Proteins in the Plasma and Urine:

Although the proteins in all fluid compartments play a major physiologic function, the plasma proteins are the most frequently analyzed. More than 500 plasma proteins have been identified.

Plasma proteins in general are referred to and collectively measured as total serum protein (TSP).

- TSP = albumin and globulin
- Globulins = α-1-globulin, α-2-globulin, β-globulin and γ-globulin proteins
- Fibrinogen

▲Plasma protein concentrations depends on several factors:

1- balance between the rate of biosynthesis and the rate of catabolism, and loss from the body.

2- the relative amount of proteins and water in the body.

3- fluid distribution between extracellular and intracellular compartment.

All plasma proteins nearly synthesized by liver with exception of immunoglobulins (Ig) which are produced by lymphocytes(B-cell), while the complement system synthesized by liver and macrophages. In addition to that, all plasma protein nearly of molecular weight that are beyond the capacity of glomeruli to filtrate them, however, the half-life of plasma protein is different, and they are catabolized by proteases.

Reference Ranges:

- Total Serum Protein: 6.5-8.5 g/dl
- Albumin: 3.5-5.5 g/dl
- Urine Protein: < 300mg/24hour
- CSF protein: 15-45mg/dl(<1%)

Function of Total Serum Protein:

- Transport small molecules
- Receptors
- Catalyze biochemical reactions
- Structural
- Nutritional source
- Oncotic pressure
- Defense against foreign antigen
- Hormones

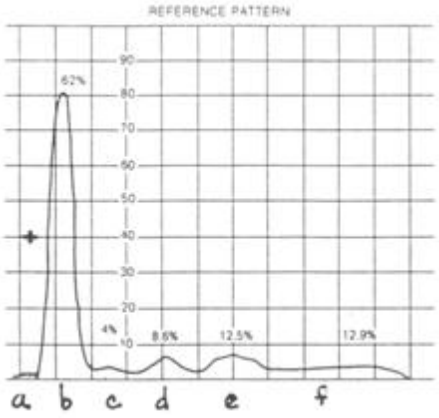
- Aids in maintenance of homeostasis
- Aids in maintenance of acid-base balance

▲The Causes of Low plasma protein levels(hypoproteinemia):

1. Chronic liver diseases(cirrhosis)
2. Malnutrition(low amino acids intake)
3. Malabsorption(low amino acids intake)
4. Increased catabolism due to illness(diseases) or surgery
5. Nephritic syndrome: when there is damage of renal glomeruli by infection or autoimmune disease, this will results in protein loss in urine, especially, light molecular weight type.
6. Gastro-intestinal loss(diarrhea, vomiting)
7. Skin loss (severe burn).
8. hypervolumia (rehydration=fluid overload).
9. blood collecting at site of IV line.

The normal plasma protein concentrations is about 6-8g/dl, usually, albumin constitutes about 60% of total plasma protein. Plasma protein components can be **separated by** salting out with *sodium or Ammonium sulphate*(*Cohn fractionation method*), *Chromatography*, *Heat Shock* method or by *electrophoresis* technique by which protein migrate in electrical field at PH 8.6 in presence of special media depending on charge and size of the protein molecules.

Typical migration zones:
a. pre-albumin
b. Albumin
c. Alpha-1-globulins
d. Alpha-2-globulins
e. Beta-globulins
f. Gamma-globulins



(**Normal SPE zone pattern**)

By electrophoresis plasma proteins are separated into 6 bands including: Pre-albumin, Albumin band, α-1globulin, α-2 globulin, β-globulin, and γ gamma-immunoglobulin(Ig) band.

Albumin is the only single protein of molecular weight(65KD), while other types are composed of several protein types. Albumin is very important fraction of plasma protein for maintenance of

normal intravascular volume by its effect on osmotic colloid pressure, so any reduction in plasma albumin concentration (hypoalbuminemia) results in edema due to migration of fluid from IV compartment into interstitial compartment.

Albumin Functions:

1. important transporting molecule for many biochemical substances including: bilirubin, fatty acids, trace elements, hormones, and drugs that are water insoluble.

2. important for limitation of biological activity of free drugs and hormones e.g. cortizol hormone 90% is protein bound, while only 5-10% free fraction (active part), and also free drug may exert double time effect of conjugated one, that's mean 25mg free effect equal 50mg effect of albumin bound drug.

Measurement of total plasma protein is of little clinical value, because sometimes, significant changes in one protein component may be masked by reciprocal changes of other component.

▲Causes of raised total protein levels:

- Bone marrow disorders, amyloidosis
- Chronic inflammatory conditions
- Posture: upright > recumbence position
- Dehydration (which may make blood proteins appear falsely elevated)

Most other important plasma protein are acute phase reactants. Usually, after tissue damage(infection) many proteins are increased as a part of inflammatory response, these referred to as **acute phase reactants**. The normal body immune response occurs in this order:

▲First phase called non-specific represented by elevated WBCs and acute phase reactants.

▲Second phase called specific reflected by raised blood lymphocytes and immunoglubulins.

Acute Phase Reactants (APR):

• ***Positive APR (+APR):***

1. Alpha-1-antitrypsin (AAT), **2.** Haptoglobin (HPT),
3. Ceruloplasmin (CER), **4.** Fibrinogen (FBG), **5.** C-reactive protein (CRP)

• **Negative APR (-APR)**:

1. Albumin (ALB), **2.** Pre-albumin (PALB), **3.** Transferrin (TRF)

Acute phase reactants of which are most important:

■ Pre-albumin:

It is rich in tryptophan and contains 0.5% carbohydrate. Prealbumin combines with thyroxine and tri-iodothyronine to serve as the transport mechanism for these thyroid hormones. Prealbumin also binds with retinol-binding protein to form a complex that transports retinol-(vitamin A).

■ C-reactive protein(CRP):

C-Reactive protein (CRP) is synthesized in the liver and appears in the blood of patients with diverse inflammatory diseases. CRP was so named because it precipitates with the C-substance, a polysaccharide of pneumococci. However, it was found that CRP rises sharply whenever there is tissue necrosis(infection), and is a best marker for acute tissue damage, and it is more specific than ESR(erythrocyte sedimentation rate) and considered as a risk factor for coronary artery disease.

■ Haptoglobin:

Haptoglobin, an α_{2}- glycoprotein, is synthesized in the hepatocytes and, to a small extent, in cells of the reticulo-endothelial system. Haptoglobin comprises two kinds of polypeptide chains: two α chains and one β chain. The function of haptoglobin is to bind free hemoglobin by its α-chain. Abnormal hemoglobin, such as Bart's and hemoglobin H, has no α-chains and cannot be bound. The reticulo-endothelial cells remove the haptoglobin-hemoglobin complex from circulation within minutes of its formation. ***Thus, haptoglobin prevents the loss of hemoglobin and its constituent iron into the urine.***

■ α1- antitrypsin:

Its main function is to neutralize trypsin-like enzymes that is secreted by neutrophils during infection (i.e., elastase, trypsin, chemotrypsin) that can cause hydrolytic damage to structural protein. α_{1}-Antitrypsin is a major component (approximately 90%) of the fraction of serum proteins that migrates electrophoretically

immediately following albumin. **Inherited deficiency of α1-antitrypsin is associated with emphysema and cirrhosis**.

▄ Immunoglobulins:

Immunoglobulins (Ig) are of five types, all of them are composed of two identical heavy chains and two identical light chains.

Types of Immunoglobulin:

1. IgM : confined only in intravascular compartment due to high molecular weight, it is the first line of body defense mechanism and its synthesis increased in response to foreign bodies introduction.

2. IgG : it is a second line of defense mechanism, which increased in response to specific structure(attack antigen specific for an organism not the organism itself).

3. IgA : synthesized and secreted in respiratory and gastro-intestinal secretion(infection).

4. IgE : it is specific for allergy and hypersensitivity reaction.

Ceruloplasmin (CER): Contains >90% serum copper

▲ Decreased levels: Associated with:

1- Wilson's disease:(Decreased serum Copper, Increased urine Copper)

2– Liver disease

▲ Increased levels:

– (+APR)

Transferrin (TRF):

It is a major component of beta-globulins and responsible for iron transport in plasma

• **Decreased levels:**

1- Liver disease
2- Renal disease
– (-APR)

• **Increased levels:** occurs in Iron Deficiency Anemia(IDA).

Urine Protein:

▲Increased urine protein

1- Increased glomerular permeability (NS, renal disease)
2- MM (paraprotein)
3- Intravascular hemolysis (hemoglobin)
4-Tubular failure (drugs, toxins)

CSF Protein:

- Proteins found in CSF originate from plasma
- Normal pattern similar to SPE, only much lower concentrations
- Adult CSF protein <45 mg/dl
- Concurrent serum analysis

▲CSF Protein Clinically measured to:

– Assess integrity of blood brain barrier
– Aid in diagnosis of disease

• **Increased levels**

1- Meningitis
2- Multiple Sclerosis (MS)

▲*Multiple Myeloma(plasmacytoma):*

It is a malignant disease of bone marrow which is the site of blood cell synthesis. The main pathological feature of this disease is rapid proliferation of plasma which are the precursor of lymphocytes(monoclonal, which mean single type of plasma cells produce single type of immunoglobulin). The IgG and IgA in this condition will increase to a very high concentrations that leads to increased viscosity of blood and as a result of that the blood flow will be sluggish, so the ESR value will markedly increased > 100 mm^3/hour. Ultimately, this leads to thrombosis and end with cerebral dysfunction, gangrene, and vision abnormality if retinal veins involved. The affected persons are easily become infected, because the normal production of other types of immunoglobulin is reduced.

Diagnosis of MM:

1- Serum Protein Electrophoresis:

We noticed the prominent abnormal band called paraprotein (M protein). This zone only formed of IgA and IgG light chains fragments(no heavy chains), so the coupling of two light chains results in the formation of a dimer (Bence-Jones protein).

2- Urine Bence Jones Protein:

The light chain molecular weight = 20 KD, so the dimer = 40 KD, which is of size easily to be filtered by renal glomeruli and excreted in urine and this confirms the diagnosis of MM. When large amounts of BJP are filtered, the accumulated protein in renal tubules and renal tissue, finally, lead to renal damage and chronic renal failure. Urine protein normally < 300 mg/24hr, while in MM urine protein will increase and may be ranged from 5-50 g/day

3- Radiological Investigation:

the classic radiographic appearance is of multiple, discrete, small, lytic lesions mainly involved skull, vertebrae, femur and humerus.

4- Hematological investigations:

- **serum urea and creatinine** were increased as a result of MM.
- **serum uric acid** is increased due to increased tissue catabolism because of chemotherapy.
- **Hemoglobin** level is decreased because of the tumor of plasma cells will inhibit the production of other bone marrow tasks.
- **Alkaline phosphatase** in serum of normal value, which is the marker of bone disease, so we can differentiate between bone tumor(↑) and MM(normal ALP).

5- Bone Marrow biopsy (histopathological changes**)**