Gamma Heavy Chain Disease in a Patient With Diabetes and Chronic Renal Insufficiency: Diagnostic Assessment of the Heavy Chain Fragment

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Heavy chain diseases are rare B-cell disorders that are characterized by an overproduction of abnormal and structurally incomplete monoclonal immunoglobulin (Ig) heavy chains and are devoid of light chains. We describe a case of a 62 year-old African-American woman with a long history of poorly controlled type 2 diabetes and subsequent probable diabetic nephropathy, hypertension, and recent onset of peripheral neuropathy involving all extremities. Routine laboratory testing revealed a distinct beta spike by urine protein electrophoresis (UPEP). No serum abnormality was noted on serum protein electrophoresis (SPEP). Serum and urine immunofixation demonstrated an IgG heavy chain

protein devoid of any corresponding light chains. IgG subclasses identified IgG1 as the predominant IgG component but when we added all the subclasses, the sum, 683.4 mg/dL, failed to come close to our total IgG of 1,770 mg/dL. Therefore, a urine IgG subclass determination was performed in-house and we identified a subclass 3 gamma chain. In conclusion, we portray a patient with an underlying monoclonal gamma heavy chain disease (HCD) who presented with a complex medical history. The evaluation of IgG subclasses in the context of a HCD may be limited by the capability of the test to recognize the particular IgG fragment. *J. Clin. Lab. Anal.* 22:146–150, 2008. © 2008 Wiley-Liss, Inc.

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INTRODUCTION

Immunoglobulins (Igs) consist of two equivalent heavy chains, either gamma (γ), alpha (α), mu (μ), delta (δ), or epsilon (ϵ), and two light chains: kappa (κ) or lambda (λ). Monoclonal gammopathies are characterized by a homogeneous Ig or an Ig fragment. A gammopathy involves an excessive amount of a protein that has either two heavy chains and two light chains, light chains only, or, rarely, heavy chains only. When the gammopathy involves only heavy chains, it is called heavy chain disease (HCD) (1). Because of their size, these monoclonal proteins are usually present in both serum and urine. HCDs are classified according to Ig isotype: gamma-HCD (Ig γ), mu-HCD (Ig μ), alpha-

HCD (Ig α), and delta-HCD (Ig δ) (2). To date there are no reported cases of epsilon-HCD (Ig ϵ) (2). The diagnosis of HCD is established by performing serum protein electrophoresis (SPEP) and urine protein electrophoresis (UPEP) followed by immunofixation using monospecific antisera to the various Ig heavy and light

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chains (3). No specific method has been instituted to subisotype the heavy chain.

Gamma-HCD, first reported by Franklin et al. (1) in 1964, is a rare disorder but it has been extensively studied because it is the second most common form of the HCDs. Approximately one-third of the patients with gamma-HCD may have an associated autoimmune disorder such as rheumatoid arthritis. The median age at diagnosis of patients with gamma-HCD is approximately 60 years. Patients with gamma-HCD may present with fatigue, weakness, and fevers. Parotid gland swelling, pharyngeal edema, and enlarged thyroid glands may also be seen (4). There may be associated lymphadenopathy, splenomegaly, and hepatomegaly (4). In our case, the patient presented with other medical problems and HCD was not clinically suspected. However, additional immunochemical studies led to the diagnosis of HCD.

The patient is a 62-year-old woman with progressive renal insufficiency and recent hospitalization for congestive heart failure. She had a history of type 2 diabetes with poor control, hypertension, and peripheral neuropathy. Her family history was significant with multiple family members having diabetes and hypertension. The review of systems revealed severe claudication.

The patient's physical examination revealed an obese female (weight 214 lbs., height 5'1") with an initial blood pressure of 200/86 and 1–2+ ankle edema. Laboratory tests included sodium 131 mEq/L (135–148), potassium 6.6 mEq/L (3.5–5.0), CO₂ 16 mEq/L (21–31), BUN 44 mg/dL (7–22), creatinine 2.1 mg/dL (0.5–1.2), and glucose 402 mg/dL (60–109). Urinalysis showed 4+protein. Ig quantitation revealed an increased IgG, and IgA and IgM values within normal limits. SPEP showed no spike, although the beta region was peaked and asymmetric (Fig. 1). Serum immunofixation (SIFE) showed a band in the IgG lane without corresponding light chain bands. These findings

Fig. 1. Serum electrophoresis (SPEP) demonstrating that gamma is decreased, beta is increased. The beta region appears to be peaked and asymmetric.

Fig. 2. Immunoelectrophoresis of the serum. Immunofixation of the serum proteins with monospecific antisera to alpha, gamma, mu, kappa, or lambda chains. One anodal band is present in the IgG lane without corresponding light chain bands. The band cannot be seen or quantitated by SPEP because it is obscured by the beta region proteins.

Fig. 3. UPEP. Protein excretion is significant. Monoclonal spike (indicated between dotted lines) is present in the beta region.

are consistent with gamma-HCD (Fig. 2). UPEP revealed a monoclonal beta spike (Fig. 3). Urine immunofixation (UIFE) studies were performed and were similar to the SIFE, confirming the presence of gamma-HCD (Fig. 4). A send out test of IgG subclasses showed all subclasses within normal limits, but their sum was in discrepancy with our total IgG levels.

Further urine immunoassay studies revealed that the heavy chain fragment was of IgG subclass 3. The patient underwent a full skeletal survey that showed osteoarthritis but the study was negative for lytic lesions. A magnetic resonance imaging study of the abdomen was unremarkable. The patient had missed all follow-up visits at the time of the writing of this article.

MATERIALS AND METHODS

Blood and 24-hr urine samples were received without preservatives or anticoagulants. The 24-hr urine volume

was measured and aliquotted. Concentration of the urine to 100 × was done using a Milliplex concentrator (Millipore, Billerica, MA). Serum Ig quantitation was performed by nephelometry (IMMAGE; Beckman, Fullerton, CA). Total serum protein was measured by a colorimetric method (Roche/Hitachi 917 modular P and D, Indianapolis, IN). Urine total protein was measured by turbidometry (Roche/Hitachi 917 modular P and D). After urine concentration, SPEP and UPEP were performed using Helena Laboratories equipment and agarose gels at an alkaline pH (Helena Laboratories, Beaumont, TX). The serum and urine proteins in the various fractions were quantified by densitometry (Helena Laboratories). The Paragon immunofixation electrophoresis kit (Beckman) was used for the immunologic identification of proteins in serum and urine. Using nephelometry, the IgG subclasses were performed at Quest Diagnostics, Nichols Institute (San Juan Capistrano, CA). The concentrated urine was used to resolve the heavy chain subclass, since it was assumed to be the dominant gamma globulin protein present in the urine. The Ig subclass of the heavy chain was assessed in the urine by enzyme immunoassay (5). In this assay, a microtiter plate was coated (10 µg/mL, 0.1 mL/well) with purified mouse monoclonal anti-human: IgG1 Fc (clones HP6070P and HP6069P), anti-human IgG2 Fc (HP6002P), anti-human IgG3 Hinge (HP6047P), anti-human IgG4 Fc (HP6023P), and anti-human IgG1 Fc (HP6043P and HP6000P). Three negative control antibodies were also used: anti-human: IgA Fc (HP6111P), anti-human IgM Fc (HP6083P), and anti-human IgE Fc (HP6061P). The monoclonal antibodies were obtained from the Hybridoma Reagent Laboratory (Baltimore, MD; www.hybridoma-reagent-laboratory.com). Plates were coated overnight at 4°C. Each

Fig. 4. Immunoelectrophoresis of the urine. Immunofixation of the urine proteins with monospecific antisera to alpha, gamma, mu, kappa, or lambda chains. One anodal band is present in the IgG lane without corresponding light chain bands.

plate was then washed once with phosphate buffered saline (PBS)-0.05% Tween, and blocked with PBS-1% bovine serum albumin (BSA). After a second wash, 0.1 mL of the concentrated urine at five- to ten-fold dilutions from 1:1,000 to 1:100,000,000 was pipetted into their respective duplicate wells. A buffer blank was analyzed as a negative control for each antibody specificity. After 1 hr, the plate was washed and mouse monoclonal anti-human IgG Fd-biotin (clone HP6045B, 1 µg/mL, 0.1 mL/well) was added to all wells for 1 hr. After an additional buffer wash, bound detection antibody was detected with 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS)-H₂O₂. Optical density was read at 405 nm and the endpoint titer was determined as dilution of specimen required to obtain an optical density of 1.0 at 405 nm. The Ig heavy chain at the highest concentration was the one with the highest endpoint dilution.

RESULTS

Serum Ig quantitation showed that IgG was elevated, 1,770 mg/dL (normal, 751–1,560), IgA was 144 mg/dL (normal, 82–453), and IgM was 66 mg/dL (normal, 46–304). SPEP with protein fractionation showed a decreased gamma region (0.51 g/dL; normal, 0.70–1.70), an elevated beta region with an asymmetric peak (1.57 g/dL; normal, 0.50–1.20), and the alpha-2, alpha-1, albumin, and total protein were within normal limits. No spike was seen on the SPEP (Fig. 1). SIFE showed one band of restricted electrophoretic mobility present in the IgG lane without corresponding light chain

Fig. 5. Immunoelectrophoresis of the urine and serum proteins to confirm the comigration of the monoclonal IgG heavy chain. Immunofixation of the urine and serum proteins either individually or mixed with monospecific antisera to gamma chain. One band of restricted electrophoretic mobility is present in all lanes. The band is strong and migrates toward the anode. There is no difference in the electrophoretic mobility of the serum or urine bands. NOTE: S: serum. U: urine at dilutions: 1/8, 1/16, 1/32, and 1/64. The number after the S or U represents the dilution of the sample tested.

TABLE 1. Evaluation of concentrated urine for isotype specificity of human immunoglobulin heavy chain

Product code (clone number)	Mouse isotype	Monoclonal antibody specificity	Reciprocal endpoint
HP6000	IgG2b	Human IgG Fc PAN (1,2,3,4)	11000000
HP6043	IgG1	Human IgG Fc PAN (1,2,3,4)	12000000
HP6045-Biotin ^a	IgG2a	Human IgG Fd PAN (1,2,3,4)	ND
HP6069	IgG1	Human IgG1 Fc	28000
HP6070	IgG1	Human IgG1 Fc	20000
HP6070	IgG1	Human IgG1 Fc	31000
HP6002	IgG1	Human IgG2 Fc	4200
HP6047	IgG1	Human IgG3 Hinge	270000
HP6023	IgG3	Human IgG4 Fc	8700
HP6061	IgM	Human IgE Fc	<500
HP6083	IgG1	Human IgM Fc	<500
HP6111	IgG1	Human IgA Fc	<500

^aHP6045-biotin was used as the detection antibody since it reacts with determinants on the Fc portion of all four subclasses of human IgG, therefore the reciprocal endpoint was not done (ND).

bands. The band was strong and migrated toward the anode. These findings were consistent with gamma-HCD (Fig. 2). UPEP revealed significant protein excretion with a monoclonal spike present in the beta region, 19.43 mg/dL (194.0 mg/24 hr) (Fig. 3). UIFE studies showed one band with restricted electrophoretic mobility in the IgG lane without corresponding light chain bands. The band was strong and it migrated toward the anode. To further confirm that the single monoclonal band in the serum and urine had identical electrophoretic mobility, serum and urine specimens were mixed in various combinations and analyzed by immunofixation (IFE) using anti-IgG antisera. Only a single band was identified in the mixing study which confirmed comigration of the heavy chain in the serum and urine (Fig. 5). Nephelometry serum IgG subclasses studies showed: IgG1 571 mg/dL (250–930), IgG2 86 mg/dL (68–525), IgG3 22 mg/dL (15–35), and IgG4 4.4 mg/dL (0–210). Although IgG1 was the predominant IgG component, when we added all the subclasses, the sum, 683.4 mg/dL, failed to come close to our total IgG of 1,770 mg/dL. Because the UIFE studies demonstrated that most of the gamma globulins excreted in the urine were of a monoclonal origin, we then decided to study the urine to find out the nature of the IgG heavy chain fragment. Human IgG subclass 3 was in highest concentration in the urine and it was assumed to represent the subclass of the gamma heavy chain excreted into the urine. Enzyme immunoassay (EIA) subtyping results are summarized in Table 1.

DISCUSSION

HCDs are rare B-cell disorders that are characterized by the production of monoclonal Igs with only heavy chain components (4). Because of their molecular size, these monoclonal proteins are usually present in both

serum and urine. Alpha-HCDs are the most common type (6). Gamma-HCDs and mu-HCDs are less common. Gamma-HCD, although rare, has been extensively studied. Approximately one-thirds of the patients with gamma-HCD may have an associated autoimmune disorder such as rheumatoid arthritis. The median age at diagnosis of patients with gamma-HCD is approximately 60 years (6).

Patients with gamma-HCD may present with fatigue, weakness, and fever but their clinical courses are highly diverse. While some patients may be asymptomatic, others may rapidly deteriorate progressing to death within months. Investigations into the pathophysiology of the HCDs have revealed that there are a variety of genetic changes that may alter the constant and variable regions of the Ig molecule. Most gamma-HCD proteins have deletions in either the V (variable) region or the CH1 (constant) domain that leads to a truncated heavy-chain length. These deletions may either leave the hinge region intact or modify the hinge region. The deletions may therefore also disrupt the disulfide linking of the light chains to the heavy chains, resulting in heavy chains devoid of light chains. The truncated chains may be 50–60% of the length of a normal heavy chain and as a result have a lower molecular weight as compared to a normal heavy chain. Due to the reduction in the molecular weight, the truncated heavy chain is often present in the urine, such as it was in our patient, and it may deposit in the kidney (7).

The diagnosis of gamma-HCD is usually accomplished using SPEP and UPEP in addition to an IFE to detect the monoclonal gamma heavy chain without an associated light chain. A spike representing the monoclonal protein may be seen in the protein electrophoresis (PEP) studies. In our patient, a protein spike was not seen in the SPEP. The band was obscured by the beta region proteins. It was only detected by UPEP.

However, both the SIFE and UIFE studies revealed the presence of a single band of restricted electrophoretic mobility that reacted only with human IgG-specific antisera, and it was devoid of any corresponding light chains. No gold standard has been established to identify the subclass of the heavy chain fragment. In reported cases in which the heavy fragment subclass has been studied, different methods have been used, ranging from Ouchterlony in the earlier cases (8), to indirect immunofluorescence staining (9), immunoblotting (7), amino acid sequence (3), immunoselection (2), and enzyme linked immunoabsorbent assay (ELISA) (10). Most of these assays have been performed on serum, tissue, and, infrequently, urine. In our case the urine proved to be a more valuable sample to determine the nature of the heavy chain fragment. The diagnostic importance of the assessment of a heavy chain fragment rests in the clinical scenario as in most diseases, but in the laboratory we aim to provide the clinician with the most accurate diagnosis possible.

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