglobulins	Viral hepatitis patients		Clinically healthy subjects
	+HAA	−HAA	
G	2383±104.0	2688±30.2	1612±78.7
M	108±8.3	127±10.5	191±33.6
A	338±15.0	242±11.6	181±20.3

Conclusion

Changes in serum proteins in clinically manifested viral hepatitis and upon hospitalization disclose a late stage of the infectious process. Thus, the epidemiologic efficiency of «early» diagnosis is reduced, and along with that the question of a more active therapeutical intervention is raised.

The constellation of the above indices, subjected to comparative study with various diseases simulating the early clinical picture of viral hepatitis, could eventually have a definite differential-diagnostical importance.

Tracing of the dynamics of the above mentioned indicators in the blood sera of contacts with viral hepatitis patients, and detection of their earliest positivation would have an essential epidemiologic and clinical bearing, and because of that further studies along this line are mandatory.

REFERENCES

В. Валцаров, И. и сътр. Диспротеинемии, Пловдив, 1973. — Зоенков, В. Хабилитационен труд, Варна, 1973. — 3. Милев, П., Биохимия, Пловдив, 1963. — Масини, М., Е. Барба. в кн. Инф. гел., Киев, 1968. — Нисевич, Н. и сътр. Вест. АМН СССР, 1972, 27, 5, 85. — 6. Пасхина, Г. В кн. Совр. методи в биохимии, Москва, 1964. — 7. Постовит, В. В кн. Инф. гел., Киев, 1966. — 8. Присс, И. *Вест. АМН СССР*, 1965, 5, 31. — 9. Рачев и сътр. Вирусен хепатит и детската възраст, София, 1961. — 10. Семендяева, М. и сътр. *Вестн. АМН СССР*, 1970, 25, 9, 54. — 11. Семерджиев, Д. Медицинска статистика, София, 1972. — 12. Тареев, Е. и сътр. Епид. гел., Москва, 1970. — 13. Ташев, Т. В кн. Болести на черния дроб и жлъчните пътища, София, 1971. — 14. Тихоненко, Г. Биохимия вирусов, Москва, 1966. — 15. Тодоров, П. Клинични лабор. изследвания в детската възраст, София, 1962. — 16. Furt, R. *J. Exp. Med.*, 1965, 122, 6, 1173. — 17. Mancini, G. et al. *Immunochemistry*, 1965, 3, 225. — 18. Pesendorfer, F. et al. *Disch. Med. Wschr.*, 1970, 6, 245. — 19. Wuhrmann, Ch. Wunderly, Les proteins du sang humain, Paris, 1961.

БИОХИМИЧЕСКИЕ АСПЕКТЫ НЕКОТОРЫХ ИЗМЕНЕНИЙ СЫВОРОТОЧНЫХ ПРОТЕИНОВ ПРИ ВИРУСНОМ ГЕПАТИТЕ

Д. Хаджилорска, П. Кокошаров, М. Радков

РЕЗЮМЕ

Изменения сывороточных протеинов при клиническом проявлении вирусного гепатита и при поступлении в больничное заведение указывает на позднюю стадию инфекционного процесса. Это уменьшает эпидемиологическую эффективность раннего диагноза и в то же время ставит вопрос о более активном терапевтическом вмешательстве.

Совокупность указанных показателей при сопоставлении их и при других заболеваниях, напоминающих начальную клиническую картину вирусного гепатита, вероятно, мьгло бы иметь определенное дифференциально-диагностическое значение.

Прослеживание динамики вышеуказанных показателей в сыворотке крови контактирующих с больными вирусным гепатитом и установление их наиболее раннего позитивирования представляло бы значительный эпидемиологический и клинический интерес, что требует необходимости дальнейшего изучения.

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

E. Yordanova, R. Marinova, R. Mishkova

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).