ABSTRACT
Multiple Myeloma (MM) is a neoplasm of B cell lineage which is characterized by excessive proliferation of abnormal plasma cells which secrete excess amount of immunoglobulin. The present paper focus on the expression of gamma globulin levels in patients suffering from multiple myeloma. SPEP is a simple lab technique where the serum is applied on a support medium and exposed to an electric current. The different fractions of the serum proteins separate usually into 5 bands, as – the albumin, α₁, α₂, β, and the γ globulin fractions. In the interpretation of SPEP, more attention is given to the gamma region, which is mainly composed of Immunoglobulin. Plasma protein levels display prominent changes in response to acute inflammation, malignancy, trauma and chemical injury. Apart from SPEP, immunofixation is used to predict result in case of very low concentration of immunoglobulin.

KEYWORDS: Multiple Myeloma, SPEP, γ-globulin, Paper Electrophoresis, Immune-fixation.

INTRODUCTION
In multiple myeloma, a type of WBC called a plasma cell multiplies unusually and releases too much protein (called immunoglobulin) into the bones and blood, ultimately damaging the organ. The plasma cells also crowd normal blood cells in the bone. They release chemicals that dissolve bone. The weak areas of bone created by this are called lytic lesions. As multiple myeloma gets worse, those plasma cells begin to spill out of the bone marrow and spread through the body. The disease has numerous consequences, including anaemia causing fatigue, bone loss resulting in weakening of the bones, bone fractures, and pain, kidney...
damage sometimes resulting in the need for dialysis, high calcium levels, altered immunity resulting in infections, and nerve damage which can cause numbness, tingling, or even pain and loss of strength.\cite{1,10}

![Figure Represents the Presence of Myeloma Cells](image)

**Classifying Myeloma**

Normal plasma cells help to defend the body against infection by producing antibodies. Antibodies typically consist of 2 heavy chains and 2 light chains. There are 5 kinds of heavy chains termed IgG, IgA, IgM, IgD, and IgE and 2 distinct types of light chain, termed kappa and lambda. In myeloma, all the abnormal plasma cells make the same antibody. Therefore the myeloma can be classified by the type of light and heavy chains produced, such as IgG kappa, IgG lambda, IgA kappa, or IgA lambda, etc. The most common type of heavy chain produced in myeloma is IgG, followed by IgA and then IgD. IgM myelomas are rare, but when IgM is elevated in the blood, the patient more likely has a related disorder, known as Waldenstrom’s Macroglobulinemia.

Multiple Myeloma (MM) is a neoplasm of B cell lineage which is characterized by excessive proliferation of abnormal plasma cells. These abnormal plasma cells secrete abnormal immunoglobulin that produces a condition called monoclonal gammopathy, which can be detected by the presence of M protein in serum and urine electrophoresis.\cite{1} It accounts for 10% of the haematological malignancies.\cite{2} It is a debilitating malignancy that is a part of a spectrum of diseases which range from monoclonal gammopathy of unknown significance (MGUS) to plasma cell leukaemia. The clinical symptoms that are suspected for a plasma cell disorder include back pain, weakness or fatigue, osteopaenia, osteolytic lesions, spontaneous fractures and recurrent infections.\cite{3}
It is very important to distinguish between MM from MGUS due to the general nature of manifestation of Multiple Myeloma and the vast difference between the occurrence of MM and MGUS. The occurrence of Multiple Myeloma is 4:100000 worldwide\(^4\) and that of Monoclonal Gammapathy of Undetermined Significance (MGUS) is approximately 1% among the population who are over 50 years of age, it is 3% among those who are over 70 years, and it is up to 10% among those who are over 80 years of age.\(^5\)-\(^7\) Moreover, the need for the therapy is also very much different in these two conditions. Therefore, Serum Protein Electrophoresis (SPEP) should be done to evaluate the general manifestations like malaise, weakness, chronic bone pain and anaemia, to detect the monoclonal gammapathy and to know the quantity of the M protein in these patients so that we can differentiate between multiple myeloma and the other causes of monoclonal gammapathy.

Serum Protein Electrophoresis(SPEP) is a simple lab technique where the serum is applied on a support medium and exposed to an electric current. The different fractions of the serum proteins separate usually into 5 bands, as – the albumin, \(\alpha_1\), \(\alpha_2\), \(\beta\), and the \(\gamma\) globulin fractions. In the interpretation of SPEP, more attention is given to the gamma region, which is mainly composed of Immunoglobulin. Many conditions can cause an increase in the gamma region, but those which cause a homogenous spike like a peak in the gamma globulin zone, are of special interest. These so called monoclonal gammapathies, result from the proliferation of a single, usually malignant clone of plasma cells which produce either a single class of intact immunoglobulins, heavy chains or light chains or both. These proteins are called para proteins or M (Monoclonal) proteins. The M protein or the M component is readily detected as a sharp symmetric spike (M spike) with \(\alpha_2\), \(\beta\), or \(\gamma\) mobility while performing the electrophoresis of serum. Multiple myeloma is the most common cause of paraproteinaemia.\(^8\),\(^9\)

The monoclonal gammapathies include malignant conditions like plasma cell dyscrasias, chronic lymphatic leukaemias and benign idiopathic forms of unknown significance. They may be associated with the drug treatment (Diphenyl hydantoin, Sulphonamide and Penicillin).

The Malignant cells may also produce free light chains, no longer bound to a heavy chain. The free light chains can be quantified in serum while the light chains are excreted in the urine. The light chains observed in urine are called M-protein or Bence –Jones. The amount of M-protein in urine is used to measure the tumour load.\(^10\)
In case of non-secreting myeloma the malignant plasma cells do not produce quantifiable amounts of monoclonal immunoglobulin or free light chains. Consequently the tumour load cannot be measured by the amount of M-protein in serum or urine, but instead is measured by the amount of plasma cells in bone marrow smears or if bone marrow smears is not done, the amount of plasma cells in bone marrow biopsy.\textsuperscript{[10]}

**Serum M-protein**

To determine the disease status in multiple myeloma, the amount of monoclonal immunoglobulin in the serum in measured in g/l. The amount of monoclonal immunoglobulin that is produced by malignant cells is much higher than the amount produced by normal cells. Immunofixation of serum is a sensitive test that can be used if there is monoclonal immunoglobulin present or not and also when the level is extremely low.\textsuperscript{[1,5]}

**Urine M-Protein**

It is the amount of free light chains in the urine. The most reliable method is by measuring the total amount in 24 hr urine collection. It is the parameter to determine disease status with light chain disease. Immunofixation another sensitive method is used to detect presence of monoclonal light chain even if the amount of urine M-protein is very low.\textsuperscript{[1,6]}

Electrophoresis is a method for diagnosis of multiple myeloma. The pattern of serum protein electrophoresis results depends on the fractions of two major types of proteins: albumin and globulins. The subsets of these proteins and their relative quantity are the primary focus of the interpretation of serum protein electrophoresis. Plasma protein levels display reasonably predictable changes in response to acute inflammation, malignancy, trauma, necrosis, infarction, burns and chemical injury.

In the interpretation of serum protein electrophoresis which mainly focus on the gamma region is composed mainly of IgG. IgG region is decreased in Hypogammaglobulinemia and agammaglobulinemia while increase in Hodgkin’s Diseases, Connective Tissue Diseases, Liver Diseases, Multiple Myeloma, Malignant Lymphoma, Chronic Lymphocytic Leukemia, Granulomatous Diseases, Waldenstrom’s Macroglobulinemia and Amyloidosis.\textsuperscript{[11,12]} In Multiple myeloma, M-protein level is usually greater than 3g/dL, appear as a narrow spike in the $\gamma$, $\beta$ or $\alpha_2$ regions.\textsuperscript{[10,13]}
MATERIALS AND METHODS

After getting the consent and authorization from the Patients, blood samples were collected from suspected cases of multiple myeloma and they were subjected to Serum Protein Electrophoresis (SPEP) from intervals in the lab of Department of Biotechnology, Rungta College of Science & Technology, Durg and School of Biological and Chemical Sciences, MATS University, Raipur. Serum was isolated from the blood samples. SPEP was performed on cellulose acetate strips by using a readymade buffer of pH 8.6. In few of the cases we, already performed Electrophoresis on agarose gel also to see any violation of the cases.

The cellulose acetate strips were initially soaked in the buffer solution and the extra amount of buffer was removed by placing them in between two Whatman Filter Papers no-1. Then, the strips were placed on the central compartment of the electrophoresis chamber. Two filter paper strips were placed on both the sides of the cellulose acetate strip to connect them with the two buffer containing chambers on both the sides of the electrophoresis chamber. Then, 10 microlitre of the serum samples were loaded on the cellulose acetate strip at the sources of the origin. Then, the electrophoresis chamber was connected to the power pack and it was subjected to electrophoresis. After one hour, the strips were removed and they were stained by using Ponceu S. After de-staining them by using the reagent which was supplied by the same company, the separated protein fractions could be visualized. The buffer, staining reagent and the de-staining reagent, all were provided by Priman Ltd. The estimation of the individual protein fraction was done by densitometer. The M band could be detected visually and the concentration of the M protein was estimated automatically by the densitometer.

RESULTS AND OBSERVATIONS

Figure showing the high level of γ globulin in multiple myeloma.
Table showing the Intensity of Bands in Paper Electrophoresis

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<tr>
<th>Types</th>
<th>Particulars</th>
<th>Serum M Protein</th>
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Figure showing the Bands of Serum Protein Electrophoresis

Figure showing the Normal Bands & M- Band in Gamma Region on SPEP Strip

Increased Complexity Of Bands In Myeloma Patients
After working in a laboratory for a number of years observed an increasing complexity of monoclonal bands in patients’ sera. Few years ago found only one monoclonal band, whereas recently we have been finding an increasing number of patients with two monoclonal bands with differing heavy chains, or three or four monoclonal bands, often with differing heavy and light chain types. Whether this finding originates from environmental factors, genetic factors or better detection is yet to be determined. From a laboratory perspective this should be kept in mind when examining the serum of a patient for myeloma.
CONCLUSION
The level of gamma globulin has been used for the diagnosis of multiple myeloma. Serum M protein and Urine M protein are estimated to detect the presence of elevated level of γ globulin. Paper electrophoresis was routinely observed to report positive result. The more sophisticated techniques of immune fixation work-up for a patient suspected of myeloma with very low level of immunoglobulin. The techniques used for serum and urine protein electrophoresis have improved significantly in both detection and resolution since many years.

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REFERENCES


