PLASMA PROTEINS AND IMMUNOGLOBULINES – An Overview

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How is blood plasma different from serum?

- Plasma is fluid portion of whole blood,
  - Plasma is obtained when whole blood containing anti-coagulant is centrifuged,
  - Plasma contains clotting factors,
- Serum is fluid portion of clotted blood,
- Serum is obtained after centrifuging clotted blood
  - Serum does not contain clotting factors that are normally present in plasma,
What are some of the proteins in blood plasma or serum?

- Plasma contains a variety of proteins with different functions and some proteins of unknown functions.
- “Total Protein” in plasma is made up of **Albumin** and **Globulins**.
- Clinical Biochemistry labs routinely measures **Total Protein** and **Albumin** usually in serum,
- **Globulin fraction** = **Total protein** – **Albumin**
• Other plasma proteins (e.g., Immunoglobulins) are measured as Classes,
• Immunochemical methods are used to measure specific plasma proteins, hormones or enzymes;
• Enzymes in serum or plasma are also measured by their activity;
• Electrophoresis can be used to separate protein components in Serum or Plasma,
  • About 5 or more major bands can be used to demonstrate the presence of Paraproteins;
What are the functions of proteins in blood plasma?

- **Some functions include:**
- **Blood clotting factors:** proteins in coagulation cascade
- **Immune defense:** Immunoglobulins, Complement proteins involved in Inflammatory responses:
  - Acute phase response proteins: C-reactive protein, alpha-acid glycoprotein (Orosomucoid);
- **Transport /binding proteins:** Albumin, Ceruloplasmin, Haptoglobin, Retinol Binding Protein, Sex Hormone Binding Globulin, Thyroid Hormone Binding Protein, Transferrin, etc.
- **Anti-proteases:** Anti-Chymotrypsin, Antithrombin, $\alpha_2$-Macroglobulin,
What are some diagnostic significance of Total Protein?

• Total Protein in plasma is about **7 – 7.5 g/dL**
  • Plasma proteins includes: Simple Proteins, Mixed or Conjugated Proteins, Glycoproteins and various types of Lipoproteins,
  • Changes in amount of Total Protein in plasma are common in some disease conditions;
  • Elevated amount of Total Protein in plasma may indicate presence of Paraproteins,
  • Decrease amount of Total Protein in plasma may indicate low level of Albumin,
What are some of the functions of Albumin?

• Albumin is one of the major plasma proteins; it is synthesized and secreted by the Liver,
  • Biological half-life of Albumin in plasma: 20 days
  • Significant decrease in amount of Albumin in plasma is usually slow to occur if it is due to reduction in biosynthesis of Albumin,

• Albumin makes the biggest contribution to plasma Oncotic Pressure,
  • Edema may occur when plasma Albumin level falls very low,

• Albumin is one of the major binding /transport proteins in blood plasma,
What are some of the possible causes of Hypoalbuminemia?

• Three main reasons for Hypoalbuminemia:

• Decreased synthesis:
  • May be due to malnutrition or mal-absorption,
  • May be a feature of advanced liver disease,

• Abnormal distribution or dilution:
  • May be induced by overhydration,
  • May be caused by increased capillary permeability as occurs in septicemia,

• Abnormal excretion or degradation:
  • May be causes by: Nephritic Syndrome, Protein-losing Enteropathies, Burns, Hemorrhage, Catabolic states,
How important are some of the Specific Serum/Plasma Proteins?

• Measurement of some specific plasma proteins gives useful information for diagnosis and management of some diseases: Examples:
  • Transferrin receptors, Ferritin,
  • Thyroid Binding Globulin (TBG)
  • Sex Hormone Binding Globulin (SHBG),
  • Haptoglobin,
  • Albumin,
  • Globulins,
  • C-reactive protein (CRP),
  • Immunoglobulins (Ig); etc.
Characteristic changes in amount of certain plasma proteins are seen after Surgery or Trauma, or during Infection or Tumor growth:

• Proteins involved are called **Acute Phase Proteins**;

• Acute Phase Protein response leads to greatly increased De Novo biosynthesis (mainly in Liver) of some plasma proteins along with decease in levels of other proteins in plasma,

• Response is stimulated by release of Cytokines: **Interleukin-1, Interleukin-6** and **Tumor necrosis factor (TNF)** and increased plasma [**Cortisol**] and [**Glucagon**]
• Acute Phase Protein response is an adaptive response to diseases;

• Example:
  • Increases in plasma levels of CRP and Complement will contain and eliminate infection,
  • Increased Coagulation Factors will aid and prevent excess blood loss,
  • Protease Inhibitors will prevent the spread of tissues necrosis when damaged cells at the site of injury release Lysosomal enzymes,

• Clinically some Acute Phase proteins are used to monitor progress of some disease condition or its response to treatment;
How useful is Electrophoresis of serum/plasma proteins (pH 8.6)?

• Electrophoresis may be used to study protein abnormalities;
  • Serum is a better choice for Electrophoresis, because the Fibrinogen of Plasma gives a discrete band, which can easily be mistaken for Paraproteins

• General pattern of electrophoresis result (Fig. 1):
  • Shows order of migration along Horizontal Axis with proteins of highest mobility closest to Anode,
  • Height of the band along the Vertical Axis shows the protein concentration,
  • Location of some major proteins are indicated underneath their Electrophoreoretic mobility peaks;
• Electrophoresis can also show gross deficiency or excess of Immunoglobulins and whether Paraproteins are present (Fig. 1);
• Quantitative measure of each protein class may be obtained by scanning Electrophoretic strip (Fig. 2)
Fig. 1: Electrophoresis of Serum proteins: (a) Normal pattern, (b) Presence of Paraproteins, (c) Presence of Paraproteins
Fig. 2: Scan of an Electrophoretic strip (Gaw et al 1999)
What are Immunoglobulins (Ig)?

• Immunoglobulins are a group of structurally related proteins that function as Antibodies;
• Immunoglobulins are produced by cells of the Lympho-reticular System,
• Immunoglobulins are also produced by Plasma Cells, which are B-lymphocytes transformed after exposure to foreign (occasionally an endogenous) Antigen;
• What is an Immunogen?
  • IMMUNOGEN is a molecule that can generate an Immune response (cellular or Humoral);

• What is Antigen?
  • ANTIGEN is a molecule that reacts with Antigen Receptors, irrespective of its ability to generate an Immune Response,
  • Antigen may, or may not be an Immunogen,
What is Hapten?

- **HAPTEN** is a small molecule that is able to react with preformed Antibodies, i.e.,
  - Hapten has Antigenicity, but is not capable, by itself, to stimulate specific Immune Response, i.e., is not Immunogenic,
  - Haptens are only Immunogenic when coupled to a large protein called a carrier;
- All Immunogens are therefore Antigenic but not all Antigens are Immunogenic,
What are Epitopes?

• Epitopes or Antigenic determinants:
  • Antigen Receptors on Lymphocytes recognize discrete sites on an Antigen called Epitopes or Antigenic Determinants,
  • Antigen recognition by B-cells and T-cells is fundamentally different and does not involve the same Epitopes,
What is the basic structure of Immunoglobulins (Ig)?

- Basic structure of Immunoglobulin (Ig):
  - 2 Identical “Heavy” Polypeptide Chains, and
  - 2 Identical “Light” Polypeptide Chains;
  - Both Chains have Inter-chain and Intra-chain Disulfide (S-S) Bonds and Non-covalent Interactions
• Two types of “Light” Polypeptide Chains:
  • Kappa “Light” Chains
  • Lambda “Light” Chains

• Five principal types of Heavy Polypeptide Chains:
  • Alpha,
  • Gamma,
  • Delta,
  • Epsilon,
  • Mu
Fig 3: Schematic diagram of an Immunoglobulin
What are the classes of Immunoglobulins?

• Immunoglobulins are named and classified by their heavy chain type;

• Five types of Heavy chains gives Five Classes of Immunoglobulins:
  • IgA,
  • IgG,
  • IgD,
  • IgE,
  • IgM
What products are obtained when IgG is hydrolyzed by (a) Papain and (b) Pepsin?

- **Action of Papain on IgG:**
  - Papain a protease enzyme acts on the **Hinge region** (in front of inter chain S-S bonds) in IgG,
  - Hydrolysis of IgG by Papain gives 3 components:
    - **Two Identical Fab** (Fragment antigen-binding) fragments,
    - **One Fc** (Fragment-crystalizable) fragment,
• **Action of Pepsin on IgG:**
  • Pepsin a protease enzyme acts on **Hinge region (behind the inter chain S-S bonds)** in IgG,
  • Hydrolysis of IgG by Pepsin produces:
    • A single divalent F(ab’)2 and
    • A pFc’ fragment
What are the regions in the structure of an Immunoglobulin?

• **V regions:** Amino-terminal portions of Heavy and Light chains show considerable **variability** in Amino Acid composition;

• **Hyper-Variable Regions** or **Complementarity-Determining Regions:** Three areas in V regions of Light and Heavy chains that have remarkably diverse amino acid sequences;

• **C region:** Parts of Heavy and Light chains that are relatively **Constant** in terms of Amino Acid composition;

• **Light chains** contain One Variable Domain (VL) and One Constant Domain (CL);

• **Heavy chains** contain One Variable Domain (VH) and 3 or 4 Constant Domains designated CH 1 – 3 or CH 1 – 4) accordingly
• Ig molecules contain **two functional areas:**
  • **Fab, or Variable end** – is the area that recognizes and binds to Antigens;
  • **Fc end** – is responsible for interaction with other components of Immune system, e.g., Complement and T helper cells;

• **Hyper-variable Loops** form Antigen-Binding Site of an Immunoglobulin molecule, i.e.,
  • Each Hyper-variable Loop contributes to the Antigenic Specific or Complementarity of the binding site for Antigen;
• Various Classes/Types of Immunoglobulins have different Tertiary structure and Functions;

• **Major Immunoglobulins in plasma are:**
  - **IgG:** neutralizes toxins, activates complement, capable of crossing Feto-placental barrier;
  - **IgA:** contains J chain and secretary component, part of defense against viral and bacterial infections;
  - **IgM:** usually first to be made in immune response, contains J chain, in presence of complement are very effective in producing Lysis of cells;
What is the significance of increase in Ig level in plasma?

• **Ig** may be increased non-specifically in a wide variety of Infections and in Autoimmune diseases

• Increase biosynthesis of Ig may be cause by **several Cell Lines**, each producing specific type of Immunoglobulins (Hyper-Gamma-Globulinemia)
  
  • Such response is said to be “**Polyclonal**” and results in diffuse increase in protein mass throughout the Gamma Globulin region;

  • Appears as broad band during Electrophoresis of Serum protein;
• Increase biosynthesis of Ig may be cause by a Single Clone of cells making Identical Ig;
  • Such is said to be “Monoclonal”
  • Ig production may increase, becomes large enough to be observed as a single discrete band on electrophoresis of the serum,
  • Such single discrete band may be due to increase in Intact Immunoglobulin or fragments called Paraproteins
What are Paraproteins?

• **Paraproteins (Monoclonal components):** discrete Ig bands, seen on electrophoresis of Serum,

• Paraproteins are due to production of Single type of Ig or Ig fragments (Light-chain or Heavy-chain fragments) by a Single clone of B cells,

• Paraproteins may arise from any of the Ig classes

• Detection of Paraprotein in blood or urine requires further investigation to determine if the Paraproteinemia is caused by Benign or Malignant condition
• Benign Paraproteinemia may occur transiently during acute infection and in autoimmune disease due to Antigen stimulation,

• Paraproteins are found in malignant conditions such as:
  • Multiple Myeloma,
  • Macroglobulinemia,
  • Heavy chain diseases, etc.
• Monoclonal Light Chains are produced in excess of Heavy chains in about 50% of cases of Myeloma, and in about 15% of cases only Light chains are found;
  • These **light chains** are small enough to spill into urine, they are known as **Bence-Jones Protein**, 
  • Serum electrophoresis may not show the presence of light chains, 
  • Urine electrophoresis after concentration may be required to demonstrate the Paraproteins,
IMPORTANT TO NOTE

• Myeloma is characterized by Bony Metastases,
• Bone pain is often the presenting symptom,
• In the face of increasing synthesis of abnormal Immunoglobulins, other bone marrow function is reduced, and there is a decline in Red and White cell and Platelet formation and decreased production of normal Immunoglobulins,
• Anemia and susceptibility to infection are the usual consequences;
REFERENCES

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