

Significance of Serum Protein Electrophoresis in the Detection of Multiple Myeloma: A Diagnostic Interpretation of Patients with Varied Immunoglobulins

Abstract

Background: To determine the relationship between serum protein electrophoresis (SPE) and serum immunoglobulin profiles of patients with multiple myeloma (MM). **Methods:** This is a retrospective study conducted at the Eric Williams Medical Sciences Complex, focusing on patients over 18 years who received diagnostic blood tests for MM. Results for SPE and serum immunoglobulin profiles were obtained using lab logbooks. Descriptive and inferential statistics techniques were used to analyze the data. **Results:** The median age of MM patients in Trinidad and Tobago is of 69 years. The ratio of male and female patients with the MM is 50:50. Out of 131 patients, 24 (18.32%) had M bands present, 60 (45.80%) had an increase in gamma globulin, and 16 (12.21%) had a decrease in albumin. In cases of M band presence and raised gamma, there was noticeable increase in IgG concentration and a slight increase in IgM concentration. There was also an increase in IgA concentration in patients with the M-band but a decrease in concentration in those with raised gamma. Patients with an elevated gamma had a significant increase in IgG diameter and IgG concentration. **Conclusions:** This study showed a relationship between the presence of M bands and serum immunoglobulins. It has proven the significance of SPE and immunoglobulins, like IgG and IGM, in the detection of MM in the early and advanced stages. Therefore, it is recommended that SPE be used, along with other laboratory tests, in the diagnosis of MM.

Keywords: Immunoglobulins, multiple myeloma, serum protein electrophoresis

Introduction

Multiple myeloma (MM) is a neoplasm of B cell lineage that accounts for 10% of all hematological malignancies.^[1,2] MM is distinguished from other diseases by the propagation of bone marrow monoclonal plasma cells (M Protein). Because of this increase, the numerous malignant cells outnumber the normal plasma cells, thereby hindering the body's ability to fight disease infection. This in retrospect leads to the presence of monoclonal immunoglobulins which can be detectable in serum or urine via electrophoresis.^[3]

The mean age for diagnosis of MM is 69 years. On average, two of every 3 MM patients are men, and the disease is most prevalent among persons of African descent.^[4,5] MM mortality rates have been drastically increasing in Trinidad and Tobago, boasting a ratio of 2.7 per 100,000 persons currently. In 2013, for every 100,000 men, 31 deaths were reported, with

the peak mortality rate for men higher than that of women, which was 15.5 per 100,000. This is cause for concern, given the fact that MM mortality rates have increased by 105.7% since 1990, an average of 4.6% per year.^[6] It is therefore imperative that we enhance the current method of MM diagnosis to facilitate a better prognosis for patients with MM.

Diagnosis of MM involves various clinical methods; complete blood count with peripheral blood smear, routine urinalysis and 24-h urine collection for electrophoresis, MRI and PET/CT scans of the humerus and femur, tests for serum levels of calcium, creatinine, albumin, lactate dehydrogenase, C-reactive protein, and serum protein electrophoresis (SPE) with immunofixation and quantitation of immunoglobulins.^[7]

Serum immunoglobulin profile is a test which quantifies the proportion of immunoglobulins that may be present in

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Access this article online

Website:
www.ijpvmjournal.net/www.ijpvm.ir

DOI:
10.4103/ijpvm.IJPVM_222_18

Quick Response Code:



How to cite this article: Nayak BS, Ojar-Taylor N, St. John S, Swann S, Thom J, Thomas B, *et al.* Significance of serum protein electrophoresis in the detection of multiple myeloma: A diagnostic interpretation of patients with varied immunoglobulins. *Int J Prev Med* 2021;12:37.

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the blood. There are five classes of immunoglobulins but generally only the three major classes are measured; IgA, IgG, and IgM.^[8] With MM, the M protein secreted by bone marrow monoclonal plasma cells comprises approximately 50% IgG, 20% IgA, 20% immunoglobulin light chains, 2% IgD, and about 0.5% of IgM.^[5] Therefore, the type of immunoglobulin present and its concentration can be useful in determining the presence and type of MM.

SPE is a laboratory examination which separates proteins according to their physical properties. There are 2 major proteins that are analyzed in the serum; albumin and globulins. The albumin is the largest protein in the serum, has the highest peak, and is found nearest to the positive electrode. The globulins, alpha 1 (α_1), alpha 2 (α_2), beta 1 (β_1), beta 2 (β_2), and gamma (γ) are smaller in size and lie close to the negative electrode, with γ being the closest.^[9] Different disorders cause different serum proteins to show an elevation or depression when compared to the normal SPE. Consequently, with MM, there is an increase in the γ curve. In clinical practice, SPE is conducted in the preliminary stage to identify the monoclonal gammopathy as well as the abundance of the M protein in patients with associated signs and symptoms of MM.^[2] This study is designed to determine the significance of SPE and immunoglobulins in diagnosing MM.

Methods

This was a retrospective cross-sectional study conducted within the Immunology and Biochemistry laboratories of the Eric Williams Medical Sciences Complex (EWMSC). It focuses on patients who received diagnostic blood tests for MM at the EWMSC in 2016 and 2017. All clinic patients, male and female, 18 years and over with blood test results including serum protein electrophoresis (SPE), total protein and immunoglobulin profile at EWMSC were considered. Patients who did both SPE and serum immunoglobulin profile blood tests were included in this study. The sample size was determined using power of 80% for a Chi-square distribution, to achieve a sample size of 250. The initial suggested sample size was 250 but starting with a pool of 419 SPE patients, cross-referencing with immunoglobulin results decreased the sample to 131 patients, of whom only 24 presented with M bands on SPE. This was due to non-availability of data which were not recorded in the patient records.

After being granted ethical approval from both campus ethics committee, the University of the West Indies and the North Central Regional Health Authority (NCRHA), a database of clinic patients were accessed through the laboratory logbooks and Vitros 4600 (dry chemistry analysers). Data was first collected on 419 patients who underwent SPE from February 2016 to May 2017. When cross-referenced with Serum Immunoglobulin Profiles, a sample size of 131 patients was obtained.

Serum protein electrophoresis

For the electrophoresis process, a cellulose acetate membrane is fully soaked in a buffer. The membrane is dried with absorbent pads and then loaded with 25 microlitres of the serum sample using applicator blocks. The electrophoresis chamber is filled with a high resolution buffer. The cellulose acetate membrane is then placed in the electrophoresis chamber and the electrophoresis is run for 20 min at 2–10 milliamperes. The membranes removed from the electrophoresis chamber and transferred to a vessel containing Ponceau S solution. Excess stain is removed with 5% acetic acid solution and the membrane is placed in clearing solution for high resolution banding.

Statistical analysis

The data collected was initially entered into MS Excel application, this allowed data to be sorted and filtered according to the required parameters. The data was then imported to IBM Statistical Package for Social Sciences Version 24 and the following techniques were used to analyse and assess the results:

1. Descriptive Statistics (Categorical Data):
Statistical tables including observed frequencies and their percentages
Summary statistic of readings distribution (means \pm standard deviation (SD))
2. Inferential statistics (Continuous Data):
Chi-squared (χ^2) including the observed and estimated results
Student independent t-test and Levene's Test. Data were expressed as statistically significant when $P < 0.05$.

Chi-squared was used to produce observed and expected frequencies of decreased, normal, and increased levels of Albumin, α_1 , α_2 , and β in the electrophoresis profiles of individuals with the γ component of their profile among patients displaying the M-band and elevated γ compared and patients who had a normal protein electrophoresis profile. The Student *t*-test was used in order to determine whether there were correlations between gender, elevated gamma, and normal SPE results and the means of various serum immunological parameters (IgG concentration, IgG Diameter, IgA concentration, IgA diameter, IgM concentration and IgM diameter). Levene's Test was also performed in order to determine whether there was homogeneity of variance. The data of individuals who had a complete electrophoresis profile and all serum immunoglobulin parameters were used to construct Table 1.

Data protection

To access the data, we were required to visit the Immunology and Biochemistry labs at the EWMSC and record the information from the records on site. At no point was the original data removed from the laboratory. Demographics such as name and age were taken to keep track of data but kept confidential on a secure document.

The data was then collated and generalized in analysis and discussion. Hence, no reference was made to specific patients in our final report.

Results

The initial results obtained from the lab logbooks consisted of the SPE results, whether each SPE value was normal, elevated, or decreased was determined by a consultant as the data was entered into the logbooks; as well as the immunoglobulin profiles (IgG concentration, IgG diameter, IgA concentration, IgA diameter, IgM concentration, and IgM diameter) of 419 patients. Of those patients, 101 (25.54%) were male and 131 were female (31.26%). The remaining 187 (44.63%) of the patients did not have their sex on record.

Of the initial 419 patients, only 131 (31.26%) had both a complete immunological profile and electrophoresis results. These were used to conduct the Student *t*-tests and Levene’s test of the various immunological parameters (IgG concentration, IgG diameter, IgA concentration, IgA diameter, IgM concentration, and IgM diameter). Additionally, from the 131 sample size, only 24 were found to have an M band present [Table 2].

Table 1 demonstrates that the median age of patients with M bands was 69 with ages ranging from 44 to 83 years. On the other hand, the median age of patients with raised gamma was found to be 57, with patients ranging from 19 to 87 years. The incidence rate of MM was equivalent for both males and females. The patients with an M band showed elevated gamma with a decrease in serum albumin levels. The study demonstrated that in cases of the presence of the M band and raised gamma, there

was noticeable increase in IgG concentration and a slight increase in IgM concentration. The data of this novel study showed a relationship between the presence of M band and serum immunoglobulins. This has proven the significance of SPE and immunoglobulins, like IgG and IGM, in detecting MM. This study therefore recommends the use of immunoglobulin profiles along with other laboratory tests in diagnosing MM.

Discussion

MM is the second most common hematological malignancy and is responsible for approximately 2% of all deaths due to cancer.^[10] SPE is an important laboratory investigation used to indicate the presence of an M band in patients with MM.^[1,2,10] Using this principle, this study used the presence of an M band to determine if the patient had MM. This criterion is consistent with updated criteria shown by other researchers.^[11] Kyle *et al.* reported that M protein can be detected by serum protein electrophoresis in 82% of patients and by immunofixation in 93%.^[12]

The study showed that in cases of the presence of the M band and raised gamma globulin, there were notable increases in IgG concentration and a slight increase in IgM concentration. However, it was revealed that in the case of IgA concentration, there is an associated increase in concentration in patients with the presence of the M band but a decrease in concentration with those with raised gamma globulin [Tables 3 and 4]. This irregularity in the IgA concentrations contrasts the established literature which indicates that noticeable increases in serum IgG, IgA, and IgM are expected with the presence of an M band or elevated gamma in SPE.^[3] This can be explained however by Bansal *et al.*^[13] who reminds us that IgA gammopathy on electrophoresis is a diverse spectrum, and to avoid misdiagnosing a patient, one should be aware of the possibility of variations. Moreover, one study^[14] found that suppression of at least one uninvolved immunoglobulin was observed in 87% of MM patients, with a slight increase in commonality with IgA myeloma.

It was also found that among patients with an M band and elevated gamma globulin there was a decrease in serum albumin levels. This was expected because hypoalbuminemia can be a symptom of MM and can be of diagnostic and

Table 1: Characteristics of patients

Characteristic	M-band (n = 24)	Raised γ (n = 80)	Normal (n = 110)
Median Age	69	57	56
Age Range	44-83	19-87	20-98
Male Sex (%)	15 (50%)	39 (49%)	48 (44%)
Isotype average			
IgG	8.06	7.33	6.26
IgA	7.56	6.40	6.50
IgM	5.50	5.67	5.42

Table 2: Number of patients, both male and female, with an increase in gamma, presence of M-band, decrease in albumin and normal results of SPE respectively

Age	Increased Gamma		M-band		Decreased albumin		Normal	
	Male	Female	Male	Female	Male	Female	Male	Female
18-20	0	1	0	0	0	0	0	2
21-40	2	4	-	-	-	3	7	16
41-60	10	12	3	4	3	4	16	18
61-80	16	12	8	5	2	3	23	25
81-100	1	2	2	2	-	1	2	4
Total	29	31	13	11	5	11	45	65

Table 3: Outputs for Student T Test and Levene's Test for Equivalence of Variances between patients with M band, normal gamma levels and serum immunological profile

	Gamma	N	Mean	Std. Deviation	Std. Error Mean
IgG diameter	M band	5	8.06	3.166	1.416
	Normal	25	6.10	.584	.117
IgG concentration	M band	3	1217.67	945.734	546.020
	Normal	25	1099.68	301.824	60.365
IgA diameter	M band	5	7.56	4.471	1.999
	Normal	25	6.36	1.324	.265
IgA concentration	M band	3	307.33	203.937	117.743
	Normal	22	276.82	137.748	29.368
IgM diameter	M band	5	5.50	3.144	1.406
	Normal	25	5.32	.675	.135
IgM concentration	M band	1	171.000	.	.
	Normal	25	92.000	41.3138	8.2628

Table 4: Outputs for Student T Test and Levene's Test for Equivalence of Variances between patients with elevated gamma globulins, normal gamma levels and serum immunological profile

	Gamma	N	Mean	Std. Deviation	Std. Error Mean
IgG diameter	Increase	21	7.33	.716	.156
	Normal	41	6.29	.611	.095
IgG concentration	Increase	21	1631.14	501.059	109.340
	Normal	41	1175.24	302.364	47.221
IgA diameter	Increase	21	6.40	1.175	.256
	Normal	41	6.52	1.219	.190
IgA concentration	Increase	21	242.08	149.041	32.523
	Normal	41	259.10	151.523	23.664
IgM diameter	Increase	21	5.67	1.078	.235
	Normal	37	5.44	.964	.158
IgM Concentration	Increase	17	119.529	64.1123	15.5495
	Normal	35	92.343	41.1289	6.9520

prognostic importance.^[15] On the other hand, Chi-squared tests showed no correlation between the presence of an M band or elevated gamma globulin with alpha1, alpha2, or beta globulins, which was also expected.^[3]

The data collected and analyzed provides evidence that there is a distinct correlation between SPE and serum immunoglobulin profiles among patients with MM. There was a significant increase in the mean levels of IgG diameter and IgG concentration in the serum immunoglobulin profiles of patients with M bands and elevated gamma. Elevated gamma is a predisposing sign of MM^[5] and can therefore be of early diagnostic importance. Hence it can be reasoned that SPE coupled with serum immunoglobulin profile can be effective tools in the preliminary and later stages of MM diagnosis, and therefore be effective tools in both screening and detection.

Limitations of methodology

Initially the desired parameters for the sample included total protein and globulin levels along with the SPE and serum immunoglobulin profile. However, due to the retrospective nature of the research, sampling was based on pre-existing data. Hence, there was no foresight into whether or not

patients would have all the desired parameters and how many of these patients would then have MM. The initial suggested sample size was 250 patients but starting with a pool of 419 SPE patients, cross-referencing with immunoglobulin results decreased the sample to 131 patients, of whom only 24 presented with the M band in the SPE. This was due to non-availability data which were not recorded in the patient records. Because of this, an inadequate number of patients correlated with other parameters for an appropriate sample size. As such total protein levels had to be exempted from the study.

Conclusions

SPE and serum immunoglobulin profiles can be used as an effective tools in the detection of MM. Hypoalbuminemia was present in patients with increased gamma globulins and myeloma band.

Recommendations

It is recommended that follow-up studies be done when larger sample sizes of MM patients are available and SPE and serum immunoglobulin profiles be used during the preliminary stages of MM diagnosis.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Received: 28 Jun 20 **Accepted:** 26 Sep 20

Published: 15 May 21

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