RESEARCH COMMUNICATION

Immunoglobulin Heavy and Light Chain Isotypes in Multiple Myeloma Patients

Nadeem A Ansari[1]*, M Owais[2], Usha[3]

Abstract

The frequency of expression of immunoglobulin (Ig) light and heavy chain isotypes was analyzed in myeloma proteins (M-proteins) from sera of 40 Indian patients with clinically established multiple myeloma. Patients samples were screened by a combination of electrophoresis, immunoelectrophoresis (IEP) and ELISA techniques in this study. We found that majority of the myeloma proteins (58%) were of the IgG isotype followed by IgA (24%) and biclonal gammopathy associated with IgG and IgA (5%). Both kappa and lambda light chains were associated with the heavy chain isotypes. We recommend the triangular combination for detection of M-proteins and biclonal gammopathy of cancerous plasma cells as biomarkers for diagnosis of myeloma.

Key Words: Plasma cells - multiple myeloma - M-proteins - biclonal gammopathy - biomarkers.

Asian Pacific J Cancer Prev, **8**, 593-596

Introduction

Myeloma and multiple myeloma are referred as cancer of antibody producing plasma cells (Longo, 2001). Although such a cancerous plasma cell, called myeloma cell has been transformed, its protein synthesizing machinery and secretory functions are not altered; thus, the cell continues to secrete specific antibody. The secreted antibodies are indistinguishable from normal antibody molecules but are called myeloma protein (M-protein, paraprotein) to denote its source. In 99% of the cases, paraproteins are secreted in the serum and/or urine (Bence Jones protein) and in the remaining 1% the paraproteins are synthesized but not secreted (Height and Treleaven,1994).

Biclonal gammopathy in which plasma cells produced two or more M-proteins, is a type of benign monoclonal gammopathy that remains stable and classified as benign, or will develop into symptomatic multiple myeloma or other related disorders (Kyle et al., 2003). The major diagnostic criterion for multiple myeloma, a type of malignant monoclonal gammopathy, is the presence of more than 10% of atypical plasma cells in the bone marrow. Prominent clinical manifestations are bone pain and anemia, in about 70% and 80% of the patients, respectively. It occurs in slightly more than 10% of hematologic malignancies in the United States.

In addition to the plasma cell disorders, M components may be detected in other lymphoid neoplasms, breast cancer, colon cancer, autoimmune conditions such as rheumatoid arthritis (Longo, 2001). The electrophoretic analysis of the serum sample is the first step for observing the altered protein concentrations and the presence of M-peak portion of proteins, which indicates myeloma but not its type. Confirmation that such an M-peak is truly monoclonal relies on the use of immunoelctrophoresis (IEP) (Kyle, 1992; Longo, 2001) that is relatively insensitive to the antigen or antibody ratio. A homogeneous M-protein forms a precipitin arc whereas normal, heterogeneous Ig forms a straight precipitin line only slightly curved at the cathodic end (Foerster, 1999).

Most of the previous studies laid emphasis on determination of single Ig heavy and light chain isotypes, there occurrence in M-proteins is similar to that of the normal polyclonal Ig (Grey and Kunkel, 1964). In this study, biomarkers other than that of single Ig heavy and light chain isotypes in M- proteins is determined by confirmatory and complementary assays for diagnosis of Indian patients with multiple myeloma.

Materials and Methods

Clinical Samples

Non-heparinized peripheral blood was collected from 40 myeloma patients attending the Immunopathology Division of Institute of Medical Sciences, Banaras Hindu University, India. Diagnosis was based on clinical criteria, including lytic bone lesions with hypercalcemia, anemia and hypogammaglobulinemia associated with recurrent bacterial infections, together with presence of more than 10% plasma cells in bone marrow smear, as previously described (Brouet and Fermand, 1995). Serum was isolated from clotted blood and stored at –20˚C until use.

Protein purification

Purification of myeloma proteins from serum was performed by ion-exchange chromatography, using DEAE-cellulose (DE-52, Whatmann, England) as

*[1]Dept. of Biochemistry, J N Medical College, AMU, Aligarh-2, [2]Interdisciplinary Biotechnology Unit, AMU, Aligarh-2, [3]Division of Immunopathology, BHU, Varanasi-7, India *For correpondence: Fax: +91-571-2720030 Email: anadeem1@rediffmail.com*

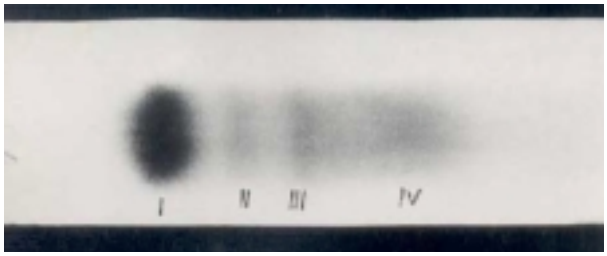

Figure 1. Serum Electrophoresis Analysis Revealed no M-peak in Normal Person. It is showing normal bands of (I) albumin, (II) α2 globulin, (III) β globulin and (IV) diffuse band of γ globulin

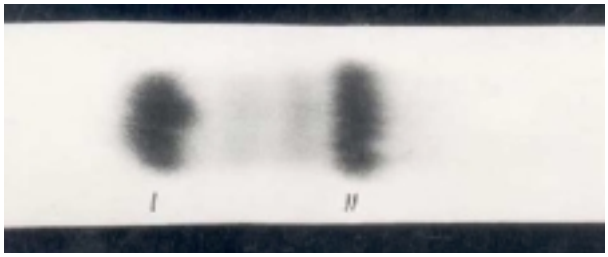

Figure 2. Serum Electrophoresis Analysis Revealed M-peak in Myeloma Patients. It is showing normal band of (I) albumin and moderate intensity M-peak in (II) β globulin region

previously described (Pruzanski, 1985). IgG was mostly eluted as breakthrough fractions with 0.01M phosphate buffer (pH 7.2).

Purification of IgA was achieved by prior precipitation with 40% ammonium sulfate followed by ion-exchange chromatography with DEAE-cellulose using 0.02-0.03 M phosphate buffer as elution buffer (Pruzanski, 1985).

Zone electrophoresis

Sera were electrophoresed on cellulose acetate paper (Helana, France) in tris glycine buffer (0.03M, pH 8.6) for 30 minutes under constant current. Papers were then stained with panceause (Merck, Germany) and destained with a 5% solution of acetic acid.

Immunoelectrophoresis

The immunoelectrophoresis technique used was evaluated by slight modifications in the published protocol as described elsewhere (Kyle, 1986). Briefly, serum proteins were first separated by electrophoresis on 1% agarose gel in tris glycine buffer (0.03M, pH 8.6). The monospecific polyclonal anti-isotype antibodies (Spinreact, Spain) were then added to the specific troughs. Separated M-proteins were permitted to interact with antibodies, followed by extensive washing, staining with amido black (Merck, Germany) and finally destaining.

ELISA

Heavy chain isotypes were determined by direct binding enzyme linked immunosorbent assay (ELISA) using polystyrene polysorp immunoplates (Nunc, Denmark) coated with 1-10µg/ml of purified M-proteins or appropriate dilutions of serum in TBS (0.15 M, pH 7.4). HRP-conjugated goat or rabbit anti-human IgG, IgA, IgM, IgD or IgE (Spinreact, Spain) was then added and the reaction revealed with orthophenylenediamine dihydrochloride (OPD) (Sigma, USA) substrate. Finally, the reaction was stopped with 20% H2SO4 and the absorbance was measured at 492 nm on an automatic microplate reader.

Results

Serum analysis for detection and location of M-peak of myeloma protein

Sera from all patients were subjected to electrophoresis. A sharp band corresponding to the M-protein was easily detectable in most of the cases. Detection of M-peak in serum was used as marker for typing of myeloma. As evident from Figures 1 and 2 that the M-peak is present in myeloma patients, while no corresponding peak was observed in sera of normal subjects. The location of M-peak varies from individual to individual. It is notable that in the majority of the myeloma cases, the total concentration of polyclonal immunoglobulin is considerably reduced and may appear as a faint diffuse band in the vicinity of M-protein.

Determination of heavy/light chain isotypes of the antibodies

Assignment of heavy or light chain isotypes were made by IEP method. A typical result obtained by IEP is illustrated in Figure 3. The overall results obtained for heavy and light chain isotypes of the myeloma samples

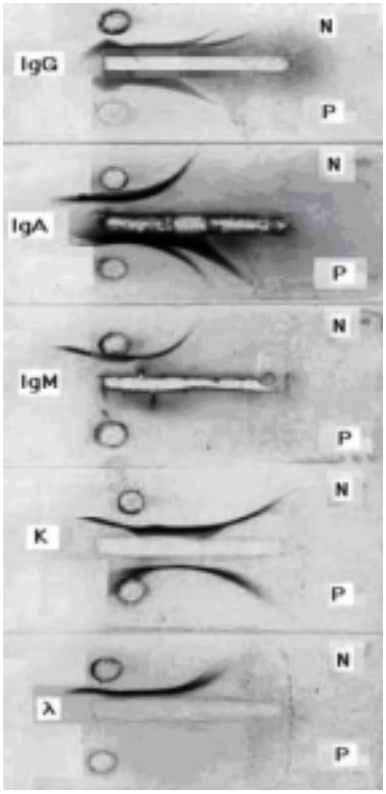

Figure 3. Immunoelectrophoresis and Staining Analysis of Normal (N) and Myeloma Patient (P) Reveals Biclonal Gammopathy of IgG and IgA-kappa type. It shows an arc of IgG, two sharp arcs one thick with bowing of IgA, absence of IgM, and sharp arc of kappa

Table 1. Typing of Myeloma by Serum IEP

Types	IgG	IgA	IgD	IgM	κ	λ	Inference
I	Present, full band	Two bands, One thick with bowing	Absent	Absent	Present, Intense band	Faint band	Biclonal IgG + IgA,κ
II	Present, full band	Two bands, One thick with bowing	Absent	Absent	Faint band	Present, full band	Biclonal IgG + IgA, λ
III	Present, Intense band	Faint band	Absent	Absent	Faint band	Present, Intense band	IgG, λ
IV	Present, Intense band	Absent	Absent	Absent	Present, Intense band	Present, Half faint band	IgG, λ
V	Absent	Present, Intense band	Absent	Absent	Present, Intense band	Absent	IgA, λ
VI	Absent	Present, Intense band	Absent	Absent	Absent	Present, Intense band	IgA, λ
VII	Present	Present	Absent	Absent	Present	Present	Indeterminate

are summarized in Table 1.

As shown in Table 1, IEP studies revealed that M-protein shows biclonal gammopathy associated with IgG and IgA heavy and kappa light chains (Figure 3) and the other found associated with IgG and IgA heavy and lambda light chains in 5% of human myeloma patients as Types I and II. The figure showed two intense bands/arcs of IgA due to dimerization. Types III and IV as IgG in 58% of patients while Type V and VI as IgA in 24% of patients. Light chain isotypes within IgG and IgA was found to be both kappa and lambda types. 13% of cases were not conclusive referred as Type VII. No IgD, IgE or IgM M-proteins were detected.

Sera from multiple myeloma and normal human subjects were tested for binding to different anti-human sera by direct binding ELISA (Figure 4). Majority of myeloma sera (35/40) showed strong binding to their respective anti-sera indicating IgG, IgA and IgG+IgA types as evident from their increase in absorbance compared to low absorbance of normal human sera at 1:100 serum dilution.

Discussion

The identification of M-proteins has been extensively used as biomarker for diagnosis of various myeloma. In fact the basis of such isotypic studies mainly rely on the fact that frequency of specific heavy or light chain isotype is proportional to the serum concentration of that isotype in the normal polyclonal pool (Pruzanski,1985; Rosen et al., 1986; Fasullo et al., 1989; Klein et al., 1994).

The sample analysed in the present studies were collected from specific region of the India. Occurrence of myeloma strictly depends on age, sex as well as socio economic background of the subjects. Myeloma is more common in patients with age of 50 years or more (Aster, 2003). The average age of the sufferers were found out to be 55 that is remarkably less as reported from other region of the world where median age was shown between 65-70 years (Rosen et al., 1986; Kyle, 2000). This could be attributed to the exposure to the mutagenic substances and other occupational health hazards. The higher prevalence of myeloma in males as compared to females is in concordance with the earlier investigations (Grey and Kunkel,1964; Thakar et al., 1997; Kyle, 2000; Longo, 2001) that again support the notion that occurrence of myeloma is mainly because of occupational hazards and females are less exposed to them.

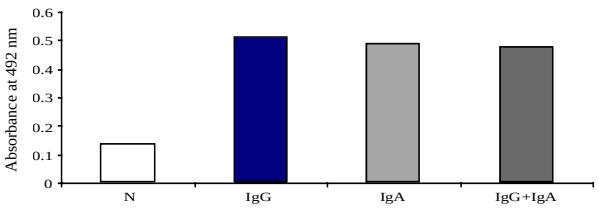

Figure 4. Direct Binding ELISA of Multiple Myeloma Serum Samples for Determination of Heavy Chain Isotypes (IgG, IgA, IgG+IgA) and Normal Human Serum (N) as Control

Our results (Figure 1 and 2) regarding the analysis of serum by zone electrophoresis for the presence and location of M-peak of M-proteins were similar to that reported by others (Grey and Kunkel,1964; Thakar et al., 1997; Kyle, 2000; Longo, 2001).

Plasma cell tumors producing two or more monoclonal proteins are found in 0.5 to 2.5% of patients (Pruzanski , 1985; Bouvet et al., 1975; Twomey and Good, 1978; Riddell, 1986; Goldsby et al., 2003). Our result (Figure 3) indicating biclonal gammopathy combination matched from those reported by investigators,(Zawadzki et al., 1977; Ando et al., 2000) while it differs from reports of other investigators (Bouvet et al., 1975; Kyle et al., 1981; Ramaiah et al., 2002; Weinstein et al., 1984; Bakta and Sutarka, 2000). The most common combinations reported are IgG + IgA (33%), IgM + IgG (24%), IgG + IgG (17%), IgM + IgA (8.5%) and IgM + IgM (8%); (Bouvet et al., 1975) occasional cases of complete Ig + heavy chains or separate light chains, and patients producing two separate monoclonal light chains also have been reported (Bouvet et al., 1975; Twomey and Good,1978). In many, but not all biclonal gammopathies, the light chain is the same for both M-components (Bouvet et al., 1975; Twomey and Good ,1978). Biclonal gammopathies have also been described in patients suffering from myeloma (Zawadzki et al., 1977).

The isotypic analysis revealed that most predominant isotype was IgG followed by IgA. Our findings regarding

the distribution of IgG M-proteins matched the reports of other studies as 60%,(Aster, 2003) 53% (Kyle, 2000; Longo, 2001) and 55.5% (Thakar et al., 1977). Distribution of IgA M-proteins was somewhat similar to that reported by others; i.e., 25%, (Longo, 2001; Aster, 2003) 20% (Kyle, 2000) and 18% (Thakar et al., 1977). IgD, IgE or IgM isotypes were indeterminate after the employment of a sensitive isotype-specific ELISA technique. This had been largely ascribed to lack of the switching- region located upstream of the genes coding for constant region of all heavy chain isotypes (Stavnezer, 1996; Spiegelberg, 1997).

Our findings indicate that the use of a combination of confirmatory and complementary assays for detection of myeloma proteins and biclonal gammopathy as biomarkers for diagnosis of myeloma, would have more reliable and precise results.

References

Ando K, Yaguchi M, Okabe S, et al (2000). IgA-lambda/IgG-kappa biclonal myeloma in which two clones proliferated in individual sites. *Intern Med*, **39**, 170-5.

Aster J (2003). Multiple myeloma and related plasma cell dyscrasias. In: Cotran RS, Kumar V, Robbins SL (ed) Robbin's Basic Pathology. Philadelphia:W. B. Saunders.

Bakta IM, Sutarka IN (2000). Biclonal gammopathy in multiple myeloma: a case report. *Gan To Kagaku Ryoho*, **27**, 544-8.

Bouvet JP, Feingold J, Oriol R, et al (1975). Statistical study on double paraproteinemias. Evidence for a common cellular origin of both myeloma globulins. *Biomedicine*, **22**, 517-23.

Brouet JC, Fermand JP (1995). Multiple myeloma. In: Frank MM, Austen KF, Claman HN, Unanue ER (ed) Samters Immunologic Diseases. New York: Little Brown.

Fasullo FJ, Herbert JR, Fritsche A (1989). IgG heavy chain subclass typing of myeloma paraproteins by isoelectric focusing immunoblot analysis. *Clin Chem*, **35**, 364

Foerster J, Paraskevas F (1999). Immunoelectrophoresis. In: Lee GR, Foerster J, Lukens J, Paraskevas F, Greer JP, Rodgers GM (ed) Wintrobes Clinical Hematology. USA: William and Wilkins.

Goldsby RA, Kindt TJ, Osborne BA (ed) (2003). Kuby Immunology. New York, Freeman and Company.

Grey HH, Kunkel HG (1964). H chain subgroups of myeloma proteins and normal 7s gammaglobulin. *J Exp Med*, **120**, 253-66.

Height SE, Treleaven JC (1994). Myeloma. In: Howrich A (ed) Oncology, Chapman and Hall, London.

Klein B, Bataille R, Harousseau JL (ed) (1994). Cytokines in human multiple myeloma. Austin, R.G. Landes Company.

Kyle RA (1986). Procedure for immunoelectrophoresis. In: Rose, Friedman, Fahey (ed) Manual of Clinical Laboratory Immunology. Washington DC: ASM Press.

Kyle RA (2000). Plasma cell disorders. In: Goldman L, Bennett JC (ed) Cecil's Textbook of Medicine. Singapore: PTE LTD.

Kyle RA, Robinson RA, Katzmann JA (1981). The clinical aspects of biclonal gammopathies. Review of 57 cases. *Am J Med*, **71**, 999-1008.

Kyle RA, Gertz MA, Witzig TE, et al (2003). Review of 1027 patients with newly diagnosed multiple myeloma. *Mayo Clin Proc*, **78**, 21-33.

Kyle RA (1992). Diagnostic criteria of multiple myeloma. *Hematol/Oncol Clin North Am*, **6**, 357-58.

Longo DL (2001). Plasma cell disorders. In: Kasper DL, Fauci AS, Longo DL, Braunwald E, Hauser SL, Jameson JL (ed) Harrison's Principles of Internal Medicine. New York: Mcgraw-Hill.

Pruzanski W (1985). Clinical conditions associated with paraproteinemia. In: Pruzanski W, Keystone EC (ed) Paraproteins in Disease. London: Churchill Livingstone.

Ramaiah SK, Seguin MA, Carwile HF,et al (2002). Biclonal gammopathy associated with immunoglobulin A in a dog with multiple myeloma. *Vet Clin Pathol*, **31**, 83-9.

Riddell S (1986). The double gammopathies, clinical and immunologic studies. *Medicine*, **65**, 135.

Rosen SN, Buxbaum JN, Frangione B (1986). The structure of immunoglobulins and their genes. DNA rearrangement and B cell differentiation, molecular anomalies of some monoclonal immunoglobulins. *Semin Oncol*, **13**, 260-74.

Spiegelberg HL (1977). Structure and biology of human IgD. *Immunol Rev*, **13**, 1-24.

Stavnezer J (1996). Antibody class switching. *Adv Immunol*, **61**, 79-146.

Thakar YS, Chande C, Pande S, et al (1997). Immunochemical studies in multiple myeloma. *Indian J Cancer*, **34**, 151-8.

Twomey JJ, Good RA (editors) (1978). In the immunology of lymphoreticular neoplasmas,comprehensive immunology. New York, Plenum Medical Book.

Weinstein S, Jain A, Bhagavan NV, et al (1984). Biclonal IgA and IgM gammopathy in lymphocytic lymphoma. *Clin Chem*, **30**, 1710-12.

Zawadzki ZA, Aizawa Y, Kraj MA, et al (1977). Familial immunopathies: report of nine families and survey of literature. *Cancer*, **40**, 2094-101.