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Original Research Article

Role of free light chains as an addendum to protein electrophoresis in expediting multiple myeloma

[1]Bhutesh Kumar Sharma, [2]Dr. Girish Konasagara Shanthaveeranna

[1]Tutor, Department of Biochemistry, St John's Medical College and Hospital, Sarjapur Road, Bangalore, Karnataka, India

[2]Associate Professor, Department of Biochemistry, Shri Atal Bihari Vajpayee Medical College and Research Institute, Shivajinagar, Bangalore, Karnataka, India

Corresponding Author:

Dr. Girish Konasagara Shanthaveeranna (girishks@outlook.com)

Abstract

Background: Multiple myeloma (MM) is a clonal plasma cell disorder with the hallmarks of bone marrow plasmacytosis and in most cases, production of an abnormal monoclonal immunoglobulin detectable in serum or urine protein electrophoresis. Assays that detect free light chain (FLC) are important in the diagnosis and monitoring of light chain only multiple myeloma (Bence Jones myeloma) and AL amyloidosis. Serum free light chain (sFLC) measured by scanning densitometry and electrophoresis lacks sensitivity in diagnosis and has a detection limit many times greater than the upper end of the normal range. Our study was done to know whether sFLC and their ratios can be used in diagnosis suspected cases of MM.

Methods: The study was conducted in a tertiary care hospital suspected of multiple myeloma. This is a descriptive analytical study involving subjects requested for Serum Protein Electrophoresis (SPE) and sFLC. Descriptive statistical analysis was carried out in the present study. To find out the association between the categorical studies variables we have used Chi square test. Pearson's rank correlation is used to find the correlation between SPE results and sFLC.

Results: sFLC was able to diagnose 30 out of 33 subjects as multiple myeloma but 11 were diagnosed as false positive. When sFLC and SPE combined, all cases of multiple myeloma were diagnosed but false positive was high with 11 cases and none were diagnosed as false negative.

Conclusions: Combination of SPE and sFLC is more sensitive in identifying nearly all patients with clinically relevant monoclonal gammopathy.

Keywords: SPE-Serum protein electrophoresis, SFLC-Serum free light chain, MM-multiple myeloma, AL-amyloidosis

Introduction

Multiple myeloma (MM) is a clonal plasma cell disorder with the hallmarks of bone marrow plasmacytosis and, in most cases, production of an abnormal monoclonal immunoglobulin detectable in serum or urine protein electrophoresis [1]. Monoclonal, immunoglobulin free light chains (FLC) are present in the serum and urine of many patients with B-cell proliferative disorders including multiple myeloma and AL amyloidosis [2].

Assays that detect FLC are important in the diagnosis and monitoring of light chain only multiple myeloma (Bence Jones myeloma) and AL amyloidosis (3). FLC can be measured in serum by scanning densitometry of serum electrophoresis gels, but this lacks sensitivity and has a detection limit many times greater than the upper end of the normal range (4). Serum immune-fixation electrophoresis is more sensitive, but it is not quantitative

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and is therefore inappropriate for monitoring. Because of these limitations, FLC are normally monitored using protein electrophoresis of urine [5-7].

Material & Methods :

Study design: Descriptive analytical study.

Place of study: Tertiary care hospital in Bangalore, Karnataka, India.

Period of study: January 2017-December 2019.

Procedure: Patient's/Subjects who requested for SPE along with sFLC were included in the study, Exclusion of samples as per the rejection criteria of Standard Operating Procedure for Protein Electrophoresis as unlabeled, wrongly labeled, contaminated specimens, turbid samples collected without refrigeration, contaminated specimens, samples which were not analyzed for both SPE and sFLC, already diagnosed and follow up cases were not included in the study. Total 57 subjects were included in the study. sFLC was analyzed by nephelometry method by siemens BN ProSpec and SPE by capillary electrophoresis method.

Ethical approval: Institutional ethical approval was obtained for the study

Statistical analysis: The data was analysed using SPSS version 23.0. Descriptive statistical analysis has been carried out in the present study. Chi-square test was used to find out the association between the categorical study variables. Pearson's Chi square was also used to find the correlation between SPE results and sFLC with p value <0.05 was considered significant.

Results:

The Study conducted was a descriptive analytical study in the tertiary care center to study the role of sFLC as an addendum to SPE by capillary zone electrophoresis in expediting light chain components of monoclonal immunoglobulins in Multiple Myeloma. A total of 57 cases were included of which 33 cases were confirmed as monoclonal gammopathy.

Demographic characteristics of the patient are shown in table 1. 90% of the subjects were from age group from 41-60 ys. The number of subjects finally diagnosed as positive are 33 and 24 subjects as negative out of 57 subjects.

The number of patients diagnosed as positive or negative by sFLC compared to final diagnosis is show in table 2. The number of patients diagnosed as positive or negative by SPE compared to final diagnosis is show in table 3. Table 4 give whether both sFLC and SPE against the final diagnosis. Table 5 describes the likelihood ratio when both the methods were used for diagnosis of multiple myeloma.

Table 1: Age Distribution

Age in years	Cases	
	No.	%
<40 yrs	1	1.7
41-60 yrs	18	31
61-80 yrs	35	61
>81 yrs	3	5
Total	57	100

Table 2: Number of subjects diagnosed by sFLC against the final diagnosis

	Final Diagnosis		
sFLC	Negative	Positive	Total
Negative	13	3	16
Positive	11	30	41
Total	24	33	57

Serum Free light chain – sFLC

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Table 3: Number of subjects diagnosed by SPE against the final diagnosis

	Final diagnosis		
SPE	**Negative**	**Positive**	**Total**
Negative	21	4	25
Positive	3	29	32
Total	24	33	57

SPE – serum protein electrophoresis

Table 4: Number of subjects diagnosed by sFLC and SPE against the final diagnosis

	Final diagnosis		
sFLC and SPE	**Negative**	**Positive**	**Total**
Negative	13	0	13
Positive	11	33	44
Total	24	33	57

Serum Free light chain – sFLC, SPE – serum protein electrophoresis

Table 5: Pearson Chi square P value and the likelihood ratio

	Pearson Chi Square p-Value	**Likelihood Ratio**
sFLC & Final diagnosis	0.000	14.42
SPE and Final diagnosis	0.000	35.696
Combined (sFLC & SPE) with final diagnosis	0.000	28.106

Serum Free light chain – sFLC, SPE – serum protein electrophoresis

Discussion:

In our study, we identified nearly all patients with multiple myeloma by clinical information and serum tests exclusively. As serum FLC concentrations were not abnormal in all the patients, it is clear that serum protein electrophoresis is a more sensitive technique for the diagnosis of intact immunoglobulin multiple myeloma (IIMM). However, serum FLC assays are more sensitive than serum electrophoresis for the identification of light chain only multiple myeloma and non-secretory myeloma [8]. Therefore, if a diagnosis of multiple myeloma is suspected, the optimum laboratory practice should be to screen sera by both SPE and sFLC assays.

The ability to detect abnormal amounts of sFLC and an abnormal FLC Kappa/Lambda is dependent on accurately determined reference intervals, so that the specificity of disease detection remains high [9]. The study showed that the percentage of identifying multiple myeloma with SPE alone was only 88% (29 out of total 33 confirmed cases of monoclonal gammopathy). It also shows 3 false positive with 4 false negative cases. The percentage of identifying monoclonal gammopathy with sFLC stood at 91% (30 out of total 33 confirmed cases of monoclonal gammopathy) with 3 false positive and 11 false negative cases.

When serum protein electrophoresis results were supplemented by serum free light chain ratio, the percentage of identifying monoclonal gammopathy was increased to 100% (33 out of 33 confirmed cases of monoclonal gammopathy). This indicates that the combination of SPE and sFLC is always better in identifying monoclonal gammopathy, than doing either SPE or sFLC alone. However, the false positive remained the same as sFLC with 11 cases. Our study finding is in line with Bakshi NA, *et al.* [7] who had also observed that the SPE results supplemented with sFLC measurement improves in diagnosing multiple myeloma.

However, a substantial number of false-positive were found with sFLC alone was used for diagnosis of multiple myeloma. When subjects end up with borderline kappa/lamda ratio, it is difficult to diagnose the case with sFLC alone and hence consideration of additional clinical and laboratory patient information to correctly interpret sFLC results is

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required [10].

Retrospective studies using stored samples of serum also indicated that FLC tests were more sensitive than existing serum and urine electrophoresis tests [10]. Several recent studies have evaluated the potential role of sFLC measurements for the diagnosis and management of patients with multiple myeloma and have shown that sFLC adds to the diagnosis of multiple myeloma cases [11-12].

As shown in table 5, the likelihood ratio of the subject diagnosing as multiple myeloma is greater than 10 when we use both sFLC and SPE in diagnosing multiple myeloma indicating that subject is more probability of having the disease.

Study by Milani P *et al*. [13] has suggested that analysis of sFLC helps in assessing the risk of progression of percusor disease to over plasma cell dyscrasias. Evaluation of sFLC is necessary for assessing the response to treatment in monoclonal light chain diseases.

Conclusion:

In conclusion, the study indicates a similar outcome as smith A. *et al*. [11], that the combination of SPE and sFLC is more sensitive in identifying nearly all patients with clinically relevant monoclonal gammopathy. Moreover, our data suggest that, in the diagnostic process for detecting multiple myeloma, it is sufficient to quantify sFLCs in patients suspected of plasma cell dyscrasia from clinical, biochemical, or SPE findings.

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