PLASMA PROTEINS

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Total blood volume is about 4.5 to 5 liters in adult human being.

If blood is mixed with an anticoagulant and centrifuged, the cell components (RBC and WBC) are precipitated.

Supernatant is plasma.

About 55-60% of blood is made up of plasma.

If blood is withdrawn without anticoagulant and allowed to clot, after about 2 hours liquid portion is separated from the clot.

Defibrinated plasma is called serum, (lacks coagulation factors) including prothrombin and fibrinogen.

Total protein content of normal plasma is 6 to 8 g/100 mL

Plasma proteins consist of:
Albumin (3.5 to 5 g/dL),
Globulins (2.5 – 3.5 g/dL)
Fibrinogen (200– 400mg/dL).
Albumin: Globulin ratio is usually between 1.2:1 to 1.5:1.

Almost all plasma proteins, except immunoglobulins are synthesized in liver.

Plasma proteins are generally synthesized on membrane-bound polyribosomes.

Most plasma proteins are glycoproteins.

In laboratory, separation can be done by salts. Fibrinogen is precipitated by 10% and globulins by 22% concentration of sodium sulfate.

Ammonium sulfate will precipitate globulins at half saturation and albumin at full saturation.

In clinical laboratory, total proteins in serum or plasma of patients are estimated by Biuret method.

Albumin is quantitated by Bromo cresol green (BCG) method, in which the dye is preferentially bound with albumin, and the color is estimated colorimetrically.

ELECTROPHORESIS

- In clinical laboratory, electrophoresis is employed regularly for separation of serum proteins.
- Electrophoresis refers to the movement of charged particles through an electrolyte when subjected to an electric field.
- Normal and abnormal electrophoretic patterns are shown in figures below

Normal Patterns and Interpretations

In agar gel electrophoresis, normal serum is separated into 5 bands. Their relative concentrations are given below:

- Albumin : 55–65%
- Alpha-1 globulin : 2–4%
- Alpha-2 globulin : 6–12%
- Beta globulin : 8–12%
- Gamma globulin : 12–22%

Albumin has the maximum and gamma globulin has the minimum mobility in the electrical field.

Gamma globulins contain the antibodies (immunoglobulins). Most of the alpha-1 fraction is made up of alpha-1 antitrypsin. Alpha-2 band is mainly made up by alpha-2 macroglobulin. Beta fraction contains low density lipoproteins.

Fig: Serum electrophoretic patterns. 1 = Normal pattern; 2 =Multiple myeloma (M band) between b and g region; 3 =Chronic infection, broad based increase in g region; general increase in a1 and a2 bands; 4 = Nephrotic syndrome; hypoalbuminemia; prominent a2 band; 5 = Cirrhosis of liver; decreased albumin; 6 = Plasma showing fibrinogen (normal condition). This may be mistaken for paraproteins

Abnormal Patterns in Clinical Diseases

Various abnormalities can be identified in the electrophoretic pattern.

1. Chronic infections: The gamma globulins are increased, but the increase is smooth and widebased.

2. Multiple myeloma: In para-proteinemias, a sharp spike is noted and is termed as M-band. This is due to monoclonal origin of immunoglobulins in multiple myeloma.

3. Fibrinogen: Instead of serum, if plasma is used for electrophoresis, the fibrinogen will form a prominent band in the gamma region, which may be confused with the M-band.

4. Primary immune deficiency: The gamma globulin fraction is reduced.

5. Nephrotic syndrome: All proteins except very big molecules are lost through urine, and so alpha-2 fraction (containing macroglobulin) will be very prominent.

6. Cirrhosis of liver: Albumin synthesis by liver is decreased, with a compensatory excess synthesis of globulins by reticuloendothelial system. So albumin band will be thin, with a wide beta fraction; sometimes beta and gamma fractions are fused.

7. Chronic lymphatic leukemia, gamma globulin fraction is reduced.

8. Alpha-1 antitrypsin deficiency: The alpha-1 band is thin or even missing.

Fig: Serum electrophoretic patterns

Fig: Normal and abnormal electrophoretic patterns

ALBUMIN

Name is derived from the white precipitate formed when egg is boiled (Latin, albus = white). Albumin constitutes the major part of plasma proteins.

Has one polypeptide chain with 585 amino acids. It has a molecular weight of 69,000 D. It is elliptical in shape.

Synthesized by hepatocytes; therefore, estimation of albumin is a liver function test.

Synthesized as a precursor, and the signal peptide is removed as it passes through endoplasmic reticulum.

Can come out of vascular compartment. So albumin is present in CSF and interstitial fluid.

Half-life of albumin is about 20 days. Liver produces about 12 g of albumin per day, representing about 25% of total hepatic protein synthesis

Half-life: Each plasma protein has a characteristic half-life in circulation; e.g. half-life of albumin is 20 days, and that of haptoglobin is 5 days.

Half-life is studied by labeling the pure protein with radioactive chromium (^{51}Cr). A known quantity of the labeled protein is injected into a normal person, and blood samples are taken at different time intervals.

Half-life of a protein in circulation may be drastically reduced when

proteins are lost in conditions, such as Crohn's disease (regional ileitis) or protein losing enteropathy.

Colloid Osmotic Pressure of Plasma

Proteins cannot easily escape out of blood vessels, and therefore, proteins exert the ‘effective osmotic pressure‘ (EOP).

If protein concentration in serum is reduced, the EOP is correspondingly decreased. Then return of water into blood vessels is diminished, leading to accumulation of water in tissues. This is called edema.

Edema is seen in conditions where albumin level in blood is less than 2 g/dL.

Transport Function

Albumin is the carrier of various hydrophobic substances in the blood. Being a watery medium, blood cannot solubilize lipid components.

Bilirubin and non-esterified fatty acids are specifically transported by albumin.

Drugs (sulfa, aspirin, salicylate, dicoumarol, phenytoin).

Hormones: Steroid hormones, thyroxine.

Metals: Albumin transports copper. Calcium and heavy metals are non-specifically carried by albumin. Only the unbound fraction of drugs is biologically active

Buffering Action

All proteins have buffering capacity. Because of its high concentration in blood, albumin has maximum buffering capacity.

Has a total of 16 histidine residues which contribute to this buffering action.

Nutritional Function

All tissue cells can take up albumin by pinocytosis. It is then broken down to amino acid level.

Albumin may be considered as the transport form of essential amino acids from liver to extrahepatic cells

Clinical Applications

Blood Brain Barrier

Albumin-fatty acid complex cannot cross blood-brain barrier and hence fatty acids cannot be taken up by brain. The **bilirubin from albumin may be competitively replaced by drugs like aspirin**. Being lipophilic, unconjugated bilirubin can cross the blood brain barrier and get deposited in brain. The brain of young children are susceptible; free bilirubin deposited in brain leads to **kernicterus** and mental retardation

- *Drug Interactions*

When two drugs having high affinity to albumin are administered together, there may be competition for the available sites, with consequent displacement of one drug.

Such an effect may lead to clinically significant drug interactions, e.g. phenytoin-dicoumarol interaction.

- *Protein-bound Calcium*

Calcium level in blood is lowered in hypoalbuminemia. Thus, even though total calcium level in blood is lowered, ionized calcium level may be normal, and so tetany may not occur. Calcium is lowered by 0.8 mg/dL for a fall of 1 g/dL of Albumin.

Edema

Hypoalbuminemia will result in tissue edema.

a. Malnutrition, where albumin synthesis is depressed (*generalized edema*)

b. Nephrotic syndrome, where albumin is lost through urine (*facial edema*)

c. Cirrhosis of liver (mainly *ascites*), where albumin synthesis is less and it escapes into ascitic fluid

d. Chronic congestive cardiac failure: Venous congestion will cause increased hydrostatic pressure and decreased return of water into capillaries and so *pitting edema* of feet may result.

Normal Value

Normal level of Albumin is 3.5-5.0 g/dL.

Lowered level of albumin (hypoalbuminemia) has important clinical significance

Hypoalbuminemia

a. Cirrhosis of liver: Synthesis is decreased.

b. Malnutrition: Availability of amino acids is reduced and albumin synthesis is affected.

c. Nephrotic syndrome: Permeability of kidney glomerular membrane is defective, so that albumin is excreted in large quantities.

d. Albuminuria: Presence of albumin in urine is called albuminuria. It is always pathological. Large quantities (a few grams per day) of albumin is lost in urine in nephrotic syndrome. Small quantities are lost in urine in acute nephritis, and other inflammatory conditions of urinary tract. In microalbuminuria or minimal albuminuria or paucialbuminuria, small quantity of albumin (30–300mg/d) is seen in urine (Paucity = small in quantity).

e. Protein losing enteropathy : Large quantities of albumin is lost from intestinal tract.

f. Analbuminemia is a very rare condition, where defective mutation in the gene is responsible for absence of synthesis.

ACUTE PHASE PROTEINS

Level of certain proteins in blood may increase 50 to 1000 folds in various inflammatory and neoplastic conditions. Such proteins are acute phase proteins.

C-Reactive Protein (CRP)

So named because it reacts with C-polysaccharide of capsule of pneumococci.

Beta-globulin and has a molecular weight of 115–140 kD.

Synthesized in liver. It can stimulate complement activity and macrophage phagocytosis. When the inflammation has subsided, CRP quickly falls, followed by ESR (erythrocyte sedimentation rate). CRP level, especially high sensitivity C-reactive protein level in blood has a positive correlation in predicting the risk of coronary artery diseases

Ceruloplasmin

i. Blue in color (Latin, caeruleus=blue). An alpha-2 globulin with molecular weight of 160,000 Daltons. Contains 6 to 8 copper atoms per molecule.

ii. Mainly synthesized by the hepatic parenchymal cells and a small portion by lymphocytes and macrophages. After the formation of peptide part (apo-Cp) copper is added by an intracellular ATPase and carbohydrate side chains are added to make it a glycoprotein (holo-Cp). The normal plasma half-life of holo-Cp is 4–5 days.

iii. Also called Ferroxidase, an enzyme which helps in the incorporation of iron into transferrin. It is an important antioxidant in plasma.

iv. About 90% of copper content of plasma is bound with ceruloplasmin, and 10% with albumin. Copper is bound with albumin loosely, and so easily exchanged with tissues. Hence, transport protein for copper is Albumin.

v. Lowered level of ceruloplasmin is seen in Wilson's disease, malnutrition, nephrosis, and cirrhosis.

vi.Increased plasma Cp levels are seen in active hepatitis, biliary cirrhosis, hemochromatosis, and obstructive biliary disease, pregnancy, estrogen therapy, inflammatory conditions, collagen disorders and in malignancies.

Drugs increasing the ceruloplasmin level are, estrogen and contraceptives.

Reference blood levels of ceruloplasmin are:

- ★ Adult Males: 22–40 mg/dL
- ★ Adult Females: 25–60 mg/dL
- ★ Pregnancy: 30–120 mg/dL

Alpha-1 Antitrypsin (AAT)

Otherwise called **alpha-anti-proteinase** or **protease inhibitor**.

Inhibits all serine proteases (proteolytic enzymes having a serine at their active center), such as plasmin, thrombin, trypsin, chymotrypsin, elastase, and cathepsin. **Ser**ine **p**rotease **in**hibitor**s** are abbreviated as **Serpins**.

AAT is synthesized in liver. A glycoprotein with a molecular weight of 50 KD. Forms the bulk of molecules in serum having **alpha-1** mobility. Normal serum level is 75-200 mg/dL.

AAT deficiency causes the following conditions:

Emphysema: The incidence of AAT deficiency is 1 in 1000 in Europe, but uncommon in Asia. The total activity of AAT is reduced in these individuals. Bacterial infections in lung attract macrophages which release elastase. In the AAT deficiency, unopposed action of elastase will cause damage to lung tissue, leading to emphysema. About 5% of emphysema cases are due to AAT deficiency.

Nephrotic syndrome: AAT molecules are lost in urine, and so AAT deficiency is produced.

Negative Acute Phase Proteins

During an inflammatory response, some proteins are seen to be decreased in blood; those are called negative acute phase proteins. Examples are albumin, transthyretin (prealbumin), retinol binding protein and transferrin.

Transferrin is a specific iron binding protein. It has a half-life of 7–10 days and is used as a better index of protein turnover than albumin.

- Plasma contains many enzymes, protein hormones and immunoglobulins.

CLOTTING FACTORS

- The word coagulation is derived from the Greek term, "coagulare" = to curdle. The biochemical mechanism of clotting is a typical example of **cascade activation**.
- Coagulation factors are present in circulation as **inactive zymogen** forms. They are converted to their active forms only when the clotting process is initiated. This would prevent unnecessary intravascular coagulation. Activation process leads to a cascade amplification effect, in which one molecule of preceding factor activates 1000 molecules of the next factor. Thus within seconds, a large number of molecules of final factors are activated. Several of these factors require calcium for their activation. The calcium ions are chelated by the gamma carboxyl group of glutamic acid residues of the factors, prothrombin, VII, IX, X, XI and XII. The **gamma carboxylation** of glutamic acid residues is dependent on vitamin K, and occurs after synthesis of the protein (post-translational modification)

Prothrombin

A single chain zymogen with a molecular weight of 69,000 D. Plasma concentration is 10–15 mg/dL.

Prothrombin is converted to thrombin by Factor Xa, by the removal of N-terminal fragment.

Thrombin

Serine protease with molecular weight of 34,000 D.

The Ca++ binding of prothrombin is essential for anchoring the prothrombin on the surface of platelets. When the terminal fragment is cleaved off, the calcium binding sites are removed and so, thrombin is released from the platelet surface.