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Original Research Article

A Study to Assess the Serum Free Light Chain Assay and Urine Protein Immunofixation in the Diagnosis of Patients with Monoclonal Gammopathy: A Retrospective Study

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Abstract

Aim: The aim of the present study was to assess the serum free light chain assay and urine protein immunofixation in the diagnosis of patients with monoclonal gammopathy.

Methods: The study included 22 patients, most of whom had monoclonal gammopathies and were hospitalized at Jawaharlal Nehru Medical College, Bhagalpur, Bihar, India for one year.

Results: The median age of the patients (13 men and 9 women) was 64 years. 3 patients were suffering only from LCDD λ and 1 patient was suffering from non-secretory myeloma with FLC κ. 11 patients had multiple myeloma (6 with IgG κ, 1 with IgG λ, and 4 without isotype determination), 1 patient had type κ bi clonal gammopathy, 1 patient had AL amyloidosis, and 2 patients had no monoclonal gammopathy. 14 patients had a positive BJP (10 free κ and 4 free λ) and of these 14 patients 100% had an abnormal κ / λ ratio. 3 patients had no BJP but had an abnormal κ / λ ratio. Bence Jones proteinuria was detected in all patients with LCDD, non-secretory multiple myeloma and in the patient with AL amyloidosis. In contrast, BJP was detected in only 50% of patients with IgG κ multiple myeloma and was not detected in the patient with IgG λ myeloma. The 11 patients with a GFR lower than 60 had an abnormal ratio □/□ and positive Bence Jones proteinuria.

Conclusion: In conclusion, even if it has been demonstrated the higher sensitivity of the FLC assay in the diagnosis of monoclonal gammopathies, BJP still remains a marker that has its importance in these pathologies.

Keywords: Multiple myeloma, bence Jones protein, light chains, kappa lambda ratio, renal failure

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, 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]

The aim of the present study was to assess the serum free light chain assay and urine protein immunofixation in the diagnosis of patients with monoclonal gammopathy.

Materials and Methods

The study included 22 patients, most of whom had monoclonal gammopathies and were hospitalized at Jawaharlal Nehru Medical College, Bhagalpur, Bihar, India for one year. The clinical diagnosis of MM was made according to international diagnostic criteria. [8,9]

The following parameters were noted at the time of diagnosis: age, gender, creatinine, glomerular filtration rate calculated from serum creatinine using the CKD-EPI (chronic kidney disease - Epidemiology Collaboration) study equation, β2-microglobulin, monoclonal protein isotype, urinary immunoglobulin/24 hours, and percentage of plasma cells in the bone marrow.

Method

Protein electrophoresis of serum and 24-hour urine, and immunofixation of serum proteins were performed on agarose gels (Hydra gel 1 Bence Jones, Hydra gel 4 Bence Jones, Hydragel 1 IF, Hydra gel 4 IF). The following antisera were used: anti-Hum. IgG, anti-Hum. IgA, anti-Hum. IgM, anti-Hum. Kappa, anti-Hum. Lambda, anti-Hum. Free light chains Kappa, anti-Hum. Lambda free light chains, anti-Hum. Ig G.A.M.

In each case, the presence of intact/fragmented immunoglobulin in the urine was confirmed by a specific positive antiserum reaction. BJP analysis was performed on unconcentrated urine. The detection level of Bence Jones protein is generally within 1 -5 mg/dL. It should be noted that the urinary protein level was not measured.

The FLC assay was performed on an immunoturbidimetric automaton, the SPA plusd™ (The Binding Site, UK), following the manufacturer's instructions, with fresh or frozen sera.

The Freelite™ assay (The Binding Site, UK) is a turbidimetric assay that uses polyclonal antibodies directed against epitopes of the FLC constant region that are normally hidden in intact immunoglobulins; therefore, only light chains that are not bound to a heavy chain are quantified. Serum is diluted 1:10 for SPA plus™ and allows quantification ranging from 4-180 mg/L and 4.5-165 mg/L for detection of FLC λ and λ respectively. The ratio of the FLC λ/FLC λ assay was compared with normal reference values (ratio κ/λ ranging from 0.26-1.65). [10]

Results

Table 1: Characteristics and biological results of patients

	n	Mean	Median (Range)
Age (Years)		64	63 (52-80)
Creatinine mg/L		36.4	24.5 (5.76-111)
Bence Jones protein positive	14		
Free κ	10		
Free λ	4		
Bence Jones protein negative	8		
Serum free light chain dosage			
κ mg/L	22	503.27	68.30 (7.91-4800)
λ mg/L	22	127.45	18.06 (0.86–1559)
Normal ratio κ / λ	5		
Abnormal ratio κ / λ	17		

The median age of the patients (13 men and 9 women) was 64 years. 3 patients were suffering only from LCDD λ and 1 patient was suffering from non-secretory myeloma with FLC κ. 11 patients had multiple myeloma (6 with IgG κ, 1 with IgG λ, and 4 without isotype determination), 1 patient had type

κ bi clonal gammopathy, 1 patient had AL amyloidosis, and 2 patients had no monoclonal gammopathy. 14 patients had a positive BJP (10 free κ and 4 free λ) and of these 14 patients 100% had an abnormal κ / λ ratio. 3 patients had no BJP but had an abnormal κ / λ ratio. Bence Jones proteinuria was detected in all patients with LCDD, non-secretory multiple myeloma and in the patient with AL amyloidosis.

Table 2: Outcome of Bence Jones proteinuria and serum light chain assay ratio κ / λ according to pathology

	Bence Jones proteinuria (positive)	Bence Jones proteinuria (Negative)	Abnormal ratio □/□	Normal ratio □/□
Light chain deposition disease (n = 3)	3	0	3	0
Non-secretory multiple myeloma (n = 1)	0	1	1	0
IgG □ multiple myeloma (n = 6)	3	3	5	1
IgG □ multiple myeloma (n = 1)	0	1	1	0
Biclonal gammopathy type □ (n = 1)	1	0	1	0
Multiple myeloma without iso type determination (n = 4)	4	0	4	0
AL Amyloidosis (n = 1)	1	0	1	0
Absence of monoclonal gammopathy (n = 2)	0	2	0	2
Patient without diagnosis (n = 3)	2	1	2	1

In contrast, BJP was detected in only 50% of patients with IgG κ multiple myeloma and was not detected in the patient with IgG λ myeloma.

Table 3: Assessment of renal activity based on Bence Jones proteinuria and free light chain assay ratio □/□

	Mean creatinine level (mg/L) and normal ratio □/□ (0.26 < □/□ <1.65) (n = 1)	Average GFR (mL/min/1.73 m2) And normal ratio □/□(0.26 < □/□ < 1.65) (n = 1)	Mean creatinine level (mg/L) and abnormal ratio □/□ (□/□ > 1.65 □/□ < 0.26)(n = 16)	Average GFR (mL/min/1.73 m2) and abnormal ratio □/□ (□/□ > 1.65 □/□ < 0.26) (n = 16)
Bence Jones proteinuria (Positive)	AP*	AP	44.64 (n = 13)	37
Bence Jones proteinuria (Negative)	8.46	98	9.79 (n = 3)	95

The 11 patients with a GFR lower than 60 had an abnormal ratio □/□ and positive Bence Jones proteinuria.

Table 4: Classification of patients who underwent myelograms according to the ratio □/□ and Bence Jones

	Normal ratio □/□ BJP(Negative)	Abnormal ratio □/□ BJP (Negative)	Abnormal ratio □/□ BJP (Positive)
Bone marrow plasma cells < 10%	1	2	1
Bone marrow plasma cells > 10%	0	0	9

Myelograms were performed in 13 patients. Four patients had a plasma cell count of less than 10% and nine patients had a plasma cell count of more than 10%, including one patient with a count of 90%.

Table 5: Comparison of sFLC assay results with the results of investigation in urine

	N	Mean (range) of serum FLC□ values (mg/L)	Mean (range) of serum FLC□ values(mg/L)	Mean (range) of □/□ ratio	Number of patientswith abnormal □/□ ratio	Number of patientswith normal □/□ ratio
Proteinuria de BJ (positive)	14	756.38 (10.48-4800)	179.82 (0.86-1559.19)	153.32 (0.008-321.74)	14	0
Proteinuria de BJ (negative)	8	60,34 (7.91-302.07)	20,70 (4.52-69.67)	6.39	3	5

Of the patients with a level below 10%, 3 patients had no BJP and of these 3 patients, only one had a normal ratio. All patients with a plasma cell count above 10% had a positive BJP and an abnormal □/□ ratio.

Discussion

Multiple myeloma (MM) is a malignant tumor of plasma cells, which are involved in the production of monoclonal immunoglobulins. A characteristic feature of this plasma cell dyscrasia is the secretion of monoclonal immunoglobulins, often called monoclonal M proteins, which can be used as a diagnostic or monitoring marker for the disease. The classification and differential diagnosis of monoclonal gammopathies is based on clinical, biological, and radiological criteria but remains difficult in some cases. MM is the most common malignant gammopathy and is associated with a broad spectrum of clinical signs and symptoms. [11] The heavy and light chain components of the M protein can be identified by immunofixation and quantified by serum protein electrophoresis and/or by a serum free light chain (sFLC) assay. [12] Immunofixation is much more sensitive than electrophoresis and can identify the monoclonal M protein involved. [13]

The median age of the patients (13 men and 9 women) was 64 years. 3 patients were suffering only from LCDD λ and 1 patient was suffering from non-secretory myeloma with FLC κ. 11 patients had multiple myeloma (6 with IgG κ, 1 with IgG λ, and 4 without isotype determination), 1 patient had type κ bi clonal gammopathy, 1 patient had AL amyloidosis, and 2 patients had no monoclonal gammopathy. 14 patients had a positive BJP (10 free κ and 4 free λ) and of these 14 patients 100% had an abnormal κ / λ ratio. 3 patients had no BJP but had an abnormal κ / λ ratio. Bence Jones proteinuria was detected in all patients with LCDD, non-secretory multiple myeloma and in the patient with AL amyloidosis. The median age of the patients (13 men and 9 women) was 64 years. 3 patients were suffering only from LCDD λ and 1 patient was suffering from non-secretory myeloma with FLC κ. This study shows that the FLC assay remains a more sensitive and earlier marker than the urine protein IF. Indeed, in the literature, several studies conducted in this direction have reported the same conclusion. Studies have shown the excellent sensitivity of the FLC assay for the detection of FLC in patients with LCDD. [14] Urine protein electrophoresis is still recommended for monitoring the response to treatment in patients with LCDD not measurable by serum protein electrophoresis. [15] It should be mentioned that some authors express somewhat different opinions.

11 patients had multiple myeloma (6 with IgG κ, 1 with IgG λ, and 4 without isotype determination), 1 patient had type κ bi clonal gammopathy, 1 patient had AL amyloidosis, and 2 patients had no monoclonal gammopathy. 14 patients had a positive BJP (10 free κ and 4 free λ) and of these 14 patients 100% had an abnormal κ / λ ratio. 3 patients had no BJP but had an abnormal κ / λ ratio. Bence Jones proteinuria was detected in all patients with LCDD, non-secretory multiple myeloma and in the patient with AL amyloidosis. The 11 patients with a GFR lower than 60 had an abnormal ratio □/□ and positive Bence Jones proteinuria. Myelograms were performed in 13 patients. Four patients had a plasma cell count of less than 10% and nine patients had a plasma cell count of more than 10%, including one patient with a count of 90%. Of the patients with a level below 10%, 3 patients had no BJP and of these 3 patients, only one had a normal ratio. All patients with a plasma cell count above 10% had a positive BJP and an abnormal □/□ ratio. Siegel et al [16] reported that FLC analysis provides a more accurate measure of light chain burden than urine protein electrophoresis. It has been proposed that the combination of serum electrophoresis and FLC assay evaluation be incorporated into an algorithm for the evaluation of monoclonal gammopathies. [16,17] Studies have shown that serum normalization of FLC concentration correlates with clinical efficacy of treatment. [18,19] Other studies show that urine test results underestimate the amount of sFLC produced and overestimate the response to therapy due to renal reabsorption and metabolism of sFLC. [20]

Conclusion

In conclusion, even if it has been demonstrated the higher sensitivity of the FLC assay in the diagnosis of monoclonal gammopathies, BJP still remains a marker that has its importance in these pathologies.

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