Article

Renal Biopsy Prognostic Findings in Children With Atypical Hemolytic Uremic Syndrome

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Abstract

Background: The aim of this study was to investigate the histopathological findings in kidney biopsies in children with atypical hemolytic uremic syndrome (aHUS) and to determine whether specific pathological findings in aHUS have a prognostic value.

Methods: Renal biopsy specimens of 29 patients who were recorded in the national Turkish aHUS registry database were available for review. Histopathological findings were compared with the clinical and laboratory features at the presentation and the final outcome.

Results: The mean age at presentation and follow-up period was 4.9 ± 3.9 and 3.9 ± 3.0 years, respectively. The median time interval from the first symptom to biopsy was 10 days. Vascular thrombosis and interstitial fibrosis were significantly related to chronic kidney disease (CKD) requiring dialysis or kidney transplantation during follow-up (5.6-fold, for both). Glomerular necrosis, cortical necrosis, and glomerular sclerosis were markedly associated with CKD without dialysis (6.2-fold, 13.3-fold, and 8.8-fold, respectively). However, presence of endothelial swelling, subendothelial widening, and fragmented erythrocytes was found to be correlated with a favorable final outcome.

Conclusions: Presence of vascular thrombosis, cortical necrosis, and glomerular sclerosis in histopathological evaluation correlated with developing CKD. Chronic changes in the interstitial compartment were also related to poor prognosis, a finding that has been shown for the first time in pediatric aHUS cases.

Keywords

atypical hemolytic uremic syndrome, cortical necrosis, glomerular sclerosis, interstitial fibrosis, prognosis, thrombotic microangiopathy, tubular atrophy

Introduction

Hemolytic uremic syndrome (HUS) is a thrombotic microangiopathy characterized by a triad of thrombocytopenia, Coombs negative microangiopathic hemolytic anemia, and acute kidney injury. It is classified as 4 subgroups: (a) infections-associated HUS mostly associated with Shiga toxin-producing *Escherichia coli* (STEC), *Streptococcus pneumonia*, influenza A, H1N1, and HIV; (b) HUS secondary to coexisting conditions such as organ transplantation, drugs, malignancy, malignant hypertension, autoimmune diseases; (c) cobalamin C defect-associated HUS; and (d) atypical HUS due to dysregulation of the alternative complement pathway.[1]

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However, HUS is practically categorized as typical (STEC-HUS) and atypical HUS (aHUS; usually stems from uncontrolled complement activation).[2] The basic patho-mechanism in STEC-HUS is initiated by Shiga toxin (or Shiga-like toxin), a potent cytotoxin that binds to the cell membrane glycolipid Gb3 on the glomerular endothelium eventually leading to microvascular thrombosis. On the other hand, however, alternative complement pathway involvement is central for endothelial damage in aHUS. Pathogenic variants of genes encoding the complement regulatory proteins of the alternative pathway result in uncontrolled activation of this pathway, which leads to the formation of membrane attack complex on the cellular surfaces and thereby widespread microtrombi in the microcirculation.[1]

Both aHUS and STEC-HUS could present variable clinical picture: from mild self-limiting disease to severe multisystemic involvement depending on underlying genetic defect in case of aHUS and virulence of the microorganism in case of STEC-HUS.[2] However, aHUS is very rare compared with STEC-HUS.[3,4] Although it is difficult to differentiate aHUS from STEC-HUS at the first presentation, some clinical features would strongly be suggestive for aHUS. These include age of disease onset (ie, <6 months of age), family history of HUS, recurrent disease in an individual, severe multisystem involvement, and identification of mutations in the genes of the alternative complement pathway. Presence of diarrhea that is mostly observed in STEC-HUS would not exclude the possibility of aHUS as 20% of aHUS patients may present with diarrhea.[1] Usually, a kidney biopsy is not indicated unless clinically aHUS is considered with associated clinical findings such as heavy proteinuria, prolonged oliguria, and unresponsiveness to initial plasma therapy (ie, exchange or infusion) or before starting eculizumab treatment in some patients. aHUS can lead to chronic kidney damage characterized by sustained hypertension or proteinuria in long-term follow-up, even without renal replacement therapy, in more than half of the patients.[5,6] Clinicians agree that severe initial presentation, persistent oliguria or anuria, need for dialysis, and central nervous system involvement are indicators of a poor prognosis.[7–9] However, the relationship between initial histopathological findings and clinical features at presentation and long-term outcomes in children with aHUS is not definitely known.[10,11]

Renal morphological features of HUS on light microscopic evaluation have been traditionally divided into early changes (ie, within 2 months from the initial symptoms), such as fibrin thrombi in capillary lumens, endothelial swelling, bloodless glomeruli, mesangiolysis, fragmented erythrocytes in the mesangium and subendothelial area, glomerular capillary tuft collapse and mucoid intimal thickening, and late changes (ie, after 2 months since first clinical presentation), such as duplication of the glomerular basement membrane, mesangiolysis, arterial intimal fibrosis, organization of luminal thrombi, and glomerulosclerosis and interstitial fibrosis.[12,13]

The aim of this study was to investigate the renal biopsy findings in children with aHUS and to identify whether specific histopathological findings have a prognostic value and show correlation with any clinical and laboratory findings at presentation.

Material and Methods

Patient Selection

Children with aHUS whose diagnoses were confirmed by the joint committee, which comprised pediatric nephrology experts are registered in a web-based national Turkish registry system (NRS) that was established in 2013 (www.ahusnet.org). Shiga toxin-producing *E. coli* (STEC) was investigated in a national centralized reference laboratory and STEC-positive patients as well as patients with depressed ADAMTS13 levels (ie, $\leq$5%), other specific infections, coexisting diseases, or those with drug-related HUS were excluded from the registry. With this approach, a refined aHUS cohort was established. Overall, the registry includes 146 aHUS patients and their demographic and clinical features as well as outcomes were published previously.[14] In the present study, we only focused on those patients who had biopsied for any reason. In this registry, 44 out of 146 pediatric patients had a renal biopsy. All centers were contacted, and renal biopsy specimens were requested for reevaluation. Twenty-nine biopsy specimens were obtained from these centers. The remaining 15 patients were excluded from the study since the histopathological slides of these patients were not available for review. Histopathologic reevaluation was performed by a single pathologist (IIG from the authors) who was blinded to clinical features of the patients.

Clinical Data Collection

Demographic, clinical, and laboratory data were recorded at presentation and at the last visit for each patient. According to the information declared by the centers in the reports, the renal biopsy indications were as follows: to confirm the diagnosis (in 21 patients), to start eculizumab administration (in 4 patients), to reveal the etiology of nephrotic range proteinuria associated with clinical HUS findings (in 3 patients), and to understand the unresponsiveness of the patient to plasma exchange or eculizumab therapy (in 1 patient).

The major clinical features such as age (year), time from presentation to biopsy (day), follow-up duration (year), the presence of severe anemia (defined by less

than 7.5 g/dL) and hypertension (defined by more than >95th percentile for age, gender and height), abnormal urine output (oliguria defined by urine output less than 1 mL/kg/h in infants and young children, and less than 0.5 mL/kg/h in older children or anuria defined by absence of any urine output), and laboratory features such as the hemoglobin and hematocrit values, the amounts of platelets, the serum level of lactate dehydrogenase, estimated glomerular filtration (using Schwartz's formula), and the amount of proteinuria (nephrotic proteinuria defined by $\geq$40 mg/m^2/h or urine protein/creatinine ratio $\geq$2 mg/mg, or non-nephrotic proteinuria defined by 4–40 mg/m^2/h or 0.2–2 mg/mg) at presentation, were recorded for each patient.

The final outcomes were defined as the need for continuous renal replacement therapy (RRT) or kidney transplantation during follow-up period or the presence of proteinuria, hypertension, and chronic kidney disease (CKD) without RRT at the last visit.

Histopathological Assessment

Histopathological findings were compared with the clinical and laboratory features at the presentation and the final outcomes. H&E, PAS, trichrome, and methanamine silver-stained sections were available for every patient's biopsy. Each biopsy specimen was reevaluated for the presence of glomerular (sclerosis, thrombosis, fragmented erythrocytes, fibrinoid necrosis, congestion, endothelial swelling, sub-endothelial widening and wall thickening, crescents, ischemic collapse, capillary double contours or reduplication of glomerular basement membranes, necrosis, mesangiolysis), vascular (mucoid intimal thickening, thrombosis, intimal proliferation, fibrinoid necrosis), interstitial (edema, fibrosis, inflammation), tubular (acute tubular injury, tubular atrophy) lesions, and the presence of cortical necrosis. Specimen adequacy was considered as 10 or more glomeruli with at least 2 arteries. All of the renal biopsies had been examined by immunofluorescence (IF) microscopy and the IF findings reported in the final pathology reports were recorded. Electron microscopic images were not available for the cases.

Genetic and Anti-complement Factor H Autoantibody Analyses

Mutation analyses were performed in 21 out of 29 patients (72%). Mutation analyses via Sanger sequencing of the coding regions of the *CFH, CFI, MCP (CD46), CFB*, *C3, DGKE*, and *CHFR5* genes were performed at the Hacettepe University Nephrogenetics Laboratory, Ankara, Turkey. In silico analyses using Polymorphism Phenotyping v2 (PolyPhen2) (http://genetics.bwh.harvard.edu/pph2/index.shtml) and Mutation taster (http://www.mutationtaster.org) softwares were applied for predicting likely effects of the variations. aHUS mutation database (http://www.fh-hus.org), The Human Gene Mutation Database Professional (http://www.hgmd.cf.ac.uk/ac/index.php), and dbSNP database (https://www.ncbi.nlm.nih.gov/snp) were used to check whether identified variations had been reported previously. Multiplex ligation-dependent probe amplification (MLPA) analysis was employed for CFHR1-3 deletion using SALSA MLPA probemix P236-A3 ARMD mix-1kit (MRC-Holland TM) according to the manufacturer's recommendations. Anti-complement Factor H autoantibody was searched using the CFH IgG ELISA Kit (Abnova™), according to the manufacturer's recommendations, with a detection limit of 0.6 AU/mL.

Statistical Analysis

Binary logistic regression was used to determine specific kidney biopsy findings that might contribute to initial clinical features and the prognosis with a P-value $< .05$ remained in the final model. Results for biochemical parameters were shown as mean $\pm$ SD. The SPSS 21.0 program was used for all statistical analyses.

Results

Of the 29 patients whose biopsies were examined, 14 were male and 15 were female. Their mean age at presentation was 4.9 $\pm$ 3.9 years, and the mean follow-up period following first presentation was 3.9 $\pm$ 3.0 years. The rate of kinship between parents was 34.5% for all patients examined. Two patients had a family history of aHUS. At the first presentation, nephrotic range proteinuria, severe anemia ($\leq$7.5 g/dL), and eGFR $<$15 mL/min/1.73 m^2 were present in 48%, 52%, and 48% of the patients, respectively (Table 1).

During follow-up, 2 patients died, 8 underwent dialysis or kidney transplantation for end stage kidney disease, 7 remained to have persistent hypertension and/or proteinuria, and complete recovery was achieved in only 3 patients. The remaining 9 patients have been following up with different stages of CKD (stages between 1 and 4; Table 2).

At the acute phase of the disease, 13 patients were treated with plasma exchange therapy followed by eculizumab, 7 patients were treated with first-line eculizumab, and 9 patients received only plasma exchange therapy.

When the clinical features of the 117 patients were compared to the 29 patients who were biopsied, there were no significant differences in terms of mean age (4.8 $\pm$ 4.4 vs 4.9 $\pm$ 3.9 years), the frequency of oliguria/anuria (67% vs 69%), and the duration of anuria and eGFR $<$90 mL/min/1.73 m^2 (94% vs 100%).

Table 1. Demographic Information and Clinical and Laboratory Findings of the Patients at the Presentation.

Demographic Information (14 Male/15 Female)	Mean ± SD Median (Range)	Clinical Findings	N (%)	Laboratory Findings	Mean ± SD Median (Range)
Age at presentation (year)	4.9 ± 3.9 4.3 (0.41–15.2)	Severe anemia (≤7.5 g/dL)	15 (52)	Hemoglobin (g/dL)	7.5 ± 1.8 7.4 (3.5–11.9)
Time from presentation to biopsy (day)	33 ± 66 10 (0–308)	Severe hypertension	10 (34.5)	Hematocrit (%)	22.5 ± 5.8 21.5 (15–35.3)
Follow-up time (year)	3.9 ± 3.0 3.3 (0.7–13.5)	Oliguria	20 (69)	LDH (U/L)	2045 ± 1450 1784 (258–5260)
		Anuria	8 (27.5)	Platelet ($\times 10^9$/L)	101 ± 50 79.5 (43–213))
		Nephrotic range proteinuria	14 (48)	eGFR mL/min/1.73 m^2	22.5 ± 15.7 17.3 (5.1–63.5)

Abbreviations: eGFR, estimated glomerular filtration (Schwartz's formula); LDH, lactate dehydrogenase.

Table 2. The Clinical and Laboratory Findings of the Patients at the Last Visit.

Clinical and Laboratory Findings	Patients, N (%)
Fully recovery	3 (10)
Death	2 (7)
CKD not requiring RRT	9 (31)
CKD requiring RRT or Transplantation	8 (28)
Peritoneal dialysis	4 (14)
Hemodialysis	1 (4)
Transplantation	3 (10)
Hypertension + proteinuria	5 (17)
Proteinuria alone	2 (7)

Abbreviations: CKD, chronic kidney disease; RRT, renal replacement therapy.

However, when the outcomes of the 2 groups at the last visit were compared, there was a significant difference in terms of complete renal recovery (77% vs 10%, $P < .01$). In addition, 2 of 3 patients who died during the follow-up period had a renal biopsy. None of 29 patients had biopsy complications.

The median time from the first presentation of the patients to renal biopsy was 10 days (IQR 5–64). The mean number of glomeruli per biopsy was 32 ± 14, and the mean number of arterioles present per biopsy was 6 ± 3. Specimens contained renal cortex and medulla in 18 cases and only renal cortex in 11 cases. Among the glomerular lesions examined, endothelial swelling was the most frequent finding (90%), followed by subendothelial widening and capillary wall thickening by light microscopy (66%) and ischemic collapse (59%). Among the vascular lesions, fibrinoid necrosis (34%) was the most common pathological finding, followed by mucoid intimal thickening (28%) and luminal thrombi (24%). The most frequently observed tubulointerstitial lesions were acute tubular injury and interstitial edema (79% and 72%, respectively). Glomerular necrosis was present in 24% of the cases and cortical necrosis was identified in 6 cases (21%). Chronic lesions including global (7 out of 29 patients; 24%) and segmental glomerulosclerosis (2 out of 29 patients; 7%), tubular atrophy (8 out of 29 patients; 28%), and interstitial fibrosis (7 out of 29 patients; 24%) were also recorded for every biopsy (Table 3). According to the time of biopsy, the lesions were distinguished as early and late. Interestingly, histopathological examination of the patients who underwent biopsy within 1 month after the first signs of hemolytic uremic syndrome showed not only early lesions but also late lesions (Table 4).

Presence of cortical necrosis and glomerular necrosis were found to increase the probability of anuria at initial presentation by 9- and 5.6-folds, respectively (Table 5). On the other hand, the presence of glomerulosclerosis, interstitial fibrosis, and tubular atrophy was associated with an increased risk of having markedly reduced eGFR (<15 mL/min/1.73 m^2) at first presentation, and this risk was 7.5-fold for glomerulosclerosis, 25.5-fold for interstitial fibrosis, and 5.5-fold for tubular atrophy (Table 5).

In terms of final outcomes, the presence of vascular thrombosis and any degree of interstitial fibrosis were found to increase the probability of developing chronic renal disease requiring dialysis or kidney transplantation in the follow-up period by 5.6-fold (Table 6). All of 7 patients with interstitial fibrosis developed CKD and 3 of them required dialysis or kidney transplantation and 1 patient died during follow-up. On the other hand,

Table 3. Histopathologic Findings Associated With Major Outcomes in 29 aHUS Children.

Histopathologic lesions	Total Number of Kidney Biopsies N = 29 (%)	Major Outcomes: Fully Recovery Yes N = 3 (%)	Fully Recovery No N = 26 (%)	CKD Yes N = 19 (%)	CKD No N = 10 (%)	Among CKD Population, Requiring RRT or Tx[a] Yes N = 8 (%)	Among CKD Population, Requiring RRT or Tx[a] No N = 11 (%)
Glomerular							
Endothelial swelling	26 (90)	3 (100)	23 (88)	16 (84)	10 (100)	6 (75)	10 (91)
Sub-endothelial widening and wall thickening	19 (66)	1 (33)	18 (69)	13 (68)	6 (60)	6 (75)	7 (64)
Ischemic collapse	17 (59)	3 (100)	14 (54)	11 (58)	6 (60)	6 (75)	5 (45)
Mesangiolysis	12 (41)	1 (33)	11 (42)	7 (37)	5 (50)	3 (37.5)	4 (36)
Congestion	12 (41)	1 (33)	11 (42)	7 (37)	5 (50)	3 (37.5)	4 (36)
Fragmented erythrocytes	10 (34)	1 (33)	9 (35)	4 (21)	6 (60)	1 (12.5)	3 (27)
Sclerosis (global and segmental)	9 (31)	0 (0)	9 (35)	6 (32)	3 (30)	4 (50)	2 (18)
Thrombosis	9 (31)	0 (0)	9 (35)	6 (32)	3 (30)	4 (50)	2 (18)
Capillary double contours	9 (31)	0 (0)	9 (35)	6 (32)	3 (30)	3 (37.5)	3 (27)
Necrosis	7 (24)	1 (33)	6 (23)	4 (21)	3 (30)	1 (12.5)	3 (27)
Crescents	5 (17)	0 (0)	5 (19)	3 (16)	2 (20)	2 (25)	1 (9)
Fibrinoid necrosis	3 (10)	0 (0)	3 (12)	1 (5)	2 (20)	1 (12.5)	0 (0)
Vascular							
Fibrinoid necrosis	10 (34)	1 (33)	9 (35)	5 (26)	5 (50)	2 (25)	3 (27)
Mucoid intimal thickening	8 (28)	0 (0)	8 (31)	7 (37)	1 (10)	3 (37.5)	4 (36)
Thrombosis	7 (24)	0 (0)	7 (27)	6 (32)	1 (10)	4 (50)	2 (18)
Intimal proliferation	4 (14)	0 (0)	4 (15)	4 (21)	0 (0)	2 (25)	2 (18)
Interstitial							
Inflammation	21 (72)	2 (67)	19 (73)	15 (79)	6 (60)	7 (87.5)	8 (73)
Edema	17 (59)	1 (33)	16 (62)	11 (58)	6 (60)	4 (50)	7 (64)
Fibrosis	7 (24)	0 (0)	7 (27)	5 (26)	2 (20)	4 (50)	1 (9)
Tubular							
Acute tubular injury	23 (79)	3 (100)	20 (77)	13 (63)	10 (100)	4 (50)	9 (82)
Tubular atrophy	8 (28)	0 (0)	8 (31)	8 (42)	0 (0)	3 (37.5)	5 (45)
Cortical necrosis	6 (21)	1 (33)	5 (19)	3 (16)	3 (30)	0 (0)	3 (27)

Fully recovery means the patients with normal renal function, no proteinuria, and no hypertension. CKD "Yes" means the patients with low estimated glomerular filtration rate. CKD "No" means the patients with normal renal function, but with proteinuria and/or hypertension. CKD requiring RRT or Tx means end-stage renal disease requiring renal replacement therapy (peritoneal dialysis or hemodialysis) or renal transplantation.

Abbreviation: CKD, chronic kidney disease.

[a]The last column indicates CKD patients only.

glomerular necrosis, cortical necrosis, and interstitial inflammation were found to be associated with developing CKD not requiring RRT. This risk was 6.2-fold for glomerular necrosis, 13.3-fold for cortical necrosis, and 6.2 fold for interstitial inflammation. Glomerular lesions including endothelial swelling, subendothelial widening along the capillary walls, and the presence of fragmented erythrocytes were not associated with poor final outcomes in these patients (Table 6).

Genetic analyses were performed in 21 out of 29 patients (72%). The remaining 8 patients (27.5%) could not be tested since blood samples of these patients were not available. Identified variations in 8 of 21 patients tested (38%) were as follows: *CFH* in 3 patients, *CFB* plus *MCP (CD46)* in 1 patient, *C3* in 1 patient, *CFB* plus *CFHR1-3* deletion in 2 patients, and *CFHR1-3* deletion in 1 patient (Table 7). Anti-CFH antibody was detected in 2 patients who had also *CFHR1-3* deletion. No variation was detected in the remaining 13 patients (62%). There was no difference between patients with genetic variations and without in terms of renal outcomes and histopathology.

Discussion

In the present study, we have analyzed biopsy findings and their relationship with short-term clinical findings and long-term outcomes in pediatric patients with aHUS. The data on whether there is correlation between histopathological findings and clinical course as well as final outcome are scarce in the literature.[10,11]

Table 4. Distribution of Early and Late Lesions According to Biopsy Timing.

Histopathologic Lesions		Total Number of Patients With Lesions N = 29 (%)	Time Interval Between Initial of Disease and Biopsy	
			≤1 Month Frequency of the Lesions N = 19 (%)	>1 Month Frequency of the Lesions N = 10 (%)
Early lesions	Acute tubular injury	23 (79)	15 (79)	8 (80)
	Intersititial inflammation	21 (72)	15 (79)	6 (60)
	Endothelial swelling and subendothelial widening	19 (65)	12 (63)	7 (70)
	Glomerular collapse	17 (59)	9 (47)	8 (80)
	Intersititial edema	17 (59)	12 (63)	5 (50)
	Mesangiolysis	12 (41)	6 (32)	6 (60)
	Fragmented red blood cells	10 (34)	7 (37)	3 (30)
	Fibrin thrombi	9 (31)	8 (42)	1 (10)
	Fibrinoid necrosis (glomerular)	3 (10)	3 (16)	0 (0)
Late lesions	Glomerular sclerosis (global or segmental)	9 (31)	7 (37)	2 (20)
	Reduplication of glomerular basement membranes	9 (31)	4 (21)	5 (50)
	Tubular atrophy	8 (28)	5 (26)	3 (30)
	Intersititial fibrosis	7 (24)	5 (26)	2 (20)

Table 5. Relationship Between Biopsy Findings and Clinical and Laboratory Features at the Presentation Using Binary Logistic Regression Test.

Clinical and Laboratory Features (Dependent Variables)	Biopsy Findings (Independent Variables)	B	OR	CI (95%)	*P*
Anuria	Cortical necrosis	2.19	9.00	1.20–37.41	.03
	Glomerular necrosis	1.73	5.66	0.02–1.10	.04
Low eGFR (<15 mL/min/1.73 m^2)	Glomerulosclerosis	2.01	7.50	1.20–44.00	.02
	Interstitial fibrosis	3.23	25.50	2.30–27.50	.008
	Tubular atrophy	1.71	5.57	0.88–35.26	.04

Abbreviations: B, logistic regression b coefficient; eGFR, estimated glomerular filtration rate (Schwartz's formula); OR, odds ratio.

Table 6. Relationship Between Biopsy Findings and Final Outcomes Using Binary Logistic Regression Test.

Final Outcomes (Dependent Variables)	Biopsy Findings (Independent Variables)	B	OR	CI (95%)	*P*
CKD not requiring RRT	Glomerular necrosis	1.83	6.20	0.94–41.51	.04
	Cortical necrosis	2.59	13.33	1.28–138.84	.03
	Glomerulosclerosis	2.18	8.88	0.92–85.65	.04
	Endothelial swelling and subendothelial widening	−1.72	0.17	0.03–0.90	.04
	Fragmented erythrocytes	−2.10	0.12	0.02–0.70	.01
CKD requiring RRT or Transplantation	Vascular thrombosis	1.73	5.66	0.89–36.08	.04
	Interstitial fibrosis	1.73	5.66	0.089–36.08	.04

Abbreviations: B, logistic regression b coefficient; CKD, chronic kidney disease; OR, odds ratio; RRT, renal replacement therapy.

This study results show that some particular kidney biopsy findings in aHUS were significantly correlated with initial clinical and laboratory features as well as poor final outcome. We observed that cortical and glomerular necrosis associated not only with a very severe presentation but also with a poor prognosis. The presence of cortical necrosis and glomerular necrosis confers 9- and 5-folds, respectively, increased risk of anuria at initial presentation. Similarly, the risk for developing CKD on follow-up was 13 times higher for cortical necrosis and 6 times for glomerular necrosis, compared to those without these lesions. We believe that particular attention should be given to the presence of cortical necrosis, since it has also been reported as a frequent finding in autopsies of aHUS patients.[12] It is postulated that ischemia due to intravascular thrombosis in glomerular capillaries and arterioles leads to these pathologic changes, reflecting the severity of vascular

Table 7. Genetic Abnormalities, Pathologic Findings, and Final Outcomes in the Patients With Mutations.

Patient No.	Gender	Genetic Abnormalities	Variant	Predicted Aminoacid Change	Zygosity	PolyPhen Prediction (Score)	Mutation Taster Prediction (Score)	Family history of aHUS	Pathological Lesion in Kidney Biopsy	Final Outcome
1	Female	Single, C3[a]	c.537_539del	p.Leu180del	Heterozygous	NA	Polymorphism (0.99)	No	Acute and chronic	CKD requiring RRT
2	Male	Single, anti CFH antibody associated with CFHR1-3 deletion	CFHR1-3 deletion	–	Homozygous	NA	NA	No	Acute	Hypertension
3	Female	Single, CFH[b]	c.3148A > T	p.Asn1050Tyr	Heterozygous	Benign (0.016)	Polymorphism (0.99)	No	Acute and chronic	CKD requiring RRT
4	Male	Single, CFH[c]	c.2127_2129del	p.Tyr711del	Heterozygous	NA	Disease causing (0.54)	No	Acute and chronic	Hypertension and proteinuria
5	Male	Single, CFH[d]	c.2850G > T	p.Gln950His	Heterozygous	Probably damaging (0.96)	Polymorphism (0.99)	No	Acute and chronic	CKD not requiring RRT
6	Female	Combined, MCP[e] and CFB[f]	MCP:c.286 + 18_27delT	–	Heterozygous	NA	NA	No	Acute and chronic	CKD not requiring RRT
			CFB: c.1697A > C	p.Glu566Ala	Heterozygous	Benign (0)	Polymorphism (0.99)			
7	Male	Combined, CFB[g] and Anti CFH antibody associated with CFHR1-3 deletion	CFB: c.397G > A	p.Asp133Asn	Heterozygous	Benign (0.014)	Polymorphism (0.7)	No	Acute	Recovery without sequelae
			CFHR1-3 deletion	–	Homozygous	NA	NA			
8	Female	Combined, CFB[h] and CFHR1-3 deletion	CFB: c.1050G > T	p.Lys350Asn	Heterozygous	Probably damaging (0.99)	Polymorphism (0.81)	No	Acute	CKD requiring RRT
			CFHR1-3 deletion	–	Heterozygous	NA	NA			

Polymorphism Phenotyping (PolyPhen) v2 (http://genetics.bwh.harvard.edu/pph2/index.shtml), Mutation taster (http://www.mutationtaster.org), and variations and predicted aminoacid changes have been named according to the guidelines of the Human Genome Variation Society using Mutalyzer software (https://mutalyzer.nl).

Abbreviations: C3, Complement 3; CFH, complement factor H; CFHR1-3, complement factor H-related genes 1 and 3; CFB, complement factor B; CKD, chronic kidney disease; MCP, membrane cofactor protein (CD46); NA, not applicable; RRT, renal replacement therapy.

[a]Besbas et al.[14]

[b]Esparza-Gordillo et al.[15]

[c]Besbas et al.[14]

[d]Caprioli et al.[16]

[e]This study.

[f]Feng et al.[17]

[g]Besbas et al.[14]

[h]Roumenina et al.[18]

involvement.[19–21] We noted that the vast majority of patients with cortical and glomerular necrosis had undergone early biopsy (within the first 20 days after initial symptoms) and presented with anuria. Therefore, it can be postulated that anuria at presentation may suggest severe histopathologic lesions, like cortical and/or glomerular necrosis, even in the absence of renal biopsy in pediatric aHUS patients, and may be an indication for the administration of eculizumab as a first-line therapy.[22]

The relationship between STEC-HUS and glomerular scleroses has been shown in previous studies.[23–27] In addition, focal global or segmental glomerulosclerosis has also been reported in cases of aHUS induced by inherited methylmalonic academia, but not in patients with complement mediated atypical HUS.[28,29] We observed segmental glomerular scleroses in only 2 patients.

In this study, we have showed that another poor prognostic histopathological finding was the presence of vascular (ie, arteriolar/arterial) thrombosis in the renal biopsy. Our patients with vascular thrombosis had a greater risk of CKD requiring dialysis or kidney transplantation. Similarly, a previous study reported that particularly arteriolar involvement was frequently associated with the occurrence of CKD in the follow-up.[30] The results of a more recent study also showed that the probability of developing CKD during follow-up was 3.5 times higher in the presence of vascular thrombosis, especially in the case of arteriolar or arterial involvement.[11]

Glomerular lesions of endothelial swelling, subendothelial widening along the capillary walls, and presence of fragmented erythrocytes were not found to be associated with poor prognosis in our study. Since these findings represent early and diagnostic changes of HUS in a kidney biopsy, they may even be associated with better prognosis which may result from earlier therapeutic intervention.

Tubulo-interstitial changes are not specific biopsy findings in aHUS. Moreover, many studies did not emphasize the prognostic importance of tubular atrophy and interstitial fibrosis in this disease.[5,9,11,12,14,19–21,23,24] However, we observed that particularly any degree of interstitial fibrosis was associated with more severe presentation and worse prognosis. It is well known that a positive correlation exists between deteriorated renal functions and chronic changes in the interstitial compartment irrespective of the underlying renal disease.[31] In addition, tubular atrophy and interstitial fibrosis usually parallel the glomerulosclerotic lesions' distribution and severity in many kidney diseases, primarily in lupus nephritis.[32] The association of histopathological changes in the tubulointerstitial compartment and disease prognosis is well described in lupus nephritis.[33] A recent study also showed that tubulo-interstitial injury has an important and active role in the process that can lead to progressive nephron loss.[34] A diminished cross-sectional area resulting from interstitial fibrosis affects the renal peritubular capillaries, and this change may contribute to a decrease in the glomerular filtration rate.[35] Similar pathological mechanisms may also result in poor prognosis in aHUS patients. In this study, we observed that the time from the first presentation of the disease to renal biopsy in vast majority of patients, who had interstitial fibrosis and tubular atrophy, was shorter than 1 month (Table 4). This is a noteworthy finding that might indicate that changes in tissue level begin before the symptoms.

Although this study gives important results about the relationship between initial histopathological findings and long-term outcomes in children with aHUS, it is important to note that this was a retrospective study of aHUS cases that were biopsied, and that these may not be representative of all aHUS cases.

In conclusion, renal pathological changes in children with aHUS may help predicting the patients' prognosis. To the best of our knowledge, this is the first study to investigate the relationship between initial kidney biopsy findings and long-term outcomes in pediatric atypical HUS cases. We showed that the presence of cortical and/or glomerular necrosis may indicate a CKD development in children with aHUS.[5,12,19–21,23] We also observed that interstitial fibrosis is important in predicting the poor prognosis of children with aHUS. Although the importance of chronic interstitial changes (ie, interstitial fibrosis) in determining of renal prognosis has been previously demonstrated in different kidney diseases, we have shown for the first time in this study that this prognostic sign is also valid for pediatric patients with aHUS.

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Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee at which the studies were conducted (IRB approval number FON10/03-22) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the ethics committee of Hacettepe University (FON10/03-22). Written informed consent was obtained from the parents of all the patients.

Statement of prior presentation

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