EDUCATIONAL COMMENTARY – PROTEIN AND ITS IMPORTANCE TO THE BODY

Educational commentary is provided through our affiliation with the American Society for Clinical Pathology (ASCP). To obtain FREE CME/CMLE credits click on Earn CE Credits under Continuing Education on the left side of the screen.

Learning Outcomes

On completion of this exercise, the participant should be able to:

- discuss the importance of protein in the growth and development of healthy cells and tissue;
- describe the methods by which protein and albumin are measured;
- identify the causes of abnormal total protein, albumin, and globulin levels; and
- explain how the albumin to globulin ratio is used to interpret diagnostic findings.

Total Serum Protein

Proteins are important building blocks for the body and are necessary for the growth, development, and health of cells and tissues. They consist of polymers of amino acids that are linked covalently through peptide bonds. Short chains of amino acids are termed dipeptides, tripeptides, tetrapeptides, pentapeptides, and for chains longer than five amino acids, oligopeptides. At a length of 40 or greater, a chain takes on the physical properties associated with proteins. Total serum or plasma protein (total protein) concentration is generally assayed as part of a health checkup to assess nutritional status or to help diagnose certain liver, kidney, and bone marrow disorders and other metabolic disease processes. The test may also be ordered if unexplained weight loss, fatigue, or edema has occurred.

The concentration of total protein in serum and plasma is relatively stable, with a reference range of approximately 6.0-8.0 g/dL. It is important that each laboratory establish its own reference range, as ranges may vary slightly due to factors such as age, sex, population, and test method. Reference ranges are slightly lower for neonates, young children, and adults older than 60 years. An increased total protein concentration may be observed during pregnancy, and a lower total protein concentration (0.4-0.8 mg/dL lower) is expected when a specimen is collected from a patient who is in a reclining position.

The total protein level is the amount of the two major protein classes in the blood, albumin and globulin. Variations in the concentration of total protein result from either a change in the plasma volume or a change in the concentration of one or more specific proteins in the plasma. Increased total protein concentrations may be found in conditions that increase protein production (hyperproteinemia), such as inflammatory disorders and multiple myeloma. Dehydration (decreased plasma volume) due to inadequate water intake or excessive water loss, as in cases of severe vomiting, diarrhea, Addison’s disease, or diabetic acidosis, causes a relative hyperproteinemia. Decreased total protein (hypoproteinemia) may be observed in conditions where production of albumin or globulin proteins is impaired, such as malnutrition or severe liver disease; conditions that accelerate the breakdown or loss of
protein as in kidney disease (e.g., nephrotic syndrome); malabsorptive disorders such as celiac disease, Crohn’s disease, and short-bowel syndrome; and conditions that increase plasma volume, thereby diluting the blood, such as congestive heart failure.

The traditional method of measuring total protein is by the use of biuret reagent. Either plasma or serum may be used for analysis but serum is preferred. Fasting samples are not necessary, but hemolyzed samples should be avoided. The peptide bonds of protein react with Cu²⁺ ions in alkaline solution to form a colored product that is measured spectrophotometrically at 540 nm. The intensity of the reddish-violet color produced by the reaction with tripeptides, oligopeptides, and polypeptides (i.e., long, continuous, and unbranched peptide chains) is proportional to the number of peptide bonds and, therefore, to the amount of protein in the reaction system. Amino acids and dipeptides do not react. Other methods of protein measurement are displayed in Table I. A total protein test result is not to be interpreted as a single result only, but must be correlated with the albumin and albumin to globin ratio (A/G) results as well as consideration of other clinical findings, supplemental tests and information.

Table I. Methods of protein measurement.

<table>
<thead>
<tr>
<th>Method</th>
<th>Measures</th>
<th>Accuracy and Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct photometric</td>
<td>Absorption of UV light at 200-225 nm (peptide bonds) and at 270-290 nm (aromatic rings of tyrosine and tryptophan)</td>
<td>Both suffer from an uneven distribution of the amino acids (tyrosine and tryptophan) among individual proteins in a mixture and from free tyrosine and tryptophan, uric acid and bilirubin.</td>
</tr>
<tr>
<td>Dye-binding</td>
<td>Ability of protein to bind with amido black and CBB; absorbance activity at 465 nm and 595 nm</td>
<td>Unequal affinity and binding capacities of individual proteins are a limitation, complicated further by the lack of a consistent calibrator.</td>
</tr>
<tr>
<td>Folin-Ciocalteu (Lowry)</td>
<td>Free and unfolded polypeptide forms of tyrosine and tryptophor reduce phosphotungstic–phosphomolybdic acid and produces a blue color</td>
<td>More useful for assay of pure protein with known composition and reactivity rather than a mixture of individual proteins with different concentrations and relativities.</td>
</tr>
<tr>
<td>Kjeldahl</td>
<td>Acid digested to convert nitrogen to ammonium ions; titration of nitrogen</td>
<td>Standardized and reproducible. Time-consuming, inconvenient, and impractical.</td>
</tr>
<tr>
<td>Refractometry</td>
<td>The refractive index (a fundamental physical property of proteins) to assess their composition or purity</td>
<td>Quick and convenient estimate of results, inaccurate at a protein &lt;3.5 g/dL, dilution is necessary for &gt; 11.0 g/dL protein.</td>
</tr>
<tr>
<td>Turbidimetric and nephelometric</td>
<td>Precipitation of protein by SSA alone or in combination with sodium sulfate and TCA, or TCA alone</td>
<td>Dependent on formation of a fine precipitate of uniform, insoluble protein particles, which scatter light in suspension.</td>
</tr>
</tbody>
</table>

Abbreviations: CBB, Coomassie brilliant blue; SSA, sulfosalicylic acid and TCA, trichloroacetic acid

Albumin

The most abundant protein in the body is albumin, comprising about 50% of total protein. It is a small globular protein, synthesized by parenchymal cells in the liver where there are vast synthetic reserves. Albumin helps keep the blood from leaking out of blood vessels and assists in transporting substances such as fatty acids, phospholipids, amino acids, and hormones through the blood. It is necessary for
EDUCATIONAL COMMENTARY – PROTEIN AND ITS IMPORTANCE TO THE BODY (cont.)

tissue growth and healing. Albumin is highly conserved across many species and has unique properties that seem functionally indispensable, such as buffering the serum ionized calcium, osmoregulation of plasma volume, solubilizing unconjugated bilirubin, and binding cationic drugs. This protein is an indicator of how well the liver and kidneys are functioning and how well the diet provides protein, and may determine the cause of excess fluid retention.

Approximately 60% of total albumin is found in the extravascular space. It is the major protein component of cerebrospinal fluid, interstitial fluid, urine, and amniotic fluid. Increased albumin levels are observed only in acute cases of dehydration and have no clinical significance. Decreased levels are seen in many clinical conditions (Table II). Albumin has a longer half-life than other proteins, so is a poor indicator of nutritional deficiency or impaired synthesis. Prealbumin and coagulation factors are more sensitive measures, as their half-lives are much shorter.

Table II. Decreased albumin.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cause</th>
<th>Albumin Level</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analbuminemia</td>
<td>Genetic (rare)</td>
<td>Serum/plasma, &lt; 0.5g/dL</td>
<td>Major clinical manifestations related to abnormal lipid transport.</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Acute/Chronic</td>
<td>Serum/plasma, Decreased</td>
<td>Hemodilution Loss into extravascular space Increased consumption by cells Decreased synthesis</td>
</tr>
<tr>
<td>Hepatic disease</td>
<td>Severe parenchymal damage or loss (95%) of function</td>
<td>Serum/plasma, Normal to decreased in advanced disease</td>
<td>Increased immunoglobulin concentrations Loss into extravascular space Direct inhibition of synthesis by toxins and alcohol</td>
</tr>
<tr>
<td>Urinary loss</td>
<td>Excess glomerular filtration, tubular damage or hematuria, or combination</td>
<td>Urine, &gt;20 mg albumin per gram creatinine</td>
<td>Physical exercise and fever may affect levels. Mildly increased levels may predict development of chronic renal disease in persons with active nephrotic syndrome or diabetes mellitus.</td>
</tr>
<tr>
<td>Gastrointestinal loss</td>
<td>Inflammatory disease of the intestinal tract</td>
<td>Serum/plasma, Decreased</td>
<td>Little concern unless excessive or persists. Chronic protein-losing enteropathy may result in loss similar to nephrotic syndrome.</td>
</tr>
<tr>
<td>Protein energy malnutrition</td>
<td>Nutritional status and absorption</td>
<td>Serum/plasma, Decreased</td>
<td>Low concentrations generally do not correlate with the degree of malnutrition and are more often due to acute phase reaction.</td>
</tr>
<tr>
<td>Edema and ascites</td>
<td>Usually secondary to vascular permeability</td>
<td>Body fluids, Varies from very low to higher than plasma</td>
<td>Swelling of ankles, abdomen</td>
</tr>
</tbody>
</table>

Albumin is most often measured by automated dye-binding methods using bromocresol green (BCG) or purple (BCP). Due to these dyes’ great affinity for albumin, the initial rate of binding usually is measured
EDUCATIONAL COMMENTARY – PROTEIN AND ITS IMPORTANCE TO THE BODY (cont.)

and related to the albumin concentration of the sample. Serum is the preferred sample because the dye-binding assays overestimate albumin in the presence of fibrinogen and heparin. An abnormal serum protein electrophoresis (SPE) pattern may also reflect an inaccuracy of albumin measurement by dye methods.

Globulin

Globulin comprises the other half of total protein and consists of multiple classes of proteins, including alpha (α), beta (β), and gamma (γ) types. The liver produces some of the globulins, and others are produced by the immune system. These proteins include enzymes, antibodies, hormones, complement, carrier proteins, and several others. Quantitative testing for globulin is not performed and the result is reported by calculation:

\[
\text{Total Protein (g/dL) - Albumin (g/dL) = Globulin (g/dL)}
\]

Globulin is calculated to determine the chances of developing an infection and to diagnose blood diseases such as multiple myeloma or macroglobulinemia. Disorders associated with high or low globulin levels are numerous. Transferrin and ferritin measurement assesses iron status; ceruloplasmin reflects copper transport and storage; and cardiac troponins reveal myocardial damage. Tumor markers such as prostate-specific antigen, α-fetoprotein, carbohydrate antigen markers, carcinoembryonic antigen, and so forth are used to detect and monitor treatment of such diseases as cancers, multiple myeloma, and α1-antitrypsin deficiency. Fibrinogen and coagulation factors are used to assess hemostasis function, and various enzymes reveal tissue damage and necrosis. Certain clinically significant globulin proteins are exhibited in Table III. When indicated, separation of globulin into its classes is done by SPE. Information is obtained about the different levels of α, β, and γ types, allowing the clinician to identify the disease process and to monitor treatment.

Table III. Significant proteins other than albumin.

<table>
<thead>
<tr>
<th>α-Globulin</th>
<th>β-Globulin</th>
<th>γ-Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1 Antitrypsin (AAT)</td>
<td>Transferrin (Tf, siderophilin)</td>
<td>Immunoglobulins</td>
</tr>
<tr>
<td>Haptoglobin (Hp, HAP)</td>
<td>Complement (C3 and C4)</td>
<td>C-reactive protein (CRP)</td>
</tr>
<tr>
<td>α2 Macroglobulin (AMG)</td>
<td>β2 Microglobulin (BMG)</td>
<td>Fibrinogen</td>
</tr>
<tr>
<td>High-density lipoprotein (HDL)</td>
<td>Low-density lipoprotein (LDL)</td>
<td></td>
</tr>
<tr>
<td>Ceruloplasmin (CER)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Albumin to Globulin Ratio

The albumin to globulin (A/G) ratio is calculated by dividing the albumin result by the globulin result (both in g/dL). As albumin and globulin each compose roughly half of the protein, the usual A/G ratio is slightly higher than 1.0. In disease, the relative amounts of albumin and globulin may be affected, resulting in an abnormal A/G ratio. This may provide a clue as to the cause of the protein change.
EDUCATIONAL COMMENTARY – PROTEIN AND ITS IMPORTANCE TO THE BODY (cont.)

A low A/G ratio may indicate:
1. Overproduction of globulins: multiple myeloma, autoimmune diseases, chronic inflammation
2. Underproduction of albumin: cirrhosis of the liver or nephrotic syndrome
3. Selective loss of albumin: kidney (nephrotic syndrome)

A high A/G ratio may indicate:
1. Underproduction of immunoglobulins: genetic deficiencies, leukemia, steroid and immunosuppressant treatment, α₁-antitrypsin deficiency

A normal A/G ratio is usually observed when a low total protein concentration is caused by a plasma expansion (hemodilution) process, because the albumin and globulin are diluted to the same extent.

Body Fluid Protein

Urine
Circulating proteins are conserved in healthy kidneys by exclusion from the glomerular filtrate and reabsorption from the renal tubules. Proteinuria occurs when either mechanism fails or the concentration of a particular protein is increased enough to cause an “overflow” that overwhelms the filtration and reabsorption systems. Proteinuria is a common symptom of nephrotic syndrome, an increased permeability of the glomerular membrane. Nephrotic syndrome results from focal segmental glomerulosclerosis, minimal change disease, and membrane damage to the glomeruli. Overflow proteinuria is most often caused by overproduction of immunoglobulins such as characteristic of multiple myeloma.

The dipstick semi-quantitative method of measuring protein in urine is not a good indicator of overflow or tubular proteinuria, as the dye-based method is most reactive to albumin. A 24-hour timed collection of urine is usually requested for quantitative total or specific protein assay and for electrophoretic separation. Protein is measured by the biuret method and results are given in milligrams per day. Other timed collections, 4, 8, and 12 hours, have been used to monitor renal transplant recipients or patients undergoing replacement therapy to compensate for acute renal loss of albumin.

Alternatively, measurement of the protein to creatinine ratios of random urine samples is common. Protein is measured in urine (and cerebrospinal fluid) with benzethonium chloride, which precipitates the protein and increases the turbidity of the sample. The turbidity is proportional to the protein concentration. This technique is very sensitive and can yield accurate results in samples with very low protein concentrations (< 20 mg/dL). The urine protein to creatinine ratio on random midday urine samples correlates well with 24-hour urine collection for quantitating urinary protein loss.
Reference interval ranges for urine protein:
  Random, 1-14 mg/dL
  24-hour quantitative, <300 mg/day

Protein to creatinine ratio, diurnal variation:
  Male (18-83 yrs.) <0.11 mg/mg
  Female (18-83 yrs.) <0.16 mg/mg

Cerebrospinal Fluid
Albumin is the predominant protein in normal cerebrospinal fluid. The reference interval for cerebrospinal fluid total protein is 15 to 45 mg/dL. For chemical analysis, the health care practitioner will usually be most interested in the protein, glucose, and, perhaps, chloride level and will occasionally request protein electrophoresis. Many diseases of the central nervous system will result in a change in the protein content of the cerebrospinal fluid.

Increased protein concentration may be caused by an increased capillary permeability in bacterial or viral meningitis, encephalitis, and poliomyelitis and brain tumor. A mechanical obstruction such as spinal cord tumor or cerebral hemorrhage may also result in increased total protein. Higher total protein levels are observed in conditions with heightened local immunoglobulin production, such as neurosyphilis and multiple sclerosis. Tuberculous meningitis and brain abscess will exhibit increased protein due to both an increase in capillary permeability and increased local immunoglobulin production.

Methods for measuring total protein in spinal fluid are limited. Commonly turbidimetric methods and versions of the Coomassie brilliant blue (CBB) dye-binding method are used. Turbidimetric methods have the disadvantage of requiring a large sample volume, 0.2 to 0.5 mL. CBB methods are sensitive with smaller sample volumes but they underestimate globulins. As cerebrospinal fluid protein is predominantly albumin, the underestimation may not be serious enough to preclude the use of CBB methods.

Peritoneal and Pleural Fluids
Disease-associated accumulations of fluid in the peritoneal and pleural cavities vary greatly in total protein content. Ultrafiltrates are characterized by low protein levels and very low amounts of high-molecular-weight proteins. Serous fluids are high in protein with significant amounts of large proteins such as immunoglobulins. Division of these fluids into transudates (protein, < 3 g/dL) and exudates (protein, > 3 g/dL) assists in categorizing disease processes. Transudates ordinarily reflect permeability of filtering membranes, whereas exudates are produced from infection or malignant neoplasms. Measurement methods of these fluids are similar to total protein measurement in serum or plasma.
Other Body Fluids
Assessment of protein in other fluids may assist the clinician in diagnosis. Though not analyzed for total protein, various protein analytes are measured in amniotic fluid, saliva, and feces. Amniotic fluid is tested for α-fetoprotein and other analytes in antenatal screening for birth defects. Saliva is analyzed for secretory immunoglobulin A (IgA) to evaluate possible immunological deficiency. Assays of α1-antitrypsin in feces may be helpful in the diagnosis of exudative enteropathy or other forms of gastrointestinal protein loss.

Summary
Proteins perform and regulate most of the metabolic functions in living organisms. Measurement of protein in the various biological fluids is a vital component of diagnosis and treatment. An array of methods and techniques for protein measurement are routinely available in the clinical laboratory. Clinical applications of new methods are on the horizon that will expand the use of protein analysis in health and disease. The information shared in this article touches the surface of the function, measurement, and relation of protein to disease.

Bibliography


Rice Memorial Hospital Laboratory. Reference interval: SPE studies. Willmar, MN: Rice Memorial Hospital; 2016.

EDUCATIONAL COMMENTARY – PROTEIN AND ITS IMPORTANCE TO THE BODY (cont.)

Total protein and A/G ratio. LabTests Online website. 


© ASCP 2016