Occurrence of Serum M-Protein Species in Japanese Patients Older Than 50 Years Based on Relative Mobility in Cellulose Acetate Membrane Electrophoresis

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We investigated the occurrence of serum M-protein species in 2,007 Japanese patients older than 50 years of age. All sera samples were analyzed by cellulose acetate membrane electrophoresis. The relative mobility of an M-protein band was calculated by dividing the migration distance of M protein by that of albumin. M proteins were found to be present in 71 of 2,007 cases (3.5%). Men 80–89 years old showed the highest occurrence of M proteins, 11.0%. The relative mobility of M-protein bands, especially the band of the IgA-type M protein, increased as the patient's age

advanced. The patients had higher levels of the IgG-type M protein than healthy Japanese subjects. We found that the occurrence of M-protein species in Japanese patients increases with their age. The IgG-type M protein was most frequently expressed among other types. The mobility of the M protein was greater in older patients probably because of aging-related changes in the carbohydrate chain of immunoglobulins composing an M-protein molecule. *J. Clin. Lab. Anal.* 14:64–69, 2000. © 2000 Wiley-Liss, Inc.

Key words: monoclonal gammopathy of undetermined significance; elderly person; incidence of M protein; IgG subclass

INTRODUCTION

M proteins are occasionally detected in healthy human subjects, elderly persons, and patients with malignant diseases. It has been reported in Sweden (1–3), the U.S. (4,5), and France (6) that approximately 3% of persons older than 70 have monoclonal gammopathy without showing any evidence for having myeloma, amyloidosis, macroglobulinemia, or related lymphoproliferative disorders. Such cases of benign monoclonal gammopathy are termed as the monoclonal gammopathy of undetermined significance (MGUS). However, Kyle et al. (7,8) have reported that 11% of MGUS patients developed multiple myeloma, macroglobulinemia, or a malignant lymphoproliferative process in 5 and 20–35 years of following up. Thus, it is expected that understanding the property of M proteins will be very useful for diagnosing and treating these MGUS-related diseases.

We have collected serum samples from 2,007 patients who were over 50 years old and who visited Gunma University Hospital and were screened for the presence or absence of serum M proteins by means of cellulose acetate membrane electrophoresis. Observed M proteins consisted of light and

heavy chains. Observed incidence of M-protein species was stratified for every 10 years of patients' age.

Even the same type of M protein showed a variety of relative mobility values in electrophoresis. Therefore, we further studied the correlation between the relative mobility of M-protein bands and aging or diseases.

MATERIALS AND METHODS

Subjects

The subjects studied were 2,007 randomly selected patients at Gunma University Hospital: 1,043 elderly males (50–97 years old) and 964 elderly females (50–96 years old). All collected serum samples were stored at –80°C until analysis.

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Cellulose Acetate Membrane Electrophoresis

All serum samples were analyzed by cellulose acetate membrane electrophoresis. A group of several serum samples was loaded onto a Separax-SP membrane (Fuji Photo Co. Ltd., Tokyo, Japan) and electrophoresed in 0.6 mol/l veronal buffer (pH 8.6,) at 0.7 mA/cm for 30 min.

Protein Fractions

After electrophoresis, the membrane was stained with 0.8% Ponceau-3R in 6% TCA solution for 2 minutes, then destained in 3% acetic acid solution and dried at room temperature. The percentage of each protein fraction was determined by scanning at 508 nm using the densitometer (Cliniscan, Helena Laboratories. Co. LTD., Beaumont, TX.).

Immunofixation

Immunofixation was carried out according to Aguzzi et al. (9). Serum samples were diluted in ratios from 1:1 to 1:19 with physiological saline depending upon the content of M proteins. Diluted samples after cellulose acetate membrane electrophoresis were immunofixed on the membrane using the specific antiserum (Dako, Glostrup, Denmark) to IgG, IgA, and IgM (kappa and lambda types for each) for 20 minutes at room temperature. The membrane was deproteinized, stained by Ponceau 3R, and destained in 3% acetic acid solution.

Quantification of M Protein

The content of each M protein was calculated by multiplying the amount of total protein by the percentage ratio of an M-protein band.

Statistical Analysis

Wilcoxon's test and Mann-Whitney's U-test were used for testing the statistical significance of intergroup differences, taking $P < 0.05$ as the level of significance.

RESULTS

Age-Related Incidence of M Proteins

As a preliminary test, approximately 400 in- and outpatients at Gunma University Hospital were divided into nine age groups; 0–19, 20–29, 30–39, 40–49, 50–59, 60–69, 70–79, 80–

89 and 90–97 years, and the appearance of M proteins was examined by means of cellulose acetate membrane electrophoresis in about 50 patients selected randomly from each age group. Any M-protein band could not be detected in patients younger than 49 years. Thus, the occurrence of M proteins was examined in 2,007 sera samples obtained only from patients (1,043 males and 964 females) at the age of 50 years or older.

As shown in Table 1, the incidence of M protein in all cases studied was 3.5% (71/2,007 cases). The group of male patients at 80–89 years of age showed the highest incidence of M proteins, 11.0% (11/100 cases). In the group of female patients at the same age range (80–89 years), on the other hand, the incidence was 3.39% (2/59 cases). Excepting females at 60–69 years of age, all age groups of males showed higher values of M-protein incidence than all age groups of females.

Identification of M-Protein Species

The type of M proteins was identified using immunofixation in 71 cases with electrophoretically detected M proteins. As shown in Table 2, IgG-type M proteins were most frequently observed in all age groups, except for the 90–97 years group which had no IgG M protein. IgG kappa-type M protein occupied 54.9% (39/71 cases), and IgG lambda-, IgA kappa-, IgA lambda-, IgM kappa-, and IgM lambda-type M proteins occupied 22.5, 8.5, 8.5, 4.2, and 1.4%, respectively. All 5 patients in the 50–59 year group had only IgG kappa-type M protein (100%, 5/5).

The mean age of patients with each M-protein type was 69.1 ± 9.65 years for IgG kappa type, 71.6 ± 7.59 years for IgG lambda type, 71.8 ± 11.1 years for IgA kappa type, 73.3 ± 8.02 years for IgA lambda type, 77.7 ± 12.7 years for IgM kappa type, and 68 years for IgM lambda type. There was no sex difference in the incidence of each M-protein subclass. The IgG kappa-type M protein was most frequently expressed in both male and female patients. The lowest incidence of IgG kappa-type was observed in 70-year-old individuals.

Relative Mobility of M-Protein Bands

The relative mobility of each M-protein band was calculated in relation to patients' age. To calculate the relative mobility, the electrophoretic migration distance of the M protein was divided by that of albumin.

TABLE 1. Occurrence of M proteins in age groups

Age	Total			Male			Female		
	No. of cases	M protein		No. of cases	M protein		No. of cases	M protein	
		No. of cases	% Incidence		No. of cases	% Incidence		No. of cases	% Incidence
50–59	451	5	1.1	203	2	1.0	248	3	1.2
60–69	775	31	4.0	402	13	3.2	373	18	4.8
70–79	599	21	3.5	321	13	4.0	278	8	2.9
80–89	159	13	8.2	100	11	11.0	59	2	3.4
90–97	23	1	4.3	17	1	5.9	6	0	0.0
Total	2,007	71	3.5	1,043	40	3.8	964	31	3.2

TABLE 2. Immunoglobulin types of M proteins

	IgG (kappa)		IgG (lambda)		IgA (kappa)		IgA (lambda)		IgM (kappa)		IgM (lambda)	
	No. of cases	% Incidence	No. of cases	% Incidence	No. of cases	% Incidence	No. of cases	% Incidence	No. of cases	% Incidence	No. of cases	% Incidence
Age												
50–59	5	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
60–69	18	58.1	6	19.4	3	9.7	2	6.5	1	3.2	1	3.2
70–79	9	42.9	7	33.3	2	9.5	3	14.3	0	0.0	0	0.0
80–89	7	53.8	3	23.1	0	0.0	1	7.7	2	15.4	0	0.0
90–97	0	0.0	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0
Total	39	54.9	16	22.5	6	8.5	6	8.5	3	4.2	1	1.4
Males												
50–59	2	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
60–69	8	61.5	3	23.1	1	7.7	1	7.7	0	0.0	0	0.0
70–79	6	46.2	4	30.8	1	7.7	2	15.4	0	0.0	0	0.0
80–89	6	54.5	2	18.2	0	0.0	1	9.1	2	18.2	0	0.0
90–97	0	0.0	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0
Total	22	55.0	9	22.5	3	7.5	4	10.0	2	5.0	0	0.0
Females												
50–59	3	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
60–69	10	55.6	3	16.7	2	11.1	1	5.6	1	5.6	1	5.6
70–79	3	37.5	3	37.5	1	12.5	1	12.5	0	0.0	0	0.0
80–89	1	50.0	1	50.0	0	0.0	0	0.0	0	0.0	0	0.0
90–97	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	17	54.8	7	22.6	3	9.7	2	6.5	1	3.2	1	3.2

Fig. 1. Correlation between the age and the relative mobility of M-protein types. Correlation between the patient's age and the relative mobility of IgG type or IgA type M protein was examined. IgG (lambda) and IgA (kappa)

were correlated significantly with the age, while IgG (kappa) and IgA (lambda) showed no such correlation.

As shown in Figure 1, values of relative mobility of IgG kappa, IgG lambda, IgA kappa, and IgA lambda M proteins correlated with the age, with positive correlation coefficients of 0.233, 0.527, 0.827, and 0.601, respectively. Among them, the relative mobility of IgG lambda and IgA kappa M proteins correlated significantly with the patients' age.

M-Protein Levels and Immunoglobulin Types in Malignant and Benign Diseases

In all patients expressing M proteins, 63 patients with established diagnosis were grouped for individual diseases, and identified M-protein types in these patients are summarized in Table 3. In these 63 patients, 35 patients had cancers and occupied 55.6% (35/63) of patients with M proteins. Of these cancerous patients, 9 had prostate cancer, 8 had alimentary (stomach, intestine, etc.) cancer, 4 had uterus/ovary cancer, 3 had lung cancer, 3 had kidney tumors, 2 had liver cancer, 2 had osteoncus, and there was 1 case each of thyroid, pituitary, gallbladder, and basal cell tumor cancers. Multiple myeloma was detected in 8 patients with M proteins, 3 of whom were complicated with cancer. Macroglobulinemia was found in 2 patients with M proteins. In the other 15 cases (23.8%, 15/63), 2 had bone fracture without ill changes such as osteomyelodysplasia, 2 had aneurysm, and there was 1 case each of diabetes mellitus, tuberculosis, eruption, rectal polyp, skin ulcer, cerebral infarction, atrial septum defect, aortic incompetence, renal insufficiency, boil, and pedal mycosis. These 15 cases were regarded as those of benign diseases. None of the malignant diseases previously mentioned appeared to express any specific type of M protein, and the pattern of incidence of each M-protein type in individual diseases was nearly similar to that in all cases studied (see Table 2). Patients with the benign diseases only had IgG- or IgA-type M protein and none of them showed IgM type.

Table 4 shows the mean M-protein level and its range in each disease. Patients with multiple myeloma and macroglobulinemia had higher mean levels of M protein (3.17 and 2.15

g/dl, respectively) than did other patients (0.78–1.22 g/dl). M-protein levels in patients with benign diseases and in patients with various cancers alone were significantly ($P < 0.05$) lower than those in other groups.

Mean values of relative mobility of all M-protein bands in individual diseases are also shown in Table 4. A difference ($P < 0.05$) was obtained between the benign disease group and the other group.

DISCUSSION

Monoclonal gammopathy of undetermined significance (MGUS) is the benign M proteinemia termed by Kyle et al. in 1978 (7). However, MGUS is now known to frequently progress to such serious diseases as multiple myeloma and macroglobulinemia, and consequently, the importance of follow-up has been emphasized (10–12).

It was found in the present study that the incidence of M proteins increases with age, and this finding is in support of previous reports from Sweden (1–3), the U.S. (4,5), and France (6). Bowden et al. (13) have investigated the incidence of multiple myeloma in 146 Japanese and 111 U.S. persons and found that the incidence of M proteins was 2.7% in Japanese, which was lower than 10% in U.S. In the present study, multiple myeloma was found in 8 of 2,007 Japanese subjects, namely, the incidence was only 0.4% and was much lower than that reported by Bowden et al. The incidence of M proteins in elderly subjects, however, was higher in our elderly Japanese subjects than that previously reported overseas. This difference may have resulted from our investigation of a population of outpatients at our hospital; hence, few healthy elderly persons and some patients with multiple myeloma and macroglobulinemia were included in the population studied.

IgG-type M proteins were most frequently detected in our cases, and the incidence of IgA- and IgM-type M proteins was found to increase with aging. The high incidence of IgG-type M proteins is conceivable, since the blood IgG concen-

TABLE 3. Immunoglobulin types of M proteins in benign and malignant disease^a

Disease	IgG		IgA		IgM		Total
	kappa	lambda	kappa	lambda	kappa	lambda	
Multiple myeloma	4	2	1	1			8
	6.3%	3.2%	1.6%	1.6%			12.7%
Macroglobulinemia					2		2
					3.2%		3.2%
Cancer associated	2	1					3
with multiple myeloma	3.2%	1.6%					4.8%
Various cancers	20	8	3	3	1		35
	31.7%	12.7%	4.8%	4.8%	1.6%		55.6%
Benign disease	9	3	1	2			15
	14.3%	4.8%	1.6%	3.2%			23.9%
Total	35	14	5	6	3		63
	55.6%	22.2%	8.0%	9.3%	4.8%		

^aThe upper and lower values are the number of cases and the percentage of patients with the captioned immunoglobulin type, respectively.

TABLE 4. Level and relative mobility of M protein in malignant and benign diseases

Disease	No of cases	M protein level (g/dl)			Relative mobility		
		Range	Mean	SD	Range	Mean	SD
Multiple myeloma	8	2.22–3.93	3.17	0.56	0.19–0.48	0.36	0.099
Macroglobulinemia	2	1.36–3.03	2.15	1.18	0.34–0.51	0.425	0.12
Cancer associated with multiple myeloma	3	0.19–3.25	1.22	0.24	0.28–0.45	0.383	0.091
Various cancers	35	0.11–2.38	0.78	0.62	0.06–0.63	0.347	0.124
Benign disease	15	0.1–1.89	0.84	0.51	0.11–0.52	0.299	0.12

tration is known to increase with aging (14–16). This may also be the case for IgA-type M protein, that is, the higher the age, the higher the blood IgA level.

For individual diseases, the incidence of M proteins was highest (47%) in cancer-bearing patients, whereas patients with benign diseases comprised one-fourth of all persons expressing M proteins. The concentration of M proteins was apparently high in patients with multiple myeloma and macroglobulinemia, but there was no clear difference in the concentration of M proteins between cancer-bearing patients and patients with benign diseases.

In our experiences with cellulose acetate membrane electrophoresis, the IgG band is situated between the fast and slow γ -globulin regions, the IgA band is located between the β -globulin region and the mid γ -globulin region, and IgM migrates to a level between the slow β -globulin region and the mid γ -globulin region. Thus, the electrophoretic migration of an M protein changes depending upon its component immunoglobulins.

As far as we are aware, the electrophoretic mobility of M proteins has been discussed only by Kyle et al. (7), but they have stated that there was no relationship between the mobility of an M-protein band and the type of disease. In the present study, however, M proteins in patients with multiple myeloma or macroglobulinemia showed significantly greater values of relative mobility, i.e., closer to the albumin band, than that of patients with benign diseases. This increase in mobility is thought to be related to the age of the patients composing these groups. The mean age was 75 years for patients with multiple myeloma and 68 years for patients with benign diseases, and the former was significantly higher than the latter. Therefore, it is conceivable that age-related greater mobility of M proteins may reflect the age-related increase of IgA level.

The relative mobility of all types of M proteins increased with aging, and this tendency was most prominent for the IgA-type M protein. IgG is known to change the structure of its carbohydrate chains with aging, and it has been reported that elderly persons have the agalactosyl-type (G0) more abundantly (17,18). Thus, the age-related change of the relative mobility of M proteins is thought to be induced by the age-related structural change of carbohydrate chains in M proteins. In general, the migration of proteins in cellulose acetate membrane electrophoresis is affected by various factors such

as the iso-electric point of the protein, the structure of carbohydrate chains, etc. In particular, age-related changes in the number of sialic acids are thought to be most influential. This is considered to be the case also for IgA and IgM.

In conclusion, it was confirmed in the present study of Japanese subjects at the age of 50 or older that the incidence of M proteinemia increases with aging. The level of M proteins was not different from its normal range in diseases other than multiple myeloma and macroglobulinemia. The electrophoretic mobility of M proteins was found to change with aging. Further studies will be carried out to elucidate the cause of this age-related change in the electrophoretic mobility of M proteins.

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