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BIOCHEMICAL INDICATORS OF THE TOTAL PROTEINS THROUGH ELECTROPHORESIS

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Abstract

The plasma contains more than 300 different proteins: enzymes, enzymes inhibitors, factors of coagulation, antibodies, transporting proteins. With the exception of the immunoglobulins and hormones, the majority of the plasmatic proteins is synthetized in hepatocytes and is unleashed in the circulatory torrent. The serum and plasma differs regarding the concentration of proteins and the type of molecules. Thus, the serum (obtained after the conclusion of the process of coagulation) doesn't contain fibrinogen and the majority of the factors involved in the "waterfall" of coagulation. This test detects the sum of the serum circulatory proteins, which can suffer variations in physiologic and pathologic conditions.

Keywords: albumin, protein, electrophorese

INTRODUCTION

The albumin is a non-glycosylate protein synthetized by the cells of the hepatic parenchyma with a rate of 14g/day. It represents the most important protean component from the plasma, the cephalorachidian liquid and urine. In the plasma, the albumin is responsible mainly for the maintaining of the oncotic pressure; also it is involved in the transport of different compounds (free fat acids, bilirubin, hormones, ions of metals and medicine).

The serum albumin has a time of bisection of 18-20 days; this period is shortened in the conditions of an increased catabolism: severe infections, hemorrhages, surgical interventions, protean losses on the kidney level, in the gastro intestine and cutaneously. From this reason, the albumin is considered a reactive of acute phase "negative" (it decreases as answer to the infectious and acute inflammatory processes).

The albumin is a global indicator of the condition of nutrition of the body, especially for the elder persons with different chronic affections. It was found

thus that for the hospitalized elder persons, the hypoalbuminemia represents a factor of risk independent in what the mortality is concearned.

The levels of albumin under 2.0-2.5 g/dL associated to the nephritic syndromes, hepatic cirrhosis or enteropathies with losses of proteins can cause edemas.

Classically, the detection and identification of the monoclonal gammopathies is made by the electrophoresis of the proteins with immunifixation (IFE) which combines the electrophoretic separation in gel of agarosis of the proteins with immune precipitation, using monospecific antiserums compared to the heavy and light individual chains. Thus, are obtained fast results, easily to be interpreted. While the appearance of the polyclonal components is due to the activity of many types of clones of lymphocytes B, the monoclonal gammopathies result after the proliferation of a single cellular clone B.

The capital electrophoresis is an alternative method for the characterization of the monoclonal gammopathies (immunotypization). Each sample is mixed with individual antiserums that are specific to the gamma heavy chains (Ig G), alpha (Ig A) and m (Ig M), respectively the light chains kappa (free and connected) and lambda (free and connected). The proteins, separated in capillary of quartz are detected directly by absorbance at 200 nm. The electrophoregrams are evaluated visually for the detecting of the presence of the specific reactions with the presupposed monoclonal proteins.

MATERIAL AND METHODS

The current method for the separation of the serum proteins used in the laboratory of medical biochemistry is electrophoresis. There are solid supports used and alkaline pH, to which all the protean components are loaded negatively and migrate to the positive pole. On the electrophoregram, after coloring, the proteins appear under the form of bands which are measured for the optical density, each band having a maximum of absorption. By electrophoresis, in the conditions mentioned above, are obtained five fractions: serum albumin, a_1, a_2, b, g – globulins. Some systems of electrophoresis can differentiate the b area in two distinct bands b_1 and b_2 resulting thus 6 protean fractions totally.

The albumin, a_1 and b appear under the form of homogenous bands, well defined. a_2, and g appear as diffuse bands and the fraction g presents in its central part an area intensively colored.

Preparing the patient – à jeun (before eating) or after the meal.

Specimen harvested – blood.
Harvesting recipient – vacutainer without anticoagulant with/without separate gel.
Necessary processing after the harvesting – is separated the serum by centrifuging.
Volume of sample – minimum 0.5 mL serum.
Causes of sample rejection – hemolysis serum.
Stability of the sample – the separated serum is stable *4 days* at the room temperature or *a week at* 2-8°C
Method – electrophoretic (in gel agar) followed by the densitometry scanning of the fractions obtained.

RESULTS AND DISCUSSIONS.

Tab.1.

Value of reference of the total proteins reported to the age

Age	***Albumin (%)***	***a_1(%)***	***a_2(%)***	***b(%)***	***g(%)***	***A/G***
< 1 year	40-65	2-5	6.6-13.5	8.5-14	10-21	1.16-2.23
1 – 16 years	50-65	2-5	6-15	8.5-15	10-22	1.06-2.48
> 16 years	52 – 68	2-5	6,6-13,5	8,5 -14	11 – 21	1.39-2.23

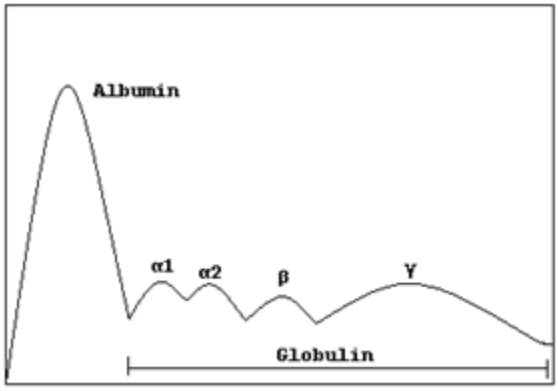

Fig.1. Electrophorese of the serum proteins

The decrease of the serum albumin (hypoalbuminemia) appears in various pathologic conditions and has different significance (malnutrition, malabsorption, nephrotic syndrome, neoplasia). The increases of albumin appears following the dehydrations. In special cases, in the area of migration of the albumin is found an additional band, due to genetic variations without

clinical relevance (aloalbuminemia and bisalbuminemia). The area a_1 reflects especially the serum concentration of alpha 1-antitripsin, thus the reduction of this fraction is met in the deficit of alpha 1 – antitrypsin associated with certain pulmonary diseases (emphysema with early debut) and hepatic (newborn hepatitis). The increases are registered in all the inflammatory reactions ("reactant of acute phase"). In rare cases the obtaining of additional wide bands in this area can suggest the presence of the alpha-fetoprotein. The area a_2 is created of 2 protean components: alpha 2 macroglobulin and haptoglobin. The increase of a_2 appears in acute infections, acute articular rheumatism, arthritis, nephrotic syndrome, diabetes, neoplasia; the decrease of this fraction can be met in severe acute pancreatitis, hepatocellular injuries, syndromes of disseminated intravascular coagulation, hemolytic anemia, megaloblastic anemia.

The area b is made of 2 distinct bands: b_1 corresponding to the transferrin and b_2 that includes the fractions of the complement C_3 and C_4. The third component of this area is beta lipoprotein, whose band can be superposed over the transferrin or C_3 or can be situated in the beta-gamma area. The serum level of b globulin increases in the iron deficiency anemia and biliary cirrhosis and decreases in autoimmune diseases in active burst (LES), nephrosis, hepatic affections, neoplasia, acute and chronic infections. In some cases of multiple myeloma of IgA type it can be seen the presence of a monoclonal component in that area. The area g Gamma-globulins are the most important globulins. In this category are include the immunoglobulins (IgG, IgA, IgM, IgD si IgE). The anomalies suffered by these are translated either by different deficits, or by the polyclonal or monoclonal hypergammaglobulinemias. The percentage of g globulins decreases in the agammaglobulinemia, hipogammaglobulinemia and nephrotic syndromes. Hipogammaglobulinemia can be congenital or achieved and can be easily detected at the electrophoresis of the serum proteins when the concentrations of the main 3 classes of immunoglobulins are decreased (IgG, IgA si IgM). The presence of hipogammaglobulinemia in adult imposes the continuing of the investigations for the finding of a possible lymphoproliferative disease (myeloma with light chains, chronic limphatic leukemia, lymphomas).

The polyclonal increases of gammaglobulins indicate a chronic immunologic process associated with hepatic affections, active chronic hepatitis, cirrhosis, collagen diseases, LES, rheumatoid polyarthritis, neoplasia in Hodgkin disease, chronic mielo-monocitary leukemia. In these cases hypergammaglobulinemia appears due to the activation of a large number of

plasmocitary clones that set free immunoglobulins. It has to be mentioned the characteristic aspect of the alcoholic cirrhosis of beta-gamma "bridge", visualized on gel as an area intensely colored in beta and gamma areas.
Sometimes, in the gamma area it can be observed some small bands, tight, named oligoclonal, met in patients with hepatitis, diseases of the immune complexes, the syndrome of the immunodeficiency achieved, the angioimmunoblastic lymphoma.

The monoclonal gammopathies include a large number of clinical and biological conditions usually associated with malign affections, multiple myeloma, Waldenström disease, amyloidosis, lymphoma, but also with some benign affections or even with the absence of a pathologic modification, monoclonal gammopathies with undetermined significance. In some cases is found at the electrophoresis of the proteins a monoclonal component (band M), resulted from the proliferation of a single plasmocitary clone. On the gel, the monoclonal gammopathies appear under the form of some tight bands, net defined, and after the densitometry scanning are obtained characteristic "peaks" that can be met anywhere between the alpha and gamma areas, due to the variable electrophoretic mobility, but the most frequently in the gamma area.

The monoclonal immunoglobulins are homogenous from the structural and biochemical point of view, being made of a single type of light chain, kappa or lambda. In circulation can be set free complete immunoglobulins or only subunits of these as would be monoclonal light chains, named Bence-Jones proteins. The presence of the monoclonal component will be specified on the final bulletin of analysis of the patient, with the recommendation to perform the electrophoresis of the serum proteins with immunofixation.

CONCLUSIONS

A small increase of the width of the albumin band can be owed to the connection of some medicine as would be the penicillin or bilirubin to patients with icterus. Also, the transitory bisalbuminemia, non hereditary, can be met as a result of a medicine treatment or of severe metabolic perturbations, met in pancreatitis.

There are cases in which the fake monoclonal bands can be given by the free hemoglobin, in case of a hemolyzed serum or an intravascular hemolysis (the band in the alpha2 – beta area). The reactive protein C in large quantity, lysosome increased, fibrinogen, in patients treated with anticoagulants, in coagulopathies, if the blood was centrifuged before the complete coagulation

and alpha-fetoprotein in large concentrations, old serums. There are situations when the visualization is difficult in case the monoclonal band is superposed over a normal band.

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