

Plasma Proteins

TOTAL PLASMA PROTEINS

- The normal serum protein level is 63-83 g/L.
- The type of proteins in serum include:
 - a. Albumin
 - b. Globulins
 - α - globulin: α_1 & α_2 -globulins
 - β - globulin: β_1 & β_2 globulins
 - γ - globulins
 - c. Fibrinogen
- Under different pathological conditions the protein levels depart from the normal range.

Functions of Plasma proteins

- **Transport:** e.g.
 - Transferrin transports iron.
 - Ceruloplasmin transports copper.
 - Albumin transports fatty acids, bilirubin calcium, many drugs etc.
 - Transcortin transports cortisol and corticosterone
 - Retinol binding protein transports retinol.
 - Lipoproteins transport lipids.
 - Haptoglobin transports free haemoglobin.
 - Thyroxin binding globulin transports thyroxin.

Functions of Plasma proteins

(contd)

- **Osmotic regulation:**

- Plasma proteins are colloidal and non-diffusible and exert a colloidal osmotic pressure which helps to maintain a normal blood volume and a normal water content in the interstitial fluid and the tissues.

- Albumin content is most important in regulation of colloidal osmotic or oncotic pressure.

- Decrease in albumin level results in loss of water from blood and its entry into interstitial fluids causing edema.

- **Catalytic function (enzymes):**

- e.g lipases for removal of lipids from the blood.

Functions of Plasma proteins

(contd)

- **Protective function:**

- Immunoglobulins combine with foreign antigens and remove them.
- Complement system removes cellular antigens.
- Enzyme inhibitors remove enzymes by forming complexes with them. e.g. α_1 antitrypsin combines with elastase, trypsin and protects the hydrolytic damage of tissues such as lungs.
- Some proteins increase during acute phase and protect the body. E.g. α_1 antitrypsin, α_2 macroglobulins

Functions of Plasma proteins (contd)

- **Blood clotting:**
 - Many factors are involved in clotting mechanism and prevent loss of excessive amount of blood. e.g. clotting factors IX, VIII, thrombin, fibrinogen etc.
 - An excess or deficiency leads to a disease. e.g hemophilia, thrombus formation. —
- **Anticoagulant activity (thrombolysis):**
 - Plasmin breaks down thrombin and dissolves the clot
- **Buffering capacity:**
 - Proteins in plasma help to maintain acid-base balance.

Specific Functions of some proteins

PROTEIN	PLASMA CONC. (g/L)	FUNCTION
Pre-albumin	0.3	Binds T3 & T4
Albumin	40.0	Transport, colloid oncotic pressure
α 1- globulin : α 1- antitrypsin	3.0	Anti proteinase
α 2- globulins	0.4	Copper transport
ceruloplasmin	1.2	Binds haemoglobin
haptoglobin		
α 2-macroglobulin	3.0	Transport, anti-proteinase

Contd.....

Specific Functions of some proteins

PROTEIN	PLASMA CONC. (g/L)	FUNCTION
β - Globulins		
Transferrin	2.5	- Iron - transport
Hemopexin	1.0	- Binds haem
Plasminogen	0.7	- Fibrinolysis
Fibrinogen	4.0	- Haemostasis
γ - Globulin		
IgA	0.9-4.5	-Ig in external secretions
IgM	0.7-2.8	- First Ab synthesised
IgG	8-18.0	-Main classes of antibody
IgE		- Involved in allergy
IgD		

MEASUREMENT OF PROTEIN FRACTIONS

- The protein fraction in plasma can be separated and estimated using the following methods:
 - Zone electrophoresis
 - Immunochemical methods
 - Chemical methods
 - Ultracentrifugation

CHARACTERIZATION, MEASUREMENT AND ISOLATION OF PLASMA PROTEINS

- **Physical Techniques**

1. Ultracentrifugation (analytical or Sedimentation velocity ultracentrifuge) at 60,000 per.min. (Refractive index the boundary between the solvent and the protein is visualized by an optical system - called Sehlieren System).

Advantage

Most useful for the determination of the mol. wt of proteins

Disadvantage

High cost of each analysis and poor resolving capacity (when applied to whole serum or plasma)

CHARACTERIZATION, MEASUREMENT AND ISOLATION OF PLASMA PROTEINS

- **Electrophoresis**

Protein in aqueous solution are charged groups (e.g. carboxylic (Asp. Glu), amino groups (Lys, Arg), they can be separated under an electric field using various stabilizing media.

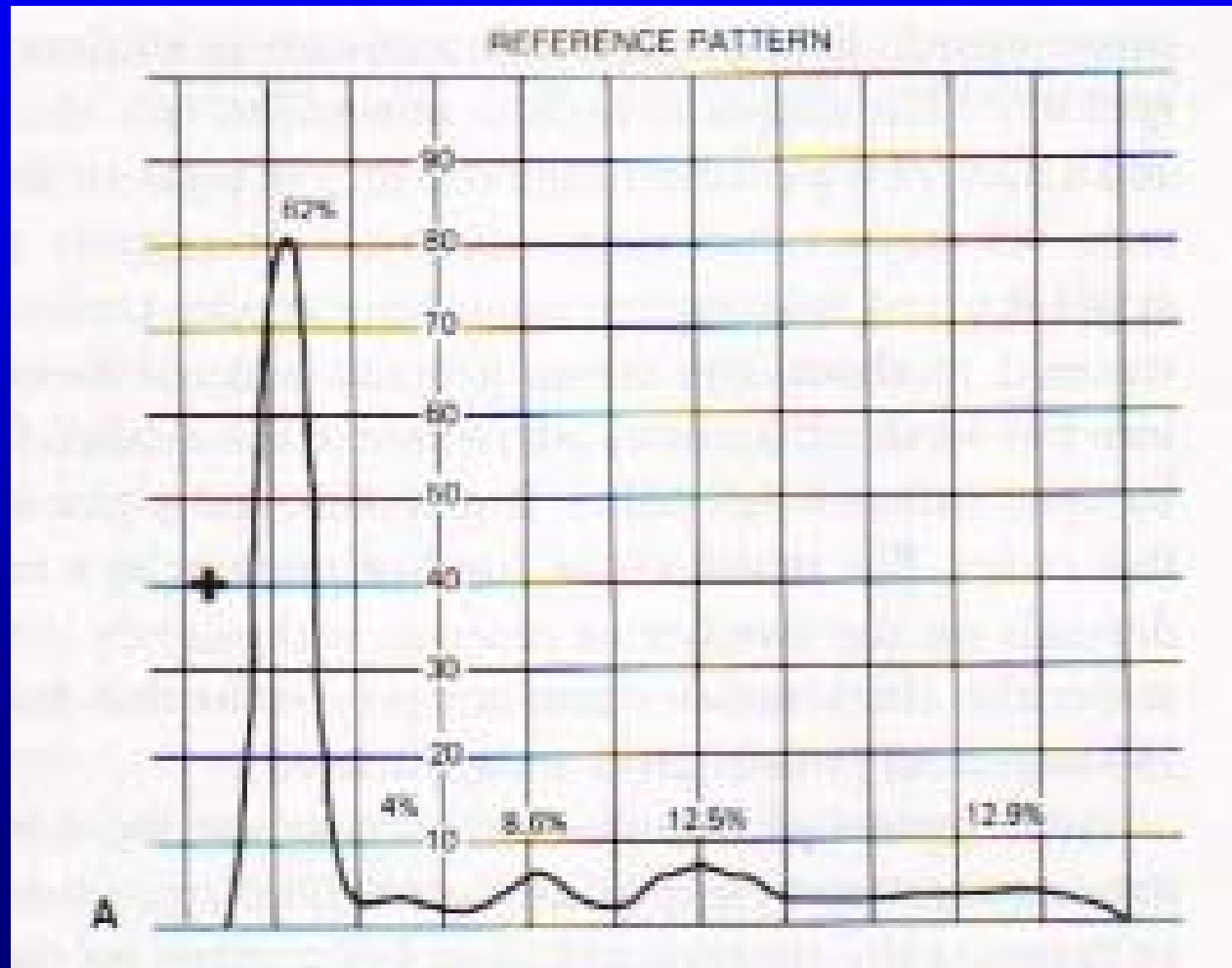
N.B. Amino groups undergo ionic dissociation at alkaline pH and carboxylic undergo dissociation at acid pH. Most proteins are -ve at pH 8.6. The pH at which +ve charges equal to -ve charges is characteristic for a protein and is called isoelectric point (PI).

- **Boundary electrophoresis: Separation in free liquid media**
- **Zone electrophoresis - Separation in stabilizing media** (e.g. Pager, Cellulose acetate, Starch, Polyacrylamide, Agarose)

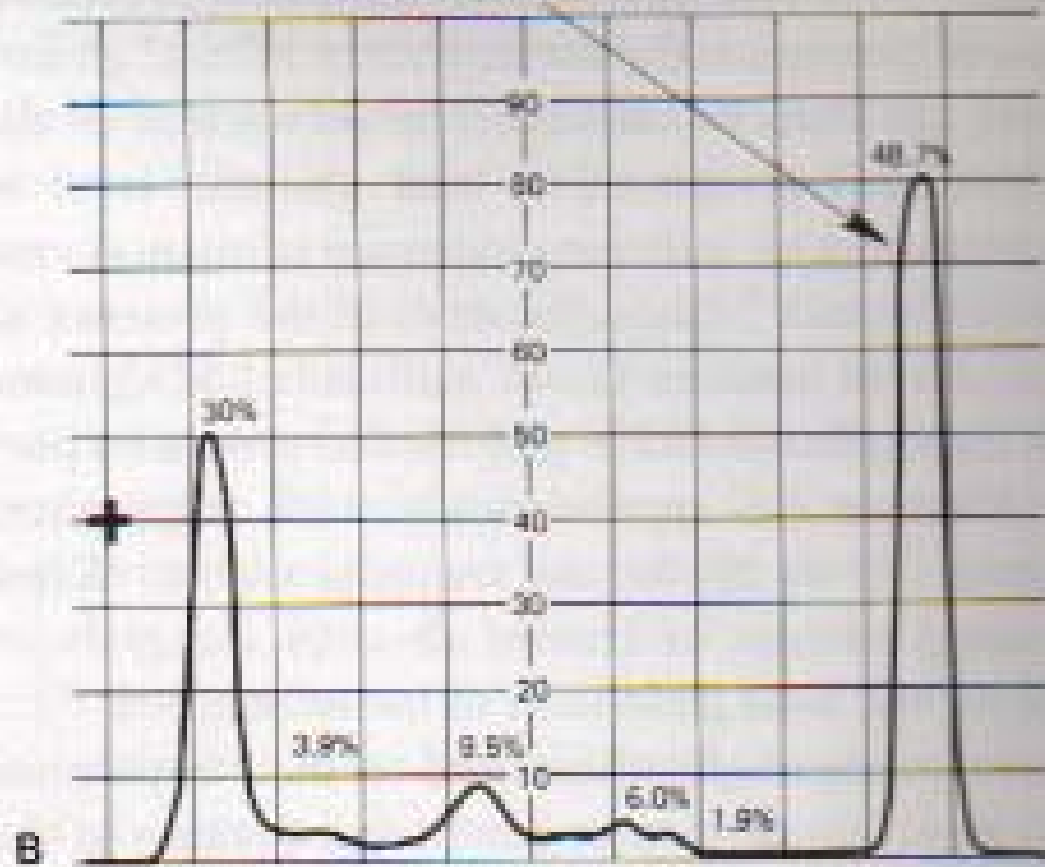
Electrophoresis

- Separates proteins on the basis of their charge.
- Types:
 - Free boundary: separation under an electric field in a fluid media. Separates plasma proteins five bands: albumin(54-58%), α 1 globulins (6-7%), α 2 globulins(8-9%), β globulins (13-14%), γ globulins (11-12%).
 - Zone electrophoresis: Separation under an electric field in a solid media e.g. paper, starch, cellulose, Acrylamide etc. Separates plasma proteins into: Albumin, α 1 globulins, α 2 globulins, β globulins, γ globulins and fibrinogen.

NORMAL HUMAN SERUM PROTEIN ELECTROPHORESIS

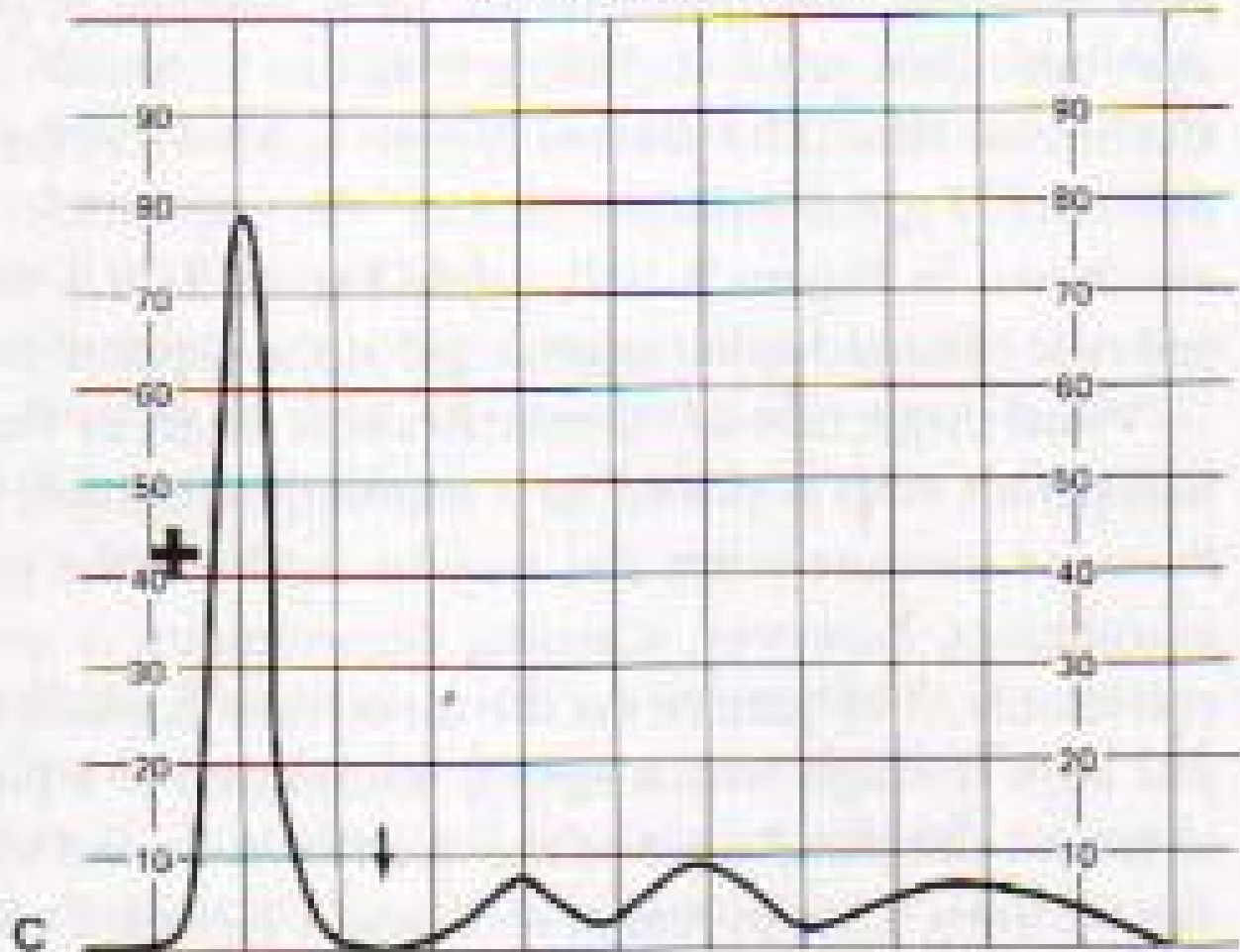


MONOCLONAL INCREASE



B

α_1 -ANTITRYPSIN DEFICIENCY



C

SERUM PROTEIN DEFECTS

- **Normal serum protein levels:**
Total serum protein level: 63-83 g/dL.
- **Hyperproteinaemia:**
Total serum protein level: > 90 g/dL.
- **Hypoproteinaemia:**
Total serum protein level: < 63 g/dL.

INDIVIDUAL PROTEIN FRACTION

ALBUMIN

- A low molecular weight protein (M.Wt= 65,000).
- Functions include:
 - **Transport**
 - **Osmotic pressure regulation**
- Synthesized in the liver.
- Deficiency: in liver disease and kidney disease.

GLOBULINS

- Heterogenous group
- Can be separated into different fractions on the basis of their electrophoretic mobility and sedimentation coefficient:

α 1-Globulin - α 1- Fetoprotein

α 1- Antitrypsin

α 2- Globulin - α 2- Fetoprotein

Haptoglobin

β - Globulin - Transferrin

Ceruloplasmin

γ - Globulin - Antibodies (immunoglobulins)

FIBRINOGEN

- A globulin of very high mol. wt.
- Can be precipitated easily.
- Can be converted to fibrin which causes the blood clot formation.
- Synthesized exclusively in the liver.

BIOCHEMICAL ABNORMALITIES OF PROTEINS

- Total protein abnormalities.
- Abnormalities of individual protein fraction:
 - Serum albumin.
 - Carrier proteins.
 - Protease inhibitors.
 - Immunoglobulins.
 - Embryonic and fetal protein abnormalities. associated with human neoplasia.

TOTAL SERUM PROTEIN ABNORMALITIES

Hypoproteinaemia may result from:

1. **Water excess** caused as a result of:
 - a. Overhydration.
 - b. Artifactual cause - blood taken from the “drip” arm.
2. **Excessive loss of protein (mainly albumin):**
 - a. Through the kidney in nephrotic syndrome
 - b. From the skin after burns
 - c. Through the skin in protein losing enteropathy.
3. **Decreased synthesis of proteins**
 - a*. Severe dietary protein deficiency e.g. in Kwashiorkor
 - b*. Severe liver disease (mainly albumin).
 - c. Severe malabsorption.

* There may be no fall in total protein if γ -globulin is raised

HYPOLBUMINAEMIA

- Normal albumin level = 32-52 g/L.
- Hypoalbuminaemia: the level of albumin <32 g/L.
- Frequently encountered.
- Consequence:
 - Oedema
 - Hypocalcaemia
 - Alteration in the levels of protein-bound substance due to loss of carrier protein.

CAUSES OF HYPOALBUMINAEMIA

- **Decrease albumin synthesis:**
 - a. Liver disease (specially chronic diseases).
 - b. Malnutrition.
 - c. Alcoholism
- **Increased albumin loss:**
 - a. Renal disease (nephrotic syndrome).
 - Loss of albumin in urine (proteinuria).
 - b. Extensive burns:
 - Loss of albumin through skin - transduction.

CAUSES OF HYPOALBUMINAEMIAContd

- Defective intake:
 - a. Malabsorption due to gastro-intestinal disease
- Protein-losing enteropathy (rare)
 - Excessive loss of protein from the body into the gut.
 - Occurs in a variety of conditions such as :
 - a. Ulceration of the bowel.
 - b. Lymphatic obstruction.
 - c. Intestinal lymphangiectasis.

CAUSES OF HYPOALBUMINAEMIAContd

- **Haemodilution**
 - a. Over hydration.
 - b. Late stage of pregnancy.
- **Artefactual**
 - a. Blood drawn from “drip” arm.
- **Non-specific causes (common)**
 - In many acute conditions including minor illnesses such as colds and boils.
 - Often in hospitalized patients.
 - Upright position when drawing blood.
 - Newborn babies.
- **Increased degradation of albumin. In:**
 - Idiopathic
 - Familial idiopathic hypercatabolic hypoproteinemia.
 - Wiscott-Aldrich syndrome

ABNORMALITIES OF CARRIER PROTEINS

α 1-globulin

- The normal serum level of α 1-globulin is 1-3g/L.
- α 1-lipoprotein transport cholesterol.
 - In a rare genetic disorder, α 1-lipoprotein deficiency (Tangiers disease), its level is reduced causing the accumulation of cholesterol esters in tissues resulting in:
 - Tonsillar enlargement.
 - Hepatomegaly.
 - Lymphadenopathy

α 1-FETOPROTEIN (AFP)

- AFP is synthesized in fetus at 14-40 weeks of gestation.
- AFP levels decline rapidly after 2 weeks of age.
- In adults it is found primarily in:
 - association with hepatocellular cancer of liver and embryonic tumor of the ovary and testes.
 - Cases of gastric and prostatic carcinoma.
 - Viral hepatitis.
 - Cirrhosis.
- AFP detection is very useful in diagnosis of primary liver cancer.

α 2-GLOBULIN

The normal α 2-globulin level is 6-10 g/L of serum.

α 2-Macroglobulin make up most of α 2-globulin fraction.

- It is a large molecule
- In nephrotic syndrome, it is retained in serum and levels are found to increase.
- Haptoglobin : binds free haemoglobin. Low levels are found in hemolytic conditions since the haptoglobin/haemoglobin complex is catabolised better than free haptoglobin.

β -GLOBULIN

Normal level of β -globulin in serum is 7-11 g/L.

- β -lipoprotein transport cholesterol in serum.
- Abetalipoproteinaemia is the complete absence of β -lipoprotein, pre β -lipoprotein and chylomicron. This causes:
 - . Inability to transport lipid from intestine or the liver.
 - . Plasma cholesterol deficiency.
- It is clinically characterized by intestinal malabsorption under steatorrhea, progressive atasia, retinitis pigmentation and crenation of erythrocytes.
- High levels of β -globulin are found in pregnancy, biliary obstruction and nephrotic syndrome.

TRANSFERRIN

- Transferrin is a β -globulin.
- It binds free iron in serum.
- Normally it is about one third saturated with iron.
- Transferrin levels are decreased in:
 - *Liver disease (e.g. cirrhosis).*
 - *Chronic infections.*
 - *Nephrosis.*
 - *Congenital atransferrinaemia.*
- Increased serum transferrin levels occur during increased transferrin synthesis caused as a result of iron deficiency anaemia.

ALTERATION OF PLASMA PROTEIN CONCENTRATION

<u>PROTEIN</u>	<u>INCREASED IN</u>	<u>DECREASED IN</u>
Albumin	Dehydration	<ul style="list-style-type: none">- Acute and chronic liver disease.- Malnutrition- Malabsorption- Cirrhosis of liver- Burns- Severe trauma- Nephrotic syndrome
Transferrin	<ul style="list-style-type: none">- Iron deficiency- In woman taking oral contraceptives.	<ul style="list-style-type: none">- Protein losing conditions- Infection; and- Neoplastic disease

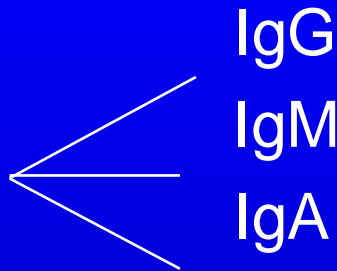
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ALTERATION OF PLASMA PROTEIN CONCENTRATIONContd

<u>PROTEIN</u>	<u>INCREASED IN</u>	<u>DECREASED IN</u>
Ceruloplasmin	- Chronic liver disease - Some infections.	Wilson disease
Haptoglobin		Haemolytic anaemia
α 1-Antitrypsin		Pulmonary emphysema.
α 2-Macroglobulin	Nephrotic syndrome collagen disorder	Liver disease in children leading to cirrhosis.
α -Fetoprotein	Hepatocellular carcinoma	
Fibrinogen		- Congenital fibrinogen def. - Shock. - Complication of pregnancy. - Major surgery - Snake bites. - Disseminated carcinoma

INFLAMMATORY RESPONSE

- Assessment of the presence and degree of inflammation can be obtained from the levels of “acute phase protein”
 - **Positive acute phase proteins:**
Increase during inflammation.
 - **Negative acute phase proteins:**
decrease during inflammation.

- Cryoglobulins
- Pure monoclonal 
 - IgG
 - IgM
 - IgA
 - Mixed.
 - Consist of complexes of immunoglobulins or altered immunoglobulins.
 - Insoluble at 4°C. Aggregate at 30°C

ACUTE PHASE PROTEINS

- Indicators of inflammatory disease with:
 - ESR
 - Leukocytosis
 - Fever
- Indicate active state of inflammation.
- Constitute: α 1-antitrypsin
- Carrier proteins:
 - Haptoglobin.
 - Ceruloplasmin.
 - Fibrinogen.
 - C-reactive proteins
 - α 1-acid glycoprotein

CLINICAL INDICATIONS FOR ASSESSMENT OF ACUTE PHASE PROTEINS

- Presence of inflammatory disease.
- Differential diagnosis of inflammatory disease.
- Estimation of the endpoint of therapy.
- Monitoring therapeutic effectiveness.
- Postsurgical follow-up in patients at risk of postoperative infections.
- Follow-up of patient with malignancy.

POSITIVE ACUTE PHASE PROTEINS

- α 1-antitrypsin.
- α 1-antichymotrypsin.
- α 1-acid glycoprotein.
- Ceruloplasmin.
- Haptoglobin.
- Complement component C3 and C4.
- Antithrombin III.

SPECIFIC INDICATIONS FOR QUATIFICATION OF SOME ACUTE PHASE PROTEINS

PROTEIN

DISEASE

α 1-antitrypsin

- Chronic obstructive pulmonary disease.
- Neonatal hepatitis syndrome
- cytogenic cirrhosis.

Ceruloplasmin

Hepatitis or cirrhosis (unexplained)

Haptoglobin

In-vivo haemolysis.
Ineffective erythropoiesis

EMBRYONIC AND FETAL PROTEIN ASSOCIATED WITH HUMAN NEOPLASIA

- Several fetal proteins are synthesized in human tumors.
- They are released in biological fluid.
- Useful in
 - diagnosis of malignancy
 - monitoring of therapy for cancer
 - evaluation of prognosis:
- The proteins often found associated with tumors are:
 - α 1-fetoproteins
 - α 2-H fetoprotein
 - β 2-S fetoprotein
 - regan alkaline phosphatase
 - fetal sulphoglycoprotein antigen
 - γ -fetoproteins
 - Carcinoembryonic antigen of the gastrointestinal tract.

INHERITED ABNORMALITIES OF THE Plasma Proteins

DEFICIENCY

ASSOCIATED ABNORMALITY

α 1-Antitrypsin

Obstructive pulmonary disease (Chronic or emphysema) liver disease.

Anti-thrombin

Thrombosis
Pulmonary embolism

Immunoglobulin
Complement

Severe recurrent or chronic infection
Severe, recurrent infection.

C1 esterase inhibitor

Recurrent non-pruritic swelling of skin and mucus membrane (hereditary angioneurotic edema)

PLASMA PROTEIN CHANGES IN LIVER DISEASES

Liver disease	HPT	A1b	C3	LDL	IgG	IgM	IgA	TRF	Pre-Alb	α 1-AT
“Pure” Biliary Obstruction	↑↑		↑↑	↑↑						
Advanced Hepatic Cirrhosis	↓↓	↓	↓		↑↑	(↑↑)	↑↑	↓	↓↓	↑
Acute Viral Hepatitis	(↓)				(↑)	↑	(↑)			↑
Infection Mononucleosis	↓				(↑)	↑	(↑)			

↓ = Decrease

↑ = Increase

(↓) = May be decreased

(↑) = May be increased

NEGATIVE ACUTE PHASE PROTEINS

- Albumin.
- Transferrin.
- Pre-albumin.