Proteins
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Learning Objectives

• Describe the electrophoresis procedure that is used to separate serum proteins and to identify a monoclonal protein
• Describe how immunofixation electrophoresis (IFE) is used to identify the heavy and light chain of the monoclonal protein
• Be able to identify a monoclonal protein from the serum protein electrophoresis and IFE patterns
• Describe the diagnostic criteria that are used to identify patients with Multiple Myeloma and MGUS

Structure of Amino Acids

ELECTROPHORESIS
Separation of a charged particle in an electric field
Rate of migration depends on:
• Charge of the molecule
• Size and shape of the molecule
• Voltage
• Support medium
• pH and ionic strength of the buffer

Optimizing electrophoresis

• Optimal electrophoretic separations must balance speed and resolution
  – Higher voltage increases speed, but heat causes evaporation of the buffer and may denature proteins
  – Higher ionic strength (buffer) increases conductivity.

Serum Protein Electrophoresis

• Apply samples 1 uL to the agarose gel
• Electrophoresis 21°C, 650v
• Dry 54°C
• Stain - Acid Blue
• Destain - Acetic Acid
• Dry 63°C
Serum protein electrophoresis

Albumin

- Most abundant protein in plasma (approximately half of total protein)
  - Synthesized in liver
  - $t_1/2 = 15-19$ days
- Principal functions
  - Maintaining fluid balance
  - Transport Protein

Clinical significance of albumin

- Hyperalbuminemia is rare and of no clinical significance
- Hypoalbuminemia
  - Increased loss (nephrotic syndrome)
  - Decreased synthesis (nutritional deficit, liver failure)
- Analbuminemia markedly decreased rare
- Bisalbuminemia, dimeric albumin with equal intensities

Alpha 1 Proteins

Alpha-1-Lipoprotein-HDL
Alpha-1-Antitrypsin-
  - Protease inhibitor that binds to and inactivates trypsin
  - Deficiency leads to destruction of the alveolar walls and is associated with pulmonary deficiency
  - Deficiency also seen in cirrhosis
  - Alpha-1-antitrypsin is an acute phase protein and is increased in acute episodes of tissue damage

Other $\alpha_1$ proteins

- $\alpha_1$-Acid glycoprotein (orosomucoid) and alpha-1 anti-chromotrypsin are acute phase proteins
- $\alpha_1$-Fetoprotein (AFP)
  - Principal fetal protein, used to screen for fetal abnormalities (neural tube defects)
**Alpha-2-Proteins**

Alpha-2-Macroglobulin - 720 Kda –

- Large non-immunoglobulin in plasma
- Synthesized in the liver
- Increased levels in nephrosis because its large size prevents passage into the urine. Also there is an increase in synthesis.
  - It is not an acute phase protein

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**BETA PROTEINS**

Transferrin - 77 Kda –

- Iron transport protein, also binds copper
- Increased in iron deficiency anemia, pregnancy and estrogen therapy
  - Decreased in acute inflammation due to decrease synthesis of transferrin by the liver
  - Negative acute phase protein

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** γ Region**

- Includes immunoglobulins (IgG, IgA, IgM, IgD and IgE)
- Single sharp peak indicates a paraprotein and is associated with a monoclonal gammopathy
- A small band is indicative of MGUS

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**Other (β) proteins**

- Beta-1 Lipoprotein 2750Kda
- Increased in nephrosis and Type II hypercholesterolemia
- C3 and C4 migrate in the β region
- Compliment proteins are decreased in genetic deficiencies, and increased in inflammation. C3 is a late acute phase protein. C3 may not be detected if the sample is kept at room temperature
  - IgA

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**Gamma Region**

- IgG migrates in the gamma and beta regions and is increased in infections, autoimmune and liver disease
- IgM migrates in the gamma region
- IgA migrates in the alpha-2, beta and gamma regions
- CRP is the most sensitive indicator of an acute phase reaction (inflammation, trauma, infection)
Acute Phase Reactants

- Other ACPs include α1-acid glycoprotein, haptoglobin, and ceruloplasmin

Nephrotic Syndrome

- Decreased albumin
- Increased α2-macroglobulin
- Decreased gamma globulins

Hepatic cirrhosis

- Decreased albumin (synthesis)
- Increased gamma globulins (polyclonal gammopathy)
  
  "β-γ bridging"

Immediate response pattern

- Decrease in albumin
- Increase in haptoglobin and alpha 1-proteins

Monoclonal gammopathy

- Albumin decreased
- Sharp peak in gamma region
**IMMUNOFIXATION ELECTROPHORESIS**

- Dilute samples with saline
- Apply sample 1 uL to the agarose gel
- Electrophoresis 21°C, 650 v
- Apply antisera
- Blot and dry 50°C
- Stain - Acid Violet
- Destain - Acetic Acid
- Dry 60°C

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**MULTIPLE MYELOMA**

Multiple Myeloma - proliferation of a single clone of plasma cells that produces a monoclonal protein

- Annual Incidence - 4 in 100,000
- Number of cases per year - 13,000
- Represents 1% of all malignant diseases
- Median age at diagnosis - 65 years
- Median survival - 3 years

**DIAGNOSTIC CRITERIA FOR MULTIPLE MYELOMA**

- Bone Marrow Plasmacytosis >10% of Plasma Cells
- Serum Monoclonal Protein
  - End Organ Damage
  - Lytic Bone Lesions
  - Renal Insufficiency
  - Anemia
  - Increased Calcium

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**CLINICAL LABORATORY IN MULTIPLE MYELOMA**

- Biochemical -
  - Serum monoclonal proteins
  - Polyclonal Immunoglobulin Decreased
  - Proteinuria, Bence-Jones Protein present in urine
  - BUN, Creatinine ↑
  - Calcium ↑, N
- Hematological -
  - Hemoglobin Decreased
  - Anemia - Normochromatic, Normocyte
  - ESR Increased
  - Rouleaux Formation

**FREQUENCY OF MONOCLONAL PROTEINS IN MULTIPLE MYELOMA**

- IgG-58%
- IgA- 24%
- Light Chains- 15%
- Biclonal- 2%
- IgD- 1%
Monoclonal Gammopathy of Undetermined Significance

Defined as the presence of a serum monoclonal protein at low levels
Number of cases per year - 750,000-1,000,000
54% Men     46% Women
Occurs in 2% of persons over 50 years, 3% over 70 years
Median age at diagnosis - 72 years
Median survival - 12 years

CLINICAL COURSE OF 241 PATIENTS WITH MGUS

Distribution Frequency of Monoclonal Proteins in MGUS

Summary

• Serum protein electrophoresis and IFE are used to identify a monoclonal protein in the serum of patients with Multiple Myeloma and MGUS.
• Patients with Multiple Myeloma and MGUS are followed by measuring the concentration of the monoclonal protein using serum protein electrophoresis.