

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS AND ACUTE KIDNEY INJURY WITH SEVERE NEPHROTIC SYNDROME IN DENGUE PATIENTS: A CASE REPORT

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Abstract. Dengue virus infection (DVI) has been rarely reported to have association with secondary hemophagocytic lymphohistiocytosis (HLH) in adults. Moreover, biopsy-proven nephrotic syndrome in the same patients has never been reported. Therefore, we describe a case of severe DVI-associated HLH and biopsy-proven renal involvement.

Keywords: dengue virus infection, hemophagocytic lymphohistiocytosis, nephrotic syndrome

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is disease of inappropriate macrophage activation. It is characterized by the presence of hemophagocytosis. This is an engulfment of blood cells by the patient's self-macrophages in the bone marrow, followed by excessive production of inflammatory cytokines, leading to a sepsis-like syndrome and multi-organ failure (Henter *et al*, 2007). Dengue virus infection (DVI) is recognized as one of the causes of secondary HLH, although seemingly rare (Rueda *et al*, 2002; De Koninck *et al*, 2014; Phuakpet *et al*, 2015). Renal involvement has been reported in both conditions but in separate case reports.

CASE REPORT

A previously healthy 23-year-old Thai man was referred to King Chulalongkorn Memorial Hospital (KCMH) due to high-grade fever and oliguria for 4 days. Two weeks earlier, he had had all-day high-grade fever without other specific symptoms

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when he first went to a referring public hospital. The physical examination at KCMH revealed high-grade fever (BT 39.0 °C) without other remarkable abnormalities. His complete blood count (CBC) showed mild hemoconcentration and thrombocytopenia (Hb 15.7 g/dl, Hct 48%, WBC 5,800/mm³, and platelet 40,000/mm³). He was suspected to have DVI. Thus, dengue NS1 antigen was requested which later returned positive on the same day. After 2 days of admission, his fever had partially resolved, and he was discharged.

He came back to KCMH again with recurrent high-grade fever, malaise, and oliguria for 4 days. On admission, he was drowsy and mildly confused. His vital signs showed fever, tachycardia, and hypertension (BT 38.5 °C, PR 110/min, RR 18/min, and BP 150/90 mmHg). He also had pale conjunctivae and a palpable spleen. At this time, he had anemia and thrombocytopenia (Hb 10.2 g/dL, Hct 31%, WBC 10,200/mm³, neutrophil 84%, lymphocyte 8.6%, and platelet 84,000/mm³). He also developed azotemia, metabolic acidosis, elevated liver enzyme and hyperCKemia (BUN 72 mg/dl, Cr 9.4 mg/dl, Na 136 mmol/l, K 3.8 mmol/l, Cl 100 mmol/l, HCO₃ 15 mmol/l, total bilirubin 1.74 mg/dl, direct bilirubin 1.36 mg/dl, AST 2913 IU/l, ALT 558 IU/l, ALP 124 IU/l, and CPK 21,779 IU/l).

The urinalysis revealed dark-colored urine with proteinuria and microscopic hematuria without

identifiable dysmorphic RBC under phase-contrast microscopy (SpGr 1.010, pH 6.5, protein 1+, glucose negative, blood 3+, RBC 10-20/hpf, and WBC 2-3/hpf). Serology studies for DVI of dengue IgG and IgM were done, both of which showed positive results.

He was diagnosed as DVI with rhabdomyolysis, hepatitis, and severe acute kidney injury (AKI). Because the patient had oliguria with uremic encephalopathy, metabolic acidosis, and refractory volume overload, sustained low efficiency dialysis was initiated at intensive care unit (ICU).

After 2 days of hospital admission, he had persistent high-grade fever with splenomegaly, cytopenia (anemia and thrombocytopenia), and liver injury following acute viral infection, which led to the suspicion of hemophagocytic syndrome. All further blood tests showing LDH 7,280 IU/l, serum ferritin 33,437 ng/ml, and fasting triglyceride 392 mg/dl supported our suspicion. We then performed bone marrow aspiration that revealed mildly increased cellularity with moderately increased hemophagocytic activities. These findings determined the diagnosis of HLH associated with the antecedent DVI.

There was no significant hemodynamic collapse that would cause either pre-renal AKI

or ischemic acute tubular necrosis (ATN) in this patient, and the urine examination also showed urine sediments together with proteinuria. His urine-protein-creatinine-ratio and urine-albumin-creatinine-ratio were 7.16 g/g creatinine and 3.42 g/g creatinine, respectively. These ratios together with hypoalbuminemia (serum albumin 2.5 g/dl) and generalized edema suggested the presence of glomerular involvement.

After correction of thrombocytopenia by platelet transfusion to raise platelet count higher than 200,000/mm³, we performed percutaneous renal biopsy under real time ultrasound guidance in ICU for the indications of delayed renal recovery and dialysis dependence. The procedure went well without any complication related to procedure. We obtained two cores of renal tissue. Light microscopic findings revealed 10 glomeruli with diffuse mild mesangial expansion, but the findings were otherwise unremarkable. The renal cortical tubules showed epithelial cell degeneration and necrosis (Fig 1).

The findings were consistent with acute tubular necrosis, which could explain the azotemia. However, these findings were not explanatory of the concomitant nephrotic proteinuria, which was proven to be mainly albuminuria. The most likely

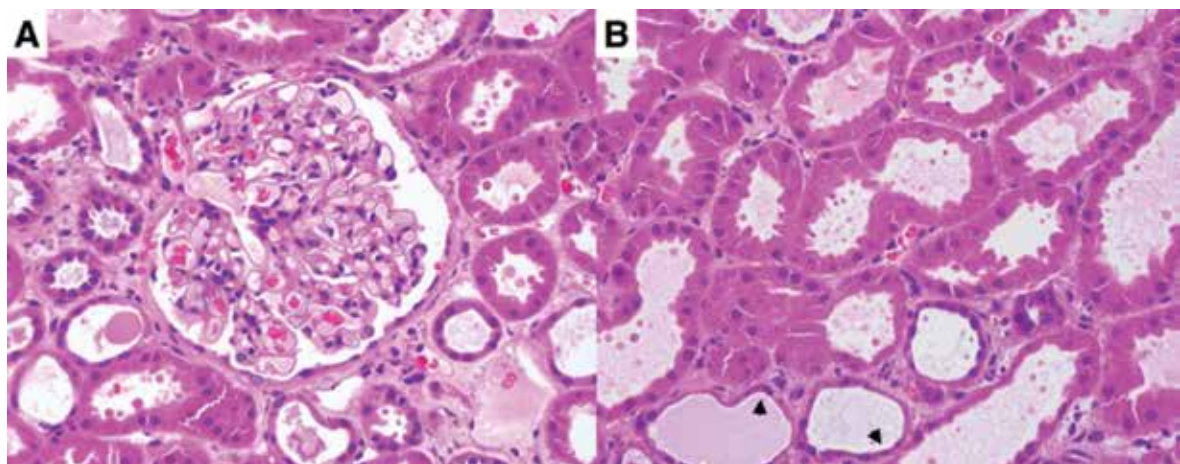


Fig 1—A: Renal specimens of the patient showing normal-looking glomerulus under light microscope (H&E staining, x 40). B: Proximal tubules were dilated and lined by flattened tubular epithelial cells (arrow heads), consistent with ATN (H&E staining, x 40).

possibility was acute podocytopathy in minimal change disease. Unfortunately, the expected diffuse foot process effacement could not be demonstrated owing to the lack of glomeruli in renal specimens submitted for transmission electron microscopy.

The patient was treated by a course of dexamethasone for HLH and best supportive care, including renal replacement therapy. He remained dialysis-dependent for 2 weeks before the recovery of renal function, anemia, thrombocytopenia, hepatitis, and rhabdomyolysis. After 4 weeks of admission, he was discharged safely with a serum creatinine of 3 mg/dl. At a follow-up visit 1 month later, his serum creatinine had returned to a normal level (0.9 mg/dl).

DISCUSSION

Our patient presented with classic clinical manifestation of DVI and HLH. Firstly, he presented with acute high-grade fever without any specific findings except for thrombocytopenia and positive dengue NS-1 antigen, both of which together made the diagnosis of DVI very likely. However, his fever persisted for more than 1 week, which was unusual for DVI. At this time, he also developed splenomegaly together with hypertriglyceridemia, elevated serum ferritin, and evidence of hemophagocytosis in bone marrow consistent with HLH by the HLH-2004 criteria (Henter *et al*, 2007).

HLH is an uncommon condition and comprises two major entities: a primary or familial HLH (FHL) mainly affecting children and secondary HLH that associated with wide arrays of diseases including infection, autoimmune disease, and malignancy (Henter *et al*, 2007). Apart from age at the onset of disease, other clinical features of both forms of HLH are similar and difficult to distinguish.

Clinical manifestations typically are fever, hepatosplenomegaly, generalized lymphadenopathy, and cytopenia while elevated liver enzyme, coagulopathy, hypertriglyceridemia, hyperferritinemia, and hypofibrinogenemia are also commonly found. According to HLH-2004 guidelines (Henter

et al, 2007), the diagnosis of HLH is made if 5 of the following 8 features are evident including 1) fever, 2) splenomegaly, 3) cytopenia affecting 2 of 3 lineages, 4) hypertriglyceridemia and/or hypofibrinogenemia, 5) hemophagocytosis in the bone marrow, spleen, or lymph nodes, 6) low or absent NK-cell activity, 7) ferritin ≥ 500 $\mu\text{g/l}$, and 8) soluble CD25 $\geq 2,400$ U/ml.

We reviewed renal involvement in DVI separately. AKI was found in up to 33.3% of severe DVI cases [dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)] (Mendez and Gonzalez, 2003; Wiwanitkit, 2005; Khan *et al*, 2008). Hemodynamic collapse seems to be responsible in most cases of DVI because AKI is typically present in association with hypotension, and a multivariate analysis from a retrospective series found that DSS was an independent risk factor for AKI in DHF patients (Lee *et al*, 2009). Other causes of AKI include rhabdomyolysis and hemolysis, leading to acute tubular injury (Lima and Nogueira, 2008).

Proteinuria is also prevalent in DHF, and it has been documented in 30% to 74% of patients, of which 1.1% even had nephrotic-range proteinuria (Garcia *et al*, 1995; Horvath *et al*, 1999). Unfortunately, renal pathology was not available in most of these cases. However, DVI can cause glomerulonephritis. In an animal model, dengue-infected mice developed mesangial and endocapillary hypercellularity with IgM deposition 2 days after the infection (Barreto *et al*, 2004). Renal biopsies from human patients with DVI with microscopic hematuria also yielded similar findings of mesangial cell hypertrophy together with glomerular deposition of immune complexes (Boonpucknavig *et al*, 1976).

Dengue antigen has also been detected in renal tissues obtained from DHF patients, but only in renal tubular epithelial cells (Jessie *et al*, 2004). This has led to the hypothesis of immune-mediated injury rather than direct viral cytopathic effects as the cause of glomerulopathy.

Our patient had generalized edema at second presentation together with nephrotic-range proteinuria and hypoalbuminemia, both of which

are compatible with nephrotic syndrome. He also had microscopic hematuria although this was not demonstrated to comprise of dysmorphic RBC, suggesting the possibility of proliferative glomerulonephritis. However, the renal pathology revealed 10 normal glomeruli with only minimal mesangial matrix expansion, which implied the presence of minimal change disease (MCD) or unsampled focal segmental glomerulosclerosis (FSGS), neither of which would not explain the presence of urinary sediments and AKI.

Thus, the concomitant findings of tubular epithelial necrosis consistent with ATN was likely responsible for the severe AKI in this patient. In our case, the pathophysiology of ATN may have been caused by tubular toxicity due to rhabdomyolysis with myoglobinuria and tubulointerstitial inflammation, resulting from the storm of cytokines in HLH because there was no clinically-evident hemodynamic collapse in this patient.

Conversely, HLH itself has been found to be associated with AKI and nephrotic syndrome. Recently, Aulagnon and colleagues (2015) reported the largest series of 95 HLH patients to date. Of 95 patients, 59 (62%) had AKI according to the KDIGO criteria (Khwaja, 2012). The major causes of AKI were ATN either by renal ischemia following hypoperfusion or nephrotoxic ATN (effect of cytokines). Glomerulonephritis as a cause of AKI was reported in 10 patients (17%), 9 of which had nephrotic-range proteinuria. Regrettably, all 10 cases were diagnosed solely on a clinical basis without renal biopsy.

There was a series of 11 patients by Thauun and colleagues (2006), which first described the glomerular complications of HLH. Renal pathology was available in all cases yielding collapsing glomerulopathy in 5 patients, minimal change disease in 4 patients, and thrombotic microangiopathy (TMA) with podocytosis in 2 patients. In this report, malignant non-Hodgkin lymphoma was the major etiology of secondary HLH.

There were 2 cases of infection-associated HLH. The first patient was a 63-year-old Caucasian female whose HLH was secondary to cytomegalovirus

infection. She experienced nephrotic syndrome with severe renal failure. The renal biopsy revealed glomerular thrombotic microangiopathy together with swollen and vacuolated podocytes, both of which were characteristic of damage to podocytes.

Another patient, a 16-year-old African female, suffered from leishmaniasis with secondary HLH and nephrotic syndrome associated with dialysis-dependent renal failure. The hyperplasia of podocytes compressing glomerular tufts and dilated tubules were noted, consistent with collapsing glomerulopathy and acute tubular necrosis, respectively.

To the best of our knowledge, there has never been a report of biopsy-proven MCD in DVI. However, this condition has been well recognized in HLH of various underlying etiologies including infection. We believe that HLH plays a major role in the development of MCD. MCD and FSGS including the collapsing variant are known to result from podocyte injury by unknown circulating permeability factors (Cho *et al*, 2007). In the setting of HLH, the hallmark is excessive release of cytokines by inflammatory cells including interleukin-6, tumor necrosis factor-alpha, and other inflammatory cytokines, one or more of which might be responsible for the injury of podocytes and contribute to the development of glomerulopathy.

In conclusion, we have presented a case of DVI whose clinical manifestation was atypical and interesting. If the patients with DVI have persistent fever of more than a week with multi-organ dysfunction not caused by leakage syndrome of typical DHF/DSS, the attending physician should raise the possibility of complication including DVI-associated HLH. This rare complication of DVI results in the storm of cytokines and can eventually contribute to AKI and nephrotic syndrome.

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