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ORIGINAL ARTICLE

Prevalence of autoimmune hemolytic anemia in multiple myeloma: A prospective study

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Abstract

***Aim*:** Autoimmune hemolytic anemia (AIHA) is frequently associated with B-cell lymphoproliferative disorders, and patients rarely develop overt clinical manifestations of AIHA. AIHA is rare in patients with multiple myeloma (MM). We conducted a prospective study to detect the presence of AIHA in MM patients and its impact on clinical presentation and outcome of the disease.

***Methods*:** Sixty-six patients were diagnosed to have MM. Seventeen of these patients who had severe anemia (hemoglobin < 6 g/dL) requiring frequent blood transfusions with or without features of hemolysis were screened for AIHA by performing direct and indirect antiglobulin (Coombs') test.

***Results*:** Seven (10.6%) of these 17 patients were found to be complicated with AIHA and carried autoantibodies in their sera. Five patients had de novo MM and two had relapsed MM. Six patients (85.7%) had stage IIIA disease and one (14.3%) had stage IIIB disease. The IgG subclass of the antibody binding to red cell membrane was compared with that of M-protein and these findings showed full correlation in all the seven patients. All of these patients were positive for subtypes of IgG and one patient had simultaneous positivity for IgA and IgG2, with presence of cold antibodies in the serum. Patients with primary disease showed remission of AIHA with therapy, whereas both the patients with relapsed disease showed no response to treatment and remained positive for antiglobulin test.

***Conclusion*:** AIHA should be suspected in MM patients with severe anemia requiring frequent blood transfusions.

Key words: AIHA, autoantibody, MM.

INTRODUCTION

Multiple myeloma (MM) is a B-cell malignancy characterized by a monoclonal proliferation of plasma cells in the bone marrow. It accounts for 1% of all malignancies and approximately 10% of all hematopoietic malignancies.[1] Anemia of variable severity is present in almost all cases of MM, either at the time of diagnosis or subsequently as the disease progresses. Several factors may be involved in the pathogenesis of anemia in MM.[1–3] Autoimmune hemolytic anemia (AIHA) is often associated with B-cell lymphoproliferative disorders such as chronic lymphocytic leukemia, non-Hodgkin's lymphoma and Waldenstrom's macroglobulinemia. AIHA in patients with MM is rare and has been reported in only a few case reports.[4–9] In a review by Pirofsky, less than 4% of total AIHA cases were due to myeloma.[4] We conducted this prospective study to detect the presence of AIHA in patients with MM and to study its impact on the clinical presentation and outcome of MM.

MATERIALS AND METHOD

Sixty-six consecutive patients were diagnosed to have MM as per the recommended diagnostic guidelines[10] at the Department of Hematology, Sanjay Gandhi Post

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Graduate Institute of Medical Sciences, Lucknow, India, over a period of 18 months. The staging of disease was done as per the Durie and Salmon clinical staging system as we were unable to estimate the serum β-2 microglobulin levels in our patients.[11]

Patients with MM having any one of the following features were screened for the presence of underlying AIHA:

1. Severe anemia (hemoglobin [Hb] < 6 g/dL) without evidence of underlying renal disease.
2. Requiring frequent blood transfusions.
3. With or without overt clinical and/or laboratory features of hemolysis.

Sera of patients who are positive with direct antiglobulin test (DAT) were subjected to antibody screening with indirect antiglobulin test (IAT) through gel technology (DiaMed Ltd., Cressier s/Morat, Switzerland) using the reagent three-cell panels (DiaMed Ltd.). For each sample, a positive control, negative control and auto-control were tested in parallel as described in technical manual AABB.[12] Samples reactive with the three-cell panels were further tested for antibody identification using gel cards and the reagent 11-cell panels (DiaMed Ltd.). Warm autoantibodies were considered to be present only when the test samples reacted optimally at 37°C with pan reactivity to the entire 11-cell panels and also with the patient's own red cells (reactive auto-control). These patients were screened for presence of antibody-coated red cells by broad spectrum nonspecific direct Coombs' test (DAT) by column agglutination technique using gel cards (DiaMed Ltd.) prior to start of therapy. Later, these samples were subjected to a more specific test to confirm the type of antibody coating on the red cells by acid elution technique and monospecific gel cards by column agglutination technique.

Treatment

All the patients received packed red cell transfusions to correct their anemia. These patients received chemotherapy and were followed up, and response to treatment evaluated.

RESULTS

A total of 17 patients fulfilled the inclusion criteria and were screened for AIHA. Seven (41.8%) of these patients with MM were found to be complicated with AIHA. Five (71.4%) of these seven patients had MM as a primary disease and the remaining two (28.6%) had a relapsed disease. Four patients (57.1%) were female with median age group of 43.3 years (Table 1). Two of them were below 40 years of age. Of the two patients with relapsed disease, one patient was previously treated with six cycles of VAD (vincristine, adriamycin and dexamethasone) regimen followed by thalidomide and dexamethasone therapy. She developed relapsed disease after 2 years of initial diagnosis. The second patient had been initially treated with six cycles of bortezomib plus dexamethasone therapy and developed relapse after 1 1/2 years of remission.

The Hb level ranged between 4.4 and 6.3 g/dL (mean 5.2 g/dL) at presentation. The leukocyte count was normal in all the patients and three patients had platelet count $<100 \times 10^9$/L at the time of first evaluation. One

Table 1 Clinical and laboratory profile of multiple myeloma patients with autoimmune hemolytic anemia at presentation

Disease parameters	Reference value	Observed value
1. Age: mean (range) years	–	48.7 (31–60)
2. Sex: total (male:female)	–	7 (3:4)
3. Hemoglobin level (g/dL)	12–14	5.2 (4.4–6.3)
4. Total leukocyte count ($\times 10^9$/L)	4–10	8.6 (5.5–14.8)
5. Platelet count ($\times 10^9$/L)	150–400	1.33 (0.7–3.38)
6. Serum bilirubin (total) mg/dL	0.1–1.3	2.7 (1.1–7.3)
7. Serum lactate dehydrogenase level (IU/L)	85–450	1016.7 (738–1200)
8. Type of myeloma: *n* (%)		
IgG	–	6 (85.7)
Biclonal (IgG + IgA)	–	1 (14.3)
9. Disease stage: *n* (%) (Durie and Salmon)		
I and II	–	0 (0)
IIIA	–	6 (85.7)
IIIB	–	1 (14.3)

patient each with primary MM and relapsed disease had overt clinical jaundice with severe anemia at presentation. The serum bilirubin level was elevated with associated reticulocytosis and increased serum lactate dehydrogenase levels. The other five patients had severe anemia with mild elevation of serum bilirubin levels. The bone marrow examination showed cellular marrow with presence of plasma cells ranging from 60 to 90%. The normal hematopoietic elements of the bone marrow were suppressed. Impaired renal function with elevated serum creatinine level was seen in one patient with relapsed disease.

Antibodies secreted by the malignant cells were of IgG type in five of six (85.4%) patients. One patient had biclonal (IgA + IgG) gammopathy. Six patients (85.7%) had stage IIIA disease and one patient (14.3%) had stage IIIB disease as per the Durie and Salmon clinical staging system. (Table 1). Compared to MM patients without AIHA, all the seven patients complicated with AIHA required multiple packed red cell transfusions to correct the anemia. The number of units transfused ranged from 5 to 33 over a period of 4–8 weeks.

The red blood cells of all seven patients were strongly coated with immunoglobulin giving a reactive DAT (Table 2). On performing elution studies, the antibody obtained in elute also showed a pan reactive status with three-cell panels. The specificity of this immunoglobulin was IgG type in almost all the patients (100%) along with IgA in one patient (14.3%) in whom serum electrophoresis and immunofixation showed the simultaneous presence of IgG along with IgA. On further testing with monospecific DAT card, the red cells of these patients were reactive for different IgG subtypes with IgG1 ($n = 2$), IgG2 ($n = 4$), IgG3 ($n = 1$) and IgG4 ($n = 1$). One patient had simultaneous positivity for IgA and IgG2, with presence of cold antibodies in the serum. Thermal amplitude of cold antibody was not elevated. Test on sera of all these patients showed pan reactive IAT with 3- and 11-cell panels. Later during the follow-up, one patient developed alloantibody anti-jK^b (Kidd-b) during the course of treatment. Kidd-b antigen negative blood was selected for transfusion to this patient.

Treatment and follow-up

Two patients with relapsed MM showed no remission of disease with second-line therapy and remained positive for antiglobulin test. Both of these patients died at 6 and 5 months after diagnosis of relapsed disease. Two patients each with primary MM were treated with VAD regimen and thalidomide plus dexamethasone regiment, respectively. One patient received MPT (melphalan, prednisolone and thalidomide) regimen. Three of these patients showed partial response to therapy and two patients had stable disease at 6 months of follow-up. All the five patients with de novo MM had remission of the AIHA with negative antiglobulin test after treatment.

DISCUSSION

Anemia is a frequent finding in patients with MM, second only to skeletal lytic lesions. Approximately 70% of the patients have anemia at diagnosis.[1] Anemia is normochromic normocytic and is multifactorial in origin. Impaired availability of storage iron, overproduction of

Table 2 Serological evaluation of autoimmune hemolytic anemia in multiple myeloma at presentation

Test parameters		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Total
DAT		3+	3+	3+	4+	3+	3+	4+	7
IAT (elute)		Pan reactive	Pan reactive	Pan reactive	Pan reactive	Pan reactive	Pan reactive	Pan reactive	7
IAT (three-cell panel)		Pan reactive	Pan reactive	Pan reactive	Pan reactive	Pan reactive	Pan reactive	Pan reactive	7
IAT (11-cell panel)		Pan reactive	Pan reactive	Pan reactive	Pan reactive	Pan reactive	Pan reactive	Pan reactive	7
Cold antibody		–	–	–	–	1+	–	–	1
Auto-control		1+	1+	1+	2+	1+	2+	2+	7
Monospecific DAT	IgM	–	–	–	–	–	–	–	0
	IgA	–	–	–	–	1+	–	–	1
	IgG1	1+	2+	–	–	–	–	–	2
	IgG2	–	–	2+	–	1+	1+	2+	4
	IgG3	–	–	–	2+	–	–	–	1
	IgG4	–	–	1+	–	–	–	–	1
	C3d	–	–	–	–	–	–	–	0
Antibody screening		–	–	–	–	Anti-jK^b	–	–	1

DAT, direct antiglobulin test; IAT, indirect antiglobulin test.

cytokines inhibiting erythropoiesis, defective production of erythropoietin and impaired marrow response to the erythropoietin have been postulated to be the main mechanisms of anemia in MM.[13,14]

The pathogenesis of AIHA in MM is still not clear due to the rarity of its occurrence in MM. There are only a few cases published in literature.[4–9] It has been suggested that MM being a B-cell malignancy is associated with a marked immune disturbance that may allow normally suppressed clones to develop and produce autoantibodies against red cell surface antigens.[6] Counter selection of VH4-36 segment in MM due to self-tolerance mechanism has also been proposed to be a mechanism in development of AIHA.[15] Several drugs have also been shown to be a causative agent for immune-mediated hemolysis in MM such as lenalidomide and interferon-α due to its immunomodulatory effects.[16,17]

Only one case reported in the literature had IgA type MM and the remaining cases were IgG type MM.[5,6,8] Both the cases reported by Wada *et al.* and Valopoulos had overt clinical and laboratory features of AIHA. However, none of the cases provided evidence that the antibody responsible for AIHA was the myeloma M-protein. These patients showed response to chemotherapy and DAT became negative.[6,8] In our series, the two relapsed patients with AIHA failed to show response to salvage chemotherapy, and antiglobulin test remained positive. All the five patients with primary MM showed negative antiglobulin test following treatment and there was decrease in the transfusion requirements.

CONCLUSION

In conclusion, we recommend that a baseline antiglobulin test should be performed in all MM patients with severe anemia and those requiring frequent blood transfusions to detect the presence of underlying AIHA. It is also probable that presence of AIHA in relapsed multiple MM is associated with poor prognosis and clinical outcome.

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