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Research Paper

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Retrospective analysis of Immuno-fixation Electrophoresis in the Detection of Multiple Myeloma: An Experience of a Corporate Laboratory

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***Abstract*—**Multiple Myeloma (M.M) is a malignant proliferation of B cell lineage mutated plasma cells, which secretes immunoglobulin as resulting in monoclonal elevations of immunoglobulins of same clonality. These monoclonal immunoglobulins can be identified by Immunofixation electrophoresis. Identification of these immunoglobulins helps to differentiate between suspected cases of multiple myeloma and benign conditions. Serum samples from 112 suspected cases of M.M were subjected to immunofixation electrophoresis on Sebia Minicap System. Monoclonal band was detected and typing of heavy chain and light chain was performed. We observed approx. 20.5 % cases had monoclonal gammopathy. Out of these approx. 60.8 % cases showed IgG type of monoclonal gammopathy. IFE is specialized non-invasive test, which can be used to identify monoclonal gammopathy where suspicion of MM is very high and where other tests are inconclusive. IFE should be recommended to the suspected cases of multiple myeloma.

Keywords— Multiple Myeloma, B-Cells, Iummunofixation Electrophoresis, Plasma Cells

I. INTRODUCTION

Multiple Myeloma (M.M) is a malignant proliferation of B cells (Plasma Cells). These abnormal cells secrete immunoglobulins resulting in increase of single type of antibodies is called as monoclonal gammopathy. Diagnosis of multiple myeloma is very important and involves skeletal imaging methods such as X-rays, CT scans and MRI scans, bone marrow cytology examination, urine Bence-Jone protein, and erythrocyte sedimentation rate along with physical evaluation, patient history, symptoms, and diagnostic testing results are routinely used for the diagnosis of Multiple myeloma.

Serum protein electrophoresis along with immunofixation electrophoresis provides an excellent non-invasive technique to identify abnormal immunoglobulins [1].

Multiple Myeloma (M.M) accounts for 10-15% of the haematological malignancies [2] and symptoms may range from back pain, fatigue, weakness or osteopenia, osteolytic lesions to spontaneous fractures and recurrent infections MM is characterized by dissemination of multiple tumor cells throughout the bone marrow. MM is a debilitating neoplasm with subtypes ranging from monoclonal gammopathy of unknown significance (MGUS) to plasma cell leukaemia[3].

MM can be easily missed or misdiagnosed. As early MM patients may not present obvious changes of bone marrow and bone in patients. Bone marrow cytology being invasive makes patients suffer and lead to limited application [4]

Urine Bence-Jone protein has also low positivity rates. Erythrocyte sedimentation rate has poor specificity in detecting MM. Therefore, We need more accurate and painless diagnostic method, which can be useful in early diagnosis of MM [4].

Serum protein electrophoresis is one of the invasive screening test now used to detect the monoclonal bands. However SPE cannot identify the characteristics of the monoclonal gammapathy like the type and extant of elevation of monoclonal protein[5]. The prevalence of monoclonal proteins has been reported to be detected by IFE in cases of hypogammaglobulinemia has been reported to be approximately 10 % which may be missed in SPE [5]. Besides, monoclonal proteins can migrate from anywhere from α region to γ region. High-resolution SPE techniques such as capillary electrophoresis can detect these small M proteins However, a significant proportion of them can be missed. Hence, a more sensitive test such as IFE can be helpful in detecting the monoclonal paraproteins [6].

The purposes of this study were to retrospectively analyse and evaluate the performance of IFE in detecting the M protein and compare with the literature.

II. METHODOLOGY

This retrospective study included a total of 112 cases of suspected myeloma patients who were advised IFE (immunofixation electrophoresis) in Health Quest Laboratories, Gurugram , Haryana, India during January 2018 to September 2019. The electrophoresis of serum proteins was carried out on fasting samples, taken on Vacutainer Tubes after centrifugation at 3000 RPM for 10 minutes. Haemolysed, opalescent and lactescent samples were excluded. Serum immunotyping were minicap Flex Piercing capillary electrophoresis system using the Minicap Protein (E) 6 kit and Immunotyping kit, respectively (Sebia, Inc.; Norcross, GA, USA). Proteins patterns were detected and analysed using PHORESIS Software. No staining was required. Results were represented as an electrophoretic curve. All assays were performed according to the manufacturer's instructions. Assays were validated and are in clinical use in a NABL compliant laboratory.

III. RESULTS

There were 110 clinically suspected cases of multiple myeloma, whose serum samples were received in for immunofixation electrophoresis during the period from Jan 2022 to September 2022.

There were 66(60%) males and 44 (40 %) females. Out of these 110 cases, 23(20.5%) were found to have monoclonal gammopathy. Figure 1 is representative serum protein curve pattern of a Monoclonal Gammopathy and Figure 2 represent as normal pattern of serum protein electrophoresis.

IFE results revealed that among these cases, 14 patients (60.8%) had showed M band with IgG monoclonal gammapathy and 7 cases (30.4%) showed IgA type monoclonal gammopathy (Table 1). Further, 1 patient showed IgM type monoclonal gammopathy. Kappa Chain restriction was observed in 10 (43.4%) Cases while lambda chain restriction was observed in 12 (52.1%) patients. Figure 3 represent a immunofixation electrophoresis showing M Band (IgA/ Lambda)

Interestingly, we also observed only lambda chain restriction in 1 (4.3 %) patient suggesting rare ?IgD/IgE monoclonal gammopathy. Due to unavailability of the specific reagents ?IgD/IgE could not be confirmed.

IV. DISCUSSION

Multiple myeloma (MM) is a dreaded diseases characterised by the accumulation of clonal, malignant plasma cells in the bone marrow. The cause of myeloma is unknown. MM accounts for about 10-15% of haematological malignancies. [2]. The median age for diagnosis is generally 65 years, with less than 3% of patients presenting at younger age (<40 years) with clinical presentation ranging from bone pain and pathological fractures, anaemia (bone marrow failure) and recurrent infections. The diagnosis of myeloma depends on detection increased plasma cells (>10%) in the bone marrow along with detection of monoclonal band on serum protein electrophoresis along with immunofixation electrophoresis [6][7].

Serum protein electrophoresis and immunofixation electrophoresis are non-invasive techniques which complements Bone marrow results [7]. In present study, out of the 110 suspected cases of multiple myeloma, 23 (20.5%) cases were found to have monoclonal gammopathy or Paraproteinaemia on IFE. These findings are in agreement with findings of Chopra et al., who reported 24.4% patients showing M protein by SPEP alone [8]. However few other studies observed presence of monoclonal band in 9.2% and approx. 4.5% cases respectively. [9] [10].

Among the 23 Monoclonal band positive cases, light chains analysis revealed, kappa (κ) and lambda (λ) chains restriction in 43.4 % and 52.1 % cases respectively. These findings are in contrast with Singh et al, who reported kappa (κ) and lambda (λ) chains restriction in in 91.6% and 8.4% cases respectively [11]. However a Spanish study reported IgG most common monoclonal gammopathy (55.8%) followed by IgA (20.8%) and IgM (13.6%) [12]. Further in our study IgM was also observed along with? IgD/IgE.

In our studies predominant IgG (60.8%) was heavy chain isotype and while IgA was in (30.4%) cases and 4.3% cases showing IgM and rare? IgD/IgE monoclonal gammopathy case. These findings are in agreement with Singh et al.[13].

V. CONCLUSION AND FUTURE SCOPE

IFE is powerful and non-invasive pain less techniques for the confirmation of Multiple Myeloma, which should be used to confirm findings of SPE , identify the clonality of Monoclonal bands. IFE has also been helpful in detecting residual/minimal residual disease. IFE can also be helpful in those cases, where M band is masked under the normal peaks. However, IFE is costly to perform but it is definitely can be more sensitive than the routine SPEP.

Table 1. Table showing the clonality pattern of M Band

M BAND CLONALITY			
IgG	IgM	IgA	?IgD/E
14	01	07	01
60.8%	4.34	30.4	4.34

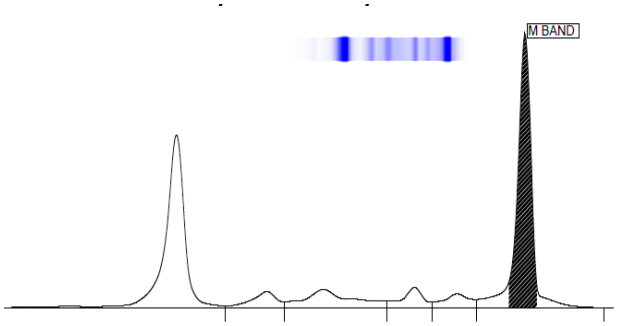

Figure 1. Protein Electrophoresis pattern of representative M Band

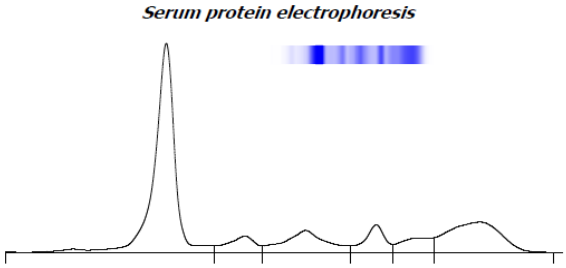

Figure 2. Protein Electrophoresis pattern of representative Normal Sample

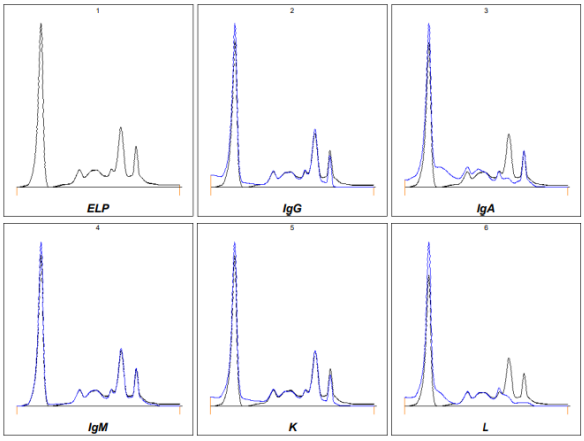

Figure 3. Immunotyping pattern of a representative M Band (IgA/ Lambda)

Acknowledgment

NA

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