Int J Pharm Bio Sci 2015 Jan; 6(1): (B) 1074 - 1080

Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

SERUM FREE LIGHT CHAIN RATIO IN CORRELATION WITH SERUM PROTEIN ELECTROPHORESIS IN MULTIPLE MYELOMA PATIENTS FROM SOUTH INDIA.

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ABSTRACT

Quantitative measurement of serum free light chains (S.FLC) has now been adopted into screening algorithms for multiple myeloma (MM). The assay indicates monoclonal free light chain production by the presence of abnormal kappa or lambda free light chain ratio (reference range 0.26-1.65). We report our experience with S.FLC assay in patients of MM in correlation with serum protein electrophoresis (SPE). Review of 50 cases of MM was undertaken. MM was diagnosed in these cases, according to the International Myeloma Working Group (IMWG) criteria. S.FLCs were measured by immunoturbidimetry method, SPE was done on cellulose acetate strip. All 50 cases of MM patients showed abnormal ratio of S.FLCs while SPE could detect monoclonal protein in 44(88%) out of 50 cases. The results indicate that serum FLC analyses have high diagnostic sensitivity and are of additional value in the detection of monoclonal proteins where there is strong clinical suspicion, especially when not detected by SPE.

KEYWORDS: Multiple Myeloma (MM), Serum Protein Electrophoresis (SPE), Serum Free Light Chains (S.FLC).

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INTRODUCTION

Myeloma and multiple myeloma (MM) are referred as cancer of antibody producing plasma cells. Although such a cancerous plasma cell, called myeloma cell has been transformed, its protein synthesizing machinery and secretory functions are not altered, thus, the cell continues to secrete specific antibody. The secreted antibodies are indistinguishable from normal antibody molecules but are called myeloma protein (M-protein, paraprotein) to denote its source. In 99% of the cases, paraproteins are secreted in the serum and/or urine (Bence-Jones protein) and in the remaining 1% the paraproteins are synthesized but not secreted[1]. The electrophoretic analysis of the serum sample is the first step for observing the altered protein concentrations and the presence of an M peak portion of proteins, which indicates myeloma but not its type[1]. Quantitation of monoclonal protein (M-protein) by serum protein electrophoresis (SPE) is recommended as a tumour marker in patients with monoclonal gammopathy. However, SPE is a semiquantitative method for detection of M proteins, and may be misinterpreted as a negative result due to lack of sensitivity, or the masking effect from other proteins when the M-protein is not present in the gamma fraction. The accuracy of urine protein electrophoresis has been questioned in patients with proteinuria. Moreover, SPE and Immunofixation electrophoresis (IFE) have limited use in the identification of patients with light chain multiple myeloma (LCMM), non-secretory multiple myeloma (NSMM) and AL amyloidosis where the M-protein may not be present in sufficient concentrations for detection[2]. However, the standard screening tests for myeloma, serum protein electrophoresis and urine Bence-Jones protein analysis are not always requested or reported promptly[3]. Quantitative measurement of serum free light chains (FLC) has now been adopted into screening algorithms for multiple myeloma[4]. International guidelines recommend a primary MM screen of SPE, serum IFE and serum FLC analyses. However, a recent report suggests this can be simplified to SPE and serum FLCs, with reflex serum IFE[5]. These FLC assays are automated and allow same day analysis and reporting of results. Immunoglobulin free light chains (FLCs) are byproducts of immunoglobulin synthesis from plasma cells & in normal subjects are released into the circulation in small quanitities[6]. In patients with multiple myeloma, the clonal proliferation of plasma cells can produce FLCs in quantities thousands of times higher than normal[7]. The use of the kappa to lambda (k/ λ) ratio enables the identification of imbalances in light chain production. With these assays, the presence of monoclonal FLCs production is indicated when the ratio of kappa (k) to Lambda (λ) serum FLCs is outside the reference range of 0.26 – 1.65[8]. We report here our experience with serum FLC assays in the diagnosis of MM in an Indian population in conjunction with SPE.

MATERIALS AND METHODS

We have reviewed the cases of multiple myeloma which are diagnosed based on the International Myeloma Working Group (IMWG) diagnostic criteria for multiple myeloma (published in 2009)[9]. This study was undertaken during period 2009 & 2010, in the department of Lab Medicine, Basavatarakam IndoAmerican Cancer Hospital & Research Institute, Hyderabad, India. SPE was carried out using cellulose acetate strips (Genio Electrophoresis, Lilac). Serum Kappa & Lambda free light chain concentrations estimated by immunoturbidimetry on Olympus AU400 analyzer using reagents from the Binding site, UK. To confirm visual hypo or hyper immunoglobulinemia on SPE and as part of estimating concentrations of monoclonal immunoglobulin's, Immunoglobulin G, A, and M levels were estimated using turbidimetry on an Olympus AU400 analyzer. International Myeloma Working Group (IMWG) diagnostic Criteria for Symptomatic multiple myeloma[9]:

All Three Required
1. Monoclonal plasma cells in the bone marrow >/=10% and/or presence of a Biopsy-proven plasmacytoma
2. Monoclonal protein present in the serum and/or urine)
3. Myeloma-related organ dysfunction (>/=1)
[C] Calcium elevation in the blood (serum calcium >10.5 mg/dl or upper limit of normal)
[R] Renal insufficiency (serum creatinine >2mg per 100 ml)
[A] Anemia (hemoglobin <10 g per 100 ml or 2 g < normal)
[B] Lytic bone lesions or osteoporosis

RESULTS

The cohort included 32 males (64%) and 18 females (36%), aged 34-73 years (mean 57.6 years). Biochemical parameters of these 50 MM patients were given in the Table 1. SPE results demonstrated the presence of an M-protein band in 44 (88%) of the MM patients. By comparison, S.FLC analyses displayed an abnormal S.FLC ratio in all 50 patients, demonstrating 100% sensitivity of the FLC assay in this disease cohort (Figure 2). The distribution of monoclonal proteins for the 50 MM patients are summarized in Figure 1. Increased ratio demonstrating presence of kFLC was seen predominantly in 80% & while decreased ratio was seen in 20% cases showing the presence of λ FLC (Figure 2).

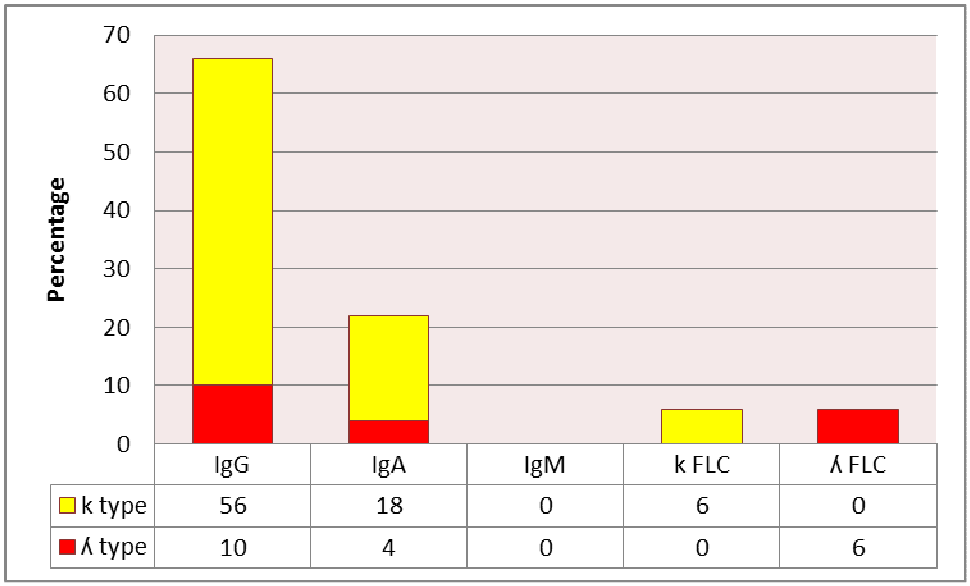
Table 1
Descriptive Statistics

Variables	***Mean ± SD, Median(range)**
Age (N=50)	57.6 ± 9.1 (yrs)
Male (N=32)	61 ± 6.2 (yrs)
Female (N=18)	51.5 ± 10.3 (yrs)
S.Total Proteins (gm/dl)	8.0 (4.3 – 15.9)
S. Albumin (gm/dl)	3.5 (1.4 – 4.8)
S. Creatinine (mg/dl)	1.9 (0.7 – 11.2)
S. LDH (IU/L)	344 (176 - 1127)
S. Calcium (mg/dl)	10.2 (9.6 – 13.7)
S. M-band conc (gm/dl)	3.5 (0 -7.1)
S. IgG (gm/L)	34.93 (3 – 84.9)
S. IgA (gm/L)	0.49 (0.05 – 73.1)
S. IgM (gm/L)	0.22 (0.12 – 1.1)
S. β_2 Microglobulin (mg/L)	5.4 (2.0 – 40.0)
k FLC (mg/L)	117.7 (3 - 2074)
λFLC (mg/L)	6.95 (3 - 11280)
k/λFLC ratio	14.53 (0.00027 – 691.3)

The Mean±SD for variables was calculated for normally distributed parameters whereas median and ranges were noted for non-normally distributed parameters.

Figure 1

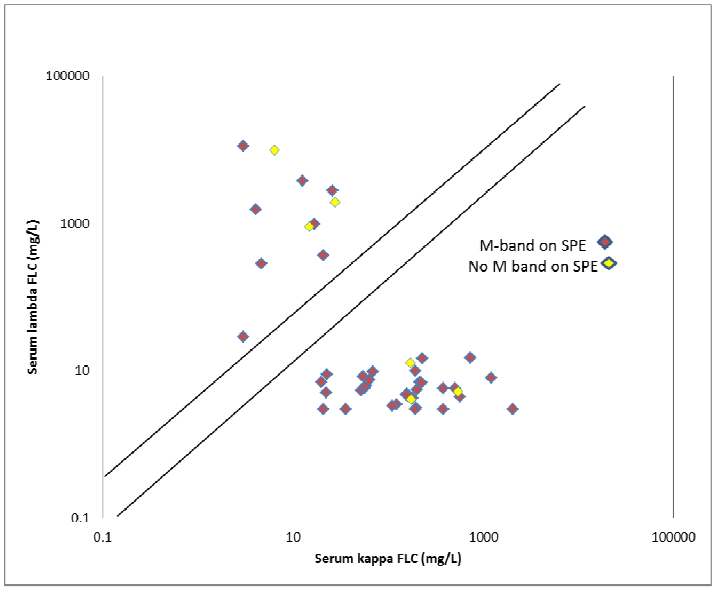
Distribution of monoclonal proteins seen in 50 Multiple Myeloma patients by quantitation of serum immunoglobulins and S.FLC. All the values are in percentages.



	IgG	IgA	IgM	k FLC	λ FLC
k type	56	18	0	6	0
λ type	10	4	0	0	6

Figure 2

Dot plot showing the FLC results from the MM cohort. 6 patients with no M-band by SPE had significantly abnormal S.FLC k/λ ratios. Diagonal lines indicate limits of a normal k/λ ratio (0.26-1.65).



DISCUSSION

Serum Protein Electrophoresis is the most common test in use for detecting monoclonal immunoglobulins, with a sensitivity that is between 500 mg/L and 2000 mg/L[10]. The wide range of sensitivity can be explained by the different migration patterns of M-proteins. In the majority of cases, the monoclonal component migrates in the γ globulin region and is easier to detect. However, occasionally the M-spike can be missed if it is masked by proteins within the

β and α globulin region. SPE to be effective it must be performed and interpreted correctly. This was confirmed by a survey in which only 29% of the participating laboratories succeeded in detecting the presence of M band in serum samples[11]. Narrow M bands in gamma region and bands in association with hypogammaglobulinaemia will be visible at 200-400mg/L, the same band in beta position, perhaps superimposed on transferrin, will be invisible[12]. Immunofixation is a more sensitive technique and can detect between 100 mg/L and 150 mg/L of M-protein[13]. A new available automated immunoassay enables quantification of serum k and λ FLCs. The serum FLC assay has also been shown to be useful to identify most patients with LCMM and non-secretory myeloma not only in diagnosis, but also in monitoring[14]. Kang et al (2005) showed that the serum kappa & lambda FLCs & k/λ FCL assay enables to detect MM with very low M protein due to early stage or after therapy[15]. In our study, all 50 patients with MM had increased serum FLCs and an abnormal k/λ ratio, although 6 patients didn't show the presence of M-protein in SPE. The light chains were increased according to the clonal type. Out of the six, four of these patients had LCMM (2 k LCMM and 2 λ LCMM) and two patients had IgA myeloma. In all these patients, the k/λ ratio was abnormal. Serum FLC assays are more sensitive than SPE in conditions, such as LCMM and MM, where the M-spike can be masked by other proteins. In IgA myeloma, monoclonal protein is known to migrate in the beta or alpha 2 region which could easily be masked. The present study showed maximum sensitivity when S.FLC ratio were used, compared to SPE (100% vs. 88%) in detecting MM. Therefore, if a diagnosis of MM is suspected, optimum laboratory practice should be to screen sera using S.FLC assay even though the electrophoresis didn't show the presence of any paraprotein.

Figure 3

SPE of a patient with k LCMM showed suppressed gamma fraction but no discernable M-spike. FLC results were highly abnormal.

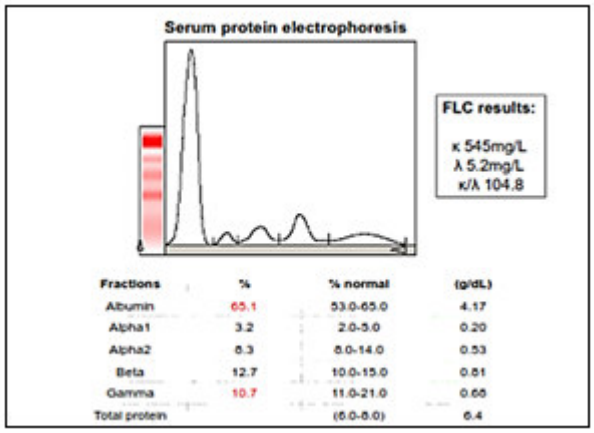


Figure 3 shows the SPE and FLC results from a κ LCMM patient. On SPE there was hypogammaglobulinemia and a raised albumin level but no monoclonal band despite highly abnormal FLC results (S.FLC ratio = 104.8). Monoclonal FLC may be polymerized to different extent and then they migrate on electrophoresis gels as diffuse bands. This is frequently found on NSMM and as well documented in LCMM. In these patients, even 5000mg/L of monoclonal FLC may be difficult to detect above the background of their plasma proteins[12].Consequently, we assume that there would be some cases of light chain myeloma that are missed by routine electrophoresis techniques, and, in these cases, FLC measurements might be extremely valuable in facilitating early detection.The study

demonstrated that all multiple myeloma patients had abnormal serum free light chain ratio. The sensitivity of the assays in this scenario (100%) was not unexpected as previous studies did demonstrate greater sensitivity for serum versus urine detection of monoclonal FLC in myeloma[16&17]. Our findings agree with previous reports highlighting the value of serum FLC analyses in a primary MM screening algorithm, consistent with International guideline recommendations[18 &19].

CONCLUSION

Using SPE alone, 6 of the 50 patients (12%) in our Indian MM cohort had no detectable M-protein, including 4 out of 6 LCMM patients. All patients had an abnormal serum FLC κ/λ ratio. This ensured maximum diagnostic sensitivity and provided a quantitative baseline value for monitoring. The results indicate that serum FLC analyses have high diagnostic sensitivity and are of additional value in the detection of monoclonal proteins where there is strong clinical suspicion, especially when not detected by SPE. Our findings in this local Indian population agree with previous reports highlighting the value of serum FLC analyses in a primary MM screening algorithm, consistent with International guideline recommendations.

CONFLICT OF INTEREST

Conflict of interest declared none.

ACKNOWLEDGEMENT

The results presented in this paper have not been published previously in whole or part, except in abstract format. Thanks to Alex Legg.

REFERENCES

1. Nadeem A Ansari, M Owais, Usha. Immunoglobulin Heavy and Light Chain Isotypes in Multiple Myeloma Patients. Asian Pacific Journal of Cancer Prevention, Vol 8, 593-596 (2007).
2. Sthaneshwar et al. Serum free light chains: diagnostic and prognostic value in multiple myeloma ClinChem Lab Med; 47(9):1101–1107, (2009)
3. Colin A Hutchison, Tim Plant, Mark Drayson, Paul Cockwell, Melpomeni Kountouri, Kolitha Basnayake1,2, Stephen Harding4,Arthur R Bradwell3 and Graham Mead, Serum free light chain measurement aids the diagnosis of myeloma in patients with severe renal failure. BMC Nephrology, 9:11 doi:10.1186/1471-2369-9-11, (2008)
4. Jerry A Katzmann, Screening Panels for Monoclonal Gammopathies: Time to Change,. Clin Biochem, August; 30(3): 105–111, (2009).
5. Katzmann JA. et al. Screening Panels for Detection of Monoclonal Gammopathies: Confidence Intervals Clin Chem; 55:1517-22, (2009).
6. Solomon A: Light chains of human immunoglobulins. Meth Enzymol, 116:101-121, (1985).
7. Bradwell AR, et al.: Serum test for assessment of patients with Bence Jones myeloma. Lancet, 361(9356):489-91, (2003).
8. Katzmann JA, Clark RJ, Abraham RS, et al. Serum reference intervals and diagnostic ranges for free kappa and free lambda immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. Clin Chem.48: 1437-1444, (2002).
9. Kyle and Rajkumar, Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma. Leukemia; 23: 3–9(2009).
10. Wolff F, Thiry C, Willems D. Assessment of the analytical performance and the sensitivity of serum free light chains immunoassay in patients with monoclonal gammopathy. Clin Biochem; 40:351–4, (2007).
11. Chopra GS, Gupta PK, Mishra DK. The evaluation of suspected monoclonal

gammopathies: the experience in a tertiary care hospital. MJAFI; 62:134-37(2006).
12. Levoguer AM, Legg A. Polymeric forms of light chain: What laboratories and clinicians need to know. Clin Lab; 57:1037-9(2011).
13. Bradwell AR, Carr-Smith HD, Mead GP, Tang LX, Showell PJ, Drayson MT, et al. Highly sensitive automated immunoassay for immunoglobulin free light chains in serum and urine. Clin Chem; 47:673–80, (2001).
14. Drayson M, Tang LX, Drew R, Mead GP, Carr-Smith H, Bradwell AR. Serum free light chain measurements for identifying and monitoring patients with non-secretory multiple myeloma. Blood; 97:2900–2(2001).
15. Kang SY, Suh JT, Lee HJ, et al.Clinical usefulness of free light chain concentration as a tumor marker in multiple myeloma. Ann Hematol, Sep; 84(9):588-93(2005).
16. Bradwell AR, et al.: Serum test for assessment of patients with Bence Jones myeloma. Lancet, 361(9356):489-91, (2003).
17. Alyanakian MA, Abba A, Delarue R, et al.: Free immunoglobulin light-chain serum levels in the follow-up of patients with monoclonal gammopathies: correlation with 24-hr urinary light chain excretion. Am J Hematol, 75:246-248, (2004).
18. Dispenzieri A, Kyle R, Merlini G, Miguel JS, Ludwig H, Hajek R, et al. International Myeloma Working Group guidelines for serum free light chain analysis in multiple myeloma and related disorders. Leukemia; 23:215–24(2009).
19. Katzmann JA, Kyle RA, Benson J, Larson DR, Snyder MR, Lust JA, et al. Screening panels for the detection of monoclonal gammopathies. Clin Chem; 55:1517–22, (2009).