AA amyloidosis associated with hepatitis B

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Abstract
We report a 13-year-old Indian boy with nephrotic syndrome caused by renal AA amyloidosis. Workup of the AA amyloidosis revealed chronic hepatitis B. Laser microdissection of the Congo-red-positive glomeruli and vessels followed by liquid chromatography and tandem mass spectrometry confirmed the presence of serum amyloid A (SAA) protein and ruled out hereditary and familial amyloidosis. Furthermore, mass spectrometry also detected a variant of SAA protein (SAA W71R).

Keywords: AA amyloid; hepatitis B; mass spectrometry; proteomics; SAA variant

Introduction
Amyloidosis is caused by extracellular deposition of proteins in an insoluble beta-pleated format. Amyloid deposits are identified based on apple-green birefringence under a polarized light microscope on Congo-red stains and the presence of rigid nonbranching fibrils 7.5–10 nm in diameter on electron microscopy [1,2]. The most common forms of systemic amyloidosis are immunoglobulin light-chain (AL) amyloidosis and systemic AA amyloidosis derived from circulating acute-phase-reactant serum amyloid A (SAA) protein in chronic inflammatory diseases [3]. AL amyloidosis is extremely rare in children, while AA amyloidosis can affect children, occurring in some with chronic inflammatory diseases, such as with familial Mediterranean fever (FMF) and juvenile rheumatoid arthritis (JRA) [3–5]. Chronic bacterial infections such as tuberculosis and osteomyelitis are also important causes of AA amyloidosis [3–5]. However, AA amyloidosis is uncommon in chronic viral infections including hepatitis B. We report a case of a 13-year-old boy with nephrotic syndrome due to AA amyloidosis associated with chronic hepatitis B infection.

Case report
A 13-year-old Indian boy presented with generalized swelling of the body and decreased urine output for 2 months. He also developed effort intolerance and palpitations while walking and running, although this improved with rest. He had a history of 3–4 similar episodes over 2 years and had partial response to treatment with frusemide and prednisolone. There was no history of joint pains, rash, fever, cough, cold, sore throat, insect bite, jaundice, abdominal pain, loose stools and red or yellowish discoloration of urine. There were no complaints of headache, chest pain or fainting episodes. On examination, his pulse rate was 100/min, respiratory rate 24/min and blood pressure 100/60 mm Hg in right arm supine position (25th percentile of height and age). He was wasted (<3rd percentile) and stunted (<3rd percentile). He had pallor facial puffiness with bilateral pitting pedal edema. Abdominal examination showed evidence of ascites and scrotal edema without any organomegaly. The rest of the systemic examination was normal. Laboratory investigations are shown in Table 1. The diagnosis of nephrotic syndrome was made based on these investigations. Ascitic fluid tap analysis was normal. Blood, urine and ascitic fluid cultures were sterile. During the hospital stay, the child developed left-sided pneumonitis, peritonitis, sepsis and shock and was treated with intravenous antibiotics, ceftriaxone and vancomycin for 14 days. The child improved and was started on prednisolone 2 mg/Kg/day; however, the child did not achieve remission even after 4 weeks. An ultrasound-guided percutaneous renal biopsy was performed to determine the cause of steroid-resistant nephrotic syndrome.

Kidney biopsy findings
The renal biopsy consisted of one core of renal cortex, which contained six glomeruli. None of the glomeruli were globally sclerosed. The glomeruli showed marked mesangial expansion with Periodic acid-Schiff-positive, silver-negative amorphous material resulting in the formation of large acellular mesangial nodules (Figure 1A). The glomerular basement membranes were thickened and spicule formation was noted along some of the capillary walls. There was moderate tubular atrophy and interstitial fibrosis present. Congo-red stain was positive and showed reddish brown material in the glomeruli and along some...
vessel walls (Figure 1B). Immunohistochemistry showed positive staining for both serum amyloid protein (SAP) and SAA protein in the glomeruli (mesangium and capillary walls) and in the walls of small arteries (Figure 1C).

Kidney biopsy diagnosis

The kidney biopsy diagnosis was amyloidosis, AA type, involving glomeruli and vessels.

Table 1. Laboratory findingsa

<table>
<thead>
<tr>
<th>Investigations</th>
<th>At presentation (SI units)</th>
<th>At 4 months (SI units)</th>
</tr>
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<tbody>
<tr>
<td>Hemoglobin (gm/dL)</td>
<td>12.3 (12.3 g/L)</td>
<td>9.2 (9.2 g/L)</td>
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<tr>
<td>White blood cells (cells/mm³)</td>
<td>35 000 (35 × 10⁹/L)</td>
<td>43 500 (43.5 × 10⁹/L)</td>
</tr>
<tr>
<td>Differential count</td>
<td>N 95L4E1</td>
<td>N90L10</td>
</tr>
<tr>
<td>Platelets (cells/mm³)</td>
<td>4.1 (4 × 10⁹/L)</td>
<td>2.6 (2.6 × 10⁹/L)</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>137 (137 mmol/L)</td>
<td>138 (138 mmol/L)</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4.8 (4.8 mmol/L)</td>
<td>3.9 (3.9 mmol/L)</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.8 (2.2 mmol/L)</td>
<td>9.2 (2.3 mmol/L)</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>5.5 (1.78 mmol/L)</td>
<td>3.8 (1.23 mmol/L)</td>
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<td>Blood urea (mg/dL)</td>
<td>20 (7.1 mmol/L)</td>
<td>24 (8.6 mmol/L)</td>
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<td>Serum creatinine (mg/dL)</td>
<td>0.8 (71 μmol/L)</td>
<td>1.0 (88 μmol/L)</td>
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<tr>
<td>Uric acid (mg/dL)</td>
<td>4.5 (268 μmol/L)</td>
<td>6.5 (387 μmol/L)</td>
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<td>Glucose (mg/dL)</td>
<td>98 (5.4 mmol/L)</td>
<td>82 (4.6 mmol/L)</td>
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<td>Total protein (g/dL)</td>
<td>3.5 (35 g/L)</td>
<td>3.8 (38 g/L)</td>
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<td>Albumin (g/dL)</td>
<td>1.3 (13 g/L)</td>
<td>2.0 (20 g/L)</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>310 (8.2 mmol/L)</td>
<td>290 (7.5 mmol/L)</td>
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<td>PT (test/control) seconds</td>
<td>11.3/13.2</td>
<td>33.8/12.1</td>
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<tr>
<td>APTT (test/control) seconds</td>
<td>27.9/29</td>
<td>50.5/28</td>
</tr>
<tr>
<td>Bilirubin (total/direct) mg/dL</td>
<td>0.9/0.4 (15/7 μmol/L)</td>
<td>2.7/1.7 (46/29 μmol/L)</td>
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<tr>
<td>SGOT (IU)</td>
<td>19</td>
<td>1537</td>
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<tr>
<td>Serum glutamic–pyruvic transaminase (IU)</td>
<td>20</td>
<td>1072</td>
</tr>
<tr>
<td>HBsAg</td>
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<td>Positive</td>
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<tr>
<td>Anti-HBe antibody</td>
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<tr>
<td>Anti-HBVcAg IgM antibody</td>
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<td>Negative</td>
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<td>HBV DNA (copies)</td>
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<td>850</td>
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<tr>
<td>Hepatitis C antigen</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Antinuclear antibody</td>
<td>Negative</td>
<td>Negative</td>
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APTT, activated partial thromboplastin time.

Fig. 1. Light microscopy: (A and B) mesangial expansion with amorphous material resulting in acellular mesangial nodule formation (A: hematoxylin & eosin, B: periodic acid Schiff × 20). (C) Congo-red stain is positive for amyloid (×20) and (D) immunohistochemistry for SAA protein shows positive staining in glomeruli and vessels (×10).

Laser microdissection and mass spectrometry studies

Laser microdissection (LMD) and mass spectrometry analysis of the amyloid were performed as previously described [6–8] (Figure 2). The licensed Patent Rights to perform protein extraction from paraffin-embedded tissue for the mass spectrometry-based amyloid testing was granted by Expression Pathology Inc. These studies were performed to rule out any familial or hereditary amyloidosis, includ-
ing leukocyte chemotactic factor 2 (LECT-2) amyloidosis [9–11]. Briefly, Congo-red-positive glomeruli and vessels were microdissected by using laser capture techniques (Figure 2A and B). Peptides extracted from the microdissected tissue were subjected to liquid chromatography and tandem mass spectrometry (MS/MS). MS raw data files were queried using three different algorithms (Sequest, Mascot and X!Tandem), and the results were combined and assigned peptide and protein probability scores in Scaffold (Proteome Software Inc., Portland, OR). For each case, a list of proteins based on peptides identified by MS was generated. Peptide identifications were accepted if they could be established at >90.0% probability as specified by the Peptide Prophet algorithm [12–14]. The most abundant peptides detected by MS/MS represented SAA protein and SAP (Figure 2C and D). The serum amyloid precursor A2 was also detected (Figure 2C). In addition, MS/MS also detected a variant SAA protein (SAA W71R) similar to that described by Møyner et al. [15] (Figure 2E). Serum amyloid precursor A3 and A4 were not detected. MS/MS failed to detect peptides representing LECT-2, fibrinogen A-α, lysozyme, transthyretin, apolipoprotein A-I or gelsolin. Apolipoprotein E, which is a common constituent of amyloid, was also present [2].

Clinical follow-up

The child was further investigated for etiological workup of AA amyloidosis. Immunodeficiency was ruled out with normal serum immunoglobulin and complement levels and a negative HIV titer. Rheumatoid factor, anti nuclear antibody, C-reactive protein, anti-streptolysin-O titer and Montoux test were negative. However, his hepatitis B surface antigen (HBsAg) titers were positive. Hence, the steroids were tapered and stopped and the child was started on enalapril, losartan and calcium supplements. Evaluation for hepatitis B infection was recommended but not completed. After 4 months, he was admitted again with complaints of fever, abdominal pain and yellowish discoloration of eyes and urine. On examination, his vital parameters were normal, icterus was present and he had hepatomegaly and bilateral pneumonia. Laboratory investigations are shown in Table 1. In particular, the liver function test was markedly abnormal: bilirubin 2.7 with direct 1.7 mg/dL, serum
glutamic oxaloacetic transaminase 1537, serum glutamic–
pyruvic transaminase 1072, alkaline phosphatase 1364,
with an abnormal coagulation profile (PT 33.8 with inter-
national normalized ratio of 2.79, activated partial thrombo-
plastin time 50.5 with control of 28). Serial blood cultures
remained sterile. Abdominal ultrasound and contrast-
enhanced computer tomography revealed the presence of
mild hepatomegaly, gall bladder wall thickening, moderate
ascites and minimal right pleural effusion. Ascitic fluid ana-
lysis was normal with sterile cultures. Viral studies revealed
presence of HBsAg, anti-HBeAg antibody, absence of anti-
HBcAg IgM antibody and HBV DNA titer was 850/mm³
pointing to chronic hepatitis B infection. Both the parents
also were found to be positive for HBsAg. The child was
treated with intravenous broad-spectrum antibiotic, albumin
infusion, losartan, enalapril, vitamin K and supportive
treatment on which he improved clinically and was dis-
charged in a stable condition. At 6 months follow-up, his
proteinuria had decreased (Up/Uc = 2.2) on enalapril and
losartan. Serum creatinine at 6-and 9-month follow-up
was 1.0 mg/dL.

Discussion
AA amyloidosis occurs during sustained acute-phase re-
response and often complicates chronic inflammatory disor-
ders. AA fibrils are derived from acute-phase-reactant
SAA protein which undergoes a process of cleavage, misfolding and aggregation to form abnormal β-pleated configuration of amyloid fibrils [2,3]. Chronic inflammation leads to persistently elevated proinflammatory cytokines resulting in high concentrations of SAA [16]. Indeed, in patients with AA amyloidosis, high levels of SAA are associated with poor prognosis compared with low to normal levels of SAA [3]. The median duration of inflammatory disease at the time of diagnosis in SAA amyloidosis was 17 years in one study [3], although typically AA amyloidosis is known to occur after 2–7 years of a chronic inflammatory process. AA amyloidosis occurs as early as 9 months of life has also been reported [17].

Common causes of AA amyloidosis in children in the developed world include chronic inflammatory conditions such as juvenile idiopathic arthritis and periodic fever syndromes such as FMF, tumor necrosis factor receptor-associated periodic syndrome, cryopyrin-associated periodic syndromes and hyperimmunglobulinemia D syndrome [3–5]. On the other hand, chronic bacterial infectious diseases such as tuberculosis and osteomyelitis are important causes of AA amyloidosis in the developing world [18]. Viral infections have been uncommonly reported with amyloidosis. A search of the literature revealed a case of hepatitis C virus-associated glomerulonephritis and AL amyloidosis, a case of Still’s disease complicated with AA amyloidosis and hepatitis B and a case of common variable immunodeficiency with hepatitis C and systemic amyloidosis [19–21]. This case report highlights the uncommon association of renal AA amyloidosis and chronic hepatitis B infection. Recently, Lannergård et al. have shown that viral infections, including hepatitis B, result in elevated SAA levels. Furthermore, SAA levels were a good marker for monitoring continuing viral infection [22].

We have recently reported on the technique of LMD- and MS/MS-based proteomic analysis as a sensitive and specific tool for the diagnosis of amyloidosis [6–8,23]. We performed LMD and MS/MS studies in this case to confirm the AA amyloidosis and rule out hereditary and familial forms of amyloidosis including LECT2. MS/MS showed the large spectra for SAA and SAP confirming the diagnosis of AA amyloidosis. Furthermore, MS/MS studies of the amyloid deposits revealed SAA2 and presence of a variant of SAA protein. The SAA variant has been described by Møyner et al., who found that the AA variant protein was larger than but otherwise very similar to other human AA proteins studied by complete sequencing. Two amino acids were found both in Position 52 (valine/alanine) and in Position 53 (tryptophan/arginine), strongly suggesting a polymorphism of AA proteins [15]. In our case, it is difficult to determine whether the SAA variant protein played a role in the development of AA amyloidosis in the setting of hepatitis B. Murphy et al. have recently reported a case of AA amyloidosis occurring as a result of a mutated form of the constitutively expressed serum amyloid A4 (SAA4) protein in the absence of any inflammatory condition. The mutated SAA4 revealed a component identical in sequence to the N-terminal portion of SAA4, except for the substitution of glycine for tryptophan at Position 22 (W22G). The authors hypothesized that the W22G alteration would profoundly affect SAA4 stability, rendering it amyloidogenic [24].

The long-term prognosis of untreated AA amyloidosis is usually poor. Prognosis is related to the degree of renal involvement and the diagnosis is often not made until substantial organ infiltration has occurred. A recent report shows that the median survival after diagnosis is 133 months [3]. In earlier studies, renal failure was the leading cause of death in 70% of cases. With availability of renal replacement therapy, infection has replaced renal failure as the leading cause of death. The aim of treatment is to eliminate the supply of precursor protein by suppressing the acute-phase response and SAA concentrations to as close to normal as possible. Anti-inflammatory agents such as chlorambucil and colchicine are beneficial in chronic rheumatologic disorders like JRA and FMF, respectively. ACE inhibitors/ARB are also useful in decreasing proteinuria in renal amyloidosis. Newer drugs such as eprodisate show great promise in slowing the progression of SAA amyloidosis. Eprodisate interferes with interactions between amyloidogenic proteins and glycosaminoglycans thereby inhibiting polymerization of amyloid fibrils and deposition of the fibrils in tissues [25].

To summarize, we report a case of a 13-year-old boy who developed nephrotic syndrome due to renal AA amyloidosis. Workup of the AA amyloidosis revealed chronic hepatitis B infection. LMD and mass spectrometry studies confirmed the presence of SAA protein, detected a SAA variant and ruled out hereditary and familial amyloidosis.

Conflict of interest statement. None declared.

References


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