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Case Report

Diacylglycerol kinase Epsilon (DGKe) Nephropathy: Rare Cause of Thrombotic Microangiopathy

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Abstract

Thrombotic microangiopathy (TMA) is defined by the triad: microangiopathic hemolytic anemia, low platelet count and organ damage in the absence of clotting disorders. The most frequent cause of TMA in children is Shigatoxin Hemolytic Uremic Syndrome (STEC-HUS). Other causes include atypical HUS (dysregulation of the alternative complement pathway), pneumococcal HUS and, rarely, mutations in diacylglycerol epsilon kinase (DG KE). We present a 12-month-old young boy with TMA, hypertension and nephrotic range proteinuria. Genetic evaluation revealed a pathogenic DGKe homozygous (c.1068_1071del) variant in the patient confirming the diagnosis of DGKe nephropathy. Further genetic analyses revealed a heterozygous DGKe variant in both parents. The renal function improved with conservative management. The report illustrates that a high degree of suspicion and genetic evaluation is required to confirm the diagnosis of DGKe nephropathy occurring in the first years of life.

Keywords: Hemolytic uremic syndrome; DGKe; Thrombotic microangiopathy; Proteinuria; complement; Consanguinity

Introduction

Thrombotic microangiopathy (TMA) is a syndrome characterized by non-immune microangiopathic hemolytic anemia, thrombocytopenia or platelet consumption, and tissue damage due to thrombosis in the microvessels in the absence of clotting disorders [1]. The causes can be hereditary (genetic) or acquired (secondary) and can occur both in children [2] and adults [3].

Histological analysis of TMA reveals endothelial cell swelling, mucoid material in the thickened intima, hypertrophy of the media, and thrombi in the vacular lumen. These changes result in partial to complete occlusion of the vascular lumen. The occlusion of the vascular lumen then results in mechanical destruction of erythrocytes via shear stress that is revealed by detection of schistocytes on the peripheral blood smear. In addition, organs undergo ischemic injury, and the severity of clinical manifestations depends on the degree of tissue ischemia, that often involves the kidney, brain, gastrointestinal tract, and heart. The most frequent cause of TMA in children is Shigatoxin

Hemolytic Uremic Syndrome (STEC-HUS)[4], followed by atypical HUS (dysregulation of the alternative complement pathway) [5], Thrombotic Thrombocytopenic Purpura (ADAMTS13 deficiency - Von Willebrand Factor-cleaving enzyme) [6], pneumococcal HUS [7] and, more rarely, vitamin B12 metabolism defect [8] and diacylglycerol kinase epsilon (DGKe) nephropathy [9]. DGKe nephropathy is characterized by TMA, hypertension, and proteinuria that is often in the nephrotic range. TMA can also result from secondary causes such as infections, autoimmune diseases, drugs and transplantation [10] (Figure 1). Since morbidity and mortality is high in TMA, early recognition and determination of the underlying etiology is of paramount importance to correctly treat the TMA and prevent irreversible organ damage (Table 1).

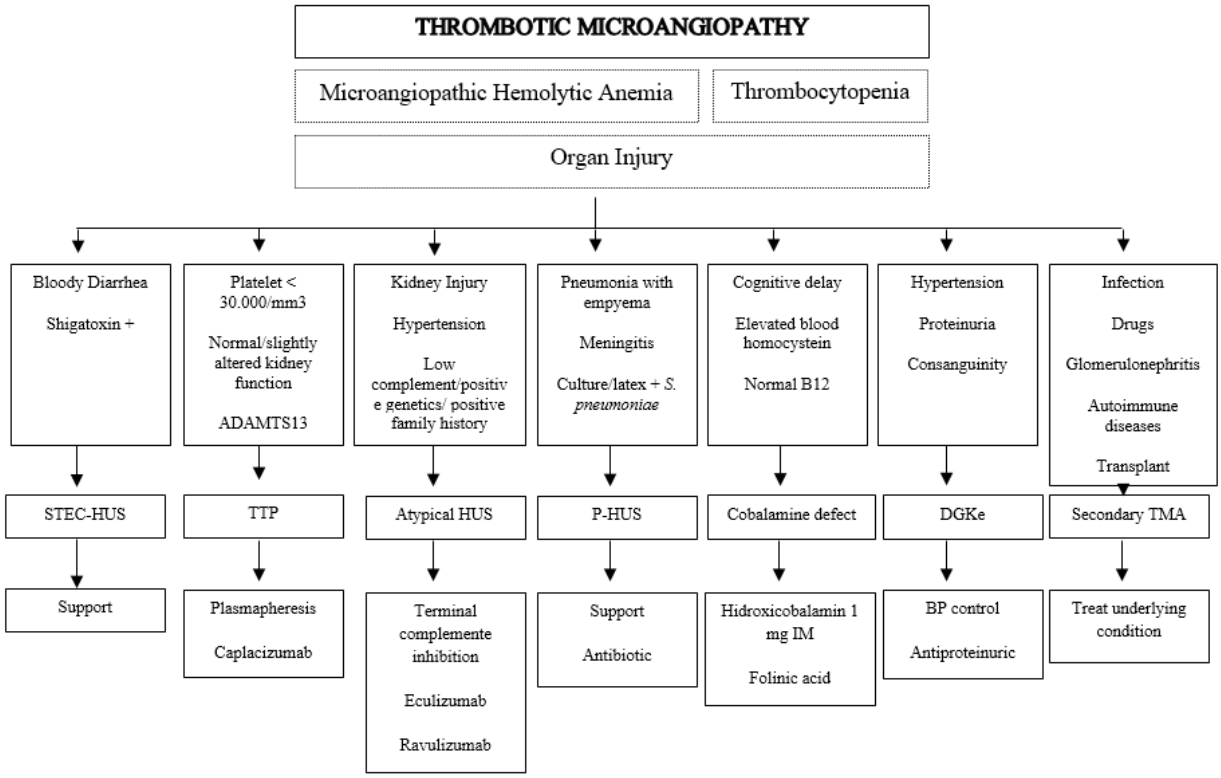

Figure 1: Definition of Thrombotic Microangiopathy and main causes in the pediatric population (clinical presentation, diagnosis and treatment).

○ Complete blood count ○ Peripheral smear (schistocytes) ○ Reticulocyte count ○ Bilirrubin ○ Lactic Dehydrogenase ○ Haptoglobin ○ Coagulation tests ○ Direct antiglobulin test (Coombs test)
○ Anamnesis and family history, consaguinity ○ Complete physical examination with Blood Pressure and Neurological Assessment ○ Kidney function ○ Urinalysis ○ Proteinúria (24 h and/or spot urine protein/creatinine ratio) ○ Hepatic enzymes ○ Pancreatic enzymes ○ Blood gases ○ Troponin, EKG, echocardiography ○ Ophtalmologic evaluation, fundoscopy
○ Stool culture and shigatoxin test ○ Activity and inhibitor to ADAMTS13 ○ Complement, blood levels ○ Cultures: blood, urine, cephalorachidic liquid, abscesses ○ Latex for *Streptococcus pneumoniae* ○ Tests for H1N1, COVID19, viral panels ○ According to epidemiology: dengue, leptospirosis, brucelosis ○ Homocystein and vitamin B12 blood levels ○ Aminoacid chromatography (blood, urine) ○ Genetic Tests: Hemolytic Uremic Syndrome panel or Whole Exome Sequencing ○ Anti-Factor H antibody ○ Kidney biospy in selected cases

Table 1: Checklist of exams and procedures to detect Thrombotic Microangiopathy, evaluate organ damage and define underlying etiology; Legend: EKG: electrocardiogram; H1N1: influenza vírus; COVID19: coronavírus 19.

We present a case of TMA occurring in a 14-month-old boy. A high degree of suspicion followed by genetic evaluation was key in making the correct diagnosis, which resulted in proper management in this rare cause of TMA. We also review the literature of this rare cause of TMA in children.

The thrombotic microangiopathy syndrome

A 12-month-old boy presented with recent history of watery diarrhea, vomiting and fever, evolving quickly with decreased urinary output and edema. In the primary care facility, exams showed anemia, elevated serum urea and creatinine and 3+ proteinuria on urinalysis (Table 2). Despite intravenous diuretic, antibiotic and fluid restriction, he progressed with development of severe hypertension, worsening of anemia (with need for packed red cell transfusion), onset of thrombocytopenia and acute kidney injury (KDIGO 1).

	Admission	D2	D3	D4 Blood transfusion	D5	D6	D7	D8	D9	D11	D20 Discharge	1 month after discharge	7 meses after discharge
Hb/Ht (%)	10.5/29.4	9.2/26.3	8.1/21.2	6.5/18.4	14.1/39.8		12.2/36	11.6/32.9	10.3/31	7.7/22.8	8.3/25	10.7/30	10.5/30
Platelets (/mm3)	100,000	79,000	101,000	98,000	159,000			86,000		32,000	358,000	405,000	427,000
Urea/Creatinine (mg/dL)			104/2.11	101/2.06	95/1.9		72/1.3	76/1.05		101/1.29	24/0.39	19/0.27	24/0.24
Urinary dipstick protein		+++	+			+++				+++	++	+++	++
Hematuria (RBC/filed) Dismorphism		+++				61\ +				100 +	29 +	80 +	
Serum Albumine (g/dL)			2.6		2.7		2.5		1.8		3.3	4.2	
Urinary protein/ creatine (g/g)									52.92	10.56		7.64	5.14
Haptoglobine (normal > 30 mg/dL)	8.4												<8
C3 serum (0,8-1,6 g/L)	0.85												1.46

Table 2: Evolution of exams during admission and at outpatient follow-up.

He was transferred to a tertiary center with a clinical diagnosis of Hemolytic Uremic Syndrome (HUS), confirmed with microangiopathic hemolytic anemia: haptoglobin 8.4 mg/dL (normal 30-230), lactic dehydrogenase 2897 U/L (normal 140-271), reticulocytes 5% (normal < 15), and the presence of schistocytes on blood smear. Additionally, he had KDIGO 1 acute kidney injury (creatinine 1.12 mg/dL and urine output 1 mL/kg/h), hematuria with dysmorphic erythrocytes (100 red blood cells/field) and nephrotic range proteinuria (urinary protein/creatinine ratio 52.92 mg/g) with hypoalbuminemia (2.2 mg/dL). On ultrasound, kidneys of normal size and echogenicity, with no other findings.

The mother had an uneventful pregnancy delivering a term baby through a cesarean section (cephalopelvic disproportion), without complications, and was discharged four days after birth. He had age-appropriate neurological development. There was no history of hospitalizations. He had a family history of consanguineous parents (first degree cousins), and his father had hypertension and autoimmune arthritis that was treated with prolonged use of corticosteroids.

Investigation tests revealed the following: negative stool culture, negative nasopharyngeal swab for bacterial growth, Hepatitis A IgG positive/IgM negative serology, negative CMV IgG/IgM negative, Hepatitis B and C negative as well as negative syphilis, normal liver enzymes, bilirubin and amylase. Further tests low limit C3 0.85 g/L (normal 0.9-1.8) with normal C4 0.27 g/L (normal 0.1-0.4), along with normal clotting tests. During hospitalization, he received amlodipine (0.3 mg/kg/day), spironolactone (2.5 mg/kg/day) and atenolol (2.5 mg/kg/day) with good blood pressure control, progressive improvement in renal function and significant decrease in proteinuria (Table 2), allowing for outpatient follow-up with good blood pressure control with amlodipine. Due to the diagnosis of TMA, nephrotic proteinuria and history of consanguineous parents, a genetic analysis was performed (Center for Research and Diagnosis of Genetic Diseases – Escola Paulista de Medicina – Universidade Federal de São Paulo) by Sanger sequencing, and a pathogenic homozygous variant was found in in the DGKe gene (OMIM *601440) (c.1068_1071del) in the patient and in heterozygosity in both parents (variant c.1068_1071del [p.Asn356Lysfs*6) classified as pathogenic, confirming the diagnosis of DGKe nephropathy.

Discussion

Patients affected with diacylglycerol kinase epsilon nephropathy (DGKe) typically present a picture of thrombotic microangiopathy with onset in the first year of age, associated with persistent arterial hypertension, microscopic hematuria and proteinuria [9], many of them in the nephrotic range. In general, the TMA in DGKe nephropathy does not respond satisfactorily to plasma therapy or to the anti-C5 terminal complement blockade. Kidney biopsy in DGKe nephropathy manifests as Membranoproliferative Glomerulonephritis (MPGN) pattern of injury with the absence of immune- and complement deposits. DGKe gene defects should thus be suspected in cases of TMA with MPGN pattern in very young children and in families with consanguinity.

In the present case, the patient presented a characteristic picture of hemolytic uremic syndrome, with a prodrome of acute diarrhea, followed by acute hemolytic anemia, with elevated lactate dehydrogenase, haptoglobin reduction, presence of schistocytes on the smear, thrombocytopenia and kidney injury, with the initial diagnosis of shigatoxin-associated HUS (STEC-HUS). However, the shigatoxin test was negative and there was a recurrence of hemolysis with the need for a blood transfusion and decreasing platelet count, and importantly developed of severe proteinuria despite stable renal function. Considering the patients young age, the presence of severe proteinuria and parental consanguinity, a preliminary diagnosis of TMA secondary to the mutation in the DGKe gene was made and then confirmed by genetic analysis. Importantly, conservative management resulted in stabilization of renal function and decrease in proteinuria.

The DGKe variant results in a premature stop codon at residue 361 (p.Asn356Lysfs*6) of the diacylglycerol epsilon kinase enzyme. According to the ACMG classification [11], it is considered pathogenic, since it is a frameshift mutation that results in a stop codon, generating a shorter and defective protein [9]. DGKe is present in the endothelium, platelets and podocytes. The pathophysiology appears to be related to protein kinase C, which promotes thrombosis and is activated by arachidonic acid containing diacylglycerols (AADAG). DGKe normally inactivates AADAG signaling by phosphorylating DAG to phosphatidic acid. Loss of DGKe function would then maintain sustained AADAG signaling, resulting in a prothrombotic state.

In endothelial cells, protein C kinase increases the production of several prothrombotic factors (von Willebrand factor, plasminogen activator inhibitor 1, platelet activating factor, and tissue factor) and antithrombotic factors (such as tissue plasminogen activator) in addition to thrombin-induced platelet activation [12].

In podocytes, a similar mechanism may occur in which DAG modifies the function of the diaphragmatic cleft with nephrin endocytosis, which could contribute to proteinuria and renal failure. Furthermore, VEGF signaling is essential for podocyte and endothelial cell survival and the loss of this signaling in the renal endothelium could culminate in thrombotic microangiopathy.

DGKe nephropathy was first described by Lemaire et al, in 2013 [9], when recessive mutations in DGKe gene were identified through exome sequencing in two unrelated families with a

diagnosis of atypical HUS. The study defined a distinct autosomal recessive Mendelian disease and with different pathophysiology from aHUS, independent of the activation of the complement system.

The association of abnormalities in DGKe and in the complement system has been reported though in isolated cases. In our case, the reduction in the level of C3 observed, which evolved with normalization in a few months, deserves discussion. The investigation for the presence of associated genetic abnormalities of the complement system was not performed in our patient, but as the clinical evolution was favorable only with supportive treatment, we believe that second mutation in complement gene in our case is unlikely. Sanchez Chinchilla et al [13], in 2014, described a case in which there was an association of DGKe nephropathy and genetic abnormality of the C3 gene. The authors stated that this association could be related to the more severe evolution observed and the favorable response obtained with the use of the complement blocker eculizumab at the time.

Miyata et al. [14] searched for complement defect in aHUS cases under 2 years of age in the Japanese Thrombotic Microangiopathy Center database to search for DGKe variants. Of 14 selected cases, one had a mutation that was not considered disease associated and another case had a splice mutation from the father and a frameshift mutation from the mother, both pathogenic for DGKe nephropathy and also showed a polymorphism in the complement Factor H (CFH) and thrombomodulin (THBD) genes. The other 12 cases did not carry variants for DGKe. The authors also describe a case of thrombotic microangiopathy caused by DGKe, with negative findings in complement genes, but which achieved control of refractory arterial hypertension and discontinuation of peritoneal dialysis with the use of eculizumab. The use of eculizumab in DGKe nephropathy is very limited and more studies are needed to define the best therapeutic approach for this condition.

Azukaits et al. [15], in 2017, described a cohort of DGKe nephropathy (10 patients from eight unrelated families). Eighty percent of patients had clinical presentation and laboratory investigations consistent with TMA. Most patients started the disease in the first year of life. All initial episodes of thrombotic microangiopathy were accompanied by arterial hypertension and proteinuria, typically at the nephrotic level. Although two-thirds of the patients needed dialysis, there was recovery of kidney function in all but one patient. The recurrent pattern was documented in 70% of patients, nearly half of whom experienced more than three relapses of the microangiopathic condition. Most relapses were accompanied by nephrotic range proteinuria. Viral infections were reported as possible triggers for the first episode in nine of ten newly identified patients. In another study, mutations in the DGKe gene were found in 9 patients from 3 families with consanguinity and proteinuria whose kidney biopsies showed a pattern of Membranoproliferative Glomerulonephritis (MPGN) [16]. More recently, a cohort of 16 patients with DGKe nephropathy from the United Kingdom was described - most had proteinuria (one at a nephrotic level) and arterial hypertension with a relapsing pattern and two patients progressed to chronic kidney disease [17].

In Brazil, de Holanda et al [18] described a case of an adult patient with TMA and arterial hypertension who was diagnosed as aHUS and was treated with eculizumab. Two years later, there were no signs of improvement in arterial hypertension, renal function and proteinuria, with the follow-up kidney biopsy showing TMA with chronicity. Genetic analysis of this patient showed a homozygous pathogenic mutation in the DGKe gene and eculizumab was discontinued [11]. The patient's past history revealed that the first episode of TMA had occurred at 6 months of age and that the parents were consanguineous.

Conclusion

We present a case of DGKe nephropathy characterized by TMA, hypertension and proteinuria occurring within the first year of life. Confirmation of DGKe nephropathy is made on genetic analysis. A high degree of suspicion, especially if there is consanguinity in the family, is needed for the diagnosis of DGKe nephropathy in children.

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