

Diagnostic Role of Serum Protein Electrophoresis

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Abstract

Introduction: Electrophoresis is a technique for separation of different charged particles. It is based on movement of charged particles through a solution when subjected to an electrical field. Since proteins are negatively charged at pH 8.6, subjecting them to electrical field will push protein particles towards anode.

Materials and Methods: Place of Study: This study was carried out in the Department of Pathology, Rampurhat Government Medical College & Hospital, Birbhum, West Bengal, India.

Period of Study: Study was conducted from January 2020 to March 2022.

Study Design: Prospective

Age Group: 1st decade to 8th decade

Control Group: 10 male and 10 female healthy persons of every decade were chosen as control groups.

Result: Cellulose acetate electrophoresis of 150 symptomatic patients performed. Major diseases diagnosed were Multiple Myeloma (24 cases), Chronic inflammatory disease (15 cases), HIV (13 cases), Hematological malignancy (10 cases), solid malignancy (10 cases), tuberculosis (7 cases) with some Miscellaneous conditions.

Among the 28 Monoclonal Gammopathy cases studied (100%), the most common case was Multiple Myeloma (24 cases, 85.71%) followed by 2 cases of Solitary Plasmacytoma (7.15%) and 1 case of Monoclonal Gammopathy of undetermined significance (MGUS) (3.57%) and 1 case of Smoldering Myeloma (3.57%).

Keywords: Serum Protein, Electrophoresis, Multiple Myeloma, Solid Malignancy

Introduction

Electrophoresis is a technique for separation of different charged particles. It is based on movement of

charged particles through a solution when subjected to an electrical field⁵. Since proteins are negatively charged at pH 8.6, subjecting them to electrical field

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will push protein particles towards anode. Weight and charge on different protein fractions separates them out into albumin (molecular wt 69000), α_1 and α_2 -globulin (molecular wt 140,000), β and γ globulin¹². Electrophoresis initially started as moving boundary electrophoresis in which boundaries of medium used to move to separate protein fractions. Later on it was improved in the form of zone electrophoresis in which electrophoretic support medium was static and after application of serum sample, different protein particles used to migrate on it⁹. Various support media are used in electrophoresis depending upon the type of electrophoresis e.g. serum proteins, enzymes, lipoproteins etc³. It includes paper, cellulose acetate, starch gel, agarose gel and polyacrylamide gel. Factors affecting mobility include size and shape of particles, ionic strength of solution, viscosity and temperature of the medium².

Separation of serum protein fractions is very important for the diagnosis of different diseases like paraproteinaemias, haemoglobinopathies, immune deficiency and genetic abnormalities. It is also helpful along with other investigations in chronic liver disease, malignancies and collagen disease. In multiple myeloma it is also helpful in monitoring the treatment¹⁷.

Materials & Methods

Place of Study: This study was carried out in the Department of Pathology, Rampurhat Government Medical College & Hospital, Birbhum, West Bengal, India

Period of Study: Study was conducted from January 2020 to March 2022.

Study Design: Prospective

Age Group: 1st decade to 8th decade

Control Group: 10 male and 10 female healthy persons of every decade were chosen as control groups.

Inclusion criteria for study:

All the suspected and diagnosed cases of dysproteinemia were included. Male and female were chosen randomly.

Exclusion Criteria:

1. Undiagnosed cases of pyrexia of unknown origin (PUO)
2. Patients receiving steroid therapy / Immunosuppressive or chemotherapy or has undergone transplantation.
3. Patients below 6 months of age or more than 80 yrs.
4. Patients with malnutrition.

Study of cases:

All, cases registered in the study were interviewed for detailed history, clinically examined thoroughly and underwent Cellulose Acetate Electrophoresis.

Selective Monoclonal Gammopathy cases were undergone immunofixation by capillary zone electrophoretic (CZE) method.

Results

A total number of 150 cases were included in this study. Different monoclonal and polyclonal gammopathy cases were studied electrophoretically. In this study maximum number of patients was in polyclonal gammopathy group(75.33%). Irrespective of clinical manifestation,6% cases were electrophoretically normal and 18.67% cases were in monolonal gammopathy group. Out of 150 cases (**Table-1**)most common case was Multiple Myeloma (20.16%) followed by Chronic inflammatory diseases (12.60%) and HIV (10.92%). single case of MGUS, Smoldering Myeloma,were studied.

In this study age range was from 1st to 8th decade(**Table-2**). Maximum cases were in 4th and 6th decade. Minimum number of cases was in 1st decade. Study shows that male female ratio is almost equal (1.22:1). Different polyclonal and monoclonal gammopathy conditions studied in our study.

Among the 28 Monoclonal Gammopathy cases(100%) in our study, the most common case was Multiple Myeloma (24 cases,85.71%) followed by 2 cases of Solitary Plasmacytoma(7.15%) and 1 case of MGUS (3.57%) and 1 case of Smoldering Myeloma (3.57%). Out of 28 cases of Monoclonal gammopathy(100%), in 24 cases (85.71%) monoclonal spike is in the gamma region. Rest 4 (14.29%) of cases monoclonal spike is in the beta region. No monoclonal spike is in alfa 2 region. We also found

that most common type of monoclonal protein was IgG Kappa.

10 Solid malignant cases studied electrophoretically. Albumin concentration decreased in 60% of cases (Table-3). But Alfa -1 and Alfa -2 protein concentration were normal in 100% and 90%

of cases. β -globulin concentration were normal in all cases. Gamma globulin concentration was raised in 60% of cases. Most of the HIV cases showed normal electrophoretic pattern except one case which showed hypogammaglobulinemia and 3 cases of polyclonal hypergammaglobulinemia(Table-3).

Table 1: Major Disease Studied (n = 119), Miscellaneous Conditions Studied (n = 31), Monoclonal Gammopathy Cases Studied (n = 28)

	Disease	Cases	
		No	Percentage
Major Disease(n=119)	Multiple Myeloma	24	20.16
	Chronic Inflammatory Disease	15	12.60
	HIV	13	10.92
	Hematological Malignancy	10	8.40
	Solid Malignancy	10	8.40
	Protein Losing Enteropathy	8	6.89
	Tuberculosis	7	5.88
	Iron Deficiency Anaemia	7	5.88
	Cirrhosis of Liver	5	4.20
	Congenital Hemolytic Anaemia	5	4.20
	Schizophrenia	5	4.20
	Total Cases	119	100
Miscellaneous Conditions(n=31)	Pregnancy	4	12.90
	Inflammatory Bowel Disease (IBD)	3	9.67
	Nephrotic Syndrome	3	9.67
	Diabetes Mellitus	3	9.67
	Chronic Osteomyelitis	2	6.45
	Autoimmune Thyroiditis	2	6.45
	Hepatitis -C	2	6.45
	Chronic Bronchitis	2	6.45
	Alfa - Anti-Trypsin Deficiency	2	6.45
	Solitary Plasmacytoma	2	6.45
	MGUS	1	3.22
	Smoldering Myeloma	1	3.22
	Epilepsy	1	3.22
	Bisalbuminemia	1	3.22
	Total	31	100
Monoclonal Gammopathy Diagnosis(n=28)	Multiple Myeloma	24	85.71
	Solitary Plasmacytoma	2	7.15
	MGUS	1	3.57
	Smoldering Myeloma	1	3.57
	Total	28	100

Table 2: Distribution of cases(n=150) according to age and gender

Age group in years	Cases			
	Male	Female	No of cases	Percentage
0-10	2	2	4	2.66
11-20	4	3	7	4.67
21-30	8	11	19	12.67
31-40	17	13	30	20
41-50	10	9	19	12.67
51-60	18	12	30	20
61-70	15	11	26	17.33
71-80	8	7	15	10
Total	82	68	150	100%

Table 3: Electrophoretic Pattern of HIV Cases Studied (n = 13) & solid malignancy Cases(n=10)

Electrophoretic Pattern of HIV Cases (n = 13)								
Parameter	Normal		Increased		Decreased		Total	
	No	%	No	%	No	%	No	%
Albumin conc.	9	69.23	0	0	4	30.67	13	100
α 1 Protein conc.	11	84.61	2	15.39	0	0	13	100
α 2 Protein conc.	11	84.61	1	7.69	1	7.69	13	100
β-globulin conc.	12	92.30	1	7.70	0	0	10	100
γ-globulin conc.	9	69.23	3	23.07	1	7.69	13	100
Electrophoretic Pattern of solid malignancy cases (n = 10)								
Albumin conc	4	40.00	0	0	6	60.00	10	100
α 1 Protein conc.	10	100.00	0	0	0	0	10	100
α 2 Protein conc.	9	90.00	1	10.00	0	0	10	100
β-globulin conc.	10	100.00	0	0	0	0	10	100
γ-globulin conc.	4	40.00	6	60.00	0	0	10	100

Discussion

This study was conducted to assess the diagnostic significance of serum protein electrophoresis, to study different monoclonal gammopathy cases, to diagnose, screen and differentiation of different polyclonal gammopathy cases on the basis of serum protein electrophoresis in proper clinical setting.

In our study, 150 Cases of suspected and diagnosed dysproteinemic cases were studied electrophoretically. All the cases studied were in the age range of 1st to 8th decade. Maximum number of cases was in the 4th and 6th decade. Minimum number of cases was in the 1st decade. The result of the study

as regard to the age incidence of patient is compatible to the study done by Gowenlocketal (1987).⁵

Out of 150 cases most common group was of polyclonal gammopathy cases (75.33%), monoclonal gammopathy cases were of 18.67% and 6.00% of patients irrespective of different clinical manifestations revealed normal electrophoretic pattern.

Nearly similar observation shown by Waqar Azimetal¹⁶ who had taken 1556 patients. Out of that 340 had normal electrophoretic pattern, 125 cases were of monoclonal gammopathy and 1091 cases were of polyclonal gammopathy. Similar observation

also made by Theodorexetal¹⁴.

In the present study Male / Female distribution was nearly 1:1.

Nearly similar sex ratio observed in a study by Dispenzieriet al (May 2001)⁴. In that study out of 148 patients 59% were female and 41% were male.

Different polyclonal and monoclonal gammopathy conditions studied in our study. Out of 150 cases most common case was Multiple Myeloma (20.16%) followed by Chronic inflammatory diseases (12.60%) and HIV (10.92%). single case of MGUS, Smoldering Myeloma, were studied.

According to Mayo clinic data (2001)¹³, 122 out of 148 patients with polyclonal gammopathy, Liver disease was the most common cause, followed by Connective tissue disease (22%), Chronic infections (6%), Hematological Disorders (5%) and Non hematological diseases (3%).

Among the 28 Monoclonal Gammopathy cases(100%)in our study, the most common case was Multiple Myeloma (24 cases, 85.71%) followed by 2 cases of Solitary Plasmacytoma(7.15%) and 1 case of MGUS (3.57%) and 1 case of Smoldering Myeloma (3.57%).

This result is not matching with Mayo clinic data of 2006⁷. They examined 1684 cases of Monoclonal Gammopathy. Out of which most common condition was Monoclonal Gammopathy of Undetermined Significance(MGUS)(55%) followed by Multiple Myeloma (16.5%) followed by Amyloidosis (11.5%), Lymphoproliferative disorders(4%), Smoldering Myeloma(3%), Solitary Plasmacytoma (2%), Waldenstorm's Macroglobulinemia(2%) and others (6%).

The cause of may be

- Our study group is small (28 patients) in respect to Mayo clinic study group (1684).
- In our study only symptomatic patients were studied, good number of MGUS patients are asymptomatic.

Out of 28 cases of Monoclonal gammopathy(100%), in 24 cases (85.71%) monoclonal spike is in the gamma region. Rest 4 (14.29%) of cases monoclonal spike is

in the beta region. No monoclonal spike is in alfa 2 region.

According to Wintrob's Clinical (2009)⁸ Hematology "A monoclonal spike is seen as a discrete band that usually migrates to the gamma or beta region of the electrophoretic strip and rarely to the alfa - 2 region.

Out of 10 Multiple Myeloma cases studied by Immunofixation method showed that the most common case was IgG (70%) Kappa (80%).

According to Alexanian R. et al (1999)¹ in Multiple Myeloma, monoclonal proteins in order of frequency are

IgG	:	61%
IgA	:	27%
Light chain only	:	25%
IgM	:	8%
Biclonal	:	5%

10 Solid malignant cases studied electrophoretically. Albumin concentration decreased in 60% of cases. But Alfa -1 and Alfa -2 protein concentration were normal in 100% and 90% of cases. β -globulin concentration were normal in all cases. Gamma globulin concentration was raised in 60% of cases.

Stephen R. Vavricka (2009)¹⁵ made nearly similar observation. They observed increase of Alfa -1 globulins and especially Alfa -2 globulins due to an increase of acute phase proteins. An increase of the gamma fraction can occasionally be observed in end-stage patients.

Graham et al (1963)⁶ also made similar observation. They did electrophoresis of 97 solid malignant cases. Albumin proportion was decreased in most of the cases. Total Alfa-2 globulin proportions were significantly elevated in all but skin cancer. In the Gamma globulin areas the effects were scattered.

Samuel Lifshitz et al (1980)¹¹ observed decreased albumin and increased Alfa-1, Alfa -2 and Beta globulins, in solid malignant cases.

Electrophoretic pattern of 13 diagnosed

HIV patients, showed that 9 cases (69.23%) of patients have normal electrophoretic patterns. 3 cases (23.07%) of patients showing polyclonal hypergammaglobulinemia and 1 patient (7.70%) showed hypogammaglobulinemia.

Gary L. Horowitz et al (2007)¹⁰ made nearly similar observation. They studied 320 HIV infected patients. Protein electrophoretic pattern (PEP) was normal in 83.8% of samples, 8.1% had subtle oligoclonal monoclonal band concentration banding, 4.4% had low concentration monoclonal band. Hypogammaglobulinemia and polyclonal hypergammaglobulinemia occurred in 1.9% each.

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