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Evaluation of Serum Immunofixation Electrophoresis And Protein Electrophoresis Data

Yeşim CİVİL[1], Birşen BİLGİCİ[2], Memiş Hilmi ATAY[3]

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ABSTRACT

Background and aim: Monoclonal gammopathies are plasma cell dyscrasias with production of homogeneous protein called paraprotein or M protein. As it can accompany many hematological malignancies, it can cause many complications in non-malignant conditions and may present with various clinical problems. Early diagnosis of patients with this entity is very important to guide clinicians in order to intervene before the disease progresses to a malignant picture. Although protein electrophoresis, which is a simple and inexpensive method, is the first step in the diagnosis of monoclonal gammopathies today, the gold standard for diagnosis is immunofixation electrophoresis.
Materials and methods: In our study, we retrospectively analyzed the results of protein electrophoresis and immunofixation electrophoresis of patients who applied to the central laboratory of our hospital with the suspicion of monoclonal gammopathy.
Result and Conclusion: We observed that immunofixation electrophoresis has higher sensitivity than protein electrophoresis. In this context, we concluded that it may be beneficial to use immunofixation electrophoresis and protein electrophoresis data together with some additional tests for further diagnosis.

Keywords: Protein Electrophoresis, Immunofixation Electrophoresis, monoclonal gammapathies

Serum İmmunofiksasyon Elektroforezi ve Serum Protein Elektroforezi Verilerinin Değerlendirilmesi

ÖZ
Amaç: Monoklonal gamopatiler, paraprotein veya M proteini adı verilen homojen protein üretimi ile plazma hücre diskrazileridir. Birçok hematolojik maligniteye eşlik edebildiği gibi malign olmayan durumlarda da birçok komplikasyona neden olabilmekte ve çeşitli klinik problemlerle karşımıza çıkabilmektedir. Bu antiteye sahip hastalarda erken teşhis, hastalık malign bir tabloya ilerlemeden müdahale edebilmek için klinisyenlere rehberlik etmesi açısından çok önemlidir. Günümüzde monoklonal gamopatilerin tanısında basit ve ucuz bir yöntem olan protein elektroforezi ilk adım olsa da tanı için altın standart immünfiksasyon elektroforezidir.
Yöntem: Çalışmamızda monoklonal gamopati şüphesi ile hastanemiz merkez laboratuvarına başvuran hastaların protein elektroforezi ve immunofiksasyon elektroforezi sonuçlarını retrospektif olarak inceledik.
Bulgular ve Sonuç: İmmünfiksasyon elektroforezinin protein elektroforezinden daha yüksek duyarlılığa sahip olduğunu gözlemledik. Bu bağlamda immünfiksasyon elektroforezi ve protein elektroforezi verilerinin bazı ek testler ile birlikte kullanılmasının ileri tanı için faydalı olabileceği kanaatine vardık.

Anahtar Kelimeler: Protein elektroforezi, immunfiksasyon elektroforezi, monoklonal gamopati

[1] Dr. Research Assistant, Ondokuz Mayıs University, Faculty of Medicine, Department of Biochemistry, Samsun/Turkey, ORCID ID: 0000-0002-9662-244X, email: yesimcivil@gmail.com **(Corresponding author)**
[2] Prof. Dr., Ondokuz Mayıs University, Faculty of Medicine, Department of Biochemistry, Samsun/Turkey, ORCID ID: 0000-0001-7783-5039, email: b_bilgici@yahoo.com
[3] Assoc. Prof. Dr., Ondokuz Mayıs University, Faculty of Medicine, Department of Internal Medicine, Division of Hematology, Samsun/Turkey, ORCID ID: 0000-00001-9666-6955, email: hilmi.atay@omu.edu.tr

INTRODUCTION

Monoclonal gammopathies are a group of diseases characterized by the proliferation of an immunologically homogeneous protein-producing plasma cell clone, often called paraprotein or monoclonal protein (Öge & Parman, 2004).

M-protein (paraprotein, monoclonal protein, M-component) is a monoclonal immunoglobulin secreted in abnormally increased amounts by a clone of plasma cells. It can be detected by immunofixation of serum, urine, or rarely other body fluids (eg, jejunal fluid in alpha heavy chain disease) or by measuring the amount of serum light chains (Bird et al., 2009).

An immunoglobulin molecule is a Y-shaped glycoprotein consisting of two identical heavy chains (α, γ, μ, δ or ε) and two identical light chains (κ or λ). These polypeptide chains are held together by non-covalent interactions stabilized by disulfide bonds (Chou, 2007). Heavy polypeptide chains are subdivided: IgG has four subclasses (IgG1, IgG2, IgG3, and IgG4) and IgA has two subclasses (IgA1 and IgA2). The M-protein may be an intact immunoglobulin (may contain both heavy and light chains), may consist of only light chains (light chain myeloma, light chain deposition disease, AL light chain amyloidosis) or, rarely, only heavy chains (heavy chain disease, heavy chain deposition disease) (Koç, 1997).

The presence of M-protein in serum or urine indicates underlying clonal plasma cell disorder or lymphoproliferative disorder. In some cases, the clonal process that produces the M-protein is malignant and this protein is associated with evidence of neoplastic disease infiltrating bone, lymph nodes, liver, spleen, or other organs (eg, Multiple Myeloma, Solitary Plasmacytoma, Waldenstrom Macroglobulinemia). However, in some cases, the M-protein is produced by premalignant small circumscribed clonal expansion and causes no symptoms (eg, Monoclonal Gammopathy of Undetermined Significance [MGUS]). Plasma cell disease accounts for 7% of all hematological malignancies (Jemal et al, 2005).

In general, premalignant disorders may cause some clinical manifestations until malignant transformation occurs. However, in the case of plasma cell disorders, even if the clonal enlargement is premalignant, fatal conditions such as cold agglutinin disease, increased viscosity, cryoglobulinemia, organ dysfunctions as a result of accumulation in the tissues, neuropathy or loss of function may occur due to some potential adverse effects of the secreted M-protein as the clonal cells secrete immunoglobulin antibodies (Merlini & Stone, 2006).

Accordingly, M-proteins may be part of asymptomatic limited clonal enlargement of plasma cells (MGUS), may be a precursor to a malignancy (myeloma, macroglobulinemia), or clonal enlargement, although in limited quantities, can lead to life-threatening complications (Primary Amyloidosis). Therefore, the clinician who notices a patient with M-protein production should make the appropriate diagnosis and initiate effective treatment in a timely manner to prevent irreversible organ damage and/or shortening of life (Merlini & Stone, 2006). A sensitive, rapid and reliable method is required to detect

the presence of M-protein in serum or urine and identify it by heavy chain and light chain type (Katzmann & Kyle, 2006).

In the evaluation of a patient for the presence of serum M-proteins or increased total serum proteins, electrophoretic techniques are classically used, supplemented by additional testing, such as protein quantification, to determine whether the protein is due to a single clone (monoclonal) (Merlini & Stone, 2006).

Electrophoresis is a method that separates proteins based on their physical properties. The serum is applied to a specific medium (such as cellulose acetate, agarose gel) and current is applied. The separation of serum proteins is provided according to the net charge (positive or negative), the size and shape of the protein.

Serum protein electrophoresis (SPE) is an inexpensive and simple screening procedure to detect M-protein. SPE is usually done by the agarose gel method (agarose gel electrophoresis). If M protein is detected, the amount of M protein can also be measured by densitometric scanning of the gel. SPE thus serves two purposes: to detect the presence or absence of an M-protein in the serum and to enable measurement of the concentration (size) of the M-protein along with the total protein concentration.

In electrophoretic methods, after electrophoresis is complete, proteins are divided into five general regions: albumin, alpha-1, alpha-2, beta, and gamma. These regions do not refer to the immunoglobulin subclass to which the M-protein may belong, and only indicate where it moves through the support medium. Various classes of immunoglobulins (IgG, IgA, IgM, IgD, and IgE) are usually found in the gamma region of SPE, but they can also be found in the beta-gamma, beta and sometimes alpha-2 globulin domain regions. A monoclonal protein is found as a single narrow peak, such as a church tower, or as a dense, discrete band in the gamma, beta, or alpha-2 region by densitometric scanning of the agarose gel. Approximately 5 percent of sera with M-protein detected have two M-proteins (biclonal gammopathy) (Kyle, Robinson, & Katzmann, 1981). Serum immunofixation should be performed to determine clonality (monoclonal, cyclonal, polyclonal).

Although SPE is considered a relatively simple laboratory method for M-protein detection, immunofixation electrophoresis is considered the gold standard for confirming the presence of these proteins and distinguishing light and heavy chains in MM (Multiple Myeloma). The combination of SPE and SIFE (Serum immunofixation elektrophoresis) techniques increases the sensitivity of detecting M-protein in MM patients by up to 97% (Aita, Arantes, Aita, & Silva, 2015). With SIFE, the type of monoclonal protein synthesized in monoclonal gammopathies is defined. This technique combines electrophoresis and immunoprecipitation techniques. Electrophoresis is based on the movement of Ig in the electrical field, which have varying charges at different pH values. Ig's have a negative charge in alkaline pH medium and move towards the anode, and in acid medium they have a positive charge and move towards the cathode. In this way, the proteins are separated from each other. In SIFE, immunoprecipitation occurs by using Ig-specific-antisera together with electrophoresis, and Ig's become visible as bands (Ercan et al, 2013).

Serum immunofixation is more sensitive than SPE and also determines the heavy and light chain type of the monoclonal protein. However, unlike SPE, immunofixation does not provide an estimate of the size (serum concentration) of the M-protein and therefore must be done in conjunction with electrophoresis. Serum immunofixation should be performed when a sharp band or peak is found in the agarose gel with SPE, or when multiple myeloma, macroglobulinemia, primary amyloidosis, solitary or extramedullary plasmacytoma, or a related disorder is suspected despite normal SPE.

Serum immunofication is critical for distinguishing the monoclonal increase in immunoglobulins from the polyclonal increase. In conventional immunofixation, the patient's serum is separated into at least five separate lanes by electrophoresis. After electrophoretic separation of serum proteins, each sample is usually coated with three different monospecific antibodies for the heavy chain component (anti-gamma, anti-alpha, anti-mu) and two (anti-kappa, anti-kappa) for the light chain component. The proteins are allowed to precipitate forming antigen-antibody complexes, then unprecipitated proteins are washed and the remaining immunoprecipitates are stained. Unlike immunoelectrophoresis, serum must be diluted before applying to the gel. Some manufacturers recommend a standard dilution for each analyte (Keren, 1999).

Immunofixation can detect the presence of M-protein at concentrations above 0.02 g/dL in serum and 0.004 g/dL in urine (International Myeloma Working Group, 2003).

While interpreting the immunofixation electrophoresis, it is first evaluated whether there is a monoclonal band in the α2, β and γ globulin regions of the protein electrophoresis in the first column. G, A, M, Kappa and Lambda regions are checked for a clear band. If there is a band, it is evaluated whether it is in the same mobility as the band in the protein region in the first column. If more than one band that migrates in different places in the same immunoglobulin class is detected, it is examined whether this is true biclonal or oligoclonal gammopathy.

In our study, it was aimed to retrospectively examine the IFE and Protein Electrophoresis data, which were studied between January 2017 and December 2019 in the Central Laboratory of Faculty Hospital, and compare the findings with the literature.

METHODS

In this study, the results of immunofixation electrophoresis of 6767 patients who applied to our hospital between 2017 and 2019 in the Medical Biochemistry Central Laboratory of Faculty of Medicine were evaluated retrospectively. 359 SPE results of these patients were evaluated retrospectively. The study protocol was approved by the School of Medicine Ethics Committee (B.30.2.ODM.0.20.08/366-393).

By examining the laboratory information system, patient outcomes were evaluated in terms of the frequency of monoclonal gammopathy, the type of gammopathy and the diagnosis they received. If there was more than one electrophoresis result of the same patient, the data obtained from the first application of the patient were included in the study.

SIFE and SPE tests were performed on the INTERLAB G26 device. SRE602K kit was used for SPE and SRE 628K kit was used for SIFE. Agarose gel containing Tris-Barbital buffer was used as the agarose gel plate. Acid Blue containing concentrated Acetic Acid solution was used as the dyeing solution.

In the SPE technique, the patient serum was applied on an agarose gel plate containing Tris-Barbital buffer at pH 8.9 and exposed to an electric field. After the electrophoretic migration step of the proteins, the agarose gel plate was denatured and stained with Acid blue, and dried after washing and clearing the dye. The shapes on the plate were scanned densitometrically using the Elfolab program and the percentage values of each fraction were obtained. Band concentrations expressed in g/dL were calculated by multiplying the sample's total protein concentration by the percent band.

In the SIFE technique, the patient's serum was applied on an agarose gel containing Tris-Barbital buffer at alkaline pH and exposed to an electric field and electrophoretically decomposed. Before the serums were studied, 1:7 dilution was applied for IgG and 1:4 dilution for IgA, IgM , □ light chain, □ light chain. After migration, the fixative solution with the code SCE240M kit was used to fix all the proteins. Then, Anti-Human-IgG, Anti-Human-IgA, Anti-Human-IgM, Anti-Human-Kappa, Anti-Human-Lambda antibodies belonging to the kit with the code SCE 323M were applied to the samples. After the formation of insoluble compound forming a precipitate band due to antigen-antibody interaction, the agarose gel was denatured (3 min at 60°C) and washed, denatured again, stained with Acid blue, and dried after the washing step (3 min at 40°C). While sharply focused bands are observed in monoclonal gammopathies, diffuse zone formation is observed in polyclonal gammopathies.

RESULTS

In our study, 6767 serum immunofixation electrophoresis and 359 serum protein electrophoresis analysis results were evaluated retrospectively. According to the SIFE results, paraprotein bands were detected in 1446 different patients. When the SPE results of the same patients were examined, the presence of monoclonal gammopathy was detected in 187 patients. In our study, we detected a paraprotein peak in 21.36% of the patients in whom SIFE was studied and in 52% of the patients in whom SPE was studied.

According to the SIFE results, of 1446 patients with paraproteinemia, 54.22% were male and 45.78% were female, with a mean age of 66.61 in men and 66.9 in women (Table 1).

Table 1. **Classification of cases by gender**

Gender	Number of patients	Percentile (%)	Average Age
Male	784	54.22	66.61
Female	662	45.78	66.91
Total	1446	100.00	66.75

When the diagnoses of the patients participating in our study were examined, it was seen that the most common multiple was Multiple Myeloma (30.01%), followed by Acute Myelofibrosis in 330 patients (22.82%), Anemia in 90 patients (13.48%), and Non-Hodgkin Lymphoma in 83 patients. (5.74%), Fever of Unknown Origin (FUO) in 60 patients (4.15%), Chronic lymphocytic leukemia (CLL) in 40 patients (2.76%), Waldenstrom Macroglobulinemia (WM) in 23 patients (1.59%), Chronic myeloid leukemia(CML) in 15 patients (1.03%), Hairy Cell Leukemia (0.41%) was diagnosed in 6 patients and Heredofamilial Primary Amyloidosis (0.27%) in 4 patients. The remaining 256 patients had non-hematological diagnoses (such as Rheumatoid Arthritis, Sarcoidosis, Ankylosing Spondylitis, Chronic kidney disease, Acute kidney failure) (Table 2).

Table 2. Classification of cases in terms of diagnosis

Diagnosis	Number of patients	Percentile (%)
Multiple Myeloma	434	30.01
Acute Myelofibrosis	330	22.82
Anemia	195	13.48
Non-Hodgkin Lymphoma	83	5.74
Fever of Unknown Origin (FUO)	60	4.15
Chronic lymphocytic leukemia (CLL)	40	2.76
Waldenstrom Macroglobulinemia	23	1.59
Chronic myeloid leukemia (CML)	15	1.03
Hairy Cell Leukemia	6	0.41
Heredofamilial Primary Amyloidosis	4	0.27
Other (RA, Sarcoidosis, CKD etc.)	256	
Total	1446	

When 1446 patients were examined in types of paraproteinemia, in order of frequency, we detected IgG-Kappa in 426 patients (29.4%), IgG-Lambda in 183 patients (12.6%), Biclonal G-Kappa in 80 patients (5.53%), Free Lambda in 74 patients (5.12), Biclonal G-Kappa+A-Kappa in 66 patients (4.56%), M- Kappa (4.4%) in 65 patients, Biclonal G-Kappa+A-Kappa in 55 patients (3.8%), M-Lambda in 52 patients (3.5%), A-Kappa in 33 patients (2.2%), and A-Lambda in 32 patients (2.2%). The results of patients with oligoclonal, triple or more band combinations and patients with other double band combinations are not reflected in the table (Table 3).

Table 3. Classification of cases according to paraprotein type

Paraprotein Type	Number of patients	Percentile (%)
G- Kappa	426	29.46
G-Lambda	183	12.66
Biclonal G-Kappa	80	5.53
Free Lambda	74	5.12

Table 3. (continued)

Biclonal G-Kappa+Free Lambda	66	4.56
M-Kappa	65	4.5
Biclonal G-Kappa+ A-Kappa	55	3.80
M-Lambda	52	3.60
A-Kappa	33	2.28
A-Lambda	32	2.21
Total	1446	

CASE 1:

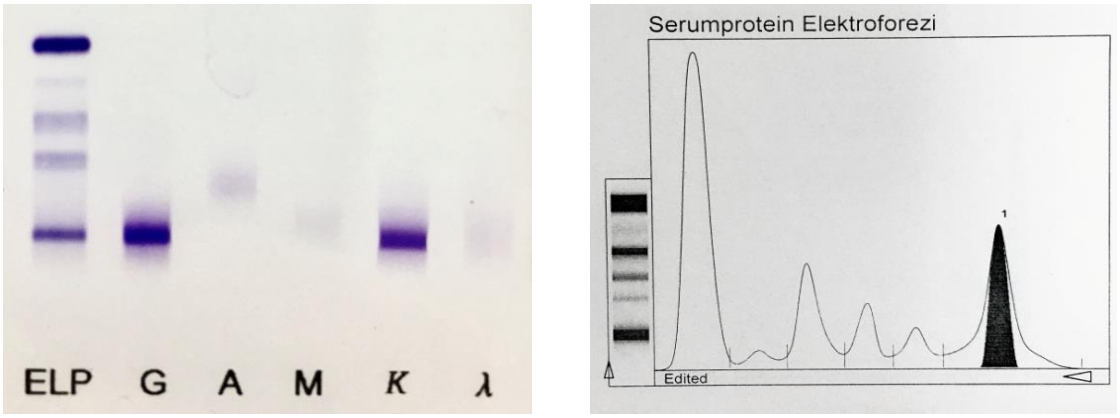

Figure 1. Serum immunofixation electrophoresis sample (left) and serum protein electrophoresis sample (right) of a patient with IgG Kappa paraproteinemia. In the left figure, a band with sharp borders is seen in the same migration in the G and Kappa regions and the protein region in the first column. In the figure on the right, a long narrow peak-shaped monoclonal gammopathy band with sharp borders is seen in the gamma region.

CASE 2:

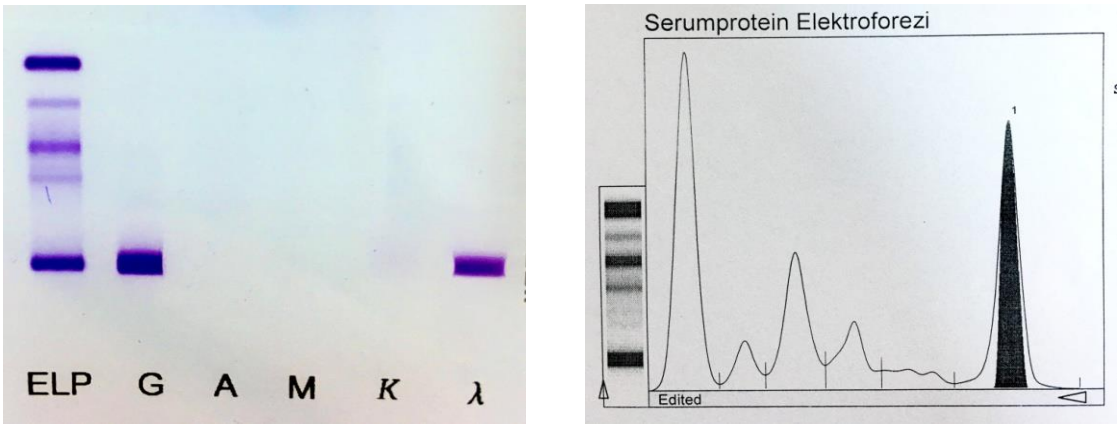

Figure 2. Serum immunofixation electrophoresis sample (left) and serum protein electrophoresis sample (right) of a patient with IgG Lambda paraproteinemia. In the left figure, a band with sharp borders is seen in the same migration in the G and Lambda regions and the protein region in the first column. In the figure on the right, a long narrow peak-shaped monoclonal gammopathy band with sharp borders is seen in the gamma region.

CASE 3:

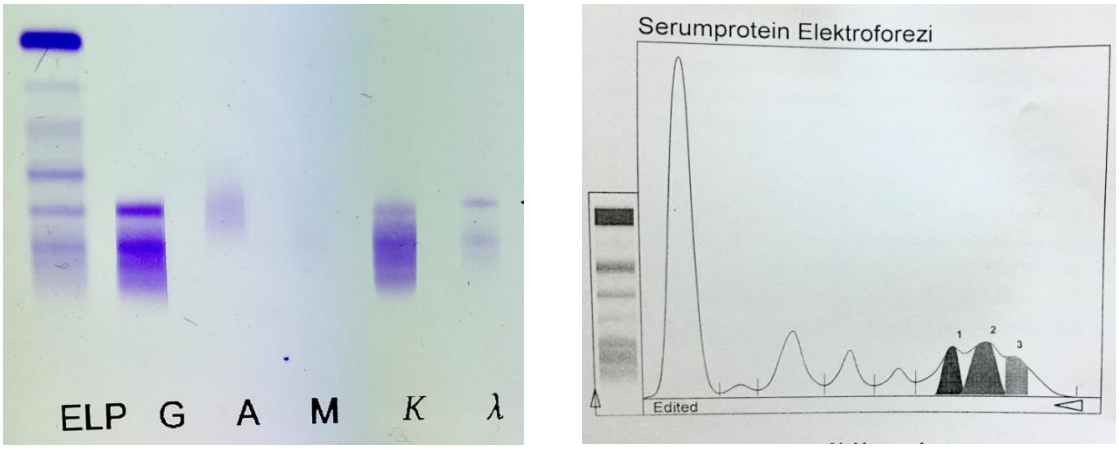

Figure 3. Serum immunofixation electrophoresis sample (left) and serum protein electrophoresis sample (right) belonging to a patient with oligoclonal gammopathy. In the figure on the left, it is seen that 4 of the bands with sharp borders in the G region have equal migration levels in the Kappa region and 2 in the Lambda region. In the figure on the right, the presence of 3 long narrow peak-shaped bands with sharp borders (oligoclonal gammopathy) is seen in the gamma region.

Table 4. Mean serum IgG concentrations of IgG Kappa cases in SPE(+) and (-) groups

Gammopathy Status in SPE	Number of patients	Mean serum IgG concentration(g/L)	
Monoclonal G Kappa with SIFE + There is monoclonal gammopathy with SPE	57	23.954	
Monoclonal G Kappa with SIFE + No monoclonal gammopathy with SPE	65	10.058	p<0.05
Total	122		

When 426 patients with monoclonal G Kappa results, which is the most common type of gammopathy according to serum immunofixation data, were examined in terms of protein electrophoresis, it was determined that SPE was studied from 130 cases. As a result of protein electrophoresis, monoclonal gammopathy was observed in the gamma band in 60 of 130 patients, while gammopathy was not found in 70 of them. When the serum IgG concentrations of these 130 patients were analyzed, the mean of 57 patients with monoclonal gammopathy in SPE was 23.954 g/L, while the mean of 65 patients without monoclonal gammopathy was 10.058 g/L. Serum IgG results of 8 patients could not be reached. Whether there was a statistically significant difference between serum IgG concentrations of these two groups was analyzed by Independent Samples Test. According to the test result, the p value was <0.05 and there was a statistically significant difference between the two groups (Table 4).

When 183 patients with monoclonal G Kappa results, which is the second common type of gammopathy according to serum immunofixation data, were examined in terms of protein electrophoresis, it was determined that SPE was studied from 55 cases. As a result of protein electrophoresis, monoclonal gammopathy was observed in the gamma band in 37 of 48 patients, while gammopathy was not found in 18 of them. When the serum IgG concentrations of these 55 patients were analyzed, the mean of 35 patients with monoclonal gammopathy in SPE was 31.79 g/L, while the mean of 16 patients without monoclonal gammopathy was 13.34 g/L. Serum IgG results of 4 patients could not be reached. Whether there was a statistically significant difference between serum IgG concentrations of these two groups was analyzed by Independent Samples Test. According to the test **result, the p value was <0.05 and there was a statistically significant difference between the two** groups (Table 5).

Table 5. Mean serum IgG concentrations of IgG Lambda cases in SPE(+) and (-) groups

Gammopathy Status in SPE	Number of patients	Mean serum IgG concentration (g/L)	
Monoclonal G Lambda with SIFE + There is monoclonal gammopathy with SPE	37	31.79	
Monoclonal G Lambda with SIFE + No monoclonal gammopathy with SPE	18	13.34	p<0.05
Total	55		

CASE 4:

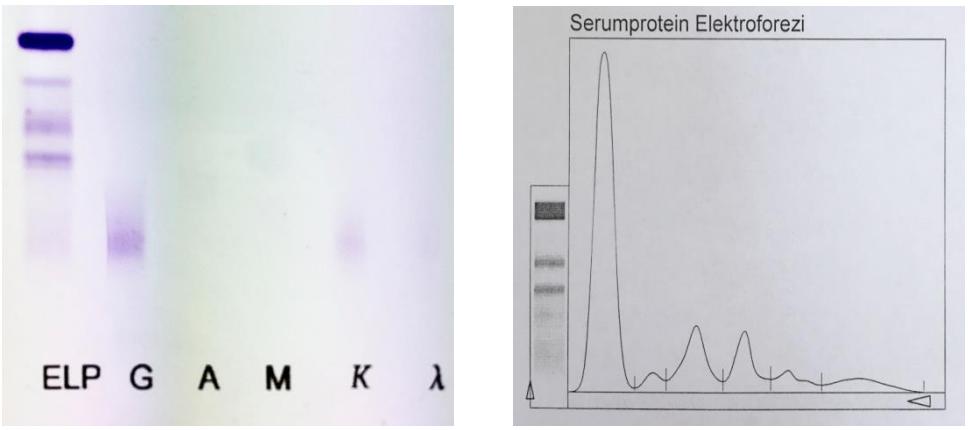

Figure 4. Serum immunofixation electrophoresis sample (left) and serum protein electrophoresis sample (right) belonging to a patient with IgG Kappa paraproteinemia. In the left figure, there is a slightly distinct band with sharp borders in the same migration in the G and Kappa regions. In the figure on the right, no peaking gammopathy band was observed in the gamma region, and a decrease in gamma band concentration (0.4 g/dl) was noted.

Urine immunofixation electrophoresis was also studied in 49 of 1446 patients who were found to have gammopathy according to the results of serum immunofixation electrophoresis, and paraproteinemia was detected in the urine IFE of 27 patients. According to this data, 55.1% of the patients whose serum was found to have paraproteinemia with SIFE, were found to have paraproteinemia with IFE in the urine sample.

Urine protein electrophoresis was applied to 18 of 1446 patients with gammopathy according to serum immunofixation electrophoresis results, and monoclonal gammopathy was detected in urine protein electrophoresis in 4 cases. According to this data, the presence of paraproteinemia was detected by protein electrophoresis in the urine sample of 22.22% of the patients whose serum was found to have paraproteinemia.

DISCUSSION

Paraprotein band was detected at a rate of 21.36% (n=1446) by serum IFE analysis of 6767 cases included in our study. When these patients were examined in terms of diagnosis, Multiple Myeloma was the most common, and when analyzed according to paraprotein type, it was noted that IgG kappa (29.46%) was the most common, and IgG lambda (12.66%) was the second most common (Table 3). Looking at the literature, the most common Ig type in MM disease was Ig G, the rarest type is Ig M (Bataille & Harousseau, 1997). In this respect, the data of our study is similar to the literature. Previously Dikker and Şahin (2018), in their study, G-Kappa was the most common with a rate of 33%, and G-Lambda was the second most common with a rate of 20%, Ercan et al. (2013) found G-Kappa as the most common with 41.3% and G-Lambda as the second most common with a rate of 35.6% and reported similar results with our study. In our study, the percentage of the two highest bands was found to be lower than in these two studies. The fact that we scanned 3-year data and included patients with monoclonal and biclonal bands as well as oligoclonal or different band combinations may have caused these percentages to be lower in our study. The rates of detecting gammopathy in these two studies were 23% and 18%, respectively, and these rates are similar to our study (21.36%).

Distribution of plasma cell proliferative diseases according to Mayo Clinic dysproteinemia database between February 2002 and December 2008 (n=1877) MGUS 27% (n=524), MM 24% (n=467), AL 30% (581), lymphoproliferative disease (3%), plasmacytoma 1.3% (n=26), asymptomatic MM 10% (n=191), POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes) 1.6% (n=31), extramedullary plasmacytoma was 0.53% (n=10), LCDD (light chain storage disease) was 0.95% (n=18) and WM was 1.3% (n=26) (Katzmann et al, 2009), while in our study (n=1446) 82.12% (n=551) of the patients were found to have MM. According to Mayo Clinic data, the percentage of MM was found to be higher in our patient group. In our study, the WM was found to be 3.43% (n=3), closer to the percentages of Mayo Clinic data (Table 2). In our study, the data of patients who applied to our hospital for a 3-year period and were studied for SIFE were evaluated. The percentage difference may be related to the fact that our study covered a period of 3 years and the

number of patients included in our study was relatively low. In addition, since our hospital is a 3rd level healthcare institution, patients are referred to us from lower level healthcare institutions for treatment. During this process, the cases may have passed the MGUS stage and arrived at a more established clinical picture and at a stage where the disease associated with gammopathy could be defined. Therefore, the percentage of MGUS diagnosis may not match the expected values. In our data, among the diagnoses associated with gammopathy, Acute Myelofibrosis is the second, and Anemia is the third, followed by hematological disorders such as Non-Hodgkin's Lymphoma, Fever of Unknown Origin, CLL, and CML. Again, this may be due to the high frequency of admission of patients with hematological malignancies for diagnosis and treatment, since our hospital is a 3rd level healthcare institution, and may be associated with the frequent accompaniment of monoclonal gammopathies with hematological malignancies.

In our study, gammopathy could not be detected by SPE in 47.91% of the patients who were found to have gammopathy with SIFE. In a previous study by Potdevin et al. (1983) in 101 patients with monoclonal gammopathy, similar to our results, they detected gammopathy in 97 cases with SIFE, while they detected gammopathy in 50 cases (49.5%) with SPE.

Paraproteinemia could not be detected in 70 (53.84%) of 130 patients with SIFE monoclonal G-Kappa paraproteinemia. Likewise, in 18 (37.5%) of 48 patients who were found to have monoclonal G-Lambda paraproteinemia with SIFE, paraproteinemia could not be detected in SPE. When we investigated the reason for this situation, it came to mind that the amount of paraprotein in some cases might be too low to be detected in SPE, so that it could not create a peak in the gamma band. There is also information in the literature that SPE detects M protein at a minimum concentration of 0.3-0.5g/dL (Gay-Bellile et al., 2003). It has been reported that M-protein can be detected at concentrations of 0.02 g/dL in serum and 0.004 g/dL in urine for SIFE (International Myeloma Working Group, 2003). In our study, when serum Ig G concentrations of SIFE (+) and SPE (+) patients were compared with serum Ig G concentrations of SIFE (+) and SPE (-) patients in both monoclonal gammopathy groups, a statistically significant difference was found (Table 4 and Table 5). These data prove to us that serum protein electrophoresis is a useful initial procedure to screen for M protein, but it may be insufficient in some cases in terms of diagnosis. Especially in cases of hypogammaglobulinemia, the presence of a peak may not be observed in SPE even if the patient has gammopathy (O'Connell, Horita, & Kasravi, 2005). In some cases, even if serum Ig concentrations are normal, a very small M protein may be present. Sometimes, the M-protein can produce a broad band in agarose gel with a polyclonal pattern. This M-protein can occur when complexed with other plasma components, forming IgM dimers and pentamers, IgA polymers, or IgG aggregates. Some patients make monoclonal light chains (Bence Jones proteinemia), which are usually present in concentrations too low to be seen as a spike in agarose gel due to rapid excretion in the urine. In these patients, only when renal failure develops, serum M protein concentrations increase and begin to make visible bands in the SPE. In patients with suspected monoclonal gammopathy, SIFE should be studied to confirm and define the monoclonal protein

(Bataille, & Harousseau, 1997).

According to these data, it is seen that the rate of catching gammopathy in SPE is lower than in SIFE. Cases in which the presence of gammopathy could not be detected by SPE gave us the opportunity to compare our findings regarding the effectiveness of tests such as SPE, SIFE, urine protein electrophoresis, urine immunofixation electrophoresis used in the diagnosis of plasma cell dyscrasias. In a previously published study, while SPE sensitivity was 87.6% and SIFE sensitivity was 94.4% in Multiple Myeloma patients, these rates were found to be 79% for SPE and 87% for SIFE in general plasma cell diseases (Katzmann et al, 2009). However, SPE only reveals the presence and amount of M protein and cannot subtyping (Leung, 2016). Although SIFE detects paraproteinemia more sensitively, it is not possible to detect the concentration of M protein with SIFE. In this case, the use of both tests together in the diagnosis, treatment response and follow-up process of the patients will be a guide for clinicians. In our study, we observed that 55.1% of the patients with gammopathy with SIFE were found to have gammopathy with urine IFE and 22.22% with urine protein electrophoresis. In a previous study conducted with patients with plasma cell dyscrasia, it was reported that 37.7% of the patients had positive results with urine protein electrophoresis (McTaggart, Lindsay, & Kearney, 2013). In our study, this rate may have been lower (22.22%) because urine protein electrophoresis was studied from only 18 patients. This result proves that in the diagnosis, treatment response and follow-up of plasma cell dicrasias, in addition to SIFE and SPE, it would be more accurate to evaluate urine immunofixation electrophoresis, urine protein electrophoresis and also the measurement of serum free light chain amount, especially in cases where kidney damage develops.

CONCLUSION

In this study, 3-year immunofixation and electrophoresis data of our hospital were analyzed retrospectively, and we found that we had similar results with the data in the literature in terms of the frequency and percentage of gammopathy. The SPE and SIFE data we obtained may be useful in guiding our clinicians in the steps to be followed in the screening and definitive diagnosis of monoclonal gammopathies. Although SPE is a useful screening test in the diagnosis process of patients who applied to our laboratory with a preliminary diagnosis of gammopathy, SIFE was found to be the gold standard method for definitive diagnosis, in line with the literature (Merlini & Stone, 2006).

Our study can be expanded to include more patients over a longer time period. A wider study can be conducted, including primary and secondary health care institutions, especially with the identification and inclusion of MGUS patients, which are the most frequently seen in the literature.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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