AN IMMUNOLOGICAL BASED STUDY OF MONOCLONAL GAMMOPATHIES AMONG SUSPECTED INDIVIDUALS IN KASHMIR REGION

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ABSTRACT

Serum and urine samples from 513 patients clinically suspected of monoclonal gammopathies over a period of five years (1992-97) were subjected to various immunological procedures viz, electrophoresis, immunoelectrophoresis and immunoglobulin estimations. Laboratory investigations confirmed gammopathies in 10.33%. It was observed that overall age of incidence for monoclonal gammopathies in both sexes was between 42-72 years with a male to female ratio of 1.4:1. Predominant paraprotein detected was IgG type (75.47%) followed by IgA (16.98%) and Bence Jones proteins (7.55%). Amongst positive patients, 64.16% were having kappa (κ) type light chains and 35.84% lambda (λ) type light chains. 69.39% patients with serum M component (IgG and IgA) had Bence Jones proteinuria. Densitometric scanning revealed that majority of IgG type paraprotein was found in the slow gamma globulin region and majority of IgA type paraprotein was found equally distributed between beta and fast gamma globulin regions. Both types had decreased albumin and alpha-2- globulin concentrations as compared to normal controls. Immunoglobulin levels in patients with paraprotein had very high levels of serum IgG (6467.0 mg%) and IgA (2714.0 mg%) in respective types of monoclonal gammopathies; the rest of immunoglobulin classes were either at normal or decreased levels.

KEY WORDS: Monoclonal gammopathy, Immunoelectrophoresis, Densitometry, Bence Jones proteinuria, Immunoglobulins.

INTRODUCTION

Diagnosis of a disease entity, benign or malignant in a subject is primarily based on clinical examination and is supported and confirmed by various laboratory investigations. A monoclonal gammopathy is a disease condition used to designate the proteins usually produced by malignant cells in multiple myelomas and other B cell lymphoproliferative lesions; presence of M-component alone however, does not indicate a neoplastic process. Monoclonal gammopathies of all types have been studied in detail by various workers in other parts of the world including some parts of India. No such study has been conducted in Kashmir, situated at an altitude of above 5000 feet, where people in general have different life styles, food habits and exposed to different environmental conditions than those from other

parts of India. Serum and urine samples of individuals suspected of monoclonal gammopathies by clinical and radiological examinations were subjected to various immunological investigations for determination and characterization of any monoclonal protein.

MATERIALS AND METHODS

Blood and urine samples from suspected patients attending the hospital and referred to Immunology Department for investigations during the period 1992-97 (5 years) were collected. In all, 513 patients were investigated. Twenty normal subjects were included in the study as controls.

Collection of blood and urine samples

Five ml of blood samples were collected by venipuncture in plain vials. The blood was allowed to clot, serum was separated by centrifugation and kept at -20°C until assayed. Urine samples

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(24hour) were collected in clean containers. Thirty ml of each urine sample was concentrated to 1.5 ml by dialysing against sucrose in dialysis bags (10,000 MW retention). The concentrated samples were preserved at -20°C until assayed.

Electrophoresis of samples

Initial screening of serum and urine samples for presence of any monoclonal gammopathy was undertaken by performing agarose gel electrophoresis (1) using a horizontal electrophoresis apparatus (Heto Frig, Denmark) followed by staining with Coomassie blue staining and destaining procedures.

Immunoelectrophoresis of samples

Serum and urine samples showing presence of a monoclonal protein were further analysed by immunoelectrophoresis (IEP) (2,3).

Densitometric scanning of electrophoresed samples

Each stained electrophoresed plate was subjected to densitometric scanning (4) for evaluation of distribution pattern of different protein bands and computed calculations of concentrations using Beckman densitometer.

Immunoglobulin estimation by single radial immunodiffusion (SRID)

Quantitative estimation of immunoglobulin classes (IgM, IgG and IgA) in all serum samples from suspected as well as control individuals was carried out using SRID plates from M/S Behring, Germany (5). Reference sera (Orion Diagnostics, Finland) of high, medium and low

concentrations of each class of immunoglobulins were used to establish the standard reference curve for estimation of respective immunoglobulin class.

RESULTS

Out of a total 513 suspected patients, only 53 patients showed presence of a monoclonal gammopathy in serum or urine or both. The details of patients screened and initial investigation by agarose gel electrophoresis are given in tables 1 and 2.

Table 1. Age and Sex Distribution of Patients

<i>Patients</i>	
A. Total nos. clinically suspected	: 513
B. Male to Female distribution	: 264:249
C. Age group	: 25-90 yrs
<i>Patients with paraproteins</i>	
D. Total samples positive	: 53
E. Percent positive (Total)	: 10.33
F. Male to female distribution	: 31:22 (1.4:1.0)
G. Age group of males	: 46-70 yrs
H. Percent positive among males	: 58.49
I. Age group of females	: 42-72 yrs
J. Percent positive among females	: 41.51

Each electrophoresed serum sample was subjected to densitometric scanning for determining the percentage of different protein fractions. The concentration of different immunoglobulins for serum samples was also estimated and the results are shown in table 3.

Table 2. Detection of Paraproteins in serum and urine by agarose gel electrophoresis in suspected patients.

Total No. of patients	No. of samples without M-protein	No. of samples Total	samples with Serum & Urine	M - protein Serum only	Urine only	Bence Jones protein
513	460	53	34	15	4	34
(100.00)	(89.67)	(10.33)	(6.63)	(2.92)	(0.78)	69.39*

Values in parentheses indicate percent of total.

*The data represents the % of serum positive M-proteins (34+15=49).

Table 3. Densitometric scanning and immunoglobulin estimations of serum samples of suspected positive patients with monoclonal gammopathies

Type of para- protein	Densitometric scanning (Mean % of total protein)							A/G Ratio	Immunoglobulin levels (mg/dl)		
	Albumin	Globulin							IgG	IgA	IgM
		1	2	β	Fγ	Sγ	Tγ				
IgG	36.9	1.6	8.5	10.6	9.0	33.4	42.4	0.62	6467	142	78
IgA	36.4	1.1	7.2	25.8	16.8	12.7	29.5	0.58	948	2714	88
Controls	44.9	2.0	15.6	14.4	8.9	14.2	23.1	0.82	1376	193	139

*1= α -1-macroglobulin, 2= α -2-macroglobulin, β = β -globulin,

F γ = Fast γ globulin, S γ = Slow γ globulin, T γ = Total γ globulin.

The samples showing presence of M-protein in serum or urine samples were further analysed by Immunoelectrophoresis and the results are depicted in tables 4 and 5.

Table 4. Prevalence of different types of paraproteins in serum and urine samples of positive patients by Immuno-electrophoresis.

Sex	No.of cases	Type of paraprotein in serum			Presence of free light chains in urine
		IgM	IgG	IgA	
Male	31	—	25	04	02
Female	22	—	15	05	02
Total	53	—	40	09	04
% of total	100.00	—	75.47	16.98	7.55

Table 5. Light chain status of patients of paraproteins evaluated by Immunoelectrophoresis

Type of Para-protein	Total	Type of light chain					
		Kappa (κ)			Lambda(λ)		
		Male	Female	Total	Male	Female	Total
IgG	40	14	12	26	11	03	14
IgA	09	03	04	07	01	01	02
Light chains	04	—	01	01	02	01	03
Total	53	17	17	34	14	05	19
% Positive	100.00	32.08	32.08	64.16	26.41	09.43	35.84

DISCUSSION

None of the positive cases had IgM type paraprotein (Waldenstrom's Macroglobulinaemia). We, however, do not conclude that IgM type paraprotein does not exist in Kashmiri community as the present study was hospital based and not community based. The densitometric scanning provided information about relative increase and decrease within protein population and homogeneity of each protein. The major quantity of IgG type paraprotein was found in slow gamma globulin region where as major portion of IgA paraprotein was distributed equally in beta and fast gamma regions. Both IgG & IgA type paraproteins had decreased albumin and alpha-2 globulin as compared to normal controls. Bailey et. al. (6) have reported that bulk of IgG is found in slow gamma region and IgA in the fast gamma globulin region. This variation with regards to IgA type could not be fully explained. This can be attributed to the fact that only 53 positive cases were available for comparison.

The immunoglobulin estimation of the positive cases revealed that the patients with IgG type paraprotein had very high serum IgG levels (6467 mg%) and normal IgA and IgM levels. Similarly, patients presenting IgA type paraproteins had very high serum IgA levels (2714 mg%) with normal IgG and IgM levels. Some of the patients with IgG type paraprotein had either low IgM or IgA levels. Similarly, in some of the patients with IgA type paraprotein IgM levels were lower than normal. The immunoglobulin levels presented in the table 3 are average values of positive and normal controls. These levels are quite in accordance with the published criteria for these two types of paraproteins.

It is evident from the results that presence of monoclonal gammopathies in suspected patients in this region do not show any major drift from the accepted data in general as depicted in table 6.

To conclude, the present study has given some information regarding prevalence of

Table 6. Comparison of the immunological parameters evaluated in the present study with the reported data

Reference	Age group	male:female ratio	predominant isotype*	predominant light chain*	Proteinuria
Rapaport & Rowe (7)	50-70	—	—	—	—
Kyle and Bayrd (8,9)	—	1.6:1	IgG (70%)	—	—
	—	—	IgA (20%)	—	—
Jacobs (10)	—	1:1	—	—	—
Longo (11)	—	—	IgG (53%)	—	67%
	—	—	IgA (25%)	—	—
Chappel & Haney (12)	—	—	IgG (60%)	—	—
	—	—	IgA (20%)	—	—
Kern (13)	—	—	—	Kappa (75%)	—
	—	—	—	Lambda (25%)	—
Ossermann et al. (14)	—	—	—	—	65%
Gandara & Mackenzie (15)	—	—	—	—	65%
Shafi et. al. (Present study)	42-72	1.4:1	IgG (75.47%) IgA (16.98%) Light Chain Disease(7.55%)	Kappa(64.16%) Lambda(35.84%)	69.39%

*The data in the parentheses indicate percentage of total positive cases

monoclonal gammopathies, light chain status and Bence Jones proteinuria among patients. This study was conducted on those individuals who reported to the hospital after being suspected of having any type of gammopathy, thus it does not reveal a true picture of the monoclonal gammopathies in the community. Due to non-availability of community based mortality data at the state level, a more detailed survey of the local population is needed to assess the

prevalance and mortality due to the disease in the community.

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