

IMMUNOCHEMICAL STUDIES IN A PATIENT WITH WALDEN-
STROM'S MACROGLOBULINEMIA

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Introduction:

Macroglobulinemia syndrome was first recognized by Waldenstrom in 1944(13) this syndrome now classified as a plasma cell dyscrasia involving those cells population normally responsible for the synthesis of M globulin. The excessive proliferation of these cells result in the elaboration of large quantities of monoclonal (Mtype), associated with a variable clinical pattern in which the most common and predominant features are: Anemia, bleeding manifestation as symptoms related to the serum macroglobulins. Presently five major classes of immunoglobulins are recognized, IgG (gamma-G), IgA (gamma-A), IgM(gamma-M), IgD (gamma-D), IgE (gamma-E). Each molecule of these immunoglobulins is thought to consist of two heavy (H) and two light (L) polypeptide chains. The heavy chains determine the biologic properties of each immunoglobulin class and are designated as gamma, alpha, mu, delta and epsilon for the IgG, IgA, IgM, IgD, and IgE classes respectively. The light chains occur in only two antigenic types, kappa and Lambda. The Bence Jones proteins are pathologic counterparts of L chains.

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IgM consist five subunits which have the general structure of all immunoglobulins namely a pair of type specific heavy chains and a pair of light chains of either the Kappa or Lambda type. Five such subunits combine to form an IgM molecule $(\text{U} 2 \text{K} 2)_5$ or $(\text{U} 2 \text{K} 2)_5$.

Case History:

A 46 years old male admitted with fatigues, widespread ecchymoses and bleeding from the gums. He had lost 14 Kg. over the four months period of his illness. His appetite was poor. The spleen was palpable to 5 cm. below the left costal margine; the liver was not felt. Examination of the central nervous system was within normal limits. He was pale, blood pressure 120/70, ascultation of the heart and lungs was within normal.

Methods

Haematology:

Routine haematological studies were performed.

Biochemistry

Biochemical studies including Blood glucose, urea, cholestrol, uric acid SGOT, SGPT, LDH, ALKALIN phosphatase and bilirubin were carried out.

Protein studies:

Electrophoresis of serum and urinary proteins was performed on cellulose acetate. Urinary protein was concentrated by ultrafiltration before electrophoresis.

Immuno-electrophoretic analysis was carried out according to Peetom (11) with anti human sera prepared by this laboratory. Immunoglobulins were identified by specific monovalent antisera against human IgG, IgA, IgM.

Quantitative tests for immunoglobulins were performed by rocket immunoelectrophoresis and immunodiffusion plates (1).

Rocket immunoselection was used to detect free mu chain protein in sera and concentrated urine (6).

The presence of the Bence Jones protein was investigated at 56 C° using acetate buffer PH= 4.7.

Family members which were available for study were mother, two sons and two daughters of the propositus.

Results

Haematology:

Haemoglobin 10g/100, haematocrit 31%, sedimentation rate 95mm after one hour, total white count 6000/mm³ consisting of 58% neutrophils, 40% lymphocytes, 2% monocytes, platelet count was 178000/mm³, red blood cell count 3, 920000/mm³.

Bone marrow examination:

Bone marrow biopsy revealed generalized medularly hyperplasia with an increased percentage of plasmocytes with vacuolated cytoplasm.

Biochemistry:

Glucose, SGOT, SGPT, LDH, alkaline phosphatase, bilirubin, blood urea, serum uric acid, cholesterol were within the normal range. Total plasma protein was 9.4 g%, cryoglobulin test was negative. Serum and urine electrophoresis:

serum protein electrophoresis revealed albumin 34%, alpha 1, 2%, alpha 2, 10%, beta 8%, gamma 46%.

The urine and concentrated urine electrophoresis did not show any protein, and there was no Bence Jones protein.

Immunoelectrophoretic analysis of the serum with polyvalent antisera to the normal human sera revealed an IgM paraprotein, the quantitation of immunoglobulins resulted IgG = 1020mg./100ml. (normal 770-1400) and IgA = 40mg./100ml. (normal 80-300) IgM paraprotein at a serum level of 3500mg./100ml.

The levels of immunoglobulins IgG, IgA and IgM in his relatives were variable, they were within normal limits in three, only one of his daughters had increase IgM level in her serum (376mg./100ml, normal

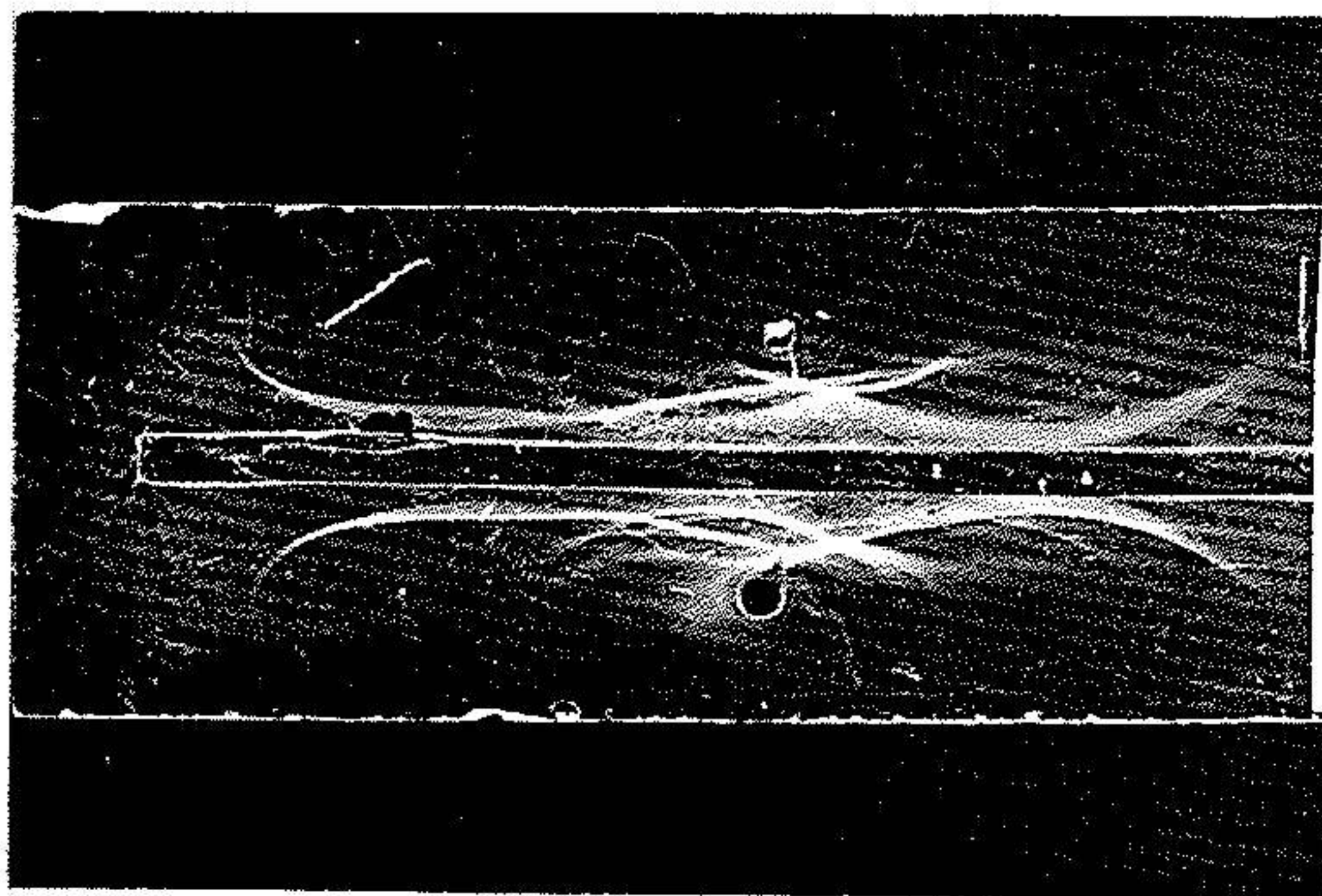


Fig. 1. Immunoelectrophoresis of the normal serum proteins (above), and from the patient with Macroglobulinemia (below). note the presence of heavy IgM band in the patient's sera (arrow)

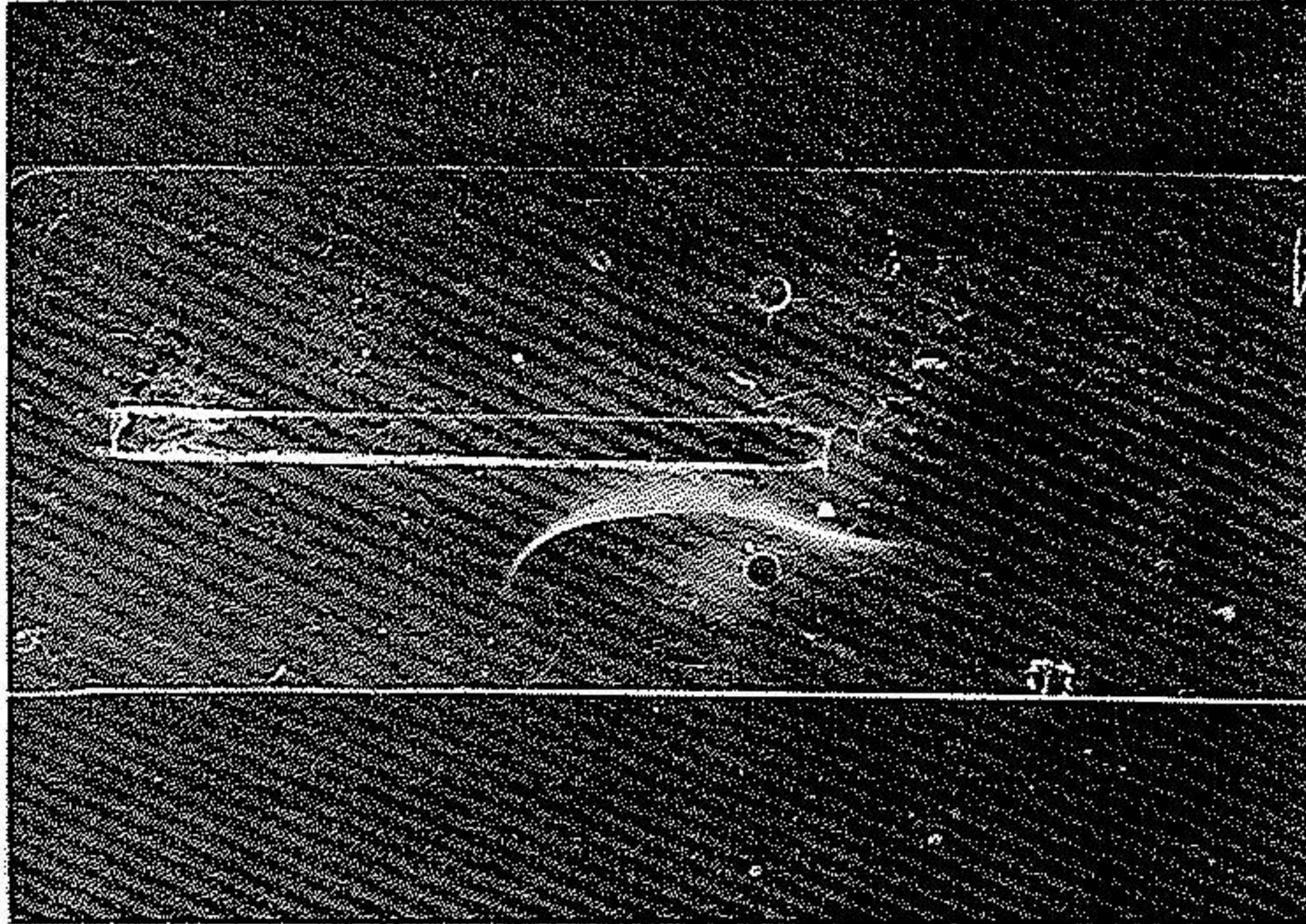


Fig. 2. Immunoelectrophoresis of the normal and the patient's sera using specific monovalent Anti IgM antisera note the heavy IgM band in the sera from the patient with Macroglobulinemia.

50-170) immunoselection test for detection of free mu chain negative.

Discussion

Waldenstrom's disease will be defined as the association of prominent macroglobulinemia with infiltration of the bone marrow, the liver, the spleen, the lymph nodes, and sometimes even the peripheral blood by malignant lymphocytic cells. Anemia occurs in over 80% of the patients with macroglobulinemia (2, 5, 9); it was a presenting feature in our case anemia may be caused by hemolysis (3), blood loss

or distributed erythropoiesis secondary to the proliferation of abnormal cells in the marrow. An elevated erythrocyte sedimentation rate accompanies the disorder (2, 5, 9) as seen in our patient. Serum electrophoresis on cellulose acetate revealed an abnormal gamma globulin band, but some others have shown in either the beta or gamma zone (4). Some authors reported the presence of the Bence Jones proteinuria in their patients (7) but in our case this was negative. This syndrome is differentiated from multiple myeloma to which it may be related to the lack of osteolytic bone lesion and the infrequent finding of Bence Jones proteinuria (7). In this syndrome circulating macroglobulin exist in large amount, and abnormal level of IgA are also frequent (high or low) but abnormalities in the IgG level are rare we found decreased level of IgA and normal range of IgG. Several workers have tried to use immunofluorescent techniques to localize the macroglobulins produced in the various proliferating cells during Waldenstrom's disease. Dutcher and Fahey (5) found macroglobulin in the cytoplasm and in the nucleus of lymphoplasmacytic cells. Waldenstrom's disease occurs in the elderly and is rare before the age of sixty (2, 5, 9), but our patient and two other cases which had been observed in this laboratory, were below 50.

Massari et al (10) found occurrence of macroglobulinemia in a family and suggested the possibility of a genetic abnormality, and also Seligman et al (12) reported the roles of genetic factor in families of patients with Waldenstrom's macroglobulinemia, in the present study we observed only the elevation of IgM level in one of the patient's daughters.

Summary

Waldenstrom's macroglobulinemia was studied in a 46 year old Iranian male with anemia, bleeding, ecchymose and splenomegaly. The diagnosis of Macroglobulinemia was established by the presence of very

high level of IgM paraprotein in sera which was detectable by immunoelectrophoresis and rocket immunoelectrophoresis, and by the presence of bone marrow infiltration of plasmocytes. Family studies revealed a high IgM level in one of the patient's daughters

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