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Changes of free light chains in urine in case of gammopathy

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SUMMARY

We have developed a latex agglutination immunoassay method for free light chains of immunoglobulins in urine and clarified its clinical significance. Bence Jones protein, which was qualitatively detected in urine with the traditional immunoelectrophoresis, could be quantitatively determined by this method. The free light chain determination was useful for diagnosis and prognosis of multiple myeloma that is associated with monoclonal free light chains. The increase in monoclonal free light chains in urine was found in patients with primary macroglobulinemia, primary amyloidosis, and systemic lupus erythematosus. These results suggest that free light chains in urine are important markers for multiple myeloma and related diseases in clinical practice.

Key words : free light chain, multiple myeloma, latex agglutination immunoassay, Bence Jones protein.

INTRODUCTION

Free light chains, which are fragments of immunoglobulins, are excreted in urine in some pathological conditions because of their low molecular mass (44,000 Da, dimer). These are minimally present in blood and urine of healthy persons, but to a lesser extent than in those with certain disorders. Free light chains are divided into two groups, monoclonal and polyclonal, which can be distinguished by immunoelectrophoresis.¹⁾ Especially, detection of Bence Jones protein, which is a monoclonal free light chain, has been widely used for diagnosis of multiple myeloma, primary macroglobulinemia, and related diseases as a tumor marker.^{2,3)} However, precise measurement of monoclonal free light chains in urine has been very difficult for several reasons. Here we report a latex agglutination immunoassay method to quantify free light chains in urine and their clinical significance.

MATERIALS AND METHODS

Materials

The early morning urine samples were collected for this study. The 114 healthy adults (male 53, female 61) were selected as controls. The subjects consisted of 42 patients with multiple myeloma (kappa type 26, lambda

type 16), 3 with primary amyloidosis, 1 with primary macroglobulinemia, and 18 with systemic lupus erythematosus. Seven of the patients with multiple myeloma underwent a clinical study to follow-up changes of free light chains in urine after therapy of the disease. Sixty patients with blood diseases and 79 with renal diseases were selected. The former consisted of 18 patients with acute myelocytic leukemia, 16 with acute lymphocytic leukemia, 5 with chronic myelocytic leukemia, 16 with malignant lymphoma, 2 with myelofibrosis, 2 with monoclonal gammopathy of undetermined significance, and 1 with myelodysplastic syndrome. The latter consisted of 20 patients with immunoglobulin A nephropathy, 4 with membranous nephropathy, 11 with nephrotic syndrome, 3 with acute renal failure, 23 with chronic renal failure with hemodialysis, 4 with chronic renal failure without hemodialysis, and 14 with chronic renal failure after renal transplantation.

Latex agglutination immunoassay

Latex agglutination immunoassay was performed according to Ohtani's method.⁴⁾ Standard solution and urine samples were analyzed by an automatic immunoanalytic apparatus LX-M (Eiken Chemical Co., Tokyo, Japan).

Immunoelectrophoresis

Identification of Bence Jones protein and monoclonal

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protein was performed by the immunoelectrophoretic method with concentrated urine (50- to 100-fold enriched) and with the light chain preparation.

RESULTS

Abnormally high values of free light chain lambda were found in 16 of 16 cases (100%) of multiple myeloma with lambda type, which was similar to the lambda type of monoclonal protein in multiple myeloma, while the kappa type showed an abnormally high value of free light chain kappa in 24 of 26 cases (92.3%) studied (Fig.1). When this method was applied to clinical measurement of free light chains in urine, a kappa value of more than 30 mg/l and kappa/lambda ratio of more than 10.0 were determined as criteria for free light chain kappa type (Fig. 2) and a lambda value of more than 30 mg/l and a lambda/kappa ratio of greater than 5.0 as criteria for free light chain lambda type (Bence Jones protein) (Fig. 3). Fifteen of the 26 cases with multiple myeloma kappa type were identified as having a monoclonal light chain kappa (Fig. 2). In contrast, free light chains were increased in all 16 patients with multiple myeloma lambda type and identified as monoclonal light chain lambda type (Fig. 3). Only one of the 31 cases with increased monoclonal free light chain showed negative result with immunoelectrophoresis.

In urine of four of 26 cases (15.4%) with multiple

myeloma kappa type and in 13 of 16 cases (62.5%) with multiple myeloma lambda type there were more than 200 mg/l of free light chains. It is noteworthy that among cases with more than 350 mg/l of free light chain kappa type and a greater than 130 kappa/lambda ratio and also more than 150 mg/l of free light chain lambda type and a greater than 10.0 lambda/kappa ratio there were only two of 26 cases (7.7%) with multiple myeloma kappa type but 13 of 16 cases (81.3%) with multiple myeloma lambda type. Furthermore, such results were found in no other diseases than multiple myeloma. In contrast, five of 26 cases (19.2%) of multiple myeloma kappa type and three of 16 cases (18.8%) of multiple myeloma lambda type showed an increase in both free light chain kappa and lambda.

Urine of one patient with primary macroglobulinemia had 300.8 mg/l of free light chain kappa type and a 125.3 kappa/lambda ratio, which was identified as monoclonal free light chain kappa type. In amyloidosis, one of three cases had 54.5 mg/l of free light chain kappa type and a 68.1 kappa/lambda ratio, indicating monoclonal free light chain kappa type.

In 12 of the 18 patients with systemic lupus erythematosus, there was an abnormal elevation of free light chains and an increase or decrease in kappa/lambda ratio. Of those cases, six showed an increase in both kappa and lambda types, two an increase in only the kappa type, one an increase in only the lambda type, and

Fig. 1. Change of free light chain in various disorders with Bence Jones proteinuria.

Fig. 2. Correlation between urinary kappa/lambda ratio and free light kappa-chain in hematopoietic disorders.

Fig. 3. Correlation between urinary lambda/kappa ratio and free light lambda-chain in MM lambda type and renal disorders.

five an increase in the kappa/lambda ratio. Of those latter five cases, one case with 134.0 mg/l of free light chain kappa type and a 25.8 of kappa/lambda ratio was found to have monoclonal light chains. All 60 patients with blood diseases and 79 with renal diseases had negative results concerning monoclonal light chains.

Values for urinary free light chains were followed after the administration of chemotherapy in three patients with multiple myeloma kappa type and in four with lambda type. Decreases in free light chains were

noted in all cases (Fig. 4). In the two patients with marked decreases, the decreases were parallel to improvement of symptoms.

The interesting clinical course of a patient with multiple myeloma is as follows. This patient (male, 57-years-old) had immunoglobulin D lambda type and Bence Jones protein lambda type, and complained of back pain, lumbago and anemia upon admission. Bone marrow was hypercellular (>10% plasma cells), free light chain lambda type of 2690.0 mg/l and kappa type

Fig. 4. Change of urinary free light chain after chemotherapy in patients with multiple myeloma.

of 2.6 mg/l in urine, and a marked decrease in the kappa/lambda ratio (a marked increase in lambda/kappa ratio). After administration of prednisolone and combined therapy of vincristine-adriamycin-dexamethasone, improvement of clinical symptoms, a marked decrease in free light chains in urine and a trend toward an increased kappa/lambda ratio were observed. After transplantation of peripheral blood stem cells, free light chain kappa type and lambda type and kappa/lambda ratio all recovered to normal ranges and a complete remission was obtained.

DISCUSSION

The latex agglutination immunoassay which we established in the present study has several advantages, i. e., good reproducibility, simplicity and rapidity.⁴⁾ Monoclonal free light chain was found in urine in 31 of 42 patients with multiple myeloma and in one patient, respectively, with primary macroglobulinemia, primary amyloidosis and systemic lupus erythematosus.

It is reported that the diseases in which Bence Jones protein was frequently found in urine are B cell malignant tumor diseases, such as multiple myeloma and primary macroglobulinemia as well as primary amyloidosis.⁵⁾ In systemic lupus erythematosus there is usually an increase in polyclonal light chains, but an increase in monoclonal light chains is not common.⁶⁾ In the present study, immunoelectrophoresis could not detect Bence Jones protein in the urine of a patient with multiple myeloma lambda type while the quantitative

method did. It may be that results of immunoelectrophoresis are influenced by the extent of urine concentration and the titer of specific anti-serum. Our method is advantageous for detection of a minimal amount of monoclonal free light chains which can not be detected by immunoelectrophoresis.

Diagnosis of B-cell related malignant tumor was suspected when free light chains were elevated over 200 mg/l for kappa type and over 150 mg/l for lambda type. In two of 26 patients (7.7%) with multiple myeloma kappa type, the free light chain kappa type was over 350 mg/l and the kappa/lambda ratio was over 130, and, therefore, they were diagnosed as multiple myeloma kappa type. Thirteen of 16 patients (81.3%) whose free light chain lambda type was over 150 mg/l and the lambda/kappa ratio was over 10 could be diagnosed as having multiple myeloma lambda type.

Free light chains in urine distinctively decreased after treatment of multiple myeloma in seven patients, and the measurement of free light chains was beneficial for monitoring the disease process and determining therapeutic results. Free light chains in urine in one patient returned to within normal values in parallel with clinical improvement after treatment and the patient achieved a complete remission. Although only qualitative detection of Bence Jones protein has been performed by heating test of urine or immunoelectrophoresis, it is noteworthy that the present method can not only measure that protein quantitatively, but is useful for monitoring therapeutic results and prognosis. These results suggest that free light chains in urine are important markers for multiple myeloma and related diseases in clinical practice.

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