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Diagnostic Utilization of Serum Protein Electrophoresis and Serum Immunofixation in Detection of Monoclonal Gammopathy

Nimra Mazhar
Ayisha Imran
Ghazala Qamar
Mahnoor Mumtaz

Chughtai Institute of Pathology, Lahore

Abstract

Objective: the aim of our study was detection of monoclonal bands in serum protein electrophoresis followed by their characterization with the help of serum immunofixation electrophoresis. Moreover, comparison between the two techniques in order to detect any discrepant findings was done as well.
Methodology: This is a prospective study carried out at Chughtai Institute of Pathology from January 2025 to June 2025. Eighty three samples revealing a distortion in gamma region on SPE were selected. Both male and female patients above 18 years of age were included in the study. Patients who had any history of renal or liver disease were excluded from the study. After selection, the samples were run for SPE and SIFE, which was followed by the statistical analysis with the help of SPSS software
Results: A total of 83 samples were analysed on SPE, out of which a monoclonal band was detected in 81 cases, two cases revealed hypogammaglobulinemia. This was followed by SIFE for characterization of the monoclonal proteins which aided in detection of monoclonal bands in the cases which revealed hypogammaglobulinemia on SPE.
Conclusion: Study revealed that SIFE has a greater sensitivity as compared to serum protein electrophoresis for detection of M bands. SPE serves as a good screening tool, SIFE acts as a gold standard as it can help in detection of monoclonal bands which might be missed if SPE was performed alone.
Key Words: Monoclonal gammopathies, Serum protein electrophoresis, Serum immunofixation electrophoresis.

Address for Correspondence
Dr Nimra Mazhar,
Chughtai Institute of Pathology, Lahore
nimramazhar9596@gmail.com

Introduction

Abnormal proliferation of plasma cells or lymphocytes can lead to secretion of aberrant proteins called Paraproteins or M-proteins.[1] Detection of a discrete monoclonal (M) band in the serum leads to a diagnosis of monoclonal gammopathy.[2] SPEP is a screening test for detection of monoclonal protein and SIFE helps in confirming the presence of monoclonal protein and hence is considered the “gold standard” for the confirmation of monoclonal protein. [2] Immunofixation electrophoresis also assists in distinguishing between monoclonal and polyclonal rise in immunoglobulins.[1] SIFE helps in determining/ detecting the type of immunoglobulin involved. [2]

Monoclonal gammopathy can present with a variety of clinical manifestations ranging from an asymptomatic condition requiring no intervention known as Monoclonal gammopathy of undetermined significance (MGUS) to Multiple myeloma (MM), which requires clinical intervention.[3]

M proteins can be detected in the serum or urine. There are various clinical modalities which can help in detection of these monoclonal proteins, one such technique is serum protein electrophoresis. Serum protein electrophoresis helps separate and identify the paraproteins and hence helps in the diagnosis of monoclonal gammopathy.[4]

Serum immunofixation electrophoresis has an improved sensitivity when compared to serum protein electrophoresis. Serum protein electrophoreis has a limit of detection of monoclonal proteins of 100 mg/dL while Serum immunofixation electrophoresis can detect monoclonal proteins as low as 10 mg/dL and hence it has a better sensitivity when compared with Serum protein electrophoresis.[5] Due to better sensitivity of SIFE, if a SPE shows a negative result it should be followed by SIFE to rule out monoclonal gammopathy.

Serum immunofixation electrophoresis can help in detection of low intensity bands which can get missed if serum electrophoresis were to be performed alone especially in cases of hypogammaglobulinemia.[5]

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When SPEP is performed proteins are divided into five regions: albumin, alpha-1, alpha-2, beta and gamma.[6] Monoclonal protein usually appears as a sharp band usually in the gamma globulin region but less frequently in the beta-globulin region and very rarely can also be detected in the alpha-2 regions.[6] Serum protein electrophoresis is usually performed as a screening test and this is followed by serum immunofixation which identifies the heavy chains (IgG, IgM, IgA) and light chains (Kappa or Lambda).[6]

Monoclonal proteins are seen in multiple myeloma and various other monoclonal gammopathies and are secreted in serum and urine and hence serve as a biomarker which can aid is diagnosis and monitoring of these diseases.[7]

Several techniques are available to detect, quantitate and characterize the monoclonal proteins (M-Proteins). Protein electrophoresis is one of such techniques and it can be performed either by an agarose gel electrophoresis or capillary electrophoresis.[7] Serum protein electrophoresis helps in the detection and quantification of M-protein in the serum and urine.[7] Serum immunofixation electrophoresis helps in the confirmation and determination of the type of immunoglobulin heavy and light chain involved.[7]

Our study was aimed at the detection of monoclonal bands in the serum with the help of serum protein electrophoresis. This was followed by the confirmation and characterization of these bands with the help of serum immunofixation electrophoresis. Moreover, we also compared between the two modalities and reported the discrepant findings encountered.

Methodology

This was a prospective study carried out at Chughtai Institute of Pathology after getting an ethical approval from institution review board. This was followed by data collection which was done with effect from January 2025 to June 2025. Our study included 83 blood samples from patients on whom serum protein electrophoresis was performed and a distortion was seen in the gamma region.

In order to obtain a blood sample, venepuncture was done under strict aseptic measures and blood was collected in serum separator tubes. Freshly drawn serum samples were used. Hemolyzed samples were rejected as hemolysis can produce a distorted band in alpha-2 region.[8] Moreover, plasma samples were also avoided as fibrinogen can appear like a band which can be misinterpreted as a monoclonal component.[8]

Capillary 3 OCTA instrument was used to perform serum protein electrophoresis. This instrument has 8 capillaries which function in parallel and allow 8 simultaneous analysis. It separates human serum proteins by capillary electrophoresis in an alkaline buffer with pH maintained at 9. This leads to separation of proteins into the following manner i.e. gamma globulins, beta-2 globulins, beta-1, globulins, alpha-2 globulin, alpha-1 globulin and albumin respectively.

Serum immunofixation was also performed with the same instrument. This instrument has silica capillaries allowing 6 simultaneous analysis. Electrophoretic pattern of sample is obtained in the first capillary once the sample is mixed with ELP solution and it leads to production of a reference pattern. Afterwards the antisera patterns are obtained in the following 5 capillaries by mixing the patient's diluted sample with specific antisera against IgG, IgA, IgM, Kappa and Lambda.

Statistical analysis was performed using SPSS software (version 25.0; IBM Corp, Armonl, NY, (USA). Categorical variables were presented as absolute frequencies (n) and relative percentages (%). The distribution of serum protein electrophoresis and immunofixation electrophoresis findings were described accordingly. Comparative evaluation of M-protein detection between serum protein electrophoresis and immunofixation electrophoresis was conducted using cross-tabulation and Pearson's Chi-square test. A two-tailed P-value of <0.05 was considered indicative of statistical significance.

Results

In the current study a total of 83 samples were analysed and subjected to serum protein electrophoresis. Out of the total patients tested 51 (61.4%) were males and 32 (38.5%) were females. Male to female ratio turned out to be 1.5:1

Upon performing serum protein electrophoresis, monoclonal peak was detected in 81 cases out of which majority were detected in the gamma region (84.3%) followed by beta region (10.8%). Apart from this two

cases revealed bifid peak with one being in the gamma region and another case revealed bifid peak in late beta and early gamma region. Moreover, two cases revealed hypogammaglobulinemia and distortion in gamma region. Figure-1 represents the data from serum protein electrophoresis revealing frequency of M-protein detection according to the region on Serum protein electrophoresis.

Table I: shows distribution of monoclonal gammopathies according to age.

Age (years)	Frequency (n)	Percentage (%)
40-49	10	12.04 %
50-59	31	37.3 %
60-69	27	32.5 %
70-79	12	14.4 %
80-89	1	1.2 %
90-100	2	2.4 %

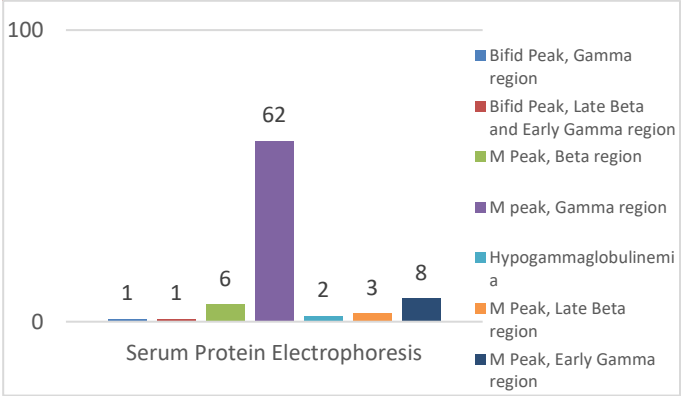

Figure 1. Frequency of Monoclonal Peaks detected by SPE

These samples were then subjected to serum immunofixation in order to determine the type of immunoglobulin heavy and light chain involved. Following results were revealed upon performing serum immunofixation. Figure 2 represents the results of serum immunofixation.

Table II: Results of immunofixation electrophoresis.

Immunophenotyping	Type κ n(%)	Type λ n(%)	Total n(%)
Type IgG	42 (50.6)	22 (26.5)	64 (77.1)
Type IgA	10 (12.04)	4 (4.8)	14 (16.8)
Type IgM	-	1 (1.20)	1 (1.20)
Light chain type	-	4 (4.8)	4 (4.8)
Total [(n(%)]	52 (62.6)	31 (37)	83 (100)

Serum immunofixation was performed on the samples which revealed a bifid peaks and the results obtained revealed one case to be IgG Kappa and another to be IgA Lambda. Moreover, there were two cases which revealed hypogammaglobulinemia and a distortion in gamma region and these patients had a significant history of backache, weight loss and anemia so based upon a very strong suspicion of a monoclonal gammopathy based on clinical history, symptoms of the patient and the distortion in the gamma region despite hypogammaglobulinemia these samples were subjected to serum immunofixation electrophoresis and the monoclonal peaks were detected. One case turned out to be IgA Kappa and another turned out to be a lambda light chain.

The results of serum protein electrophoresis revealed that out of 83 patients tested a monoclonal band was detected in 81 patients and got missed in two cases. The detection rate of M protein by immunofixation electrophoresis turned out to be 100% which turned out to be higher than serum protein electrophoresis.

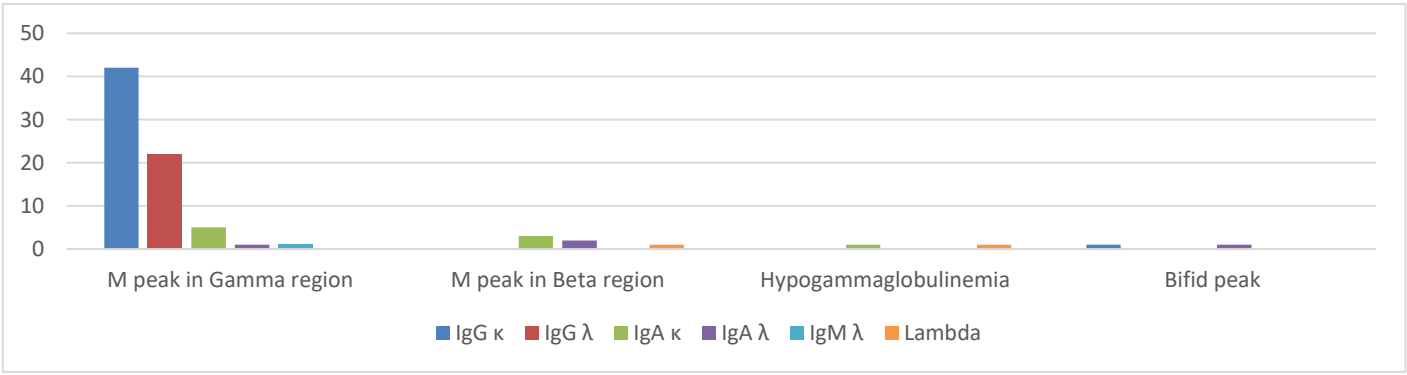

Figure 2. Results of Serum Immunofixation Electrophoresis.

Results of immunofixation electrophoresis revealed that IgG type (64 cases, 77.1%) was the most common including 50.6% kappa and 26.5% lambda) and this was followed by type IgA (14 cases, 16.8%). This can be seen in table II.

Hence, it was found that sensitivity of immunofixation was more as compared to serum protein electrophoresis. As

two cases were missed on protein electrophoresis which on immunofixation turned out to be monoclonal bands.

Discussion

Aberrant proliferation of abnormal plasma cells lead to a condition known as monoclonal gammopathy which can be detected by Serum protein electrophoresis as an M-band.9 Diagnosis of monoclonal gammopathy requires detection of a monoclonal band by Serum protein electrophoresis or by serum immunofixation electrophoresis. Serum protein electrophoresis serves as a screening test and this screening test is followed up by immunofixation electrophoresis. Immunofixation electrophoresis is a definitive method for both identification as well as for the characterization of the heavy and light chains involved.9 Moreover, immunofixation electrophoresis also helps in the distinction of monoclonal peaks from a polyclonal rise in immunoglobulins.[1]

Serum protein Electrophoresis is a technique in which the proteins are separated based upon their molecular weight and electric charges.8 SPE distributes the proteins into peaks with largest one being of albumin and this is followed by the proteins of globulin group which are subsequently divided into α1, α2, β1, β2, and γ globulins.[10] These immunoglobulin molecules are glycoprotein which comprise of two identical heavy chains (α, γ, μ, δ, or ε) and two identical light chains (κ or λ).[10] Once monoclonal proteins are detected it is important to measure them as it aids in diagnosis, treatment and for follow-up monitoring of the patients.[10]

A study was conducted by Dogan at Basaksehir Cam and Sakura City Hospital, Istanbul, Türkiye which included patients aged 18 years and above who had applied for serum protein electrophoresis and serum immunofixation electrophoresis. Data obtained from this study revealed that monoclonal gammopathies were detected in male patients predominantly and it was associated with increasing age.[10] Both of these findings were consistent with the results obtained from our study. Similarly, most common immunoglobulin detected by serum immunofixation electrophoresis turned out to be IgG Kappa which was followed by IgG Lambda being the second most common and then by IgA Kappa, all of these results of immunofixation electrophoresis are similar to the data which was observed in our study.[10]

These monoclonal proteins act as a tumor marker. Moreover, it helps clinicians in the monitoring of patients and aids in an early detection of a plasma cell clone in case of known cases of paraprotenemia.[11] Serum immunofixation electrophoresis is more sensitive than Serum protein electrophoresis in the detection of monoclonal gammopathies as it determines the type of heavy and light chain involved. Moreover it also helps is differentiating monoclonal peak from a polyclonal increase in immunoglobulins.[12] Serum immunofixation electrophoresis (SIFE) can detect the presence of monoclonal protein at a concentration above 0.02 g/ dl in serum as compared to serum protein electrophoresis which can only detect the proteins above 0.004 g/dl.12 Literature has shown that prevalence of monoclonal gammopathy increases with increasing age and with male gender. [10, 13]

A study was conducted in Turkiye by Civil at the Medical Biochemistry Central Laboratory of Faculty of Medicine from 2017 to 2019 in which patient samples were analysed retrospectively and results reported by Civil revealed that the most common paraprotein detected was of the IgG Kappa type and this was followed by IgG Lambda which was the second most common type with the rarest type being IgM.[12] Data from our study reveals similar results with IgG Kappa being the most common type followed by IgG Lambda to be the second most common type of paraprotein detected. Similar IgM was the rarest paraprotein detected in our study with only one case being of the IgM type out of the total 83 cases examined.

Study done by Civil also revealed that in 47.9% of the cases, gammopathy was not detected by serum protein electrophoresis and a monoclonal band was detected when those samples were subjected to serum immunofixation electrophoresis.[12] These findings are also similar to data from our study in which two cases which revealed no monoclonal band on serum protein electrophoresis had a monoclonal protein present in them only for it to be detected by a better modality i.e. serum immunofixation electrophoresis.

Serum protein electrophoresis can detect monoclonal proteins at a concentration of 0.3 to 0.5 g/dL. On the other hand serum immunofixation can detect monoclonal proteins at much lower concentration as low as 0.02 g/dL.[12] Therefore sometimes the concentration of a

monoclonal protein is so low that it can go undetected if the sample is subjected to serum protein electrophoresis alone. Hence, data from study done by Civil and our study proves that in cases of hypogammaglobulinemia special attention needs to be made and these cases must be subjected to serum immunofixation electrophoresis, as these bands might go undetected if serum protein electrophoresis were to be performed alone.

Similarly Ramasamy conducted a study at the Worcester Royal Hospital in Worcestershire UK and found out that males comprised the majority of the patients in whom a monoclonal protein was detected and that the most common type of immunoglobulin detected was IgG.[13] These findings are also similar to the results of our study in which majority of the patients with a monoclonal protein are males with the most common immunoglobulin being detected to be of IgG type.

Siddiqui IA conducted a study in India in which a total of 123 samples with a strong suspicion of paraproteinemia were analysed. Serum protein electrophoresis was performed and this was followed by serum immunofixation electrophoresis. In the study conducted by Siddiqui IA a male predominance of 70.9% was observed and the most common age group to be affected by a monoclonal protein turned out to be from 51 to 60 years (47.3%). These findings are similar to data observed from our study which revealed the most common age group to be impacted with monoclonal protein to be of 50-59 years (37.3%) and a male predominance of 61.4% was seen as well.[1] Likewise, immunofixation electrophoresis data from Siddiqui revealed that the most commonly detected paraprotein was of IgG type.[1]. These findings are quite similar to the results obtained from our study in which serum immunofixation electrophoresis performed revealed a total of 77.1% of cases to be of the IgG type which was followed by 16.1% of IgA cases and in 4.8% of the cases only light chain was detected.

A study was conducted by Zhu in Baoding, China at the department of clinical laboratory of an affiliated hospital of Hebei University. This study was done to look into the diagnostic significance of serum protein electrophoresis and serum immunofixation electrophoresis in detection of multiple myeloma and a total of 105 patients were investigated. Data from the study done by Zhu revealed that serum immunofixation is better than serum protein electrophoresis in detection of monoclonal bands. In study conducted by Zhu it was found that monoclonal band was not detected in 4 cases (3.81%) and when these samples were subjected to serum immunofixation electrophoresis, monoclonal bands were detected. Immunofixation results revealed one case to be of IgA type and the rest 3 cases turned out to be of light chain type.[14] Hence, we saw that these results are also similar to the ones of our own study in which two cases (2.4%) got missed on serum protein electrophoresis and upon serum immunofixation electrophoresis, one of the cases turned out to be of IgA Kappa type and another turned out to be of the Lambda type. Moreover, this study also revealed a male preponderance in pateints with monoclonal proteins.[14]

Similarly, a study was performed by the American society of clinical pathology in which they analysed a total of 160 samples collected. Out of these 160 collected samples, 17 cases revealed hypogammaglobulinemia and these samples were then subjected to serum immunofixation electrophoresis. Thirteen out of the total 17 cases revealed a positive immunofixation electrophoresis with a different zone of restriction which turned out to be either a monoclonal immunoglobulin (most commonly IgG Kappa or Lambda) or a monoclonal light chain (kappa or lamda).[15] These findings are similar to our study in which cases which revealed hypogammaglobulinemia on Serum protein electrophoresis revealed a zone of restriction once subjected to serum immunofixation electrophoresis. However, findings reported by Strobel at the American society of clinical pathology differed from our study in one regard, it was found in their study that the remaining 4 patterns were negative for monoclonal immunoglobulins of any kind.[15]

A study was conducted at the laboratory of Ankara Etlik City Hospital with an aim to predict the frequency and type of monoclonal gammopathy. The data from the study revealed that among the samples tested for serum immunofixation electrophoresis most common type of immunoglobulin detected was IgG Kappa and the second most common type of paraprotein detected was IgG Lambda.[16] These results are in consensus with the results reported in our data where most common type of paraprotein detected turned out to be IgG Kappa which has been followed by IgG Lambda.

A study was conducted by Fadili at the laboratory of Hôpital Universitaire International Cheikh Khalifa Ibn Zaid, Casablanca, Morocco from January 2023 to January 2025 in which serum samples were collected from patients who had a strong clinical suspicion of monoclonal gammopathy. A total of 200 patient samples were analysed in the study which revealed a male preponderance with 103 patients (51.5%) being males and 97 (48.5%) females. The most common bands detected was of IgG Kappa followed by IgG Lambda.[17] These findings are consistent with the ones reported in our study.

Serum immunofixation electrophoresis is superior to serum protein electrophoresis as it is useful in detection of low-concentration of proteins and hence helps in detection of early relapsed disease.[17] While serum protein electrophoresis serves as a very good screening tool it is very important to confirm those cases with the help of serum immunofixation electrophoresis.[17] Moreover, it has also been seen that false negatives can be revealed by serum protein electrophoresis when the concentration of paraproteins are low, as these low concentration paraproteins can co-migrate with normal protein fractions and this is most commonly seen in light chain disorders.[17]

Conclusion

In this study it was found that SIFE has a greater sensitivity as compared to SPE when it comes to detection of M-bands. The results of our study are similar to data in literature in terms of frequency percentage age and gender distribution of gammopathy. While SPE acts as a good screening test SIFE is to be considered gold standard as it will help in detection of monoclonal gammopathies in cases where paraprotein concentrations are low to a concentration which can go undetected if SPE were to be performed alone.

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