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Case Report

Hemoglobin cast nephropathy: a rare but serious complication of hemolysis in a pediatric patient

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ABSTRACT

Background. Intravascular hemolysis is a serious and rare condition in children and causes the release of hemoglobin and heme into circulation, which have proinflammatory properties. These substances lead to inflammation, oxidative stress, apoptosis, and organelle dysfunction that lead to acute kidney injury (AKI). We report a pediatric case diagnosed with hemolysis-associated hemoglobin cast nephropathy due to autoimmune hemolytic anemia.

Case. A 4-year-old boy, who was admitted to another hospital with complaints of fever and dark urine for one day, developed anemia and kidney failure in the follow-up, was referred to our hospital. In physical examination, pallor and icterus on the sclera were noted. The patient had low hemoglobin and haptoglobin levels concomitant with high levels of serum lactate dehydrogenase, urea and creatinine. A peripheral blood smear showed marked spherocytes without schistocytes. A kidney biopsy was performed due to ongoing overt hemolysis and dialysis requirement, which showed findings consistent with hemoglobin cast nephropathy. Although the initial polyspecific direct antiglobulin test (DAT) was negative, due to persistent intravascular hemolysis DAT was studied monospecifically and showed IgM antibody positivity. Therefore, a diagnosis of autoimmune hemolytic anemia was made, and corticosteroid treatment was started. Hemolysis immediately ceased and the need for erythrocyte transfusion and dialysis disappeared.

Conclusions. Acute kidney injury associated with hemoglobin cast nephropathy is an extremely rare condition in childhood. Although the initial course is severe and potentially life-threatening, the prognosis is favorable with the treatment of the underlying cause and management of AKI. Therefore, pediatricians should be aware of this rare clinical entity during clinical practice.

Key words: acute kidney injury, autoimmune hemolytic anemia, intravascular hemolysis, hemoglobin cast nephropathy.

Intravascular hemolysis, which is characterized by red blood cell (RBC) lysis in circulation is an unusual but life-threatening condition and causes the release of proinflammatory hemoglobin and heme into circulation.[1] The underlying etiology is diverse, including autoimmune hemolytic anemia, infections, medications, toxins, transfusion of incompatible blood groups, paroxysmal nocturnal hemoglobinuria (PNH), mechanical, disseminated intravascular coagulation, thrombotic microangiopathy (TMA), and hemoglobinopathies.[2]

Hemolysis-associated acute kidney injury (AKI) occurs due to acute tubular injury as a result of inflammation, oxidative stress, apoptosis, and organelle dysfunction caused by molecules

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such as hemoglobin, heme, and iron released from lysed RBCs.[3] Here, we report an extremely rare condition hemoglobin cast nephropathy, in a pediatric patient due to massive intravascular hemolysis that is associated with autoimmune hemolysis.

Case Report

A previously healthy 4-year-old boy was admitted to another hospital with complaints of fever and dark urine for one day. Two weeks before the presentation, he had a fever, cough, runny nose, and watery diarrhea, and all these symptoms resolved within 3-4 days. No medication was taken. Within 24-hour of admission, he had become pale and anuric, and a rapid decrease in the hemoglobin level and acute increase in serum urea and creatinine levels (from 0.72 mg/dl to 1.29 mg/dl within 9 hours) were observed. The patient was referred to our hospital for further investigation and management with a preliminary diagnosis of hemolytic uremic syndrome (HUS).

At admission, the patient was afebrile, his blood pressure was 120/60 mmHg (95th percentile for age, gender and height 113/70 mmHg), heart rate 107/min, respiratory rate 24/min, and oxygen saturation was 95%. The physical examination was unremarkable except for pallor and icterus that was visible on the sclera. Laboratory tests were as follows: hemoglobin 7.6 g/dl, mean corpuscular volume (MCV) 78.9 fl, reticulocyte count 3.04%, white blood cell count 16,500/mm^3, platelet count 359,000/mm^3, total bilirubin 1.9 mg/dl (normal: 0.3-1.2), direct bilirubin 0.3 mg/dl (normal: 0-0.2), lactate dehydrogenase (LDH) 2,843 U/L (normal: 110-295) and creatinine kinase 355 U/L (normal: <171). Acute phase reactants were high namely C-reactive protein 14.2 mg/dl (normal: 0-0.8) and procalcitonin 161 ng/ml (normal: 0-0.1). AKI was diagnosed with high blood urea nitrogen (49.6 mg/dl, normal: 5-18) and serum creatine levels (1.9 mg/dl, normal: 0.26-0.50). Estimated glomerular filtration rate was calculated as 24.3 ml/min/1.73m^2 by the modified Schwartz formula.[4] Microscopic examination of the peripheral blood smear revealed marked spherocytes without schistocytes. Serum haptoglobin level was <5.83 mg/dl (normal: 36-196), and direct antiglobulin test (DAT) and indirect antiglobulin tests studied via the polyspecific antiglobulin test were negative. Urine analysis demonstrated 1+ protein and trace blood with a specific gravity of 1013. In the microscopic evaluation, five red blood cells per high power field were seen. No pathological findings were found in the kidney ultrasound. On the day of admission, hemodialysis was performed due to anuria and progressive deterioration of kidney functions. The patient needed intermittent hemodialysis for two weeks and received seven RBC transfusions due to ongoing hemolysis. A comprehensive study was performed to detect the etiology of hemolysis including ADAMTS13 activity, coagulation studies, direct and indirect agglutination, hemoglobin electrophoresis, and enzyme assays for pyruvate kinase deficiency and glucose-6-phosphate dehydrogenase deficiency, osmotic fragility testing, and flow cytometry for PNH. All of these tests were found to be within normal limits.

Respiratory pathogens panel for bacteria and viruses as well as SARS-CoV-2 PCR were negative. Serologic tests for cytomegalovirus, Epstein-Barr virus, hepatitis B and C viruses, human immunodeficiency virus (HIV), anti-nuclear antibody, and anti-double-stranded DNA were negative. However decreased levels of complement 3 (59.2 mg/dl, normal: 79-152) and complement 4 (8.50 mg/dl, normal: 16-38) were detected. Due to concern about a possible malignancy, a bone marrow aspiration was performed, and any hematological malignancy was excluded.

HUS was considered in the differential diagnosis because of the presence of anemia, elevation of LDH, and AKI in the initial evaluation of the patient, however absence of both thrombocytopenia and peripheral smear findings of TMA (i.e. schistocytes, fragmented erythrocytes) ruled out HUS. A kidney biopsy

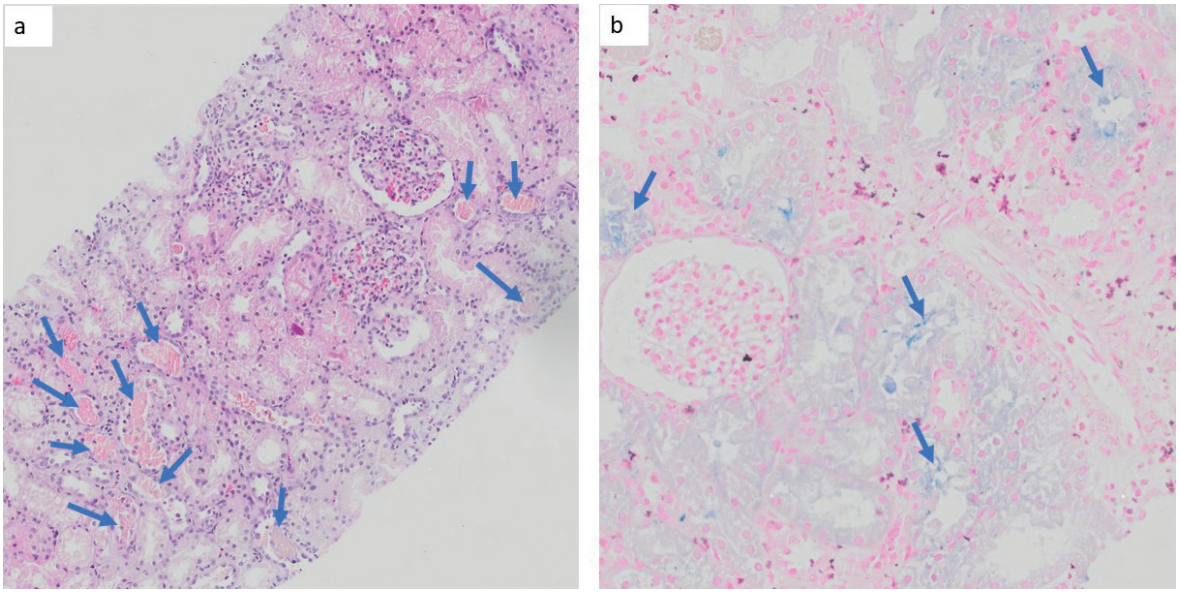

Fig. 1. Pathological findings from kidney biopsy. (a) Histopathologic examination of the renal biopsy revealed abnormal casts (arrows) in the tubules of the kidney. The casts appeared granular, eosinophilic with hematoxylin and eosin (H&E) stain, pale on periodic acid-Schiff (PAS) stain, there were some fragmented cell nuclei in the casts. There was no giant cells or inflammatory cells (H&E stain). (b) Presence of iron in the casts (arrows), consistent with hemolysis in the etiology (Prussian blue stain).

was performed on the 7th day of admission due to lack of improvement in kidney functions and ongoing dialysis requirements. Forty glomeruli were sampled. Glomeruli were otherwise normal except for segmental mesangial cellular proliferation alone. Despite extensive clinical overlap with HUS, there was no histological evidence of acute vascular endothelial damage or TMA. However, tubular findings were most striking including vacuolar degeneration, loss of brush borders, and granular, sometimes globoid-shaped pigmented casts that were predominantly observed in the proximal tubules (Fig. 1a). Interstitial fibrosis and tubular atrophy were absent. Prussian blue staining clearly demonstrated hemosiderin accumulation in the casts (Fig. 1b). Immunohistochemical staining for hemoglobin could not be performed due to unavailability of the dye in Türkiye. Immunofluorescence examination, using IgA, IgG, IgM, C1q, C3, C4, kappa, albumin, and fibrinogen was negative. Taken together, the biopsy was reported as a hemoglobin cast nephropathy with presence of intratubular hemoglobin casts that led to acute tubular necrosis.

Because of rapid declining of hemoglobin levels and of laboratory findings suggesting intravascular hemolysis, DAT was performed manually and mono specifically. Although repeated polyspecific antiglobulin tests were negative, the monospecific antiglobulin test showed IgM antibody positivity. Therefore, autoimmune hemolytic anemia was considered, and corticosteroid (prednisolone 2 mg/kg/day) treatment was started on the 15th day of admission. Hemoglobin level immediately stabilized within 1 day after initiation of corticosteroid concomitant with a rapid decline in blood LDH levels and rapid improvement in kidney function. As such, requirement of both erythrocyte transfusion and dialysis ended. Serum creatinine level decreased to 0.4 mg/dl within 2 weeks (Fig. 2).

Written informed consent was received from the parents of the patient for publication.

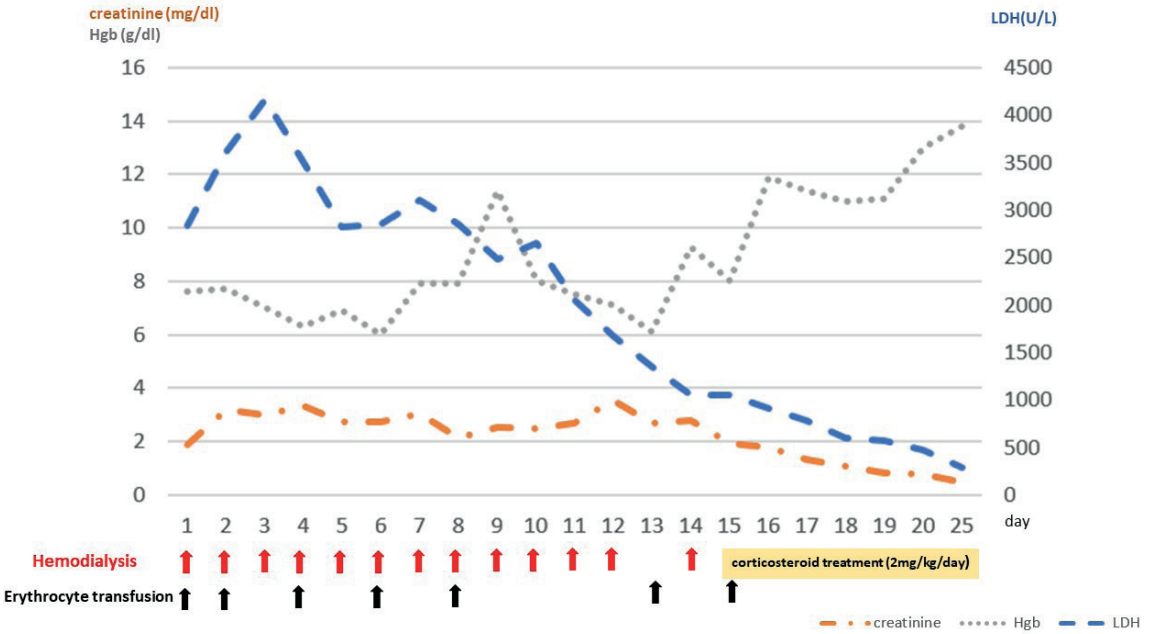

Fig. 2. Levels of hemoglobin (Hgb), lactate dehydrogenase (LDH), and serum creatinine from admission to diagnosis and treatment. Red arrows show hemodialysis sessions and black arrows show erythrocyte transfusions.

Discussion

Here we present a pediatric patient with a hemoglobin cast nephropathy, which is an extremely rare condition that requires special attention in pediatrics practice.

Acute hemolytic anemia is usually a rapidly progressing, severe, and life-threatening condition. The first step is to determine whether the disease is immune-mediated or not. Autoimmune hemolytic anemia is an acquired form of hemolytic anemia, which is caused by the host's immune system acting against its RBC antigens and is mostly defined as hemolytic anemia with a positive DAT. Most commercial DATs are routinely performed by a polyspecific method, which is able to detect IgG and complement (C3d), but not IgA or IgM antibodies. Therefore, it may cause false negative results as was the case in our patient. Despite the obvious clinical and laboratory findings of severe intravascular hemolysis, DAT was persistently negative in our patient. Therefore, monospecific DAT was studied manually and was found to be positive, which was the rationale for starting corticosteroid therapy that resulted in rapid improvement. Our observation emphasizes the importance of monospecific DAT in the presence of unexplained intravascular hemolysis.

Although acute and chronic nephrotoxicity of hemoglobin and heme have been defined, the exact mechanism of nephrotoxicity has not been fully elucidated, but it is most likely to be multifactorial.[3] Cell-free hemoglobin, heme, and iron, which are released from lysed RBCs have pro-inflammatory properties. Normally hemoglobin binds to haptoglobin in circulation. In overt hemolysis, large amounts of hemoproteins exceed the capacity of the endogenous scavengers; these free hemoproteins in plasma are filtrated by the kidney. Free hemoglobin, heme, and iron cause oxidative stress, nitric oxide (NO) depletion, inflammation, and eventually cell death in kidney tissue. Experimental studies have shown that mitochondria are particularly vulnerable to heme-mediated damage and

heme accumulation within mitochondria results in organelle dysfunction and concomitant kidney injury. In addition, hemoproteins cause endothelial activation and vascular injury. Ultimately, tubular necrosis, the formation of intratubular casts due to the interaction of hemoglobin with Tamm-Horsfall protein, and, decreased kidney perfusion due to lack of depletion have been suggested to be responsible for hemoglobin cast nephropathy.[5-7]

Clinically hemolysis-associated nephropathy can resemble other etiologies of AKI, such as acute tubular necrosis, acute interstitial nephritis, and TMA. The main cause of TMA in children is HUS. HUS is the most important differential diagnosis due to its similar clinical presentation and laboratory findings such as anemia, elevation of LDH, and a decrease in haptoglobin level. While the presence of schistocytes suggests the diagnosis of HUS, spherocytosis is present in autoimmune hemolytic anemia in the peripheral smear. The antiglobulin test is therefore critical in determining whether hemolytic anemia is immune-mediated or not. Direct antiglobulin test is negative in HUS except for *Streptococcus pneumoniae*-associated HUS. Kidney biopsy is the gold standard for diagnosis and is necessary to rule out TMA, as there are significant differences in the management of these two distinct entities. Histopathologically, TMA is characterized by microvascular damage in the form of endothelial swelling, fibrinoid necrosis and/or fibrin thrombus in capillaries and other small-sized blood vessels, whereas hemoglobin cast nephropathy is characterized by the presence of prominent intratubular casts, especially in the proximal tubules, and signs of acute tubular injury as in our case.

Several histopathologic entities can mimic hemoglobin cast nephropathy. Histological differential diagnosis includes myoglobin casts, degenerating RBC casts, bile casts, light chain type casts, and acute tubular necrosis.[8] Myoglobin and hemoglobin are structurally similar molecules, therefore cast formation of these molecules is indistinguishable by light microscopy; specific staining with immunohistochemistry (IHC) is the definite way to differentiate them. Clinical data can be a guide if hemoglobin IHC stain is unavailable, as was the case in our patient. We thought that the casts observed in our patient were most likely hemoglobin casts as there was strong evidence of intravascular hemolysis. In contrast, myoglobin casts are typically seen in the setting of rhabdomyolysis and those patients frequently have elevated creatinine kinase levels. RBC casts can also be seen in the presence of glomerular hematuria and are often accompanied by glomerulonephritis. Both RBC casts and hemoglobin casts stain positive for hemoglobin by IHC. The most important feature that distinguishes RBC casts from hemoglobin casts is the presence of residual fragmented RBCs, so-called RBC 'ghosts'. We excluded this possibility with kidney biopsy findings. Other reasons of cast nephropathy are intratubular bile casts that are also associated with liver failure and in this case, total bilirubin is often >20 mg/dl. Light chain cast nephropathy accompanies plasma cell dyscrasia, therefore, is often observed in adulthood.[9]

Hemolysis-associated hemoglobin cast nephropathy is a rare condition and has been reported mostly in adults. Dvanajscak et al.[10] reported the largest case series including 27 adult patients. The remaining data are in the form of case reports in the literature. To the best of our knowledge, among these reported cases in the literature, only three biopsy-proven pediatric patients are present. The First patient was a 2-year-old girl with PNH and AKI who required hemodialysis[11], the other was a 17-year-old boy who presented with AKI and acute hemolysis after exercise[12], and the last patient was a 7-year-old girl with Evans syndrome who developed AKI in the setting of intravascular hemolysis.[13] In the first and second patients, renal functions returned to normal within 2 weeks and 1 month, respectively after controlling of hemolysis. Unfortunately, the last patient died in the acute disease period.

The primary treatment of hemoglobin cast nephropathy is to eliminate the triggering factor of intravascular hemolysis and to prevent ongoing hemolysis. Conditions such as autoimmune hemolytic anemia, drugs, toxins, PNH, and disseminated intravascular coagulation (DIC) that may cause intravascular hemolysis should be quickly reviewed and the underlying cause should be treated. This is critical for the recovery of kidney functions. In addition, supportive treatments such as blood transfusion and management of AKI are essential steps in the treatment. Although most patients need kidney replacement therapy, the prognosis is often favorable and complete resolution is usually expected. All 27 patients in the report of Dvanajscak et al.[10] presented with AKI and 57% of them required temporary hemodialysis. Kidney functions returned to normal at 78% during a mean follow-up period of 9 months. Likewise, although our patient initially presented with severe AKI requiring hemodialysis, kidney functions returned to normal within two weeks following cessation of hemolysis after starting specific treatment for autoimmune hemolytic anemia.

In conclusion, acute intravascular hemolysis is a severe and life-threatening condition. Concurrent acute kidney injury is also an extreme condition in the pediatric age. Despite severe initial clinical course and mostly requirement of dialysis, the prognosis is favorable with the treatment of the underlying condition causing hemolytic episodes and with appropriate AKI management. Therefore, in the case of AKI accompanied by severe hemolysis, hemoglobin cast nephropathy should be considered in the differential diagnosis of HUS if there are no specific TMA findings such as schistocyte, fragmented erythrocyte in the peripheral smear and thrombocytopenia is not accompanied. In this case, the absence of acute vascular endothelial damage or TMA findings in the kidney biopsy and the presence of acute tubular damage findings accompanied by intratubular casts stained with hemoglobin immunohistochemically were diagnostic. Although the inability to perform hemoglobin staining is a limitation, our case is a good example of this extremely rare condition with its clinical, laboratory, and histopathological findings. Moreover, this case report also highlights that monospecific antiglobulin tests should also be run when antiglobulin tests give negative results in cases whereby there is presence of clinical and laboratory evidence of persistent hemolysis.

Ethical approval

Informed consent was obtained from the family for the publication of the case report.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: DB, FÖ; data collection: DB, NAO, SK, RT, OİÖ, TA, DO, FÖ; analysis and interpretation of results: DB, FÖ; draft manuscript preparation: DB, FÖ. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Rother RP, Bell L, Hillmen P, Gladwin MT. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. JAMA 2005; 293: 1653-1662. https://doi.org/10.1001/jama.293.13.1653
2. Van Avondt K, Nur E, Zeerleder S. Mechanisms of haemolysis-induced kidney injury. Nat Rev Nephrol 2019; 15: 671-692. https://doi.org/10.1038/s41581-019-0181-0

3. Tracz MJ, Alam J, Nath KA. Physiology and pathophysiology of heme: implications for kidney disease. J Am Soc Nephrol 2007; 18: 414-420. https://doi.org/10.1681/ASN.2006080894

4. Schwartz GJ, Muñoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol 2009; 20: 629-637. https://doi.org/10.1681/ASN.2008030287

5. Nath KA, Grande JP, Croatt AJ, Likely S, Hebbel RP, Enright H. Intracellular targets in heme protein-induced renal injury. Kidney Int 1998; 53: 100-111. https://doi.org/10.1046/j.1523-1755.1998.00731.x

6. Gonzalez-Michaca L, Farrugia G, Croatt AJ, Alam J, Nath KA. Heme: a determinant of life and death in renal tubular epithelial cells. Am J Physiol Renal Physiol 2004; 286: F370-F377. https://doi.org/10.1152/ajprenal.00300.2003

7. Sponsel HT, Alfrey AC, Hammond WS, Durr JA, Ray C, Anderson RJ. Effect of iron on renal tubular epithelial cells. Kidney Int 1996; 50: 436-444. https://doi.org/10.1038/ki.1996.334

8. Sakthirajan R, Dhanapriya J, Varghese A, et al. Clinical profile and outcome of pigment-induced nephropathy. Clin Kidney J 2018; 11: 348-352. https://doi.org/10.1093/ckj/sfx121

9. Dvanajscak Z, Cossey LN, Larsen CP. A practical approach to the pathology of renal intratubular casts. Semin Diagn Pathol 2020; 37: 127-134. https://doi.org/10.1053/j.semdp.2020.02.001

10. Dvanajscak Z, Walker PD, Cossey LN, et al. Hemolysis-associated hemoglobin cast nephropathy results from a range of clinicopathologic disorders. Kidney Int 2019; 96: 1400-1407. https://doi.org/10.1016/j.kint.2019.08.026

11. Hothi DK, Bass P, Morgan M, Acharya J, Humphries SE, Gilbert RD. Acute renal failure in a patient with paroxysmal cold hemoglobinuria. Pediatr Nephrol 2007; 22: 593-596. https://doi.org/10.1007/s00467-006-0352-7

12. Khalighi MA, Henriksen KJ, Chang A, Meehan SM. March hemoglobinuria-associated acute tubular injury. Clin Kidney J 2014; 7: 488-489. https://doi.org/10.1093/ckj/sfu078

13. González I, Rais R, Gaut JP, Dehner LP. Evans Syndrome Complicated by Intratubular Hemoglobin Cast Nephropathy. Case Rep Pediatr 2017; 2017: 5184587. https://doi.org/10.1155/2017/5184587