Oligoclonal Banding Detected by Urinary Protein Electrophoresis and Immunofixation in Two Patients with the Acquired Immune Deficiency Syndrome and Proteinuria

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Monoclonal and oligoclonal banding has been observed in electrophoretograms of serum, cerebrospinal fluid, and urinary protein from patients infected with the human immunodeficiency virus (HIV). This is the first report of kappa oligoclonal banding in the protein electrophoretograms for urine but not for serum of two patients with the acquired immune deficiency syndrome (AIDS). Both patients had proteinuria, but only one had the nephrotic syndrome and renal failure. Serum oligoclonal banding in HIV-infected patients occurs much more frequently than in age-matched controls and may be detected before AIDS or lymphadenopathy syndrome evolves. The use of oligoclonal banding as a marker for HIV infection is currently under investigation. Urine as well as serum samples should be included in this research.

Monoclonal and oligoclonal banding has been observed in serum protein electrophoretograms of patients infected with human immunodeficiency virus (HIV) (I–10). Oligoclonal banding in the cerebrospinal fluid protein electrophoretogram of a patient with acquired immunodeficiency syndrome (AIDS) and encephalitis (II) has been reported, as has a monoclonal kappa band in the urine protein electrophoretogram of an AIDS patient with a corresponding IgG kappa band in serum (6). Here I report the first two cases in which kappa oligoclonal banding was detected in the urine but not in the serum electrophoretograms of HIV-antibody-positive patients with proteinuria.

Case 1

A 23-year-old black woman was admitted to the emergency department with lower abdominal pain, nausea, vomiting, oral thrush, and ankle edema. Her history included gonorrhea and herpesvaginitis. The patient denied use of intraavenous drugs.

Results of initial laboratory studies included serum urea 9.6 mmol/L, serum creatinine 248 µmol/L, creatinine clearance 0.48 mL/s, and 4+ proteinuria. The erythrocyte sedimentation rate was 114 mm/h, serum cholesterol 7.65 mmol/L, and complement component C3 0.6 g/L. Test results for hepatitis B surface antigen, surface antibody, and core antibody were negative. The concentration of protein in serum was 43 g/L, albumin was 8 g/L, and the α2-globulins were increased, consistent with the nephrotic syndrome. In the urinary protein electrophoretogram there was severe nonselective proteinuria, 9.68 per day. Results of the HIV ELISA test (Abbott Laboratories, Diagnostic Division, Irving, TX) were positive, but the Western blot (SmithKline Bio-Science Laboratories, Los Angeles, CA) was reactive only for p24 and gp160, which was considered nonreactive for HIV.

Throughout the hospital course, the patient experienced episodic fevers as high as 40 °C and persistent watery diarrhea. Results for multiple bacteriological cultures, including stool cultures, were negative. There was mild segmental mesangial proliferation and mesangial sclerosis in a renal biopsy specimen, consistent with early focal sclerosis. The patient was treated with salt-poor albumin and ethacrynic acid for two weeks and then discharged.

She returned to the hospital a month later with persistent nausea, vomiting, diarrhea, oral thrush, and a temperature of 40 °C. There was increased leg edema and increased abdominal girth. There was 3+ proteinuria and the following values for serum: creatinine 566 µmol/L, urea 22.1 mmol/L, total protein 38 g/L, albumin 10 g/L, and cholesterol 10.24 mmol/L. Her hemoglobin concentration was 115 g/L, hematocrit 0.35, and platelet concentration 141 × 10⁹/L.

The patient was treated with diuretics and intravenous salt-free albumin followed by ethacrynic acid. She had persistent fevers as high as 40 °C. Peritoneal fluid from a paracentesis specimen yielded a leukocyte count of 4.58 × 10⁹/L, an amylase value of 768 U/L (reference interval: 20–120 U/L), and negative results for bacteriological cultures. The serum amylase values remained increased for two weeks, reaching activities as high as 570 U/L. Serum lipase activity was also increased to >900 U/L (reference interval: 40–240 U/L). A diagnosis of acute pancreatitis was additionally supported by computed tomography. The patient was treated with cefotaxin and clindamycin.

The patient’s renal function worsened and she required hemodialysis three times per week. A 550-mL, 24-h urine collection contained 56.4 g of protein. A week later a second 24-h urine collection contained 43.4 g of protein, and discrete kappa bands were identified by protein electrophoresis and immunofixation.

Multiple bacteriological cultures were obtained. Enterobacter aerogenes was cultured from the urine, and a stool specimen was positive for Clostridium difficile toxin. The patient was anergic as judged from results of a cutaneousnergy panel. A repeat Western blot test was reactive for p24, gp41, and gp160, which was considered indeterminate for HIV by our reference laboratory, but reactive for HIV by the criteria of the Centers for Disease Control.

The patient’s hemoglobin concentration continued to decline and she required multiple blood transfusions. Her platelet concentration decreased to 62 × 10⁹/L. Except for the presence of reactive plasmacytosis, the bone-marrow biopsy findings were within normal limits. The patient suddenly developed low blood pressure, tachycardia, and decreased right-sided breath sounds. A chest tube was
inserted, which drained a massive amount of blood, and shortly afterwards the patient died. Her death occurred about two and one-half months after her initial admission.

Autopsy confirmed the cause of her death to be from spontaneous hemorrhorax, secondary to a hemodialysis-related coagulopathy. There was focal segmental sclerosis of the glomeruli, which was consistent with the histologic findings of AIDS-associated nephropathy described by Rao et al. (12).

Case 2

A 27-year-old black homosexual man was admitted to the emergency department with fever, sweating, diarrhea, and vertigo. He had been diagnosed with AIDS a month earlier and therapy with azathioprine was begun then. He was also taking phenytoin for seizures, which first occurred during his childhood. He denied use of intravenous drugs.

Pertinent physical findings included a rectal temperature of 39 °C; hepatosplenomegaly; and inguinal, cervical, and axillary lymphadenopathy. Thrush was present in his oral cavity and tonsilar area.

Pertinent clinical chemical values for serum at the time of admission included albumin 16 g/L, gamma-glutamyltransferase 72 U/L (normal 10–50 U/L), aspartate amidotransferase 55 U/L (normal 1–36 U/L), alanine amidotransferase 17 U/L (normal 1–45 U/L), alkaline phosphatase 143 U/L (normal 35–115 U/L), lactate dehydrogenase 499 U/L (normal 110–220 U/L), bilirubin 8.6 μmol/L, and direct bilirubin 3.4 μmol/L.

Results of the hepatitis B surface antigen and core antigen tests were positive, but the hepatitis B surface antibody test was negative. Urea nitrogen and creatinine in serum were within normal limits.

Hematological studies included a leukocyte concentration of 1.7 × 10⁹/L, with a differential of neutrophils 0.66, band neutrophils 0.09, lymphocytes 0.16, atypical lymphocytes 0.04, monocytes, 0.04, and eosinophils 0.01; hemoglobin 77 g/L; hematocrit 0.26; haptoglobin 0.1 g/L; ferritin 1930 μg/L; and a platelet concentration of 148 × 10⁹/L.

Over the following week, the patient's total bilirubin increased to 123 μmol/L, the "direct" fraction being 99.2 μmol/L, and the aminotransferases also increased: aspartate aminotransferase 166 U/L, alanine aminotransferase 75 U/L. Gamma-glutamyltransferase activity was 519 U/L. The alkaline phosphatase activity was 406 U/L, and lactate dehydrogenase was 674 U/L. The albumin concentration remained low and the prothrombin time increased to 14.7 s (poled normal control 11.7 s). The patient's hemoglobin concentration continued to decline and he required multiple blood transfusions. He had diffuse lymphadenopathy, and an axillary lymph node biopsy was performed after he was transfused with platelets and fresh frozen plasma. Multiple noncaseating granulomas with acid-fast bacilli were identified in the lymph node. The bone-marrow biopsy was hypercellular, with an increased proportion of megakaryocytes and erythroid precursors, and there were poorly formed granulomas with acid-fast bacilli. Therapy with isoniazid, rifampin, and ethambutol was initiated.

Serum protein electrophoresis with densitometric quantitation showed decreased albumin and α₂-globulins, and increased gamma globulins. Results of high-resolution protein electrophoresis with immunofixation were negative for oligoclonal bands. Although values for serum urea nitrogen and creatinine also remained within normal limits, trace proteinuria was detected. High-resolution protein electrophoresis and immunofixation were performed on an untimed urine specimen that contained 850 mg of protein per liter, and on a 24-h urine collection with a total volume of 2160 mL and containing 2396 g of protein. Oligoclonal kappa bands were detected in the electrophoretograms of both specimens.

After a week of antimycobacterial therapy, the patient's symptoms abated, and he signed out of the hospital against medical advice. No further follow-up has been obtained.

Materials and Methods

Total serum protein was determined by the biuret method in a Hitachi 736-30 Automatic Analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN). Total protein in urine was determined by spectrophotometric measurement of protein–dye complexes with Coomassie Brilliant Blue G 250, in a System 4 spectrophotometer (Gilford Instrument Laboratories Inc., Oberlin, OH), at a wavelength of 595 nm. For zone electrophoresis we used ultra-thin agarose gels with the Casset Electrophoresis System (Corning Medical, Palo Alto, CA). Densitometric scanning was performed on the Corning 710 Densitometer/Fluorometer. High-resolution protein electrophoresis was performed on Titan Gel High Resolution Protein Electrophoresis System (Helena Laboratories, Beaumont, TX). Antisera from Atlantic Antibodies (Scarborough, ME) were used for immunofixation.

Results

In Case 1, oligoclonal banding was detected in the protein electrophoretogram of the patient's third 24-h urine specimen. These bands were identified by high-resolution protein electrophoresis and immunofixation as kappa light chains (see Figure 1). Retrospectively, protein electrophoresis was performed on a serum specimen that was collected with the urine, but oligoclonal bands were not detected.

In Case 2, oligoclonal banding was present in the electrophoretograms of the patient's untimed and 24-h urine specimens. These bands were identified by high-resolution protein electrophoresis and immunofixation as kappa light chains (see Figure 2). Oligoclonal bands were not detected in the serum by high-resolution protein electrophoresis and immunofixation.

Discussion

There are several recent reports of paraproteinemias in HIV-infected patients, and both monoclonal and oligoclonal banding patterns have been described (I–10). The class of immunoglobulins most commonly involved has been IgG kappa; however, IgM heavy chains and lambda light chains have also been observed.

Serum oligoclonal immunoglobulins reportedly were present in 69% of all AIDS patients tested, and were more frequently detected in those with Kaposi's sarcoma (89%) than in those without Kaposi's sarcoma (13%) (1). Heriot et al. (5) detected paraproteins in 53% of their patients with AIDS and in 67% with lymphadenopathy syndrome. In other investigations, these bands were seen in patients infected with HIV but lacking the clinical manifestations of AIDS or lymphadenopathy syndrome. Kouns et al. (2) detected seven such cases, and Lefrere et al. (3) reported six cases. Crapper et al. (4) studied 130 homosexual men, and detected abnormal bands in the serum of six, all of whom were seropositive for anti-HIV. Of these six men, three
were clinically asymptomatic.

Severe renal disease, including the nephrotic syndrome and AIDS-associated nephropathy, has been described in patients with AIDS (12-17). Typically, there is focal segmental sclerosis of the glomeruli (13); however, other abnormalities reported include acute tubular necrosis, focal interstitial nephritis, nephrocalcinosis, mesangial hypercellularity and adhesions of glomerular tufts, renal cell carcinoma, and cryptococcal abscesses (13). In the study done by Gardenvsartz et al. (13) all patients with renal failure experienced proteinuria, had a higher incidence of esophageal and oral candidiasis, and had a more unfavorable short-term prognosis than those lacking renal disease.

Rao et al. (12) described AIDS-associated nephropathy as a syndrome in which >3 g of urinary protein is excreted per day and there is focal segmental sclerosis (12); this syndrome results in end-stage renal disease within a few weeks. They subsequently reported that 70% of their AIDS patients with renal disease demonstrated AIDS-associated nephropathy and all but two of 55 of these subjects died within six months after the onset of chronic uremia. They also identified a group of 18 subjects in which AIDS was diagnosed after initiation of hemodialysis; all of these patients were black, had a history of intravenous drug abuse, and presented with the nephrotic syndrome. All died within three months after they were diagnosed with AIDS (16).

The patient in Case 1 had massive proteinuria as high as 56.4 g/d and oral candidiasis, and rapidly developed renal failure that required hemodialysis. Massive proteinuria with >10 g/d of protein in the urine of AIDS patients has been previously reported (13, 14). The etiology of this proteinuria is unknown, and understanding its relationship to AIDS will require further investigation. The kappa oligoclonal bands detected in her urine appeared about the same time the repeat Western blot test was positive by the criteria of the Centers for Disease Control. Except for the oral candidiasis, there was no clinical evidence of immunosuppression or HIV infection. She died 10 weeks after her initial presentation, one week after the appearance of oligoclonal bands and a positive Western blot.

The patient presented in Case 2 had normal concentrations of serum urea nitrogen and creatinine, but also had moderate proteinuria. Kappa oligoclonal bands were detected in his urine protein electropherogram approximately one month after a diagnosis of AIDS had been established. These bands were probably not detected by serum protein electrophoresis because light chains are readily filtered into the urine. Because paraproteinemias are detected in 2.5% of HIV-infected patients and in only 0.15% of the population as a whole, the finding of oligoclonal banding may be an indication for further testing for HIV (3). Papadopoulos et al. (18) have obtained positive reactions with HIV-antibody-positive sera against HIV antigens by the immunoblot technique and positively identified the oligoclonal IgG bands with these antigens. They suggested that oligoclonal bands may be useful markers for following the course of HIV infection. Studies must be conducted to determine whether or not detection of oligoclonal bands has any prognostic value. Urine and serum samples from HIV-infected patients should be included in this research.

References
6. Ng VI, Hwang KM, Reyes GR, et al. High titer anti-HIV