FREQUENCY OF MONOCLONAL GAMMOPATHY IN HYPERPROTEINAEMIC PATIENT

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Abstract

Background: Paraproteinemia or monoclonal gammopathy is the presence of excessive amounts of a single monoclonal gammaglobulin (In this case denominated "paraprotein") in the blood. It is usually due to an underlying immunoproliferative disorder and sometimes considered equivalent to plasma cell dyscrasia. To determine the frequency of monoclonal gammopathy in hyperproteinemic patient, to interpretate serum protein electrophoresis, distribution of monoclonal gammopathy in relation to age and sex.

Materials and methods: An observational, descriptive, cross sectional study was taken place at Armed Forces Institute of Pathology (AFIP) Dhaka Cantonment, Dhaka from May 2017 - October 2017. A total of 165 hyperproteinemic patients at all age and sex were included to the study and hypoproteinaemic, normoproteinaemic patients and pregnancy were excluded. Six millilitres venous blood was collected asceptically from each patient.

Results: About 12.1% of monoclonal gammopathy was found in hyperproteinemic patients . Among them 66.1% were men and 33.9% were female. Common age group was 41-60 and 61-80.

Conclusion: The treatment, monitoring and prognosis of monoclonal gammopathy depends on the early detection of M protein band in electrophoretic pattern. This study reveals a high incidence of monoclonal gammopathy in patients with hyperproteinemia and frequency is increasing with higher age. So serum protein electrophoresis should be done for all hyperproteinemic patients.

Key words : Monoclonal; Paraproteinaemia; Gammopathy.

Introduction

Paraproteinemia or monoclonal gammopathy is the presence of excessive amounts of a single monoclonal gammaglobulin (In this case denominated "paraprotein") in the blood. It is usually due to an underlying immunoproliferative disorder. It is sometimes considered equivalent to plasma cell dyscrasia.[1] Paraproteinemia may be categorized according to the type of monoclonal protein found in blood: i) Light chains only (or Bence Jones protein). This may be associated with multiple myeloma or AL amyloidosis ii) Heavy chains only (Also known as "heavy chain disease" iii) Whole immunoglobulins. In this case, the paraprotein goes under the name of "M-protein" ("M" for monoclonal). Proliferation of a single clone of plasma cells that produce a monoclonal protein resulting extensive skeletal involvement with osteolytic lesion, aneamia which can cause renal failure and life-threatening infections due to nephrotoxic monoclonal immunoglobulin production.[2] Annual incidence of multiple myeloma is 4 per 100,000 which represents approximately 1% of all and 15% of haematological malignancies. It is more frequent in men than women and median age is 65-70 years. Normal differentiation from early B cells to plasma cells is characterized by three B-cell-specific DNA remodelling mechanisms that modify immunoglobulin genes: VDJ rearrangement, somatic mutation and class switch recombination. Pathogenesis includes: Cellular origin of myeloma cell, Genomic abnormality, IGH translocations, Gains and losses of chromosomal material, Mutations detected by whole-genome sequencing, Epigenetic modifications, Late genetic events, Interaction between plasma cells and their microenvironment. Criteria for the diagnosis of symptomatic MM: M-protein in serum and/or urine, Bone marrow (Clonal) plasma cells or plasmacytoma, Related organ or tissue impairment (end-organ damage, including bone lesions. The term 'Smouldering Multiple Myeloma' (SMM) was first defined by the presence of a serum M-protein (>30 g/L) and 10% or more plasma cells in the bone marrow in the absence of lytic bone lesions or clinical manifestations due to the monoclonal gammopathy. More recently, the IMWG considered

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that the term 'asymptomatic myeloma' could be more appropriate. Presence of an M protein (≥30 g/L) and/or 10% or greater bone marrow plasma cells in the absence of symptoms or organ or tissue impairment due to the monoclonal gammopathy and present in 10% of patients. Waldenström's macroglobulinemia is characterized by an uncontrolled clonal proliferation of terminally differentiated B lymphocytes with unknown etiology. There has been an association demonstrated with the locus 6p21.3 on chromosome 6 and increase risk of developing WM in people with a personal history of autoimmune diseases with autoantibodies and particularly elevated risks associated with hepatitis, human immunodeficiency virus, and rickettsiosis.[3-5] Some genetic factors, first-degree relatives have a highly increased risk of also contracting Waldenström's.[6] Exposure to farming, pesticides, wood dust, and organic solvents may be cause of development of Waldenström's.[7] A mutation in gene MYD88 has been found to occur frequently in patients.[8] WM cells show only minimal changes in cytogenetic and gene expression studies. Their miRNA signature however differs from their normal counterpart. It is therefore believed that epigenetic modifications play a crucial role in the disease.[9] The protein Src tyrosine kinase is overexpressed in Waldenström macroglobulinemia cells compared with control B cells.[10] Inhibition of Src arrests the cell cycle at phase G_1 and has little effect on the survival of WM or normal cells. Weakness, fatigue, weight loss and chronic oozing of blood from nose and gums are the prominent feature as well as Peripheral neuropathy (10%). Lymphadenopathy, splenomegaly and/or hepatomegaly are present in 30-40% and blurring or loss of vision, headache and (Rarely) stroke or coma are rare. This is attributed to the IgM monoclonal protein increasing the viscosity of the blood by forming aggregates to each other, binding water through their carbohydrate component and by their interaction with blood cells.[11] Significant monoclonal IgM spike evident and malignant cells consistent with the disease in bone marrow biopsy.[12] Blood test, flow cytometry and bone marrow biopsy, CT, CAT of chest, abdomen, pelvis and skeletal survey can distinguish WM and MM. Anaemia typically found in WM, as well as leukopenia and thrombocytopenia, neutropenia may also found.[13,14] Five-year survival rates for these categories are 87%, 68% and 36% respectively. The IPSSWM as well as Rituximab-based treatment regimen shows good outcome.[15] Amyloid Light-chain (AL) amyloidosis, Primary Systemic Amyloidosis (PSA) or just primary amyloidosis is the most common form of systemic amyloidosis in the USA.[16] About 10% to 15% of patients with multiple myeloma may develop overt AL amyloidosis.[17]

Materials and methods

An observational, descriptive, cross sectional study was taken place at Armed Forces Institute of Pathology (AFIP) Dhaka Cantonment, Dhaka from May 2017-October 2017. The necessary ethical issue has been considered before commence the study. A total of 165 Hyperproteinemic patient at all age and sex were included to the study and hypoproteinaemic patient, Normoproteinaemic patient and pregnancy were excluded. Six millilitres venous blood was collected asceptically from each patient. i) For the determination of serum total protein: 2 ml of blood in a vacutainer without anticoagulant. ii) For serum protein electrophoresis: 2 ml of blood in a vacutainer without anticoagulant. Serum taken from all tubes after collection and analyzed within 2 hours. Quantitative determination of total protein in human serum is done by using Siemens Dimension clinical chemistry analyzer. Serum protein electrophoresis is designed for the separation of human serum in alkaline buffer (pH 9.9) by capillary electrophoresis. The Capillarys performs all sequences automatically to obtain a protein profile for qualitative or quantitative analysis. With this technique, charged molecules are separated by their electrophoretic mobility in an alkaline buffer with a specific pH. Proteins are detected in the following order: gamma globulins, beta-2 globulins, beta-1 globulins, alpha-2 globulins, alpha-1 globulins and albumin with each zone containing one or more proteins.

Data from the study entered initially into Microsoft Office Excel program then into the Statistical Package for the Social Sciences (SPSS) program. Data will first be summarized in the form of descriptive, paired sample t test and Independent samples t test. The SPSS Version 16 was used for performing the statistical analysis which included: i) Basic descriptive statistical analysis was undertaken to compute the mean and standard deviation

for the variables. ii) Paired samples t test was used to calculate differences between mean of the total protein concentration, monoclonal gammopathy. Data was expressed as mean ± SD. $p< 0.05$ was regarded as statistically significant.

Results

The results of this study are presented in four major sections. The first section includes the subject characteristics (Age, sex distribution) and the second section deals with the results of the measured serum protein. The third and fourth, sections show the results of the frequency of monoclonal gammopathy.

One hundred and sixty five patients, 109 males (66.1) and 80 girls (33.9) with hyperproteinemic patient were included in this study. The age ranges of patients were 20 year to 90 years. The mean±SD age of males and females were 58±13.53 years and 59.18±12.57 years respectively, resulting in an overall mean±SD age of 58.41±13.19 years. The maximum number of patients 76 (46.1%) was found in the age group of 60-80 years, followed by 67 (40.6%) and 17 (10.3%) in the age group of 41-60 and 21-40 respectively.

Table I : Age distribution of the study population (n=165).

Age group in year	Male	Female	Total
1-20	2	-	2
21-40	9	8	17
41-60	48	19	67
61-80	48	28	76
81-100	2	1	1
Total	109	58	165
Mean ± SD	58.02 ± 13.53	59.8 ± 12.57	58.41 ± 13.19

Among 165 patients, males were 109 (66.1) and females 58 (33.9) with male to female ratio being 1.6:1.

Table II : Serum total protein of the study population according to sex (n=165).

Sex	Mean ± SD	Range
Male	87.02 ± 4.62	82-104
Female	85.91 ± 3.50	82-102

Table II shows the serum total protein according to sex. The mean ± SD total protein concentration in case of male was 87.02 ± 4.62 g/L and in case of female 85.91 ± 3.50 g/dL.

Table III : Comparison of total protein level between monoclonal gammopathy and without monoclonal gammopathy.

Parameters	Hyperproteinemic patients With monoclonal gammopathy (n=20) Mean ± SD	Without monoclonal gammopathy (n=145) Mean ± SD	p value
Total protein gm/L	94.7 ± 5.61	85.53 ± 2.57	0.002

Above table shows comparison of total protein concentration between with monoclonal gammopathy and without monoclonal gammopathy group. Among hyperproteinemic patient who had monoclonal gammopathy had higher total protein concentration 94.7 ± 5.61gm/L then who had without monoclonal gammopathy 85.53 ± 2.57. Total protein concentration showed statistically significant difference ($p<0.05$) between monoclonal gammopathy group of hyperproteinemic patient and without monoclonal gammopathy group of hyperproteinemic patients (Table III).

Table IV : Frequency of of monoclonal gammopathy in hyperproteinemic patients (n=165).

Monoclonal gammopathy	Number	Percentage (%)
With monoclonal gammopathy	20	12.1
Without monoclonal gammopathy	145	87.9
Total	165	100.0

Table IV shows monoclonal gammopathy was found to be present in 12.1% (20 out of 165) of hyperproteinemic subjects.

Table V : Distribution of monoclonal gammopathy in hyperproteinemic subjects according to age group and sex.

Age group in year	Sex	Hyperproteinemic patients With monoclonal gammopathy (n=26)	Without monoclonal gammopathy (n=147)	Total	p value
1-20	Male	0	2	2	
	Females	0	0	0	
21-40	Males	0	9	9	
	Females	0	8	8	
41-60	Males	07	41	48	1.0
	Females	02	17	19	
61-80	Males	7	41	48	1.0
	Females	4	24	28	
81-100	Male	0	2	2	
	Females	0	1	1	
	Total	20	145	165	

Table V shows the maximum number of monoclonal gammopathy subjects 11(55%) was found in 61-80year age group, followed by 09(45%) in the age group of 41-60 years and no monoclonal gammopathy was found rest of the group. When monoclonal gammopathy was compared between males and females in 61-80 year, 41-60 year age groups, statistically insignificant p values of 1.0 and 1.0 was found indicating no significant difference of monoclonal gammopathy was noticed in different age group with sex.

Discussion

Monoclonal Gammopathies (MGs) are B-cell lymphoproliferative disorders caused by a clonal proliferation of B lymphocytes that produce a homogeneous immunoglobulin called M-protein. Their clinical spectrum ranges from Monoclonal Gammopathy of Undetermined Significance (MGUS, a benign disorder characterized by monoclonal immunoglobulin level of <30 g/L and a percentage of plasma cells in bone marrow of <10%) to the full-blown disease Multiple Myeloma (MM). Other B lympho proliferative disorder associated with M-proteins include: waldenstrom s macro globulinemia, plasmacytoma, nonhodgkin lymphoma, chronic lymphocytic leukemia, primary and heavy and light chain amyloidosis diseases. MGUS are much more common than MM and their incidence is age dependent. The prevalence of MGs is about 1% in individuals up to the age of 60 and about 10% in people older than 80 years of age.[18] A total 165 subjects with hyperproteinemia were included in this study among them 109 were males (66.1% and 58 were females 33.9%) with male: female ratio 1.94: 1. The age range of patients was from 19 year to 90 years. The mean±SD age of study subjects were 58.41 ± 13.19 years.

In this study, the majority 76 (46.06%) patients belonged to the 61-80 year age group, followed by 67 (40.60%) patients in the 41-60 year age group. 17(10.30%) patent in 21-40 year followed by 2(1.21%) in 1-20 year. The rest 3(1.81%) were noted in the age group of 81-100 years age group. Serum total protein level of the patient in hyperproteinemic patient was ranged from 82-104. Total protein concentration in age group 1-20 years, 21-40 years, 41-60 years, 61-80 years and 81-100 years was 83 ± 1.41 g/L, 85.59 ± 3.2, 86.4 ± 3.64, 87.22 ± 5.02 and 85.67 ± 2.08 respectively.

Serum total protein concentration in case of male was 87.02 ± 4.62 g/L and in case of female 85.91 ± 3.5 g/dL. In this study, Among hyperproteinemic patient who had monoclonal gammopathy had higher total protein concentration 94.7+5.61 gm/L then who had without monoclonal gammopathy 85.53+2.57. Total protein concentration showed statistically significant difference ($p<0.05$). In this study monoclonal gammopathy was found to be present in 12.1% (20 out of 165) of hyperproteinemic subjects. In case of male 14(70%) and in female 6(30%).

In this study, frequency of monoclonal gammopathy was found 12.1% among hyperproteinaemic pateint. The result of this study correlated well with M.T Ageyein et al study.[19] Out of 90 samples of hyperproteinemia,11(12.2%) were positive for monoclonal gammopathy. Paricaud et al found 48.6% monoclonal gammopathy among hyperproteinemic patient.[20] Mean total protein concentration in my study was 86.64 ± 4.29 gm/L whereas in Paricaud study total protein concentration was much more higher 106 ± 6.9 gm/L.

In this study higher incidence of monoclonal gammopathy in males compared with females. Among the monoclonal gammopathy subjects 14 (12.84%) were male and the rest 06 (10.71%) were females. this finding was consistent with Afrouzi et al & Tamimi et al.[18,21] Afrouzi study shows that the rate of monoclonal gammopathy in men was approximately 1.5 fold of women (4.9% vs.3.15%)[18]. Study of monoclonal gammopathy in a tertiary referral hospital by Tamimi at al found that 7% monoclonal gammopathy among 6624 subjects in which 59% were males and 41% were females.[21] In contrast to this study M.T Ageyei et al in Ghana found higher incidence of monoclonal gammopathy in females as compared to males.[19]

Highest frequency of monoclonal gammopathy was found in the 61-80 year age group where the frequency was 11 in 20 (55%). This finding was consistent with the study Afrouzi et al and M.T Ageyei et al.[18,19] Afrouzi MM reported monoclonal gammopathy frequency increase with age. In age group 0-29 was 0.29%, 30-39year 0.53%, in 40-49 year 3.16%, in 50-59 year 4.79%, 60-69 year 6.78%, >70 year 6.2%.[18] M.T Ageyei study shows the rising incidence of monoclonal gammopathy

with age. One out of 11 patients with paraproteinemia (1.1%) was within the age group of 30-39, 2(2.2%) within the age group 40-49, 3(3.3%) in 50-59 and 5(5.5%) within the age group 60-69 years.[19] Ranjan Dash et al also found the similar type of result. 92% monoclonal gammopathy cases were detected in the age group 50-79 years with the peak incidence in the age group 60-69 years.[22]

The present study revealed 12.1% monoclonal gammopathy in hyperproteinemic patients and frequency was increased with rising of age. Higher frequency of monoclonal gammopathy was in males compared with female. Although the present study amply proves that increase frequency of monoclonal gammopathy is present in hyperproteinemic patients in a sizable fraction of the study group, a larger sample pooled across several centers catering to different strata of the society would have been more representative of increase frequency of monoclonal gammopathy in hyperproteinemic subjects.

Limitation

It was a single centered study with less number of data due to shortage of time.

Conclusions

The treatment, monitoring and prognosis of monoclonal gammopathy depends on the early detection of M protein band in electrophoretic pattern.

This study reveals a high incidence of monoclonal gammopathy in patients with hyperproteinemia. The increase incidence of the monoclonal band with age is higher among males specialy 40 to 60 years age group indicating that male, 40 to 60 years age group are at risk of developing monoclonal gammopathy. It is recommended that serum protein electrophoresis should be performed on all hyperproteinemic samples. So, this study will be very helpful for the treating physicians to enable early diagnosis and treatment of monoclonal gammopathy.

Recommendations

The present study revealed high incidence of monoclonal gammopathy among hyperproteinemic patients. With the background of prevailing monoclonal gammopathy in Bangladesh, this study recommends that Serum protein electrophoresis should therefore, be routinely performed on all samples found to have high protein level. This will enable early treatment of patients with monoclonal gammopathies.

Contribution of authors

MSR-Conception, data collection, data analysis, drafting & final approval.

MMU-Design, data analysis, interpretation of data, critical revision & final approval.

MMR-Interpretation of data, data collection, drafting & final approval.

Disclosure

All authors declared no competing interest.

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