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Immunological evidence of monoclonal gammopathy in North India: a hospital based study

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Background: Monoclonal gammopathy of unknown significance (MGUS) is a condition in which a paraprotein is found in the blood during standard laboratory tests. It is age-related and characterized by accumulation of bone marrow plasma cells derived from a single abnormal clone. The aim of this study was to investigate the pattern of MGUS in North Indian urban population.

Methods: Serum and urine samples were collected from 320 suspected cases of gammopathy, were analyzed by sensitive immunological technique based protein electrophoresis followed by immunofixation for detection and type of monoclonal/polyclonal gammopathies. Twenty-five healthy subjects were included as controls.

Results: Gammopathies were observed in 38 (11.88%) patients. Out of these 7.5% were monoclonal and 4.3% were polyclonal. Overall age of presentation of these monoclonal gammopathies in both sexes was between 21 and 76 years. Gender-related ratio (men:women) for these gammopathies was 1:1.18. Predominant heavy chain isotype was IgG (62.5%) followed by IgA (37.5%). Among light chains, kappa (κ) and lambda (λ) chains appeared in 91.6% and 8.4% gammopathies respectively. Paraprotein fractions obtained were IgGκ (58.3%), IgGλ (4.16%), IgAκ (33.3%), and IgAλ (4.16%) with 25% samples being positive for Bence Jones proteinuria.

Conclusions: Clinical laboratories play an important role in confirming the immunological diagnosis of gammopathies. Determination of nature of paraproteinemia and its associated diseases calls for more extensive studies in India.

Keywords: monoclonal gammopathy, immunoelectrophoresis, multiple myeloma, bence jones protein, immunoglobulins

Introduction

Disturbance in synthesis of immunoglobulins leading to increased production of antibody activity in the blood is termed as gammopathy. It can be polyclonal (heteregeneous increase in immunoglobulins involving more than one cell line) or monoclonal (characterized by the proliferation of one or more clones of differentiated B lymphocytes that produce an immunologically homogeneous immunoglobulin commonly referred to as a paraprotein or monoclonal (M) protein). The presence of an M protein alone does not indicate a neoplastic process. The term monoclonal gammopathy of unknown significance (MGUS) denotes the presence of a monoclonal immunoglobulin (Ig) without evidence of multiple myeloma (MM), Waldenström macroglobulinemia (WM), amyloidosis (AL) or other lymphoproliferative disorders.[1] MGUS is the most common of a spectrum of diseases called plasma cell dyscrasias and requires differentiation from

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the other monoclonal gammopathies because patients with MGUS are conservatively treated and do not need chemotherapy.

In the case of monoclonal gammopathy the circulating M-protein may consist of an intact immunoglobulin, the light chain only (either κ or λ), or (rarely) the heavy chain only (one of the five immunoglobulin classes G, A, M, D, or E). The prevalence of monoclonal gammopathies, which is about 1% in the general adult population, increases with age and some pathological conditions like hepatitis C virus infection, where it may exceed 10%.[2] Confirmation of diagnosis is based on immunological evaluation (demonstration of M-protein), radiological visualization of lytic bone lesions, biochemical investigations (light-chain proteinuria), or excessive marrow plasma cells. Multiple myeloma (MM) is characterized by proliferation of a clone of plasma cells. It is one of the most common hematologic malignancy that manifests by the presence of one or more lytic bone lesions, monoclonal (M) protein in the blood/urine and bone marrow involvement.[3] It is nearly always preceded by a premalignant plasma cell disorder characterized by the presence of monoclonal gammopathy of undermined significance (MGUS).[4]

Biochemical and immunological investigations have gained tremendous importance over years to help in establishing the diagnosis of diseases. Clinical laboratories play an important role in the study of monoclonal gammopathies, since it is the only location where all M-protein patients are observed. Increased amount of both electrophoretically and immunologically homogeneous M component is the main characteristic of monoclonal gammopathies (MG). Detection of M protein through characterization of the immunoglobulin type, is therefore of fundamental importance for a definitive diagnosis of MM.

Monoclonal gammopathies have been studied extensively in different parts of the world however no data on the pattern of MGUS in urban North Indian population is available. The present study aims to determine the prevalence and pattern of presentation of M proteins in monoclonal gammopathies in a tertiary care hospital in North India.

Materials and methods

Selection of patients

The present study was a prospective, hospital based study involving 320 patients, referred to the Department of Biochemistry of a tertiary care 500-bed hospital over a period of two years from April 2007 to March 2009. Out of 320, 216 patients were referred by medicine department, 69 from orthopedics department, 5 from gastroenterology, and 1 from neurosurgery. Twenty-nine known cases of multiple myeloma were referred by radiotherapy department. The common presenting complaints were: backache, pathological fractures and vertebral collapse, unexplained anemia, or renal failure. Twenty-five normal subjects were included as controls.

Sample collection

Five milliliters of venous blood was drawn from the patients. The serum was separated by centrifugation after the sample had clotted. Spot urine samples (approximately 20 mL) were collected in sterile containers and immediately transported to the laboratory. Urine and serum were stored at −20°C until analyzed.

Protein electrophoresis and immunofixation

Protein electrophoresis and densitometry

Preliminary screening of serum and urine samples for presence of any monoclonal gammopathy was carried out by serum protein electrophoresis (SPEP) performed by agarose gels (Hydrasys and Hydragel from Sebia, UK) followed by Coomassie blue staining and destaining procedures. The diagnosis of polyclonal hypergammaglobulinemia was made by visual inspection of the SPEP patterns on agarose gel. The stained electrophoresed gel was scanned on densitometric scanner (Beckman densitometer, USA) for estimation of the protein bands.

Immunoelectrophoresis

Serum and urine samples that tested positive for paraprotein were further investigated by immunoelectrophoresis. Immunofixation electrophoresis (IFE) with antisera to IgG, IgM, IgA, κ, and λ was performed (Hydrasys and Hydragel, Sebia).

Bence Jones proteinuria

Bence Jones proteinuria was detected by the heat coagulation test where the protein precipitates when heated to 50–60°C and redissolves at 90–100°C.

Result

A total of 320 patients (age ranging from 18 to 78 years) reported for serum protein electrophoresis in the Department of Biochemistry over a period of 2 years. On SPEP, gammopathy was detected in 38 new patients (11.88%) and 29 known patients of multiple myeloma. However, in the control group (25 healthy subjects), normal protein electrophoresis pattern was observed.

Table 1 Age and gender-related distribution in patients suffering from monoclonal (n = 24) and polyclonal (n = 14) gammopathy

Age group (years)	Male		Female	
	Monoclonal	Polyclonal	Monoclonal	Polyclonal
21–30	1	3	nil	2
31–40	1	nil	1	nil
41–50	1	1	3	1
51–60	2	nil	2	3
61–70	4	2	3	1
>70	2	nil	4	1
Total	**11**	**6**	**13**	**8**

Out of 38 new diagnosed cases of gammopathy, 24 had monoclonal gammopathy (7.5%), whereas 14 patients had multiple bands on SPEP indicating polyclonal gammopathy (4.3%). The prevalence of monoclonal gammopathies was found to be higher in older subjects (17 out of 24 cases were more than 50 years of age whereas only 1 case was in the age group 21–30 years). However, for polyclonal gammopathies, no age-specific pattern was observed as shown in Table 1. In the patients with monoclonal gammopathy, 11 were males and 13 were females (male: female ratio being 1:1.18). Median age of presentation was 56 years. In case of polyclonal group, out of total 14 patients males and females were 6 and 8 respectively (male: female ratio being 1:1.33) (Table 1). Amongst the patients presenting with monoclonal gammopathy, backache was the most common presenting symptom followed by vertebral collapse and anemia. In patients with polyclonal gammopathy, hepatitis was the most common presenting complaint (Table 2).

Serum of all of these patients showing presence of M band on electrophoresis was subjected to immunofixation electrophoresis. The most frequent M-protein isotype found was IgG (62.5%), followed by IgA (37.5%). Amongst the light chains, 91.6% were κ chain and 8.4% had λ light chain. The paraprotein fractions obtained and reported according to our data were IgG κ (58.3%), IgG λ (4.16%), IgA κ (33.3%), and IgA λ (4.16%) as shown in Table 3. No IgM type paraprotein was seen.

In 29 known (old) cases of multiple myeloma 58.62% (17 cases) had IgG κ, 10.34% (3 cases) had IgG λ, 20.68% (6 cases) had Ig A κ, and 10.34% (3 cases) had Ig A λ antibodies. No IgM band was found in these cases.

Visual inspection of electrophoresis gel revealed that majority of IgG type paraprotein was in the slow gamma globulin region and majority of IgA type paraprotein was found in beta and fast gamma globulin regions. Densitometric scanning of electrophoresis gel was done to quantify serum proteins. The monoclonal isotypes were found to have decreased albumin and alpha-2-globulin concentrations as compared to normal controls.

Urine samples of all patients were analyzed for Bence Jones proteinuria. Six out of 24 (25%) patients with monoclonal gammopathy demonstrated the presence of Bence Jones proteins in their urine samples.

Discussion

Hypergammaglobulinemia results from overproduction of immunoglobulins by plasma cells. This study aims to present the data collected at the tertiary care hospital in North India reflecting the prevalence of both polyclonal and monoclonal gammopathies along with the isotypes commonly seen in North Indian population.

In the population screened, monoclonal gammopathy was observed in 7.5% of patients. Average specific incidence rates are increasing sharply with age, independent of gender or race with a slightly lower rate being reported in UK, Eastern Europe, South America, India, and Japan.[5] The largest and most frequently cited survey in the white population studied in Olmsted County, Minnesota found the prevalence of MGUS to be 3.2% in persons over 50 years of age and 7.5% in those over 70 years.[6] Geographical variation tends to exist with monoclonal gammopathies. Reports on the prevalence of gammopathies varies from 5.84% in Ghanaian population[4] to 2.4% in Japan.[7] Cohen et al[8] have reported prevalence of monoclonal gammopathy as 6.1% with a greater than 2-fold difference between blacks (8.4%) and whites (3.8%). Although

Table 2 Common clinical manifestations in newly diagnosed cases of gammopathy

Polyclonal gammopathy (n = 14)		Monoclonal gammopathy (n = 24)	
Symptoms	**Number of cases**	**Symptoms**	**Number of cases**
Tuberculosis	3	Vertebral collapse	6
Hepatitis	4	Backache	7
Sarcoidosis	1	Anemia	6
Rheumatoid arthritis	2	Generalized weakness	3
Cirrhosis	2	CRF	2
Lymphoma	1		
Could not be gathered	1		

Table 3 Paraprotein fractions in patients with positive M band (by immunofixation)

		IgG κ	IgG λ	IgA κ	IgA λ	IgM (κ/λ)
Total no. of cases	24	14	1	8	1	nil
% of total	100	58.3	4.16	33.3	4.16	nil
Male	11	7	nil	3	1	nil
Female	13	7	1	5	nil	nil

prevalence observed in the North Indian urban population in this study is slightly higher than seen in other parts, it could be due to short time frame and less population studied rather than racial and environmental differences only.

The median patient age observed in the present study was 56 years, which was found to be lower as compared to that reported in earlier studies. The median age as reported by earlier studies was 70 years at diagnosis (range: 53–90)[9] and 73 years in a study done at Spanish health center.[10] In the present study the presence of monoclonal gammopathy was found to be slightly higher in women (men: women ratio being 1:1.18). This observation is consistent with a study conducted by Enrique et al (in Spanish population) where it was reported to be 1:1.3.[10] Our observation is, however, in contrast with the findings by Shafi et al (in Indian Kashmiri population) where a higher prevalence was observed in men as compared to women(1.4:1).[11]

In this study, the most common M-protein was of IgG or IgA type (62.5% and 37.5% respectively) with 91.6% having κ chain and 8.3% λ light chain. None of the samples showed the presence of IgM. In a study conducted in Kashmiri population in India, Shafi et al detected IgG type (75.47%) as predominant paraprotein followed by IgA (16.98%).[11] The findings by Shafi et al is consistent with observation from our study in which 64.16% patients had κ type light chains and 35.84% λ type light chains, though IgG isotype was more in Kashmiri population as compared to the patients visiting our hospital in Delhi. Also light chains were present in different proportion. The appearance of Bence Jones proteins in urine samples was observed only in 25% patients as compared to approximately 69.39% patients in monoclonal gammopathy patients in earlier studies,[11] the variation could be due to ethnic differences.

In another study in Japan,[7] the heavy chain isotypes of immunoglobulin were IgG in 73.6% of patients suffering from MGUS, IgA in 17.7%, IgM in 7.5%. A Spanish study has also reported IgG (55.8%) as the most frequent M-protein isotype followed by IgA (20.8%) and IgM (13.6%).[10]

Landgren et al[4] observed that in Ghanaian men with evidence of MGUS, the isotype of the monoclonal immunoglobulin was IgG 74%, IgA in 19% and IgM in 6%. The serum light chain type was κ in 48% and λ in 50% of cases.[4] Olmsted County survey[6] reported IgG in 68.9%, IgM in 17.2%, IgA in 10.8%, and biclonal in 3.0% percent with κ light chain in 62.0% λ in 37.9%. IgG was most common as detected by us though the pattern of other isotypes was different, percentage of IgA paraprotein being more and no IgM paraprotein detected in our population. Other differences include almost equal percentage of κ and λ light chains where as we detected higher κ chain (91.6%) as compared to λ chain (8.3%). However, IgM isotype was found to be more frequent in studies conducted in other parts of globe.[12–13]

Thus, it is evident that the pattern of monoclonal gammopathy observed in the North Indian urban population does not show much difference from the data available around the world. The only difference observed was the lack of IgM in this population, which was found to be present in other studies.

Our study had several limitations; only 25 normal healthy adults could be screened because of the financial constraints; study was hospital based and thus cannot be generalized for entire North Indian population. Hence, screening of healthy Indian population is recommended to have more conclusive data on the prevalence of MGUS in India.

Conclusion

We conclude that the clinical laboratories should play a more pro-active role in the study of monoclonal gammopathies in community, since all M-proteins can be detected and confirmed by the immunological techniques. However, future screening studies are needed in India.

Disclosure

No conflicts of interest were declared in relation to this paper.

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