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ORIGINAL RESEARCH ARTICLE

A Study on Free Light Chain Assay and Serum Immunofixation Electrophoresis for the Diagnosis of Monoclonal Gammopathies

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Abstract Demonstration of monoclonal immunoglobulin molecule in serum forms the mainstay in the diagnosis of monoclonal gammopathies. The major tests that help in this regard are serum protein electrophoresis (SPEP), serum immunofixation electrophoresis (sIFE) and serum free light chain assay (sFLC). Our objectives were to study the accuracy of sFLC and sIFE in the diagnosis of monoclonal gammopathies and also to study the role of combination of SPEP + sIFE + sFLC in the diagnosis of the same. 46 patients who attended the hemato-oncology clinic with signs and symptoms suggestive of monoclonal gammopathy were enrolled in this study. SPEP, sIFE, sFLC and pre-treatment serum beta-2 microglobulin levels were analysed among the study population. Both SPEP and sIFE were performed in the Interlab Genios fully automated machine. Serum beta-2 microglobulin and sFLC were estimated by immunoturbidimetry in Beckman Coulter AU 2700 analyzer. The accuracy of sIFE came to be 80% with respect to sFLC assay. Sensitivity, specificity, positive and negative predictive value of sIFE with respect to sFLC were 81.3, 78.6, 89.7 and 64.7% respectively. It was observed that a combination panel of SPEP + sIFE + sFLC could detect all the cases of myeloma included in this study. Further testing in large samples is required for generalising the findings of this study. The pre-treatment beta-2 microglobulin levels were significantly higher in the group which was positive for myeloma. A combination panel of SPEP + sIFE + sFLC prove to be more useful than individual tests for the detection of myeloma.

Keywords Monoclonal immunoglobulin · Serum protein electrophoresis · MGUS · Serum immunofixation electrophoresis · Plasma cell dyscrasias

Introduction

Monoclonal gammopathies cover a spectrum of disorders associated with the monoclonal proliferation of plasma cells. These group of disorders are unique in the fact that, they are characterized by the secretion of immunologically and electrophoretically homogeneous monoclonal or M proteins [1]. Monoclonal gammopathies can vary from premalignant conditions like monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM) to malignant types like multiple myeloma (MM), plasmacytoma, plasma cell leukemia and Waldenstrom's macroglobulinemia (WM). Low tumor burden diseases include, light chain deposition disease (LCDD), primary amyloidosis (AL) and POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes) syndrome. Of these,

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multiple myeloma is the most commonly encountered condition [2].

Screening for monoclonal gammopathies historically comprises methods like SPEP and urine protein electrophoresis (UPEP). Inability to detect low levels of monoclonal protein has been a major limitation of SPEP, due to its low analytical sensitivity [3].

IFE had been in use as a technique for the study of protein polymorphism and identification of serum protein fractions since the mid 1970's. By early 1980's, the technique was also utilized for the detection of monoclonal gammopathies [4]. IFE is about ten times more sensitive for free light chain detection than SPE but considerably less sensitive than sFLC immunoassays. The major drawback of IFE is that monoclonal immunoglobulins cannot be quantified because of the presence of precipitating antibody. Moreover, the procedure of IFE is tedious to perform and visual interpretation of the results can be subjective [5].

sFLC analysis emerged as a more objective and direct measurement of M-protein overproduction, overcoming the difficulties of 24-h urine collection for urine immunofixation electrophoresis (uIFE). These are latex enhanced immunoassays and measures concentrations as low as 1.5 and 3 mg/L for κ and λ FLCs, respectively.

Interpretation of sFLC analysis requires the measurement of both κ and λ FLCs as well as κ/λ ratio estimation. If serum κ, λ and κ/λ ratio are all within the normal ranges along with normal serum electrophoresis it is unlikely that the patient has a monoclonal gammopathy. But, if the κ/λ ratios are abnormal, along with an increase in either κ or λ FLC, it supports the diagnosis of a monoclonal gammopathy and further investigations are needed. Borderline abnormal κ/λ ratios can occasionally be seen in patients with renal impairment and in patients with polyclonal hypergammaglobulinemia caused by infections or inflammatory disorders [6]. κ/λ ratio is a sensitive numerical indicator of clonality. Excessive clonal production of only one FLC type often leads to highly abnormal κ/λ ratios in patients with plasma cell dyscrasias [7].

Very few studies have compared the role of FLC assays with sIFE, in the diagnosis of monoclonal gammopathies. The purpose of this study was to compare sIFE with sFLC assays in the detection of monoclonal gammopathies and to suggest a panel for the screening of these disorders.

Materials and Methods

This cross sectional study was conducted over a period of 1 year from March 2015 to May 2016, amongst patients attending the outpatient and inpatient wings of Department of Medical Oncology, Amrita Institute of Medical Sciences and Research Centre (AIMS), Kochi. Forty-six patients were enrolled in the study. The study was conducted as per the approval and guidelines of the ethical committee of AIMS and with the informed written consent of the participants.

Subject Selection

Patients who attended the Medical Oncology clinic with signs and symptoms suggestive of monoclonal gammopathy like—anemia, raised ESR, bone pain due to lytic lesions, hypercalcemia and excessive fatigue were enrolled in this study.

Inclusion Criteria

- Patients with symptoms suggestive of monoclonal gammopathy, including men and women, who are 30 years or above.

Exclusion Criteria

Following patients were excluded from the study.

- Patients with serum creatinine > 1.4 mg/dL.
- Patients who are immunocompromised—those with HIV infection, underwent renal transplant or who are under immunosuppressants/corticosteroid therapy.
- Patients with any other known primary malignancies or those with metastasis from unknown primary.
- Patients with known autoimmune diseases like rheumatoid arthritis, SLE/DLE, myasthenia gravis, inflammatory sero-negative polyarthritis, polymyositis, angioneurotic oedema etc.
- Patients less than 30 years of age.

Measurements

The venous blood samples were obtained under aseptic precautions. Blood samples for estimating beta-2 microglobulin and FLC, as well as for performing SPEP and sIFE were collected in vacutainers without anti-coagulant. The samples were then centrifuged at a speed of 3000 rpm for 5 min and the separated serum was transferred into labelled vials and stored at 2–8 °C. The tests were performed within 21 days of collection.

SPEP was performed in the Interlab Genios fully automated machine, using Tris-barbital as the buffer. Proteins are separated at alkaline pH by zone electrophoresis on agarose gel plates. If the total protein concentration exceeded 100 g/L, the serum was diluted with normal saline to achieve a final concentration in the range of

60–80 g/L. After separation of the bands the agarose gel plate was dried, stained with acid blue dye and finally destained. The bands were then scanned using a densitometer [8].

Serum Immunofixation electrophoresis was also performed in the Interlab Genios fully automated machine using Tris-barbital as the buffer. The principle is based on the visualization of specific proteins through antigen–antibody precipitin formation following protein separation by electrophoresis at alkaline pH. Samples whose total protein concentration exceeded 15 g/L was diluted with the immunofixation diluent to attain a concentration of 5 g/L. After separation of bands, one lane is treated with a fixative solution to fix all proteins to provide a reference pattern. The other lanes are treated with antisera displaying different binding specificities to protein domains of human immunoglobulins. The interaction between antigen which is the immunoglobulin in the sample and the antisera antibody will result in the formation of an insoluble complex that produces a band of precipitate. The agarose gel plate is then denaturated and washed to remove any excess of proteins that have not precipitated and stained with acid blue followed by destaining and drying [9].

Estimation of sFLC utilized the principle of immunoturbidimetry on Beckman Coulter AU 2700 analyzer. It involves the addition of test sample (antigen) to a solution containing appropriate antibody in a cuvette. As the antigen–antibody reaction proceeds, a beam of light is passed through the cuvette. Light scatter is monitored by measuring the decrease in the intensity of incident beam of light, which will proportional to the concentration of the test sample (antigen) [10].

Serum beta-2 microglobulin was also assayed on Beckmann Coulter AU 2700 analyzer using the principle of immunoturbidimetry. When the sample is mixed with the buffer and latex solution, human beta-2 microglobulin reacts specifically with anti-human beta-2 microglobulin antibodies coated on the latex particles, to yield insoluble aggregates. The absorbance of these aggregates is proportional to the beta-2 microglobulin concentration in the sample [11].

Statistical Analysis

Statistical analysis was done using IBM SPSS 20 (SPSS Inc, Chicago, USA). For all the continuous variables, the results are given in mean ± SD, and for categorical variables as percentage. To obtain the association of categorical variables, Chi square test was applied. To find out the efficacy of two methods, McNemar test was used. To compare the difference in means 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

The 46 patients included in the study were categorized into two groups, namely:

- 30 patients who were diagnosed with a monoclonal gammopathy (mainly myeloma).
- 16 patients who were negative for monoclonal gammopathy.

Out of the 30 patients diagnosed with a monoclonal gammopathy, there were 17 males and 13 females. Among the 16 patients, who were negative for the disease, 11 were males and 5 were females.

Discussion

Monoclonal gammopathies are conditions where excessive and abnormal amounts of immunoglobulins are produced by a clone that is developed from a single pro-B germ cell. Thus, they result from an overproduction of a single abnormal clone of a plasma cell or B lymphocyte. The M-protein/M-component is usually seen as a band of restricted migration on serum or urine electrophoresis [12]. Agarose gel electrophoresis is the usual method used for screening of M-protein, with IFE performed to confirm it's presence and to determine the immunoglobulin heavy chain class and light chain type. However, IFE does not quantify the immunoglobulins. Quantification of immunoglobulins can be performed by either nephelometry or densitometry of the M-protein [13]. Measurement of sFLC is considered among the patients in whom serum and urine M-protein levels are low. The FLC assay measures the levels of free/unbound κ as well as λ light chains in serum. The free light chain κ/λ ratio is used to identify monoclonal elevations that occur in clonal plasma cell disorders. Patients with κ/λ ratios < 0.26 are said to be having a monoclonal λ free light chain, whereas, those with ratios > 1.65 are considered to be having a monoclonal κ free light chain [10].

In our study no significant differences were observed among the age group and gender of the study subjects with and without monoclonal gammopathy (Tables 1, 2). The mean value of κ/λ in the group which was positive for monoclonal gammopathy was higher than in the group which was negative for monoclonal gammopathy (Table 3). Accuracy of sFLC and sIFE in the diagnosis of monoclonal gammopathies were studied. The accuracy of sIFE came to be 80% with respect to sFLC and there was

Table 1 Comparison of age among the two groups

Group	Age (mean ± SD)	*p* value
Monoclonal gammopathy + ve (n = 30)	62.86 ± 08.67	0.856
Monoclonal gammopathy − ve (n = 16)	63.43 ± 12.46	

Table 2 Association between gender and disease

Gender	Monoclonal Gammopathy + ve	Monoclonal Gammopathy − ve	*p* value
Males (n = 28)	17 (60.7%)	11 (39.3%)	0.533
Females (n = 18)	13 (72.2%)	5 (27.8%)	

Table 3 Levels of serum kappa and lambda and kappa/lambda ratio

Group	Kappa (mg/L) mean ± SD	Lambda (mg/L) mean ± SD	κ/λ ratio mean ± SD
Monoclonal gammopathy + ve (n = 30)	1232.39 ± 3402.68	200.52 ± 664.51	593.04 ± 2653.93
Monoclonal gammopathy − ve (n = 16)	46.87 ± 41.72	39.88 ± 69.55	1.70 ± 1.11

Table 4 Accuracy of sFLC and sIFE in the diagnosis of monoclonal gammopathies

Test	sFLC + ve Monoclonal gammopathy + ve	sFLC − ve Monoclonal gammopathy − ve	*p* value	Sensitivity (%)	Specificity (%)	Accuracy (%)
sIFE + ve	26	3	0.508	81.3	78.6	80
sIFE − ve	6	11				

no significant difference between the accuracy of either of these techniques when performed singly in the detection of monoclonal gammopathies (Table 4). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of sIFE with respect to sFLC came to be as 81.3, 78.6, 89.7 and 64.7% respectively. These findings suggest that both of these techniques possess their own advantages and limitations and one cannot be considered superior over the other. The study by Katzmann et al. [15] emphasized the importance of including sFLC in the diagnostic panel for gammopathies. Wood et al. [14] concluded that sIFE should be carried out in patients suspected with monoclonal gammopathies for screening purposes.

Addition of either sIFE/sFLC or both of these tests, to SPEP detected more number of cases. A combination of SPEP + sFLC were able to detect 28 out of those 30 patients with monoclonal gammopathy. Whereas, addition of sIFE to SPEP could detect 29 cases. And a combination of all three tests, SPEP + sIFE + sFLC, detected all 30 cases of monoclonal gammopathy (Fig. 1). Thus, for the screening of monoclonal gammopathy, a panel of 2 or 3 tests could be more effective than each one performed alone. This observation from our study is in conjunction with the results obtained from similar studies conducted by Piehler et al. [6], Katzmann et al. [15], Hill et al. [16], Holding et al. [17] and Robson et al. [18], where omission of either sFLC or sIFE resulted in loss of sensitivity.

Levels of beta-2 microglobulin were significantly higher in the group which was positive for myeloma, when compared to the group which was negative for myeloma (Table 5). Studies done by Bataille et al. [19, 20], Brenning et al. [21] Alexanian et al. [22] and Norfolk et al. [23] support our findings where they all have observed an increase in the levels of beta-2 microglobulin in patients with myeloma.

Conclusion

Based on our findings, we conclude that a combination panel of 2 or 3 tests (SPEP, sIFE and sFLC) has a higher diagnostic potential in the identification of patients with monoclonal gammopathies than when performed alone. However, we have not included any urine samples in our study and therefore the advantage of analyzing serum SPEP/IFE/FLC over urine SPEP/IFE/FLC could not be established. Also, we could not assess the prognostic utility of estimating serum beta 2 microglobulin in our patients due to time constraints. Certainly more research is needed

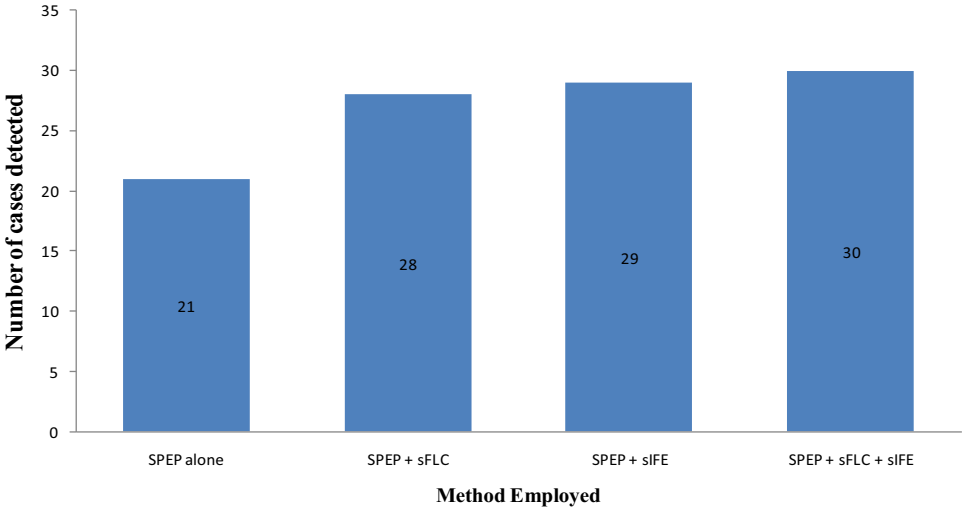

Fig. 1 Diagram showing the number of cases detected by each method

Table 5 Comparison of serum beta-2 microglobulin among the two groups

Group	Mean ± SD	*p* value
Monoclonal gammopathy (+ ve)	5.10 ± 3.16	< 0.001
Monoclonal gammopathy (− ve)	2.13 ± 1.00	

for the better understanding of different combination panels used in the setting of screening for monoclonal gammopathies.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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