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Serum Protein Electrophoresis and Its Clinical Applications

Satish Ramanathan and Chakravarthy Narasimhachar Srinivas

Abstract

This chapter focuses on the principle of electrophoresis and its utilization in a clinical laboratory. A sincere attempt has been made to discuss about clinical applications of serum protein electrophoresis, throwing light on the significance of serum protein electrophoresis in the management of multiple myeloma. Emphasis has been made on quality assurance in terms of accuracy and precision in electrophoresis to ensure reliability of patient results. A note on issues with lack of standardization of reporting of electrophoresis and an insight into global efforts to standardize the reporting of the assay has been included in this chapter.

Keywords: electrophoresis, gamma globulins, polyclonal, oligoclonal, bclonal, myeloma

1. Introduction

Serum protein electrophoresis is an electrophoretic method of separating proteins present in the serum to various fractions based on their molecular weight and electric charges. Electrophoresis had been widely used in clinical medicine for aiding in diagnosis of various clinical conditions like acute and chronic inflammations, monoclonal gammopathies, nephropathy, liver diseases, etc. This chapter discusses the clinical applications of serum protein electrophoresis [1] including the quality control practices and its implications [2].

2. Principle

The separation of proteins by electrophoresis is based on the fact that charged molecules usually migrate through a matrix/medium upon application of an electrical field [3]. The rate at which proteins move in an electric field is determined by a number of factors of the electrophoretic system and the nature of proteins itself. Some factors to mention are the strength of the electric field, temperature of the system, pH of the ions, concentration of buffer etc. [4]. Proteins vary in their size and shape and have the charges determined by the dissociation contents of their amino acids. Smaller proteins usually migrate faster, and larger proteins take a longer time. This physical property of proteins is exploited for its separation by employing the electrophoretic technique.

The most commonly employed variant of electrophoresis for serum protein separation is zone electrophoresis in which the serum proteins are separated into zones or

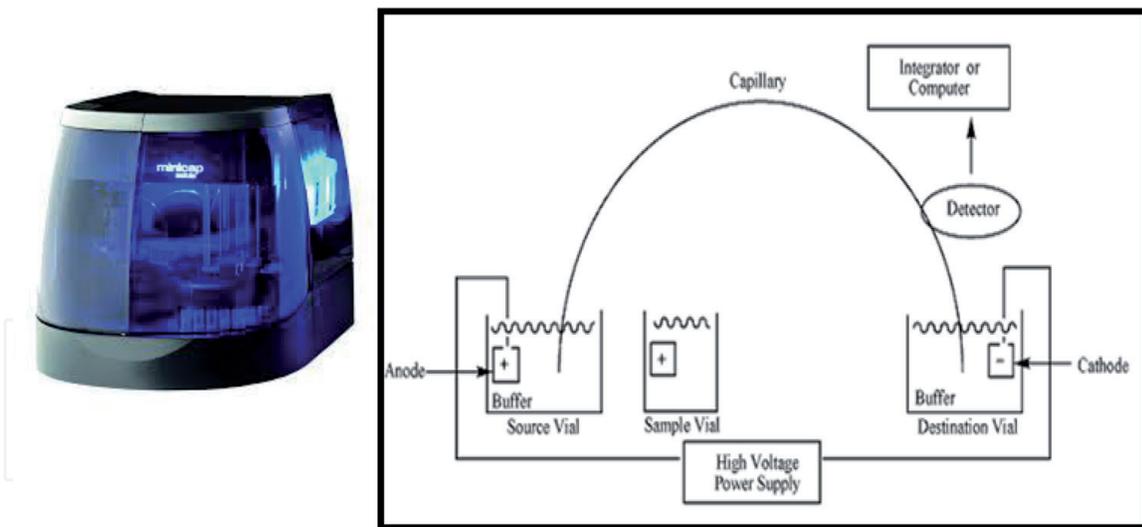


Figure 1.
Sebia Minicap flex piercing capillary electrophoresis.

fractions and interpreted accordingly [5]. There are several support mediums available for separation of serum proteins including agarose, cellulose acetate, capillary medium etc. [6]; when a capillary medium is used, the technique is known as capillary zone electrophoresis (CZE). Capillary electrophoresis is the preferred method when compared to its competitors including agarose gel electrophoresis due to the following reasons. CZE provides an improved resolution due to the following factors:

- a. The use of “electroendosmosis” principle which improves the resolution of separation
- b. Employing a “high-voltage” electric current which aids in improving the throughput (the processing time) and the resolution of protein separation.

Below is an illustration of capillary electrophoresis (Sebia Minicap Flex Piercing) (**Figure 1**). Sebia Minicap Flex Piercing capillary electrophoresis works on the principle of capillary electroendosmosis under high-voltage electric current. The Flex Piercing model of Sebia CZE aids in testing of human blood with capped tubes which in turn eliminates the biohazard associated with handling of uncapped samples.

3. Revisiting the basics: an insight into the protein family

Serum proteins are a family of albumin and globulins. Albumin is the major fraction synthesized from human liver endogenously and available through various dietary sources exogenously including egg, meat, pulses, milk etc. Globulins are a group of proteins subclassified into alpha-1, alpha-2, beta-1, beta-2, and gamma globulins based on the electrophoretic mobility (**Figure 2**). The normal biological interval of serum total proteins in a healthy adult ranges between 6 and 8 g/dl which includes Serum Albumin: 3.5–4.5 g/dl and Globulins: 2.5–3.5 g/dl.

3.1 Albumin

Albumin is a 69 kDa protein. It is the most abundant protein in serum. Albumin is synthesized in the liver and functions as a transport protein of various substances like bilirubin, enzymes, hormones, drugs etc. It also maintains fluid volume within the vascular space. Albumin is the first protein fraction to appear near the anode in

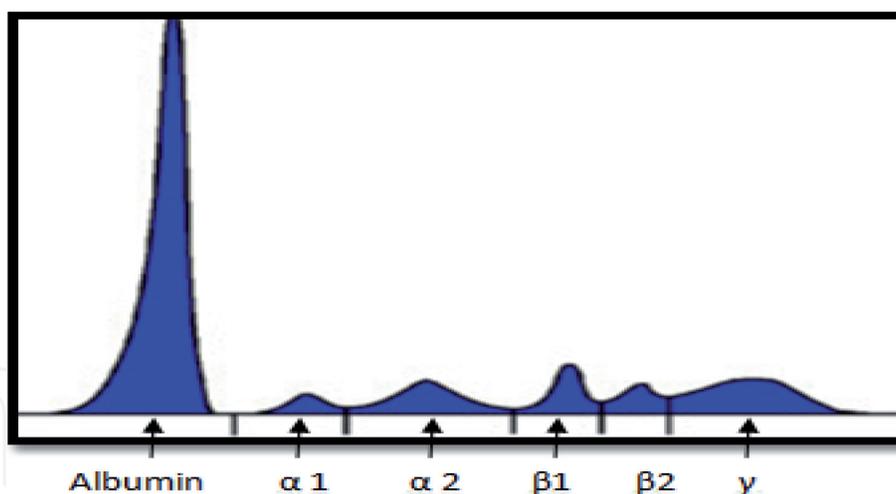


Figure 2.
Serum protein family with fractions.

SPE. Altered levels of serum albumin are associated with various clinical conditions. Low levels of albumin are clinically significant and are termed as hypoalbuminemia.

Decreased concentration of serum albumin (hypoalbuminemia) indicates either a poor dietary intake (malnutrition) or a decreased production or an increased loss. Chronic liver disease is a common clinical condition associated with decreased albumin production, and chronic kidney disease (CKD) is the most common disease associated with an increased loss of albumin in urine (proteinuria). This clinical condition is otherwise known as nephropathy. Other causes of hypoalbuminemia include acute and chronic inflammation, critical illness, pregnancy etc.

Apart from hypoalbuminemia which is commonly observed in an electrophoretogram, there are a few variations which can be observed in the in albumin peak including:

- a. Bilirubin, Triglycerides if present in high levels in serum may appear as a blunt peak which is seen adjacent to the cathode near the albumin peak.
- b. Prealbumin (transthyretin)—increased levels of pre albumin, if present due to various clinical conditions including several inflammatory diseases is seen as a blunt anodal peak distinctly separated from the peak of albumin.
- c. A rare variant observed in the albumin peak is bisalbuminemia which is a rare condition, with no clinical features, in which the serum contains two albumin variants of different electrophoretic mobilities, usually in equal concentrations, though the total concentration of albumin is normal. Bisalbuminemia may be hereditary or acquired. The acquired type has been more frequently reported in chronic renal disease and pancreatitis and in patient with chronic renal disease. Two (rather than one) albumin bands may represent bisalbuminemia. Hereditary condition is a rare anomaly caused by a genetic lesion in the albumin gene usually a point mutation.
- d. Analbuminemia (absence of albumin) is another genetically inherited metabolic disorder and was first described in 1954. This disorder is rare and affects less than 1 in 1 million births.

The most important aspect of such albumin variants lies in quantification of an albumin peak in such scenarios followed by interpretation and clinical correlation (**Figure 3**).

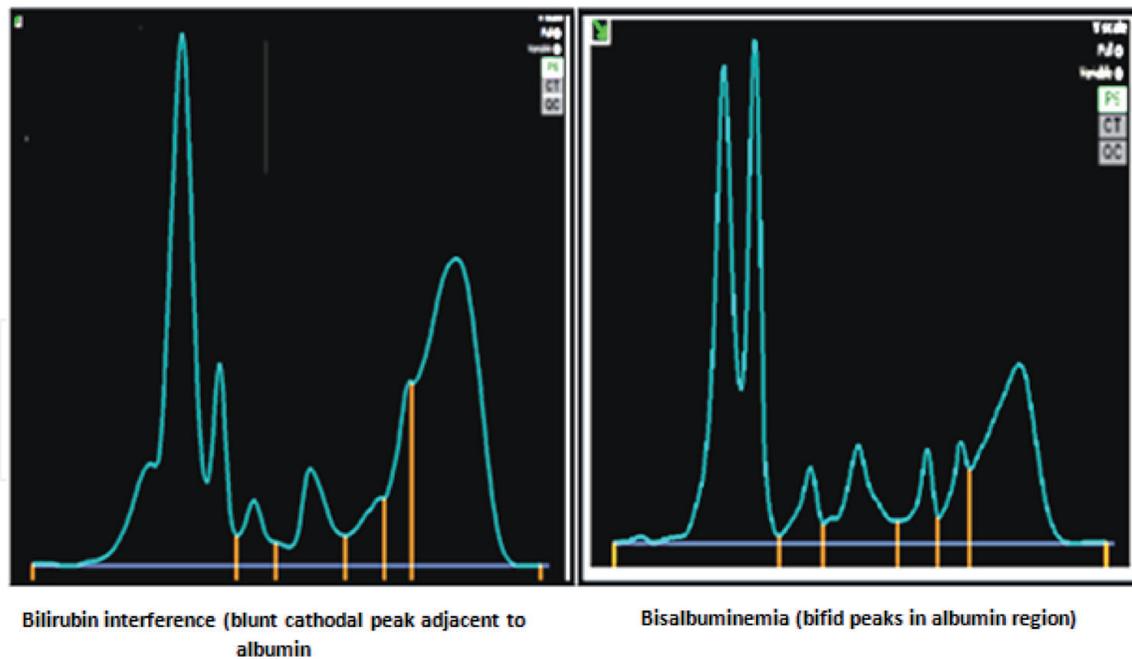


Figure 3.
Abnormal electrophoretic patterns of albumin zone.

3.2 Alpha fraction

As electrophoresis proceeds toward the negative portion of the gel (cathode), the alpha zone is the next band after albumin. The alpha zone is subdivided into two zones: the alpha-1 peak and alpha-2 peak.

The alpha-1 peak consists of alpha-1 antitrypsin (AT), alpha-1-chymotrypsin, and thyroid-binding globulin. Alpha-1 antitrypsin is an acute-phase reactant. The concentration of alpha-1 antitrypsin increases in conditions of inflammation and is usually decreased in patients with alpha-1 antitrypsin deficiency or decreased production of globulin in patients with severe liver disease. A rare variant of alpha-1 antitrypsin is encountered occasionally characterized by a split peak pattern of alpha-1 globulins.

The alpha-2 peak consists of alpha-2 macroglobulin, haptoglobin, and ceruloplasmin. Alpha-2 macroglobulin accounts for about 3% of the total protein in the serum. Because of the variable migration of the haptoglobin types, alpha-2-macroglobulin is often adjacent to, or co migrating with, haptoglobin and is therefore not seen as a discrete band.

A distorted pattern of alpha-2 region in electrophoresis is seen commonly in conditions of hemolysis, including *in vivo* and *in vitro*. The pathophysiology behind this pattern is the formation of hemoglobin-haptoglobin complexes in these conditions. This is a physiological adaptive response by human physiology to conserve hemoglobin released as a result of RBC breakdown into circulation and hemoglobin being a smaller globular protein is bound to be lost in urine. Hence to preserve it, haptoglobin is consumed to form complex with hemoglobin which results in the formation of a macromolecular protein which is retained in circulation making hemoglobin available for the production of RBCs and prevention of anemia.

Haptoglobin and ceruloplasmin are acute-phase reactants, and hence increased in acute inflammatory states.

Alpha-2-macroglobulin is increased in nephrotic syndrome and cirrhosis of the liver. The elevation of alpha-2 macroglobulin is distinctly evident in nephritic syndrome, since it is a bulky molecule, and hence retained in circulation to compensate

for the loss of other proteins in urine which is evident in form of proteinuria in urine microscopic examination.

Ceruloplasmin is an important copper-binding transport protein produced by the liver. Ceruloplasmin concentrations are markedly decreased in conditions of Wilson's disease. The disadvantage of serum protein electrophoresis is that it will not aid in the detection of a decreased ceruloplasmin.

3.3 Beta fraction

The beta zone usually is subdivided into two peaks, beta-1 and beta-2 in CZE. Beta-1 zone comprises proteins like transferrin and low-density lipoprotein (LDL).

Transferrin functions to transport non-heme ferric iron from the gastrointestinal tract. Each Transferrin molecule can bind two molecules of free iron. An increased beta-1 band is observed in iron deficiency anemia due to an increased level of free transferrin and also in pregnancy. Determinations of the transferrin levels are useful in distinguishing between iron deficiency anemia (inadequate intake or chronic hemorrhage with loss of iron stores) and hemolytic anemia, in which transferrin levels are low resulting in a beta-1 peak of low amplitude. Transferrin is usually decreased in alcoholic cirrhosis. Transferrin is also decreased during renal disease and thermal injuries.

The beta-2 band is mostly composed of complement proteins, C3 and C4. Elevated beta-2 zone can be caused in inflammatory states due to activation of complement cascade which include C3 and C4 too.

A reduced beta-2 peak intensity can be encountered in an aged sample, since the immune complexes are used up and low serum levels of complements are evidenced.

Fibrinogen is a protein with molecular weight of 340 kDa protein. Sometimes a small fibrinogen band can be seen in serum protein electrophoresis due to the insufficient clotting or failure to remove the serum from the clot. This fibrinogen band is seen between beta-1 and beta-2 regions. This band is also seen in patients who are receiving heparin therapy. It is also an important indicator of the sample type being analyzed. When plasma is used in the place of serum for protein electrophoresis, fibrinogen present in plasma appears in the beta-2 region, and this has the potentiality to interfere with the detection of monoclonal gammopathies in such patients (**Figure 4**).

3.4 Gamma fraction

One of the main clinical implications of serum protein electrophoresis is to aid diagnosis of disorders associated with alterations of gamma globulins. Gamma region comprises mainly of serum immunoglobulins. The five major classes of immunoglobulins are IgG, IgA, IgM, IgD, and IgE. The immunoglobulins are characterized by the presence of two protein moieties named as heavy chain and light chain. The classification of immunoglobulin had been made based on the composition of heavy chains, while the light chains are of two types including kappa or lambda. Physiologically, kappa forms the major light chain fraction among the two.

Various clinical conditions are associated with alteration of gamma globulins including:

- a. Hypergammaglobulinemia (increased serum gamma globulin levels)
- b. Hypogammaglobulinemia (decreased serum gamma globulin levels)

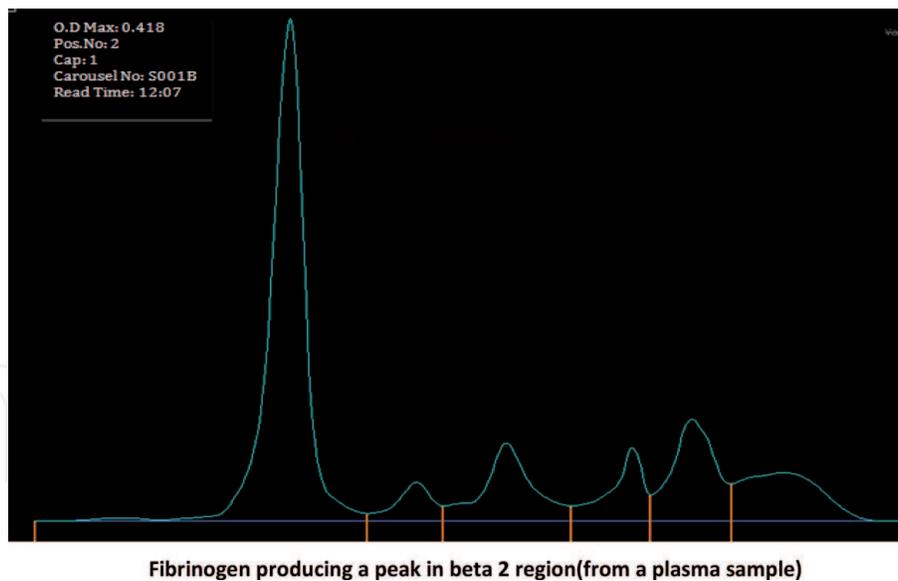


Figure 4.
Fibrinogen producing a peak in beta 2 region (from a plasma sample).

Hypergammaglobinemia (gammopathies):

Gammopathy is defined as abnormal proliferation of the lymphoid cells producing immunoglobulins. There are four types of gammopathies: polyclonal, monoclonal, biclonal, and oligoclonal.

Polyclonal gammopathies are defined as heterogeneous increase in immunoglobulins involving more than one cell line, commonly caused by a variety of inflammatory conditions (chronic inflammation), infections, chronic liver diseases (cirrhosis), chronic kidney diseases, etc.

Monoclonal gammopathies are characterized by a homogenous increase produced by clonal population of mature B cells, most commonly plasma cells. Monoclonal immunoglobulins seen in these conditions are also known as Para proteins. The classic interpretative terminology used in clinical laboratory medicine for describing a monoclonal immunoglobulin in SPE is “M” band where M stands for monoclonal. Common clinical disorders producing “M” Band in SPE include multiple myeloma and plasmacytoma in usually 60% of cases and Waldenström’s Macroglobulinemia, lymphomas, and leukemia in approximately 10% of cases. Certain monoclonal gammopathies produce “M” band in electrophoretic regions other than in gamma regions, commonly being beta region especially in case of IgA and IgG myeloma.

Biclonal gammopathies are characterized by a double peak in the gamma region. This electrophoretic pattern is seen when there is a biclonal proliferation of immunoglobulins encountered in multiple myeloma. A biclonal pattern is also seen in monoclonal gammopathies associated with IgA and IgG. In such scenarios, these immunoglobulins appear as polymerized and monomerized forms which elute as biclonal peaks in gamma region or in beta region, respectively (**Figure 5**).

The oligoclonal pattern of gamma region is characterized by more than two peaks evident in the gamma region. This pattern is commonly seen in autoimmune disorders, light chain myelomas (characterized by clonal proliferation of light chains), amyloidosis, etc. (**Figure 5**).

Apart from serum immunoglobulin, C-reactive protein (CRP) also is evident in the gamma region. C-reactive proteins levels usually increase during inflammatory responses.

Apart from the common causes of altered electrophoresis picture specific to the particular zones, a sharp distinct peak when evident especially in beta or alpha region

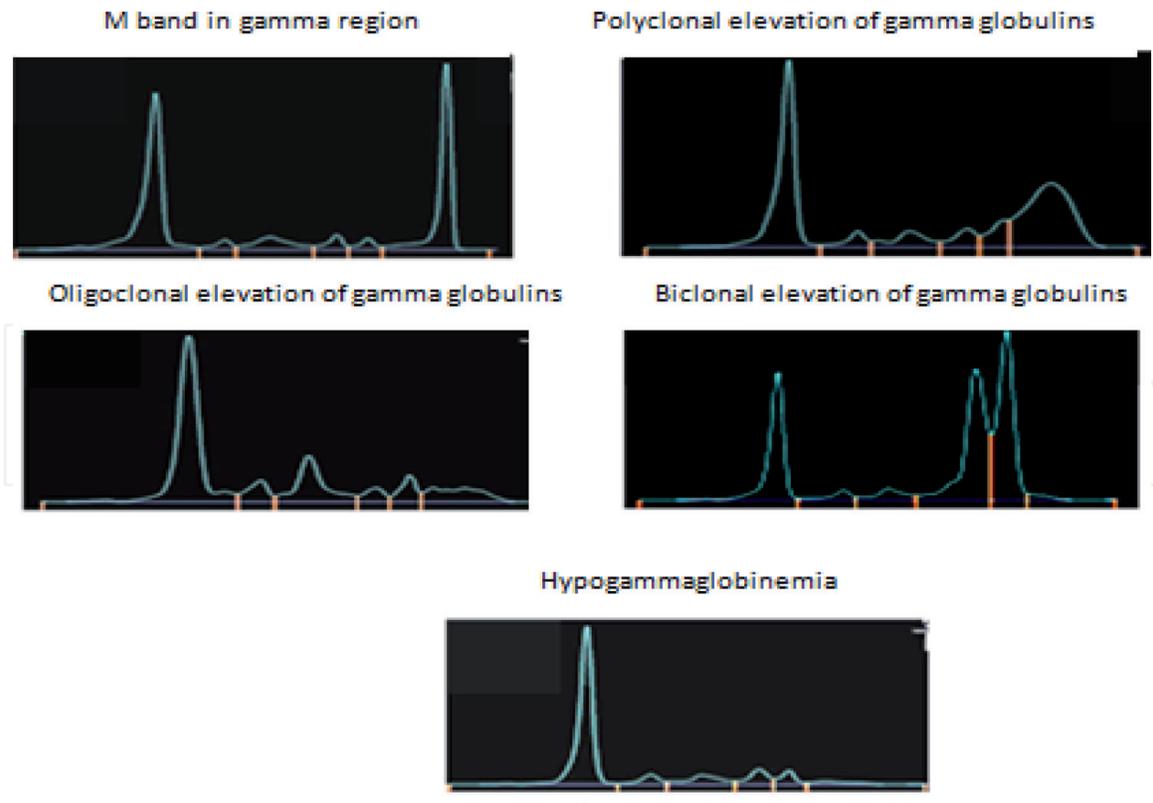


Figure 5.
Abnormal electrophoretic patterns of gamma zone.

should raise a high index of diagnostic suspicion of multiple myeloma since a few monoclonal immunoglobulins shall migrate in these zones too, in contrary to the classical gamma zone M protein pattern, which is commonly reported in these conditions.

4. Role of SPE in multiple myeloma work-up

According to the International Myeloma Foundation, plasma cell dyscrasias are group of plasma cell disorders involving a wide spectrum of pathologies including:

1. MGUS—monoclonal gammopathy of undetermined significance
2. MGRS—monoclonal gammopathy of renal significance
3. Smoldering myeloma
4. Multiple myeloma (which includes various subtypes including nonsecretory myeloma (NSMM), light chain myeloma, secretory multiple myeloma)

Criteria for diagnosis and differentiation of the plasma cell disorders based on International Myeloma Working Group (IMWG) criteria:

1. Monoclonal gammopathy of undetermined significance (MGUS)

- M protein (Monoclonal band)—<3 g/dl
- Bone marrow biopsy—<10% plasma cells seen

- No clinical symptoms/signs
- Normal free light chain ratio in serum

2. Monoclonal gammopathy of renal significance (MGRS)

- M protein (monoclonal band)—<3 g/dl
- Bone marrow biopsy—<10% plasma cells seen
- Renal disease with elevated serum creatinine
- Normal Free light chain ratio in serum

3. Smoldering myeloma

- M protein (monoclonal band)—<3 g/dl
- Bone marrow biopsy—>10% plasma cells seen
- Abnormal free light chain ratio in serum
- Clinically significant. Clinical diagnosis includes a tetrad of “CRAB” which stands for (one of the four shall be present):
 - C—hypercalcemia
 - R—renal abnormalities (elevated creatinine)
 - A—anemia
 - B—bone lesions

4. Multiple myeloma

- M protein (monoclonal band)—>3 g/dl
- Bone marrow biopsy—>10% plasma cells seen
- Abnormal free light chain ratio in serum
- Clinical diagnosis includes a tetrad of “CRAB” which stands for (one of the four shall be present):
 - C—hypercalcemia
 - R—renal abnormalities (elevated creatinine)
 - A —anemia
 - B—bone lesions

There are exceptions in SPE findings in certain cases of multiple myeloma wherein the SPE does not reveal any significant alteration or a clue toward the diagnosis.

These variants of multiple myeloma characterized by an abnormal bone marrow (increased plasma cells) but a normal SPE are termed as nonsecretory myelomas

which account to 1–2% of multiple myelomas. In such cases, an immunoassay of free light chains (FLC) in serum provides a diagnostic clue toward NSMM which show a significant disproportionate elevation of usually a clone of light chains (kappa or lambda) with an alteration in kappa/lambda ratio (normal Ratio is between 0.60 and 1.65). A commonly encountered phenomenon with laboratory testing of FLC includes “prozone” effect or “hook” effect which occurs due to antigen excess and requires appropriate dilution to obtain reliable results.

Bence-Jones protein estimation in urine is an antique piece of laboratory evidence toward multiple myeloma, which is characterized by detection of light chains in urine. But since the methodology of testing is manual and does not provide standardization, this has been replaced by urine FLC analysis in laboratories practicing good clinical laboratory practices (GCLP).

One more valiant laboratory investigation which is an essential requisite for multiple myeloma work-up includes immunoelectrophoresis.

One common principle employed in immunoelectrophoretic technique involves the use of specific antihuman immunoglobulins (e.g., Anti-IgG, Anti-IgA, Anti-Kappa, etc.) as a preprocessing step which results in precipitation of immunoglobulins if present and disappearance of the band/peak contributed by that specific immunoglobulin. Hence this technique is also known as immunosubtraction. This technique aids in typing the specific type of immunoglobulin (including the type of light chain) contributing to myeloma. This technique is supplemented by quantification of serum immunoglobulins by an immunoassay.

4.1 SPE and its clinical significance

SPE is a semiquantitative investigation which involves technical expertise to recognize the specific electrophoretic patterns and associate with various clinical conditions. This requires a laboratory practice integrated across various divisions of laboratory and with respective clinical and ancillary divisions of clinical medicine [1].

With respect to SPE, the laboratory professionals shall act as consultants to the clinical consultants. This is possible in scenarios where the clinician does not arrive at a provisional diagnosis of a gammopathy and the laboratory picks up the diagnostic clue toward gammopathy through an increased serum total protein level (<8 g/dl) and an altered serum albumin globulin (AG) ratio (which is usually altered in gammopathy). A normal AG ratio ranges between 1.2 and 1.8, while there is a significant reduction in the ratio in patients with gammopathy. This becomes an incidental finding which leads to a concept of “reflex” testing for multiple myeloma work-up including SPE, upon consent from the treating clinician and the patient.

These are some of the common SPE patterns associated with various clinical conditions:

- **Inflammation:** Increased intensity of alpha-1 and alpha-2 with a sharp leading edge of alpha-1 may be observed, but with chronic inflammation the albumin band may be decreased with increased gamma zone due to the polyclonal gammopathy.
- **Nephrotic syndrome:** The albumin band is decreased due to hypoalbuminemia. In addition, the alpha-2 band may be more distinct.
- **Cirrhosis or chronic liver disease:** A low albumin band due to significant hypoalbuminemia with a prominent beta-2 band and beta-gamma bridging

is a characteristic feature. In addition, polyclonal hypogammaglobinemia is observed.

- **Malnutrition:** Decreased albumin levels [1].
- **Alpha-1 antitrypsin deficiency:** Inflammatory condition, pregnancy.
- **Hemolysis:** Altered electrophoretic pattern of small indistinct peaks in alpha-2 region.

4.2 Quality assurance in SPE

Quality assurance in SPE is an essential prerequisite to ensure reliability of an SPE result [2]. There are two major aspects of analytical quality including precision (measure of precision) and accuracy (measure of trueness).

Good clinical laboratory practices demand processing of an internal quality control (IQC) for assessment of precision and external quality assurance (EQA)/ proficiency testing (PT testing) for accuracy assessment. IQC is a material which can be prepared in house (patient sample) or available commercially and is to be processed before a patient sample is taken up for processing.

The clinical laboratory has its responsibility to select and use an IQC which has a matrix comparable to patient sample, preferably covering the clinical decision point (cut off value that differentiates between a normal and abnormal result). EQA is an external assessment of the analytical quality wherein the laboratory processes a blinded sample and the results are compared against a reference method and/or against the consensus value of other participant laboratories for that specific sample.

The laboratory has to hold responsibility in selecting a suitable EQA provider who shall preferably be accredited to ISO 17043. If an EQA program is not available, the laboratories shall participate in exchange of samples with referral laboratories with a similar methodology and a comparable quality of testing standard.

5. Reporting of results and its standardization

Reporting SPE requires interpretation of the electrophoretic pattern which is followed by comments of such an interpretation along with the piece of advice to the clinician if indicated. There is a big lacuna in the format of reporting of SPE, each laboratorian using his/her own means of interpreting and communicating. It is the need of the hour to have a standardized format of reporting SPE for ensuring patient safety and clinician follow-up. There are no international guidelines, though the working party on standardized reporting of protein electrophoresis which is an initiative of the Australasian Association of Clinical Biochemists has come out with a standardized format of reporting SPE.

6. Conclusion

In the current scenario, it becomes the responsibility of each and every laboratory to ensure that all relevant information is available in a SPE report, easily read, understood, and interpreted by a clinician. This becomes the core of a clinical laboratory practice.

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