RESEARCH COMMUNICATION

Immunoglobulin Heavy and Light Chain Isotypes in Multiple Myeloma Patients

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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.

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
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.
Table 1. Typing of Myeloma by Serum IEP

<table>
<thead>
<tr>
<th>Types</th>
<th>IgG</th>
<th>IgA</th>
<th>IgD</th>
<th>IgM</th>
<th>κ</th>
<th>λ</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Present, full band</td>
<td>Two bands, One thick with bowing</td>
<td>Absent</td>
<td>Absent</td>
<td>Present, Intense band</td>
<td>Faint band</td>
<td>Biclonal IgG + IgA,κ</td>
</tr>
<tr>
<td>II</td>
<td>Present, full band</td>
<td>Two bands, One thick with bowing</td>
<td>Absent</td>
<td>Absent</td>
<td>Faint band</td>
<td>Present, full band</td>
<td>Biclonal IgG + IgA,λ</td>
</tr>
<tr>
<td>III</td>
<td>Present, Intense band</td>
<td>Faint band</td>
<td>Absent</td>
<td>Absent</td>
<td>Faint band</td>
<td>Present, Intense band</td>
<td>IgG, λ</td>
</tr>
<tr>
<td>IV</td>
<td>Present, Intense band</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Present, Intense band</td>
<td>Present, Half faint band</td>
<td>IgG, λ</td>
</tr>
<tr>
<td>V</td>
<td>Absent</td>
<td>Present, Intense band</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Present, Intense band</td>
<td>IgA, λ</td>
</tr>
<tr>
<td>VI</td>
<td>Absent</td>
<td>Present, Intense band</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Present, Intense band</td>
<td>IgA, λ</td>
</tr>
<tr>
<td>VII</td>
<td>Present</td>
<td>Intense band</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
<td>Indeterminate</td>
</tr>
</tbody>
</table>

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.

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, 1977).

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