Immunological Typing and IgG Subclass Distribution in Plasma Cell Dyscrasias Diagnosed Among Jordanian Patients

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Abstract

Objective: This study was carried out to determine types of plasma cell dyscrasias among Jordanian patients according to immunoglobulin class and whenever applicable subclass and determine characteristics of plasma cell dyscrasias with respect to demographic data and laboratory findings.

Methods: One hundred patients with different types of plasma cell dyscrasias newly diagnosed at the hematology and oncology clinics of four major hospitals in Amman; Al-Basheer Hospital, King Hussein Medical Center, King Hussein Cancer Center and the Jordan University Hospital during the period between March 2001 and October 2002 were included in the study. Serum specimens were assayed by three different methods; serum protein electrophoresis for the detection of monoclonal gammopathies, immunofixation electrophoresis for the identification of immunoglobulin class and a sandwich type ELISA to determine IgG subclasses.

Results: Results of this study demonstrated that 65 cases (65%) of plasma cell dyscrasias were Multiple Myeloma (MM), 29 (29%) were Monoclonal Gammopath of Unknown Significance (MGUS), 5(5%) were Solitary Bone Plasmacytoma (SBP) and one case (1%) was Waldenstrom's Macroglobulinemia (WM). The overall frequency distribution of paraprotein classes of the 65 multiple myeloma cases was: 48 (74%) IgG, 12 (18%) IgA and 5 (8%) light chain, whereas the distribution of IgG subclasses in multiple myeloma was 34 (70.8%) IgG1, 4 (8.4%) IgG2, 2 (4.2%) IgG3, 1(2%)IgG4 and 7(14.5%) belonged to more than one subclass. In the 29 cases of monoclonal gammopathy of unknown significance, the frequency was 17(59%) for IgG, 7 (24%) for IgA and 5(17%) for IgM , whereas the distribution of IgG subclasses was 10 (58.7%) for IgG1, 6 (35.4%) for IgG2 and 1 (5.9%) for IgG3. All types of plasma cell dyscrasias were significantly more common among male patients than female patients.

Conclusions: Multiple Myoloma is the most common type of plasma cell dyscrasia encountered in Jordan and the frequency distribution of paraprotein classes and IgG subclasses among Jordanian patients with plasma cell dyscrasias parallel internationally reported normal serum concentration for them. Moreover, more than one IgG subclass may be found in IgG gammopathies.

Keywords: Plasma cell dyscrasias, Immunological typing, IgG Subclasses, Multiple myeloma.

(J Med J 2006; Vol. 40 (3): 161- 167)

Received	*Accepted*
July 13, 2005	*February 14, 2006*

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Introduction

Plasma cell dyscrasias are a group of clinical disorders characterized by a monoclonal expansion of plasma cells, which elaborate monospecific immunoglobulin molecules or fragments their. [1] These proteins can be detected in serum and urine. Plasma cell dyscrasias include: multiple myeloma, Monoclonal Gammopathy of Undetermined Significance (MGUS), solitary plasmacytoma of bone, extramedullary plasmacytoma, amyloidosis, Waldenstrom`s macroglobulinemia, plasma cell leukemia, smoldering myeloma and heavy chain disease. [2]

Studies were carried out worldwide to establish the frequency distribution of paraprotein classes and IgG subclasses among plasma cell dyscrasias. [3-13] Frequency distribution of paraprotein classes and IgG subclasses have been reported to vary from one country to the other, but no reports have been published about them from the Arab world. According to Foerster (1999), the class distribution of M-components among multiple myeloma patients worldwide was found to be 61 % for IgG, 19 % for IgA, 2% for IgD, 17 % for light chain and 1 % for others. In the USA, the frequency distribution was found to be 52% for IgG, 21% for IgA, 16% for light chain, 0.5 % for IgM, 2% for IgD, 2% for biclonal and about 7% were negative for M-protein. [5]

Frequencies of 61.3 % for IgG, 16.5 % for IgA and 22.2% for light chain were reported from China. [6] Furthermore, a review of the literature from different countries revealed that the frequency distribution of paraprotein types among MGUS in Italy was 72.8 % for IgG, 11.2 % for IgA and 16% for IgM, [7] whereas it was in the USA 74% for IgG, 11 % for IgA and 14 % for IgM. [8]

Differences in the frequency distribution of IgG subclasses among plasma cell dyscrasias varied from one study to another even within the same country.

In Germany, Withold and Rick (1993) reported frequencies of 75 % for IgG1, 16 % for IgG2, 6% for IgG3 and 3 % for IgG4 as compared to 68 % for IgG1, 13 % for IgG2, 16 % for IgG3 and 3 % for IgG4 in another study from Germany. [9] Likewise, the frequency distribution of IgG subclasses among multiple myeloma varies from one country to the other. In Germany, the distribution of IgG subclasses was 66 % for IgG1, 19 % for IgG2, 11% for IgG3 and 4 % for IgG4. [9] In Texas, USA, they were 56 % for IgG1, 28 % for IgG2, 11 % for IgG3 and 5% for IgG4, [4] while in Denmark they were 83 % for IgG1, 6.6% for IgG2, 4.7% for IgG3 and 5.6% for IgG4. [10] They were 64.4 % for IgG1, 18 % for IgG2, 5.6% for IgG 3 and 6.9 % for IgG4 in Belgium, [11] whereas frequencies of 73 % for IgG1, 10 % for IgG2 and 17 % for IgG4 were reported from Iran. [3] In essential monoclonal gammopathies, frequencies of 53% for IgG1, 10 % for IgG2, 35% for IgG3 and 2 % for IgG4 were reported from Germany [9] as compared to 24 % for IgG1, 20 % for IgG2, 46 % for IgG3 and 10 % for IgG4 in the Netherlands. [13] It has been shown that more than one IgG subclass may be present in a paraproteinemia. Klouche *et al.* (1995) reported that these multiple bands were present in 24 out 92 (26%) serum specimens examined. Similarly, more than one IgG subclass was present in 10.4% of multiple myeloma specimens from China. [6]

Determing the type of plasma cell dyscrasia is important from a clinical point of view since interesting clinical correlations have been established for the different types of plasma cell dyscrasias. Multiple myeloma for example, has been associated with increased frequency of infection, hypercalecmia and amyloidosis, whereas WaldenStorm macroglobulinamia has been linked with haemolytic manifestations, neurologic symptoms and visual disturbances. [12] This study was carried out to determine the types of plasma cell dyscrasias among newly diagnosed Jordanian patients, type them according to immunoglobulin class and whenever applicable subclasses, and characterize them with respect to patients' demographic data and laboratory findings.

Patients, Materials and Methods

Patients: A total of one hundred Jordanian patients newly diagnosed with plasma cell dyscrasias receiving treatment in different Jordanian hospitals were included in this study. Patients' age ranged from 30 to 94 years with a mean age of 63 years and there were 65 (65%) males and 35 (35%) females. Diagnosis was made by the hospital pathologist on the basis of Durie and Salmon classification. [11] Information regarding patients' demographic characteristics as well as clinical and other relevant data were gathered from three sources: the medical files of patients, laboratory request forms and bone marrow biopsy report forms.

Collection of Blood Samples: Blood samples were obtained from patients attending the hematology and oncology clinics of four major hospitals in Amman: Al-Basheer Hospital, King Hussein Medical Center, King Hussein Cancer Center and the Jordan University Hospital. Blood samples were obtained during the period between March 2001 and October 2002. After clot formation, serum was separated by centrifugation, aliquoted and kept in labeled cryotubes at -70° C until tested.

Laboratory Tests: Serum specimens were assayed for typing of plasma cell dyscrasias by three different methods: serum protein electrophoresis for detection of monoclonal gammopathies as described by Tiselius (1937), immunofixation electrophoresis for identification of immunoglobulin class according to Afonso (1966), and a sandwich type ELISA to determine IgG subclasses as described by Papadea, Reimer, and Check (1989).

Statistical Analysis: The statistical method employed was the Fisher's exact test and Chi-square (X^2). Chi-square method depends on the degree of freedom *(df)* test and compares the variations between two variables. The differences in the distribution of paraprotein classes and IgG subclasses among plasma cell dyscrasias were checked by Chi-square differences and were considered significant at a *P* value of <0.05. The Statistical Package for Social Sciences (SPSS) version 10, 2000 (SPSS, INC, USA) software was used for analysis.

Results

Plasma Cell Dyscrasias: Overall Frequency Distribution : One hundred patients with plasma cell dyscrasias were included in this study. Multiple myeloma was the most common as it represented (65%) of the cases being followed by MGUS (29%), SBP (5%) and WM (1%).

Frequency Distribution According to Age Group: The majority of cases of plasma cell dyscrasias were observed among the elderly as 64% of the cases affected those above 60 years of age and the overall median age at diagnosis was 63 years. Out of the 65 cases of multiple myeloma, 3% of the patients were younger than 40 years of age and 63% were 60 years of age or older with a median age at the time of diagnosis of 65 years. Of the 29 patients with EMG (MGUS), 69% were above 60 years of age and the median age was 60 years. Of the patients with Solitary Bone Plasmacytoma (SBP), 60% were above 60 years of age and the median age was 57 years. The age distribution among the study population is shown in table (1). There was a highly significant correlation ($p<0.001$) between the increase in age and occurrence of disease. Of the patients with plasma cell dyscrasias, 65% were males and 35% were females, a difference that was statistically significant as shown in table (2).

Table 1: Frequency distribution of plasma cell dyscrasias by age group.

Age group	*Plasma cell dyscrasias*				
	MM	***EMG (MGUS)***	***SBP***	***WM***	***Total***
30-39	2(3%)	1(3.5%)	2(40%)	0%	5(5%)
40-49	6(9%)	2(6.8%)	0%	1(100%)	9(9%)
50-59	16(25%)	6(20.7%)	0%	0%	22(22%)
60-69	32(49%)	11(38%)	1(20%)	0%	44(44%)
70-79	7(11%)	6(20.5%)	2(40%)	0%	15(15%)
Above 80	2(3%)	3(10.5%)	0%	0%	5(5%)
Total	65(100%)	29(100%)	5(100%)	1(100%)	100(100%)

P< 0.001

Table (2): frequency distribution of plasma cell dyscrasias by gender.

Age group	*Plasma cell dyscrasias*				
	MM	***EMG***	***SBP***	***WM***	***Total***
Male	*41(63%)*	*20(69%)*	*3(60%)*	*1(100%)*	*65(65%)*
Female	*24(37%)*	*9(31%)*	*2(40%)*	*0(0%)*	*35(35%)*

P=0.003

Overall Frequency Distribution of Immunoglobulin Classes Among Plasma Cell Dyscrasias: Immunological typing of paraproteins among patients with plasma cell dyscrasias revealed that the overall frequencies were 69% for IgG, 20% for IgA, 6% for IgM and 5% for light chain. As shown in table (3), the frequency of Kappa light chain was higher among plasma cell dyscrasias than Lambda light chain. This difference was statistically significant with a *P* value of 0.001.

Overall Frequency Distribution of IgG Subclasses among Plasma Cell Dyscrasias: The overall frequency distribution of IgG subclasses among the 69 patients with IgG paraproteinemia was: 68.1% for IgG1, 14.5% for IgG2, 4.3% for IgG3, 1.4% for IgG4 and 11.6% for more than one subclass. IgG subclass typing by ELISA revealed that 8 patients (11.6%) with IgG paraproteinemia had more than one IgG subclass. Five patients (7.2%) had IgG1and IgG4, two patients (2.9%) had IgG1 and IgG3 and one patient (1.4%) had IgG1and IgG2 as shown in table (5).

Table (3): Frequency distribution of paraprotein classes and light chains among plasma cell dyscrasias.

		Paraprotein and light chains types					
		IgG	***IgA***	***IgM***	***Light chain***	***Kappa (κ)***	***Lambda (λ)***
Diagnosis	***MM***	***48(74%)***	***12(18%)***	***0%***	***5(8%)***	***41(63%)***	***24(37%)***
	EMG (MGUS)	***17(59%)***	***7(24%)***	***5(17%)***	***0%***	***21(74%)***	***8(26%)***
	SBP	***4(80%)***	***1(20%)***	***0%***	***0%***	***3(60%)***	***2(40%)***
	WM	***0%***	***0%***	***1(100%)***	***0%***	***0%***	***1(100%)***
	Total	***69(69%)***	***20(20%)***	***6(6%)***	***5(5%)***	***65(65%)***	***35(35%)***

P=0.001

Table (4): Overall frequency distribution of IgG subclasses among plasma cell dyscrasias.

		IgG subclasses					
		IgG1	***IgG2***	***IgG3***	***IgG4***	***Multiple IgG subclasses***	***Total***
Diagnosis	***MM***	*34(70.8%)*	*4(8.4%)*	*2(4.2%)*	*1(2.1%)*	*7(14.5%)*	*48(100%)*
	EMG (MGUS)	*10(58.7%)*	*6(35.4%)*	*1(5.9%)*	*0%*	*0%*	*17(100%)*
	SBP	*3(75%)*	*0%*	*0%*	*0%*	*1(25%)*	*4(100%)*
	WM	*0%*	*0%*	*0%*	*0%*	*0%*	*0%*
Total		*47(68.1%)*	*10(14.5%)*	*3(4.3%)*	*1(1.4%)*	*8(11.6%)*	*69(100%)*

Table (5) frequency distribution of multiple IgG subclasses among IgG paraproteins.

Multiple IgG subclass	***Patients number***	***Frequency within IgG***
IgG1-IgG4	*5*	*7.2%*
IgG1-IgG3	*2*	*2.9%*
IgG1-IgG2	*1*	*1.4%*
Total	*8*	*11.6%*

Discussion

Frequency distribution of plasma cell dyscrasias by age revealed that the prevalence of disease rose progressively with age as demonstrated by studies carried out worldwide. [5, 6, 8, 3, 7, 12] Data presented in this study showed that the minority (3%) of patients with multiple myeloma were younger than 40 years of age and the majority (63%) of them were above the age of 60 years which is in agreement with the results reported by Kyle et al. (2003). A statistically significant difference was observed in sex distribution among plasma cell dyscrasias as 65% of patients were males and 35% were females. A similar sex distribution was found in studies carried out elsewhere. [3, 5, 9]

The overall frequency distribution of paraprotein classes among plasma cell dyscrasias was 69% for IgG, 20% for IgA, 6% for IgM and 5% for light chain. These results are in agreement with the findings of Bartl et al. (2001) who reported frequencies of 60.3% for IgG, 17.3% for IgA, 14.8% for IgM, 0.3% for IgD, 0.1% for IgE, 1.8% for biclonal gammopathy and 5.4% for light chain. The frequency distribution of light chain in monoclonal paraproteins among multiple myeloma patients was found to be 63% for kappa and 37% for lambda, findings that are identical with those of Kyle et al. (2003).

This study revealed that all paraproteins of the light chain type and most of those of the IgA class were associated with multiple myeloma, whereas those of the IgM class were found to be associated with EMG. Paraproteins of the IgG class, on the other hand, which are more common, are not associated with any particular type of plasma cell dyscrasias as they predominate in all types of plasma cell dyscrasias. These findings are consistent with those of Attaelmannan and Levinson (2000), Shustik, Bergsagel and Pruzanski (1976), Foerster (1999), Baldini et al. (1996) and Radl, Wels and Hoogeveen (1988) but in contrast to those of Kyle (1973) who also reported that light chain disease may be associated with benign idiopathic Bence Jones proteinuria.

Results of this study showed that the frequency distribution of IgG subclasses among patients with plasma cell dyscrasias was 68.1% for IgG1, 14.5% for IgG2, 4.3% for IgG3 and 1.4% for IgG4. Such results are comparable to those of Withold and Rick (1993) who reported that the frequency distribution among German patients with plasma cell dyscrasias was 75% for IgG1, 16% for IgG2, 6% for IgG3 and 3% for IgG4 and to those of Klouche et al. (1995) who found that the distribution of IgG subclasses among German patients with plasma cell dyscrasias was 68% for IgG1, 13% for IgG2, 16% for IgG3 and 3% for IgG4.

Frequency distribution of IgG subclasses in multiple myeloma is in accordance with results published by Klouche et al. (1995) and Fasullo et al. (1989) but is in contrast to the results reported from Denmark by Djurup et al. (1988), from Belgium by Mangnusson et al. (1984) and from Iran by Gharazoloo et al. (1996), which demonstrated higher frequency for IgG4 than that of IgG2 and IgG3. These contradictory findings may be due to genetic diversity among populations. The IgG subclass frequency distribution among paraproteins in EMG was found to be 58.7% for IgG1, 35.4% for IgG2 and 5.9% for IgG3 which is in agreement with those of Kyle and Gleich (1982) who reported that subclass frequency distribution of IgG paraproteins in EMG was parallel to the normal serum IgG subclass distribution. The findings of this study are in contrast with those of Klouche et al. (1995) and Radl, Wels and Hoogeveen (1988) who reported that IgG3 was expressed at a higher frequency than IgG2 and IgG4. Frequency distribution of multiple IgG subclasses in the present study revealed that 7.2% of IgG paraproteins have IgG1 and IgG4, 2.9% have IgG1 and IgG3 and 1.5% have IgG1 and IgG2. Similar findings have been reported previously. [6, 9]

In conclusion, the Frequency distribution of paraprotein classes and IgG subclasses among Jordanian patients with plasma cell dyscrasias parallel the internationally reported normal serum concentration of immunoglobulins [24] and multiple IgG subclasses among IgG paraproteins may coexist. It is recommended that the phenomena of biclonal gammopathies, the presence of multiple IgG subclasses and the correlation between classes of paraproteinemias and subclasses of IgG and disease variables be investigated further.

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