Utility of serum protein electrophoresis, free light chain analysis, immunofixation electrophoresis and immunosubtraction for the diagnosis of plasma cell dyscrasias

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Abstract

Background: Monoclonal gammopathies (MG) are a spectrum of diseases, which if diagnosed early can improve the disease prognosis. Serum Protein Electrophoresis (SPE) was the preferred screening test for MG, but the low sensitivity of the same emphasizes the necessity of going for more sensitive and accurate tests. SPE is complemented with tests like serum Free Light Chain assay (sFLC), Serum Immunofixation Electrophoresis (sIFE) and Serum Immunosubtraction Electrophoresis (sISE).

Aim: Our aim was to find out the utility of each of these tests in diagnosing MG and propose a panel of tests for the diagnosis of MGs

Material and methods: 30 patients with features of MG were recruited and SPE, sFLC, sIFE and sISE were performed.

Results: Accuracy of sIFE with respect to sISE was 100% and accuracy of sFLC with respect to SPE came to be 90%. SPE, sFLC and sIFE panel and SPE, sFLC and sISE panel were able to detect all cases. Combination of SPE + sFLC + sIFE and SPE + sFLC + sISE tests were able to detect all 24 cases of MG and found to be efficient than any of these tests performed alone.

Discussion: The diagnostic accuracy of sIFE and sISEin MM patients was compared and was found to be statistically insignificant thus proving that both tests contribute equally for the diagnosis of MM.

Key words: Multiple myeloma, sISE, sIFE

Introduction

Monoclonal gammopathies (MG) are β-cells that secrete a single clone of immunoglobulins that are termed paraprotein or M-protein[1]. They include a wide range of conditions that range from pre-malignant MGUS (Monoclonal Gammopathy of Unknown Significance) and SMM (Soldering Multiple Myeloma) to clear cut malignant conditions like multiple myeloma (MM), plasma cell leukemia, plasmacytoma and Waldenstrom'smacroglobinemia (WM)[2].

Multiple myeloma is the second most common hematological neoplasm after leukemia in the world[3]. There are numerous subtypes of MM, the treatment and prognosis of which vary widely, making the correct diagnosis crucial[4]. The diagnosis of MM depends on the clinical criteria set by the International Myeloma Working Group and laboratory tests also play a significant role in the same. The most commonly employed lab tests includes Serum Protein Electrophoresis (SPE), Serum Immunofixation Electrophoresis (sIFE), Serum Free Light Chain Assay (sFLC) and Serum Immunosubtraction Electrophoresis (sISE). Though it has been the most widely used age old test for detecting and quantifying M proteins, SPE has the disadvantage that it has low sensitivity in identifying low levels of monoclonal proteins (M-protein)[5]. In addition, quantification of β-migrating M-protein is affected due to the overlapping non-immunoglobulin proteins[6]. sIFE is generally used for identifying serum protein fractions and is much more

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sensitive than SPE for detecting free light chains even though it is less sensitive than sFLCimmunoassays[7]. A main drawback of sIFE is that atypical patterns termed oligoclonal bands that rises following allogenic/ autologous transplantation and chemotherapy are observed which requires expertise for correct interpretation[7,8]. sFLC analysis are useful for detecting MM that secretes only light chains and has been proved to be superior over urine studies[9,10,11]. However sFLC was found to be having high false positive rates and variable analytical performance[12]. 799 patients with suspected plasma cell dyscrasias were tested by alternative diagnostic testing strategies consisting of serum protein electrophoresis (SPEsISE which works on the principle of capillary electrophoresis is used to subtract out the immunoglobulin subtypes in serum, thereby limiting the masking effect of normal serum proteins[6].

Thus it is evident that each diagnostic modality poses its own advantages and drawbacks and it is necessary that a proper algorithm be developed for better detection and monitoring of plasma cell dyscrasias. The novelty of the study lies in that though there are many literatures that have compared the role of each of these diagnostic tests in diagnosing MM, only few studies have attempted to compare the combined significance of all of these four tests in diagnosing plasma cell dyscrasias.Thus the aim of this study was to analyse the role of the tests- SPE, sFLC, sIFE and sISE in diagnosing plasma cell dyscrasias and also to compare the diagnostic accuracy of sIFE and sISE.

Material and methods

This case control study was performed amongst the patients visiting the OP and IP sections of a tertiary care centre over a period of 1.5 years from February 2022 to July 2023. The study was carried out in accordance with the permission and guidelines of the ethical committee of Amrita Institute of Medical Sciences (ECASM-AIMS-2021-009), as well as with the participants' informed written consent.

Subject selection

Patients who attended the Medical Oncology and clinical Hematology departments with features suggestive of MG were recruited in the study.

Inclusion criteria

Patients, both males and females, between the ages of 18 and 70, with or without signs and symptoms indicative of MG like anaemia, elevated ESR, lytic lesions causing pain in bones, hypercalcemia and increased tiredness were included in the study.

Exclusion criteria

Immunocompromised individuals, those patients having primary cancers or metastasis from indefinite primary, those diagnosed with autoimmune diseases were all excluded from the study.

Measurements

Under strict aseptic precautions venous blood samples were collected. Samples for estimation of sFLC, SPE, sIFE and sISE were collected in red vaccutainers with no anti-coagulant. To isolate serum, samples were centrifuged for 5 minutes at 3000 rpm and the separated serum were kept in 2-8°C in labelled vials. All tests were run within 21 days of sample collection. Lipemic, icteric and hemolytic samples were avoided and care was taken to avoid repeated freeze and thaw cycles of the samples.

The principle of immunoturbidimetry was used to determine the sFLC levels and the test was done in the 502 module of the Cobas 8000 I series. The test sample was mixed with an appropriate antibody in a cuvette which results in the formation of insoluble immune complexes. A light beam when passed through this cuvette would get scattered due to these immune complexes and the strength of the incident light would be proportional to the antigen concentration. Results were interpreted in comparison to a calibration curve obtained by testing a set of calibrators with recognised antigen concentration[13].

SPE was performed using the Sebia MINICAP system, operating on Capillary Electrophoresis principle. Charged particles were separated in an alkaline buffer based on electrophoretic mobility at a particular pH. Sample was diluted in a dilution buffer and aspirated into the anodic end of capillaries filled with separation buffer. High voltage protein separation was done followed by direct recognition of different protein fractions at 200nm at the cathodal capillary end. Protein concentration of each band was calculated in g/dL and concentration of each fraction was determined using total protein concentration.

sIFE was done using SebiaHydrasys 2 scan system, utilising the principle of gel electrophoresis. Most proteins were separated through electrophoresis at alkaline pH. After migration, fixative was added in one track to obtain reference pattern and different antisera were added to the remaining lanes, which include ones for IgG, IgA and IgM heavy chains and kappa and lambda light chains. The antigen-antibody interaction led to the formation of insoluble complexes which gave a band of precipitates in proportion to the concentration of antigen and antibody. After migration agarose gel plate was denatured and washed

to remove extra proteins, stained with acid blue, destained and dried. Positions of monoclonal bands in suspected immunofixed bands was compared to reference pattern to identify the abnormal proteins present in the sample[14,15].

sISE was performed using Sebia MINICAP system, operating on Capillary Electrophoresis principle. Charged particles were separated in an alkaline buffer based on electrophoretic mobility at a particular pH. Sample was diluted in a sample diluent and aspirated in to the anodic end of capillaries filled with separation buffer. Patterns of antisera were acquired with the following five analytes, by injecting in the capillaries, diluted samples mixed with particular antisera against IgG, IgA, IgM heavy chains, and free and bound kappa and lambda light chains. Reference pattern (ELP pattern) was acquired by injecting the sample plus ELP solution into a capillary, resulting in an electrophoretic pattern of all sample proteins. High voltage protein separation was done, followed by direct recognition of different protein fractions at 200nm at the cathodal capillary end. Electrophoretograms were visually interpreted to find pattern anomalies.

Statistical analysis

IBM SPSS 20 (SPSS Inc, Chicago, USA) was used for statistical analysis. Based on the results with an accuracy of 80% of two important variables, namely, sIFE with respect to sFLC observed in a publication[2] and with 95% confidence and 20% allowable error, the minimum sample size came to be 24. The results for all continuous variables are presented as mean±SD, and the results for categorical variables are presented as number and percentage. To test the statistical significance of the difference in the proportion of categorical variables, Chi square with Fisher's exact test was applied. To test the statistical significance of the efficacy of four methods, McNemar test was used. To test the statistical significance of the difference in mean of median comparison of numerical variables between groups, independent two-sample t test was applied for parametric data and Mann-Whitney U test for non-parametric data. A p value <0.05 was considered as statistically significant.

Results

Thirty patients who satisfied the inclusion and exclusion criteria were included in the study and were grouped into two- 24 patients who were diagnosed positive with MG and 6 patients who were negative of MG. Amongst the 24 patients diagnosed with MG, 15 were males and 9 were females. Amongst the 6 patients without MG, there were 3 males and 3 females.

Table 1 Comparison of demographics of the two groups

Parameter		MG +ve (n = 24)	MG -ve (n = 6)	p value
Age (years)		63.58 ± 11.26	58.67 ± 12.03	0.353
Gender	Males	15	3	0.660
	Females	9	3	

Table showing the comparison of demographics (age and gender) amongst MG positive and MG negative groups.

Table 2 Proportion of cases identified by each method

Test n=30	Positive n(%)	Negative n(%)
SPE	23 (76.7%)	7 (23.3%)
sFLC	22 (73.3%)	8 (26.7%)
sIFE	24 (80%)	6 (20%)
sISE	24 (80%)	6 (20%)

Table showing the number of cases and its proportion in percentage by each method

Table 3 Comparison of sIFE and sISE in diagnosing MG considering sIFE as the gold standard

Test	sIFE		Total	p value	Sensitivity (%)	Specificity (%)	Accuracy (%)
	MG +ve	MG -ve					
sISE +ve	24 (100%)	0 (0%)	24	1.000	100	100	100
sISE -ve	0 (0%)	6 (100%)	6				
Total	24	6	30				

Table showing comparison of sIFE and sISE in diagnosing MG keeping sIE as the gold standard. The sensitivity and specificity of sISE with respect to sIFE came to be 100% respectively and the diagnostic accuarcy was also 100%.

Table 4 Comparison of sFLC and SPE in diagnosing MG considering SPE as the gold standard

Test	SPE		Total	p value	Sensitivity (%)	Specificity (%)	Accuracy (%)
	MG +ve	MG -ve					
sFLC +ve	21 (95.5%)	1 (4.5%)	22	1.000	91.3	85.71	90
sFLC -ve	2 (25.0%)	6 (75%)	8				
Total	23	7	30				

Table showing comparison of sFLC and SPE in diagnosing MG considering SPE as the gold standard. This does not show any significant results.

Table 5 Comparison of the panel SPE, sFLC and sIFE with the panel SPE, sFLC and sISE

Category		Combination of SPE, sFLC and sISE		p value
		All positive n(%)	Any one positive n(%)	
Combination of SPE, sFLC and sIFE	All tests positive n=21	21 (100%)	0(0%)	1.000
	Any one test positive n=3	0 (0%)	3 (100%)	

Table compares the panel SPE, sFLC and sIFE with the panel SPE, sFLC and sISE which does not show statistical significance. This shows that both panels contribute equally for the diagnosis of MM.

Table 6 Mean values of kappa, lambda and kappa/ lambda ratio in the MG positive and MG negative groups

Group	MG +ve (mean ± SD)	MG -ve (mean ± SD)	p value
Kappa(mg/L)	268.65 ± 449.62	15.91 ± 2.5	0.178
Lambda (mg/L)	362.43 ± 617.765	15.95 ± 4.12	0.604
Kappa/ Lambda ratio	33.2 ± 50.51	1.02 ± 0.14	0.534

Table showing comparison of mean values of kappa, lambda and kappa/lambda ratio in the MG positive and negative groups and does not show any significance.

Discussion

MGs are neoplasm of terminally differentiated B-lymphocytes known as plasma cells that secrete Igs[16,17]. They are known for their poor prognosis with 5-year survival rates being 48.5%[18,19]. MG is the result of excessive production of a single abnormal plasma cell or B-lymphocyte and laboratory plays a crucial role in detection of the same. The diagnosis of MG is rooted on the identification of M-band through agarose gel electrophoresis in SPE[20]. SPE is the usual screening method for MG diagnosis though later studies have found sIFE to be superior to SPE in terms of sensitivity[21]. Further developments in this field have identified sISE also to be more sensitive than SPE and hence can be included in the diagnosis of MG[22]. Both sIFE and sISE helps in the qualitative analysis of M-proteins and quantification of the Igs can be performed by nephelometryor turbidimetry[18]. sFLCanalysis quantifies the amounts of free kappa and lambda light chains present in serum and is useful in cases where the monoclonal proteins in serum and urine are low[19]. Ratios of the free light chains are also commonly utilized to find out the type of monoclonal rise and thus monitor the patients with non-secretory myeloma or light chain type myelomas[23].

We did not find any association between age and gender in our study (Table 1). We analyzed the proportion of cases that were identified by each technique and found that SPE alone could identify 23 cases (76.7%), sFLC alone diagnosed 22 cases (73.3%), sIFE alone detected 24 cases (80%) and sISE alone could detect 24 cases (80%). (Table 2) This is in conjunction with those studies done by Singhal S et al[24] and Katzmann et al[25]. The sensitivity and specificity of sISE with respect to sIFE came to be 100% respectively and the diagnostic accuracy was also 100%. (Table 3) We compared sFLC and SPE in diagnosing MGs and the sensitivity, specificity and diagnostic accuracy of these came to be 91.3%, 85.71% and 90% respectively. (Table 4) Thus our data proves that sIFE and sISE could detect the maximum number of cases than SPE and sFLC and these tests have good sensitivity and specificity. Katzmann et al shows almost similar results where sIFE detected maximum number of cases, 400(93.5%) followed by sFLC 367 (85.7%) and sIFE 400 (93.5%)[25]. Katie L T et al showed that sISE yields comparable results to sIFE in M-protein identification[26]. However, a study by Litwin CM et al showed sISE to be less accurate than sIFE in determining the immunotype of MG[27]. Comparison of the serum free light kappa and lambda chains amongst the MG positive and negative patients did not reveal any significant results. (Table 4)

A comparison of 2 panels- SPE + sFLC + sIFE and SPE + sFLC + sISE was done for testing the diagnostic

efficacy. The 21 cases that were found to be positive through SPE + sFLC + sIFE were also found to be positive through SPE + sFLC + sISE and the comparison was not statistically significant (Table 5). This shows that one of these panels is not above the other and that both contribute equally for the diagnosis of MM. Similar observations were made in a study done by Kuriakose E et al which showed that a combination of the panel SPEP + sIFE + sFLC could detect all the cases of myeloma included in their study[2]. Another study by Miyazaki K et al observed that sIFE and sISE combined with sFLC was able to detect more cases that any of these tests done alone for AL amyloidosis diagnosis[28]. We did not include any urine samples in our study since sFLC measurement has already been proven to have advantages over 24-hour urine protein electrophoresis and urine IFE[29].

Our data suggest that sIFE and sISE techniques possess their own merits and drawbacks and one test cannot be considered superior over the other. Though sISE seems to be better than sIFE in terms of improved sensitivity there are still arenas where sIFE has to be done as a complementary technique. One situation is when there is a need to detect a free light chain and the other is to detect a second small monoclonal band in a sample with biclonalgammopathy. One of the drawbacks that have been raised with regards to sISE is its inability to detect low concentration bands but this can be avoided with better training and also by utilizing sIFE as a complementary technique[26].

Conclusion

MG is a spectrum of diseases, which if diagnosed early can improve the prognosis of the patient thus being able to lead a better quality of life. SPE that was the preferred screening test for MG is now complemented with more sensitive and accurate tests like sIFE and sISE. The screening panel of SPE + sFLC + sIFE and SPE + sFLC + sISE has turned out to be efficient in the diagnosis of MG than any of these tests performed alone. The diagnostic accuracy of sIFE and sISE in MM patients was compared and was found to be statistically insignificant, allowing us to reach the conclusion that one of these tests is not above the other and that both contribute equally for the diagnosis of MM.

Recommendations

Due to time and financial constraints we could do this study in a population which was only a little above the calculated sample size. Hence our recommendation is to conduct the same on a larger sample size.

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