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International Journal of Clinical Biochemistry and Research

Journal homepage: https://www.ijcbr.in/

Original Research Article

Comparision of the diagnostic sensitivity of biochemical tests in the screening of monoclonal gammopathy

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ARTICLE INFO

Article history:
Received 17-03-2023
Accepted 27-03-2023
Available online 05-04-2023

Keywords:
Serum protein electrophoresis
Serum free light chain ratio
Free light chain
Immunofixation electrophoresis
Multiple myeloma

ABSTRACT

Introduction: Monoclonal gammopathy include several clinical variants ranging from asymptomatic MGUS, asymptomatic smouldering myeloma, multiple myeloma to aggressive plasma cell leukemia. The characteristic property of myeloma cells is the production and secretion of M protein. Due to its diverse structure, no single test can identify M protein accurately in all patients. A simple, non-invasive combination of tests is necessary for the screening of monoclonal gammopathy.
Aim: The present study aims to evaluate the diagnostic sensitivity of a panel of biochemical tests used in the screening of monoclonal gammopathy.
Material and Methods: This study was conducted retrospectively on the newly diagnosed cases of monoclonal gammopathy screened with SPE and ISUB/IT using sebia capillary electrophoresis and serum free light chain ratio using immunoturbidimetry method.
Results and Discussion: Out of the 142 patients included in the study, 120 had M band in SPE, 125 had monoclonal gammopathy by ISUB/IT and 121 patients had abnormal sFLCR. The diagnostic sensitivity obtained was 84.5% 88% and 85.2% for SPE, ISUB/IT and sFLCR respectively. Addition of sFLCR to SPE and ISUB/IT could identify 20 more patients who had no abnormality in either of these tests. The combined sensitivity of SPE and ISUB/IT was 88%, while that of SPE and sFLCR and a combination of all the three tests was 98.6%.
Conclusion: The simple, non-invasive, cost-effective screening panel of SPE plus sFLC ratio could be used as the initial screening method for patients with suspected monoclonal gammopathy with increased reliability.

1. Introduction

Monoclonal gammopathy are disorders characterised by clonal neoplastic proliferation of terminally differentiated B lymphocytes (plasma cells or myeloma cells) that primarily involve the skeletal system in a multifocal fashion. It includes several clinical variants ranging from MGUS, asymptomatic smouldering myeloma, non-secretory myeloma to an aggressive form of myeloma termed plasma cell leukemia.[1] Clinical features of monoclonal gammopathy result from the abnormal accumulation of clonal plasma cells within the bone marrow, which produce monoclonal protein with a reduction of normal immune function, as well as cause destructive bone lesions. The monoclonal protein / M protein / paraprotein is an immunoglobulin or a component/fragment of an immunoglobulin.[2] The detection and quantification of monoclonal proteins (M-proteins) is central to the diagnosis and monitoring of monoclonal gammopathy.

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https://doi.org/10.18231/j.ijcbr.2023.015

Rather than conferring a specific diagnosis, the detection of M-proteins is an indication for further investigation.[3]

Protein electrophoresis is the first choice of investigation to detect M protein in serum. Serum protein electrophoresis detects the presence of an M-protein in the serum and also enables the measurement of the concentration of the M-protein present in the M band along with the total protein concentration.

Immunofixation electrophoresis is considered the gold standard test for confirming the presence of these M proteins.[4] Serum immunofixation electrophoresis is more sensitive than serum protein electrophoresis and also determines the heavy and light chain type of the monoclonal protein. Gel-based serum immunofixation (IFE) and capillary-based immunotyping (ISUB/IT) methods are FDA-approved for detecting and isotyping M-protein.[5] In monoclonal gammopathy, intact monoclonal immunoglobulins are accompanied by variable concentrations of free light chains in blood which appear in the urine as Bence Jones proteins.

Turbidimetric immunoassays detect and quantify the kappa and lambda free light chains using monospecific antibodies directed against epitopes that are exposed only when the light chains are free (unbound to heavy chain) in solution. The approach to diagnosis is to quantitate both the Kappa and Lambda free light chain concentrations and use the ratio of Kappa to Lambda to detect unbalanced light chain synthesis.[6] A number of studies have assessed the use of sFLC analysis in the detection of plasma cell disorders. Studies have found that sFLC testing has superior diagnostic sensitivity to UPE for detecting plasma cell dyscrasias, particularly AL amyloidosis, light chain deposition disease, and nonsecretory myeloma.[7,8] International Myeloma Working Group guidelines 2014 recommends the use of sFLC assays as a first-line test along with SPE for MM and related plasma cell disorders. They also have clinical utility in serial monitoring of patients without measurable disease by other methods, as a valuable prognostic tool and as a component of the stringent complete response criterion in multiple myeloma.[1]

There are many studies showing the diagnostic accuracy of various investigations used in work up of myeloma and their clinical utility.[8]

2. Aim of our Study

To evaluate the diagnostic sensitivity of a panel of biochemical tests used in the screening of monoclonal gammopathy.

3. Materials and Methods

This was a retrospective study conducted from the data of patients investigated for the suspicion of monoclonal gammopathy in the Biochemistry department of Basavatarakam Indo-American cancer hospital and research institute.

3.1. Inclusion criteria

All the newly diagnosed, untreated myeloma suspected patient's samples received in the biochemistry laboratory for three tests serum protein electrophoresis, serum free light chains and serum immunofixation using ISUB/IT capillary electrophoresis over a period of four years, Jan 2019 to December 2022.

3.2. Exclusion criteria

Patient data lacking results of any one of the three tests were excluded from the study. Patients with a repeat of these three tests during the study period were also excluded.

3.3. Positive criteria

Presence of M band in SPE, disappearance of the M band in the antiserum treated serum for ISUB/IT using capillary electrophoresis; abnormal sFLCR of >1.65 or <0.26.[7]

Serum protein electrophoresis was performed using automated sebia capillarys system which separates serum proteins in alkaline buffer by capillary electrophoresis. After SPE was performed, samples were run for serum immunotying by immunosubstraction method using sebia immunotyping kit where serum proteins were mixed with specific antisera.

Immunoglobulins specifically react with their corresponding antiserum. At the end of the analysis, each antiserum pattern (IgG, IgA, IgM, κ and λ) is automatically overlaid with the ELP (Protein Electrophoresis) reference curve. Disappearance of the abnormality in the antiserum-treated pattern indicates the presence of a monoclonal protein.

Serum free light chains were measured using Freelite® Human Kappa and Lambda kit (The Binding site Ltd., Birmingham, UK) which evaluates the concentration of soluble antigen by immunoturbidimetry method. Statistical analysis was done using Microsoft excel to calculate the sensitivity of individual test and various combination of tests.

4. Results

The total number of newly diagnosed plasma cell neoplasm with the three investigations SPE, ISUB/IT using capillary electrophoresis and sFLCR were 142 during our study period. 91(64%) among them were male patients and 51 (36%) were females. Their age was ranging from 35 to 91 years with a median age of 61 years.

Majority of patients had gamma bands in SPE while 13% (n=15) had beta bands. In ISUB/IT using capillary the predominant type of gammopathy was IgGK(41%) followed

Table 1: Results of SPE, serum ISUB/IT using capillary electrophoresis and serum free light chain ratio of the newly diagnosed cases of monoclonal gammopathy

Test	Number of patients (percentage)
SPE using capillary	
M band present	n=120 (84.5%)
M band absent	n=22 (15.5%)
ISUB/IT using capillary	
Gammopathy present	n=125(88%)
Gammopathy absent	n=17 (12%)
Abnormal sFLCR	n=121(85.2%)
Kappa type	n=81(57%)
Lambda type	n= 40(28.2%)

by IGGL(22%), IGAK(10%), IGAL(5%), IGMK(2%) only Kappa (2%) and only Lambda(6%).

There were 22(15.5%) patients of monoclonal gammopathy with no band in SPE. Five among them obtained a gammopathy when ISUB/IT was run. While the rest 17(12%) patients didn't show any abnormality even with ISUB/IT. 15 patients among these which were missed by SPE+ISUB/IT had altered serum free light chain ratio with involved chain concentration >100 mg/dl. Remaining two patients were showing no abnormality in all the three tests. 24 hour urine for screening of monoclonal gammopathy with all these three tests was done for these patients. Results of urine were also negative. There were 21 patients from our study who had a normal sFLCR inspite of the presence of M band in SPE and gammopathy in ISUB/IT.

Results of diagnostic sensitivity of each test and combination of tests are as follows:

Table 2: Diagnostic sensitivity of biochemical tests in the screening of monoclonal gammopathy

	True positive (n)	False negative (n)	Diagnostic sensitivity (%)
SPE alone	120	22	84.5
ISUB/IT alone	125	17	88.0
sFLCR alone	121	21	85.2
SPE + ISUB/IT	125	17	88.0
SPE + sFLCR	140	2	98.6
SPE + ISUB/IT + sFLCR	140	2	98.6

5. Discussion

Monoclonal gammopathy are characterized by clonal expansion of plasma cells which secrete monoclonal proteins. Monoclonal proteins can be intact immunoglobulins, monoclonal light or heavy chains, or both. Detection of the monoclonal immunoglobulin or its fragments plays a central role in the diagnosis of monoclonal gammopathy. The diversity in the structure and the concentrations of M-proteins make it challenging to diagnose and monitor plasma cell disorders.[6]

SPE is the first choice of test for the screening of monoclonal gammopathy. The sensitivity of SPE alone in identifying monoclonal gammopathy was found to be 74% from a study by Mayo group,[8] while it was 94% and 90% by Mc Taggart et al.[3] and Bakker et al.[9] respectively. In our study, the sensitivity is 84%. Among the 22 patients of monoclonal gammopathy with no M band in SPE, 5 patients showed monoclonal gammopathy by ISUB/IT with a sensitivity of 88%, making it more sensitive than SPE. ISUB/IT confirms the presence of monoclonal protein and also determines the immunoglobulin heavy chain class and light chain type associated with it.

Studies have shown the higher sensitivity of IFE or ISUB/IT compared to SPE in the detection of monoclonal gammopathy.[3]

The sFLCR is mainly affected by the dominant production of one type of light chain by clonally proliferated plasma cells, and the suppressed production of normal light chain of the other type altering the ratio. Altered sFLCR was observed among 121 patients of monoclonal gammopathy with a sensitivity of 85.2%. Out of 121 patients, 81 patients (67%) had kappa type and 40 patients (33%) had Lambda type of monoclonality showing the predominance of kappa type of gammopathy.

In patients with intact immunoglobulin gammopathy (n=114), 82% (n=94) had altered sFLCR.

These results indicate that excessive production of free light chains was observed in the majority of patients who had intact monoclonal immunoglobulin detected in ISUB/IT. These findings are consistent with a study by Katzman et al[6] in which abnormal serum FLCR was detected in 90%–95% of intact immunoglobulin MM. A study by Maciej Korpysz et al., found abnormal FLCR in 86.4% and 88.9% of all patients who had κ or λ band detected on sIFE.[10]

However, 18% (n=20) patients had normal sFLCR despite kappa or Lambda band being detected on ISUB/IT along with a heavy chain band. Similar findings were reported by Singhal et al.[11] who found sFLCR normal in 34% of cases with positive sIFE.

When the sensitivity of different combination of tests was calculated (Table 1) in our study, SPE and sFLCR combined was 98.6% and that of a combination of SPE, ISUB/IT using capillary and sFLCR was also 98.6%. When sFLCR was omitted and sensitivity of combined SPE and ISUB/IT using capillary was calculated, it was 88%. A panel with all three serum assays plus urine IFE detected 98.6% of the cases by the Mayo Clinic group. A combination of SPE and sFLC were able to detect 28 out of those 30, addition of sIFE to SPE could detect 29 cases. And a combination of all three tests, detected all 30 cases of monoclonal gammopathy

in their study.[8] In another study by Mc Taggart et al[3] sensitivity of SPE was 94.4% and addition of sFLC testing alongside SPE increased the sensitivity to 100.0%, while the addition of UPE to SPE gave a smaller increase in sensitivity to 96.1%. Addition of either sIFE/sFLC or both of these tests, to SPE detected more number of cases as was observed from studies conducted by Piehler et al.,[12] Katzmann et al., Hill et al.,[13] where omission of either sFLC or sIFE resulted in loss of sensitivity.

Addition of sFLCR could identify patients of monoclonal gammopathy which could not be detected by SPE or ISUB/IT using capillary. LCMM accounts for around 20% of all cases of MM characterised by the presence of monoclonal FLCs in the serum or urine, in the absence of intact monoclonal immunoglobulins, alongside clonal bone marrow plasma cells and the presence of end organ damage. Diagnostic sensitivity of 100% of sFLCR for LCMM has been found in various studies.[14–17] In our study sFLCR could identify the patients of LCMM who were not showing a band in SPE or ISUB/IT using capillary. There were two patients who had negative findings in all these three tests. Addition of urine panel also did not give any conclusive result. Only biopsy of the lesion could identify them as solitary plasmacytoma and renal AL amyloidosis respectively. Solitary plasmacytoma are characterised by the absence of M protein in serum or urine.[1] Abnormal sFLCR are found only in 88–100% of patients with LCDD, of which approximately 1/3 of patients are negative by sIFE. Definitive diagnosis of AL amyloidosis or LCDD is established by a tissue biopsy and histological examination.[14]

In view of a comparable overall sensitivity for the combination of SPE and sFLCR and combination of all the three tests, a simple first line screening panel of tests would be SPE and sFLCR for the identification of monoclonal gammopathy.

6. Conclusion

In conclusion, the diagnostic sensitivity of combined sPEP and sFLC ratio is 98.6%, which is equivalent to combined sPEP, sFLC & ISUB/IT in detecting the presence of M-protein in MM patients. SPE plus sFLCR algorithm could be used as the initial screening method for patients with suspected MM with increased reliability. This simple screening algorithm can be performed in most of the laboratories as it seems cost-effective & less time-consuming for the diagnosis of monoclonal gammopathy.

7. Abbreviations

SPE/sPEP: Serum protein electrophoresis, ISUB/IT: Immunosubstraction or immunotyping using capillary electrophoresis. sFLCR: Serum free light chain ratio, MGUS: Monoclonal gammopathy of undetermined significance; MM: Multiple myeloma; FLC: Free light chain; IFE: Immunofixation electrophoresis; LCMM: Light chain multiple myeloma; LCDD: Light chain deposition disease.

8. Source of Funding

None.

9. Conflict of Interest

None.

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Cite this article: Anjum A, Bagade S, Boyella PK. Comparision of the diagnostic sensitivity of biochemical tests in the screening of monoclonal gammopathy. *Int J Clin Biochem Res* 2023;10(1):96-100.