Absence of Beta-Globulin Band in the Serum Protein Electropherogram of a Patient with Liver Disease

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Agarose-gel electrophoresis of serum of a 72-year-old woman with liver cirrhosis showed virtually no beta-globulins two weeks before the patient's death. There was marked decrease in the concentrations of transferrin, beta-lipoproteins, hemopexin, complement component C3, beta-glycoprotein I, and cholesterol in serum. Absence of a beta-globulin band appears to signify an ominous prognosis.

During the last two decades, serum protein electrophoresis has become a routine test in many clinical laboratories. This basic clinical tool is used for screening purposes (e.g., for the presence of paraproteins) or as an adjunct diagnostic test. Separation of serum proteins into electrophoretic fractions allows resolving a protein mixture into components of clinical diagnostic value. Concentrations of different fractions can change in various diseases and signal the necessity of performing additional studies.

Cellulose acetate and agarose gel are now the most common supporting media used for routine serum protein electrophoresis. With these media, usually five to six bands or electrophoretic zones are separated, each composed of a multitude of individual proteins with similar electrophoretic mobilities. Alterations in these fractions are mainly quantitative (increase or decrease) and seldom diagnostic. Qualitative alterations (absence of a band or presence of additional bands), however, have more clinical significance.

We report here a patient whose serum electropherogram in agarose gel showed a virtual absence of beta-globulins.

Case History

A 72-year-old woman was admitted to The Buffalo General Hospital for slight jaundice, ascites, black stools, and hypotension. She was anorexic and had not eaten for the last three days. Five years before this admission, liver cirrhosis was diagnosed from a liver biopsy. She had had multiple accidental fractures, and a 15-year history of alcohol abuse was noted.

At admission, her blood pressure was 102/70 mmHg, she had moderate pitting edema, ascites, and oliguria. Hemoglobin concentration was 85 g/L, hematocrit 27%, and maximum corpuscular volume 108 μm3 (normal, 80–105 μm3). Values for other analytes were: bilirubin 41 mg/L, cholesterol 690 mg/L, creatinine 18 mg/L, urate 85 mg/L, serum urea nitrogen 130 mg/L, ferritin 3862 μg/L (normal, 20–120 μg/L), total protein 56 g/L, albumin 33 g/L, lactate 133 mg/L, phosphate 30 mg/L, calcium 76 mg/L, sodium 141 mmol/L, potassium 3.9 mmol/L, bicarbonate 12.6 mmol/L, alkaline phosphatase 108 μmol/min per liter at 37 °C (normal, 30–105), amylase 54 U/L (normal, 20–110), creatine kinase (MM fraction only) 480 U/L (normal, up to 120 U/L), lipase 10 μmol/min per liter at 37 °C (normal, 10–80), aspartate aminotransferase 1500 U/L (normal, <40 U/L), thyroxin 17 μg/L (normal, 50–120), thyrotropic hormone (TSH) 17.7 milliunits/L (normal, 1–6), and triiodothyronine resin-uptake 47% (normal, 24–35%). The blood pH was 7.35. The urinary sediment contained hyaline casts and many bacteria. The plasma prothrombin time was 16.6 s (control, 12 s) and the partial thromboplastin time was 44.8 s (control, 45 s).

During the hospital course the patient's condition did not improve. Her blood pressure decreased to 80/60 mmHg, serum albumin concentration was 30 g/L, bilirubin concentration increased to 52 and later to 200 mg/L (two-thirds being of the "indirect" type), the prothrombin time increased to 20 s, and the aspartate aminotransferase activity decreased to 138 and later to 54 U/L. The total protein concentration decreased to 50 g/L, serum potassium to 2.5 mmol/L, and the serum creatinine concentration increased to 23 mg/L.

The patient developed aspiration pneumonia and died two weeks after admission to the hospital.

Methods of Further Study

Serum protein electrophoresis was performed at admission on a thin layer of agarose gel in sodium barbital buffer, pH 8.8, ionic strength 0.05 (Corning ACI, Palo Alto, CA 94308). For higher resolution electropherograms, we used a thicker layer of agarose in sodium barbital buffer with higher ionic strength (0.08), higher voltage (200 V instead of 90 V), and a heat-removing plate (Panagal, Worthington Diagnostics, Freehold, NJ 07728). With the first technique the serum protein electrophoresis routinely shows five bands, but with the second technique at least eight bands are usually observed in normal sera. The agarose plates were stained with Amido Black and scanned with a densitometer at 575 nm (Corning ACI, Model 740). This densitometer does not automatically select the valleys of the tracings. Serum was concentrated about threefold with a Minicon B15 concentrator (Amicon Corp., Lexington, MA 02173).

Immunelectrophoresis was performed in agar gel (Agar Noble; Difco, Detroit, MI 48232) by the micro-method of Scheidegger (1) or in commercial plates of agarose gel (Corning ACI) as previously described (2). Antisera to whole
Fig. 1. Serum protein electrophoresis in agarose gel
Upper and lower patterns are normal; middle pattern reveals the absence of
beta-globulin band in a patient with liver cirrhosis

human serum, complement component C3, C3 proactivator, IgA, IgM, beta-lipoprotein, transferrin, and hemopexin were obtained from Cappel Laboratories, Downingtown, PA 19335, or Behring-Calbiochem, La Jolla, CA 92037. The concentrations of transferrin, beta-lipoprotein, hemopexin, beta2-glycoprotein I, and complement C3 were determined by radial immunodiffusion (3) with commercial reagents (Behring-Calbiochem, and Meloy Laboratories, Springfield, VA 22151).

Results

Serum protein electrophoresis in agarose gel of an unconcentrated serum sample virtually did not reveal a beta band, and the other bands showed decreased staining, i.e., a decreased concentration of their protein components (Figure 1). The proportions of the electrophoretic fractions were: albumin 63% (normal, 50-69%), alpha1-globulins 5% (normal, 3-5%), alpha2-globulins 8% (normal, 7-13%), and gamma-globulins 24% (normal, 9-22%). Seven samples from other patients, applied on the same plate, all showed beta-globulin bands, thus ruling out technical artifacts. High-resolution electrophoresis of a three-fold concentrated serum sample from this patient showed a marked decrease in the two bands usually observed in the beta-globulin region (Figure 2). Immunelectrophoresis showed a marked decrease of C3, hemopexin, beta-lipoprotein, and C3 proactivator, as well as a decrease in transferrin (Figure 3).

The C3 concentration was 550 mg/L (normal, 870-1800 mg/L), transferrin 0.72 g/L (normal, 2-3 g/L), and hemopexin 390 mg/L (normal, 500-1100 mg/L). Serum beta2-glycoprotein I was <7.8 mg/L (normal, 150-300 mg/L) and beta2-lipoprotein concentration was 1.43 g/L (normal for the age, 5-7 g/L).

Discussion

The virtual absence of the beta-globulin band in agarose-gel serum protein electrophoresis of the patient just described was...
striking. This pattern has not been seen before in the more than 55,000 electrophorograms examined at this institution during the past six years.

Absence of one of the five common bands in serum protein electrophoresis is a rare finding, but it has a more definite significance than a mere increase or decrease in the staining intensity of a band. \(\alpha_1\)-Globulin band is absent in some patients with severe \(\alpha_1\)-antitrypsin deficiency (usually with Pi phenotype ZZ). Indeed, the accidental finding of the absence of \(\alpha_1\)-globulins in a serum electropherogram led to the discovery of the clinical manifestations of \(\alpha_1\)-antitrypsin deficiency (4). Cases of absence of the albumin band (analbuminemia) are very rare (5). The absence of \(\gamma\)-globulins is seen more often and usually signifies a congenital or acquired decrease of immunoglobulins as found in various types of immune deficiencies.

The \(\beta\)-globulin fraction is considered to be rarely altered in the routine electrophoresis (6), except for monoclonal spikes, and this fraction seems to be the least frequently associated with specific pathological disorders. Although an increase in \(\beta\)-globulins in some liver diseases is usually mentioned (7), a decrease of \(\beta\)-globulins is not commonly discussed (8). The \(\beta\)-globulin fraction of serum protein electrophoresis consists of many proteins, most of them in concentrations below 0.5 g/L, generally considered as the minimum amount of a protein that can be detected in routine electrophoresis. Essentially, three proteins—transferrin, beta-lipoprotein, and complement component C3—determine the electrophoretic pattern of \(\beta\)-globulins. Among other proteins in the beta fraction are hemopexin, plasminogen, clotting factors, other complement components (e.g., C2, C6, C7), and \(\beta_2\)-glycoproteins I and II.

Serum proteins are mostly synthesized in the liver, and liver damage is associated with a decrease in their concentration, especially albumin. \(\beta\)-Globulins represent about 9.5% of the total proteins in a normal electropherogram (range, 7.5–11.5%) and the amount of \(\beta\)-globulins is roughly proportional to the cholesterol concentration of the serum (9). The patient just described had a very low concentration of cholesterol, as seen in advanced liver failure. The concentrations of transferrin, C3, and hemopexin in serum can also be decreased in patients with liver failure. In general, the \(\beta\)-globulin fraction tends to decrease in liver cirrhosis, particularly noticeable when cellulose acetate is used as a support medium in electrophoresis (10). However, the most characteristic electrophoretic finding in cirrhosis is “beta-gamma bridging,” i.e., the beta and gamma regions cannot be clearly resolved. In 1966, Riegel and Thomas reported an 83-year-old woman with anemia who did not show \(\beta\)-globulins on a serum protein electrophoretogram on filter-paper (11). This patient was considered to have atransferrinemia. However, the resolution obtained with paper electrophoresis is known to be lower than that of agarose gel electrophoresis.

Although the three major proteins composing the \(\beta\)-globulin region were markedly decreased in the serum of our patient, they were still present and their cumulative amount exceeded the limit of detection in agarose electrophoresis, which makes the absence of the \(\beta\)-globulin band on agarose-gel electrophoresis puzzling. However, it is known that in the case of immunoglobulins or albumin, for example, densitometric values are lower than the values as measured with use of radial immunodiffusion. Perhaps these proteins stained much more weakly with the commonly used protein stain Amido Black, which would suggest alterations of their structure. Absence of the \(\beta\)-globulin band was not transitory; the same pattern was found in three serum samples collected at different times during the two-week hospital course of the patient. However, the absence of the \(\beta\)-globulin band was not the result of a genetic defect, because patterns obtained at previous hospital admission were normal. At the time of admission the patient had various dysfunctions (e.g., hypothyroidism) besides the liver failure. However, we believe that liver failure was the main reason for the abnormal electrophoretic pattern. In any case, the absence of the \(\beta\)-globulin band in serum protein electrophoresis cannot be related only to atransferrinemia and may signal a poor prognosis.

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References