Hemolytic uremic syndrome outbreak in Turkey in 2011

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SUMMARY: Ekinci Z, Candan C, Alpay H, Canpolat N, Akyüz SG, Gündüz Z, Dursun İ, Bek K, Dursun H, Işıyel E, Öktem F, Tabel Y, Akil İ, Delibaş A, Gülleroğlu K, Akıncı N, Dinçel N, Özkaya O, Söylemezoğlu O. Hemolytic uremic syndrome outbreak in Turkey in 2011. Turk J Pediatr 2013; 55: 246-252.

The aim of this retrospective multicenter study was to define the epidemiological and clinical features and prognostic factors of the first diarrhea-related hemolytic uremic syndrome (D+HUS) outbreak in Turkey in 2011.

All pediatric nephrology centers in Turkey were asked about D+HUS patients via e-mail. Seventy D+HUS patients (median age: 5.7 years) participated. The seasonal peak was around the 7th, 8th and 9th months with 44 cases, centered in the east Marmara region. No causative agent could be identified. The rate of neurological complications and mortality was 21.4% and 4.2%, respectively. Eculizumab was used in four cases. Two of them had severe neurological complications despite plasma exchange. Elevated polymorphonuclear leukocyte count during hospital admission was the predictor of both severe disease and poor outcome. Duration of prodrome was the predictor of poor outcome ($p<0.05$). In conclusion, the median age of the affected children was greater than in the previous reports, while clinical features and outcome were similar.

Key words: hemolytic uremic syndrome, diarrhea-related, outbreak, children, epidemiology, eculizumab.

Although hemolytic uremic syndrome (HUS) is reported as a leading cause of acute renal failure in children, that is not the case in Turkey. The total number of reported HUS cases was 18/year in 2010[1,2]. The outbreaks reported from Germany and France in 2011 apprehend a spread of the *Escherichia coli* (*E. coli*) O104:H4 to Turkey[3,4]. An outbreak of HUS occurred during late summer and early autumn in 2011 in the east Marmara region of Turkey. Here, we report the outbreak information collected during 2011.

Material and Methods

In summer 2011, an unexpected number of HUS cases in the east Marmara region were observed. The Turkish Society for Pediatric Nephrology (TSPN) prepared a form and asked all 40 pediatric nephrology centers in Turkey to send the data of diarrhea-associated HUS (D+HUS) patients diagnosed during 2011 via e-mail. The collected data included age, gender, residence, presenting symptoms, time from the onset of symptoms to hospitalization (duration of prodrome), presence of diarrhea and/or bloody diarrhea and/or vomiting during prodrome, use of antimicrobial and anti-diarrheal medication during prodrome, clinical characteristics, date of hospitalization, date of discharge, laboratory data on admission, date

of stool culture, need for transfusions (type and amount), use of dialysis, type of dialysis, use of plasma exchange, use of eculizumab, outcome during discharge, and outcome after a minimum of three months follow-up. If a stool specimen was submitted for shiga toxin-producing *E. coli* (STEC) testing, the results were obtained from the Refik Saydam Sanitation Center, National Enteric Pathogens Reference Laboratory. Patients with a diagnosis of atypical HUS were excluded.

Definitions

D+HUS was defined as microangiopathic hemolytic anemia (with evidence of red cell fragmentation), thrombocytopenia (platelet count $\leq 150x10^9$ / mm^3) and evidence of renal involvement (presence of acute kidney injury and/or abnormal urinary sediment as evidenced by cylinderuria, proteinuria or hematuria) that progressed after gastrointestinal symptoms (diarrhea and/or vomiting).

Estimated glomerular filtration rate (eGFR) was based on the calculation by the Schwartz formula k x height (cm) / plasma creatinine (mg/dl).

Hematuria was defined as ≥5 red blood cells/ high power field (rbc/hpf).

Proteinuria was classified as negative, subnephrotic >0.2 mg/mg and nephrotic >2 mg/mg in the first obtained urine with protein/ creatinine.

Hypertension was defined as systolic and/ or diastolic blood pressure ≥95th percentile for age, gender and length on three separate measurements with auscultation method.

Oliguria was defined by a urine flow of <0.5 ml/kg/h in children <30 kg and <400 ml/ day in children >30 kg during the first day of admission.

Outbreak zone was used for the three cities in the east Marmara region (İstanbul, Kocaeli and Sakarya) where many unexpected D+HUS cases were seen.

Patients were classified as having a poor long-term outcome if they died before or after they were discharged or if any of the following conditions were present at or after hospital discharge: blindness, hemiparesis, chronic renal failure, or need for anticonvulsant therapy, kidney transplantation or colonic resection[5,6]. A good long-term outcome was defined as the absence of any of the preceding conditions. Patients were also classified as having severe disease if they died or met two of the following criteria: hospitalization for more than two weeks, dialysis for more than 10 days, and presence of at least one severe complication. Severe complications included all the poor outcome variables, shock, cardiac arrest, coagulopathy, and the need for mechanical ventilation[5].

The results were analyzed by using the Statistical Package for the Social Sciences (SPSS) for Windows ver. 13.0 (SPSS, Chicago, IL), and descriptive statistics are presented as the mean ± standard deviation (SD) if equally distributed or as the median and range if unequally distributed. Univariate analyses for group comparisons were performed using independent samples t test or chi-square test. Multivariate analysis was performed to assess whether there were significantly independent predictors of severe disease and poor prognosis. Age at onset, time from the onset of symptoms to hospitalization (duration of prodrome), presence or absence of bloody diarrhea, abdominal cramps, vomiting, fever, use of antimicrobial therapy during the prodromal illness, and polymorphonuclear leukocyte count in the peripheral blood on admission were evaluated as predictors of outcome and disease severity by multiple regression analysis.

A p value <0.05 was considered statistically significant.

Results

Seventy patients with D+HUS from 15 centers participated in this study. These patients were enrolled retrospectively between 1 January 2011 and 31 December 2011. Follow-up time was 116.30±48.71 (5-300) days with a median of 90 days. The patient with the minimum follow-up time of 5 days was one of the deaths.

Forty-four patients (62.9%) were from three cities in the east Marmara region (outbreak zone), whereas 26 patients were from 21 cities dispersed throughout Turkey. Demographic characteristics of these patients are presented in Table I according to the inhabitation region. The seasonal distribution of D+HUS

Table I. Demographic Characteristics of 70 Patients with HUS in Turkey in 2011

	Outbreak zone n	Outside of outbreak zone n	p	Total n (%)
Sex			0.391*	
Female	25	12		37(52.9%)
Male	19	14		33(47.1%)
Age**(years)	7.30±4.31 (6)	6.70±5.11 (5)	0.330***	7.07±4.60 (5.75)
Residence			0.606*	
Urban	40	24		64(91.4%)
Rural	4	2		6(8.6%)
Seasonal distribution			0.001*	
July, August, September	36	11		47(67.1%)
Other months	8	15		23(32.9%)

* Chi-square test
** Age is presented as mean ± SD (median)
*** Mann-Whitney U test

Table II. Clinical Characteristics of 70 Patients with HUS in Turkey in 2011

Symptom	Outbreak zone n=44 (%)	Outside of outbreak zone n=26 (%)	p*	Total n= 70 (%)
Diarrhea☼	37 (84)	25 (96)	0.124	62 (88.6)
Bloody diarrhea	21 (47)	14 (53)	0.402	35 (50)
Vomiting	40 (90)	23 (88)	0.521	63 (90)
Abdominal cramp	30 (68)	24 (92)	0.018	54 (77.1)
Fever	16 (36)	16 (61)	0.043	32 (45.7)
Respiratory symptoms	4 (0.09)	5 (19)	0.195	9 (12.9)
Use of antimicrobials	16 (36)	10 (38)	0.866	26 (38.2)
Requirement for dialysis	32 (72)	19 (73)	0.601	51 (72.9)
CNS involvement	10 (22)	5 (19)	0.489	15 (21.4)
Severe disease	20 (45)	11 (42)	0.498	31 (44.3)
Poor prognosis	5 (0.11)	2 (0.07)	0.479	7 (10)
Number of deaths	3 (0.06)	0 (0)	0.234	3 (4.2)
Duration of prodrome (days)**	5.05±2.42 (1-10)	8.52±7.51 (2-30)	0.134***	6.45±5.34 (1-30)
Duration of oligoanuria (days)**	12.75±22.61 (2-130)	7.05±6.15 (0-20)	0.305***	10.43±17.95 (0-130)
Duration of dialysis (days)**	16.10±23.27 (0-130)	12.89±9.08 (1-31)	0.701***	14.92±19.24 (0-130)

☼Eight patients had abdominal cramps and vomiting.
* Chi-square test
** Data are presented as mean±SD (min, max)
***Mann-Whitney U test

Table III. Laboratory Findings of 70 Patients with HUS in Turkey in 2011

	Outbreak zone	Outside of outbreak zone	p*	Whole group
Minimum hemoglobin (g/dl)	6.84±1.21 (4.5-11.5)	6.31±1.07 (4.4-8.8)	0.081	6.65±1.18 (4.4-11.5)
Minimum platelet (/μl)	58354±44037 (8000-149000)	54030±31436 (10400-128000)	0.932	56748±39636 (8000-149000)
PNL** on admission (/μl)	10155±7500 (1500-35300)	9469±5889 (2400-24000)	0.925	9891±6884 (1500-35300)
Predialysis maximum serum creatinine (mg/dl)	5.50±3.27 (0.72-14.3)	4.88±2.83 (0.60-10.40)	0.473	5.27±3.11 (0.6-14.30)
Time elapsed before stool specimen was taken (days)**	9.26±4.26 (3-18)	12.10±9.48 (1-27)	0.765	10.24±6.51 (1-27)

Data are presented as mean±SD (min, max)
*Mann-Whitney U test
**Polymorphonuclear leukocyte

cases was significantly different according to the inhabitation region (p=0.001). Clinical characteristics of D+HUS cases are presented in Table II according to inhabitation region. No significant difference was recorded regarding symptoms according to inhabitation region. However, the number of patients with abdominal cramps and fever was significantly high outside of the outbreak zone.

No patient developed colon necrosis or perforation and/or insulin-dependent diabetes. According to the severe disease criteria, 31 patients were classified as having severe disease. According to the poor long-term outcome criteria, 7 patients were classified as having poor long-term outcomes. Distribution of patients according to both criteria was similar according to inhabitation region. Fifty-one (72.9%) patients required dialysis. Of these, 27 patients were followed up with peritoneal dialysis (PD), 19 patients with hemodialysis (HD) and 5 patients with PD and HD. Twenty patients went on to plasma exchange. Of these, only 10 had central nervous system (CNS) involvement. All of the 3 deaths had CNS involvement. Two of them died in the first week with cardiogenic shock, and the third one died on the 37th day with lung bleeding. Four patients were treated with eculizumab. At the end of the 3rd month, two of them had chronic renal disease, one of them had hypertension, and the fourth one fully recovered. One of these patients developed a generalized tonic-clonic convulsion and lost consciousness during the 10th plasma exchange. Although complement C3 level was normal, plasma exchange was terminated and eculizumab was started on the day of convulsion. The girl's neurological condition improved dramatically in 24 hours; however, anuria resolved slowly. Nephrotic range proteinuria (63 mg/m^2/h) and low GFR (44.9 ml/min/1.73 m^2) were persisting in her last follow-up in the 6th month. The other girl presented with severe bloody diarrhea in which PD was insufficient in controlling her uremic symptoms, and intermittent HD was performed concomitantly with PD. After 45 days, in an effort to protect from chronic renal failure, eculizumab was started weekly at a dose of 900 mg for four weeks. She is still anuric and was diagnosed as chronic renal failure.

Laboratory findings of the D+HUS cases are presented in Table III according to inhabitation region. Of 70 patients, complement C3 levels were reported in only 56, and 22 (39%) of them were found to be low.

Stool specimens of only 29 cases were sent for evaluation of STEC in a mean of 10.2 ± 8 (range: 1- 27) days after the onset of diarrhea. From these, only four were positive: two *E. coli* O104:H4 (from the outbreak zone), one *E. coli* O145: H (-), and one *E. coli* ONT: H5 were identified. It was not possible to identify the definitive causative agent or the source of the causative agent. Only one case from

Table IV. Clinical and Laboratory Characteristics According to Disease Severity

	Non-severe disease	Severe disease	p*
Age	7.40±4.51 (1.60-16)	6.68±4.75 (1-16.20)	0.336
Duration of prodrome (days)	7.56±5.37 (3-30)	5.27±4.71 (1-26)	0.015
Minimum hemoglobin (g/dl)	6.59±0.95 (5.1-9.5)	6.73±1.43 (4.4-11.5)	0.825
Minimum platelet (/μl)	53848±41274 (8000-149000)	60396±37825 (10400-149000)	0.230
PNL** on admission (/μl)	8281±5130 (1500-24000)	11770±8183 (2300-35300)	0.062
Minimum haptoglobin (mg/dl)	7.71±9.28 (0.07-50)	37.39±58.78 (0-220)	0.011
Maximum LDH (U/L)	2243±1432 (648-7094)	2708±1221 (473-5984)	0.024
Transfused erythrocyte suspension (n)	1.97±1.32 (0-6)	2.73±2.27 (0-8)	0.201
Transfused platelet suspension (n)	0.85±2.18 (0-8)	1.23±3.27 (0-16)	0.581
Plasma exchange (n)	2.38±7.20 (0-43)	1.97±3.84 (0-14)	0.373

*Mann-Whitney U test
**Polymorphonuclear leukocyte

central Anatolia had a history of travel from Germany. No common food, visits to markets, restaurants or events, animal contact, or leisure activity was identified in the outbreak zone based on responses to a special questionnaire of the Turkish Ministry of Health. None of the cases reported eating sprouts.

Final results of the patients were: 44 full recoveries, 5 lost to follow-up, 3 exitus, 4 with chronic renal disease, 8 with proteinuria, 4 with hypertension, and 2 with hypertension and proteinuria.

Comparison of clinical and laboratory characteristics of patients according to the severity of disease are shown in Table IV. Duration of prodrome (p= 0.015), minimum haptoglobin level (p=0.011) and maximum lactate dehydrogenase (LDH) level (0.024) were significantly different between the groups according to disease severity.

Minimum haptoglobin level and maximum LDH levels were significantly high in the severe disease group. It was decided to use these parameters as indicators in predicting disease severity. However, according to multiple regression analysis, they were insignificant. Evaluation of the parameters with multiple regression analysis revealed that elevated polymorphonuclear leukocyte count during hospital admission was the predictor of both severe disease (p=0.032) and poor outcome (0.016). Duration of prodrome was found to be the predictor of poor outcome (p=0.023).

Discussion

Diarrhea-related hemolytic uremic syndrome (D+HUS) is one of the rare causes of acute kidney injury in Turkey. In the 2010 acute kidney injury report of the TSPN, the total number of HUS cases was 18 from 17 centers[1]. In another report about the etiology of renal

failure from southeast Anatolia in Turkey, HUS accounted for only 2% of all renal failures during a five-year period. In that report, the very low incidence of HUS was explained by the rarity of eating hamburgers, well-developed conventional cooking practices, and a possible low incidence of enterohemorrhagic *E. coli* (EHEC) infections[2].

After the German outbreak in May and June 2011, the Turkish Ministry of Health published an informative report about the prevention and diagnosis of STEC HUS and distributed it at the end of June[3]. At the end of August, the TSPN mailing group discussed the increased number of D+HUS cases, and a data collection form was distributed. Evaluation of the data revealed a seasonal and regional outbreak of D+HUS (Table I). However, the number of bloody diarrhea cases was not increased according to the local health department's declaration. Somehow, the number of HUS cases was significantly increased throughout Turkey throughout the year, and also a local outbreak was accepted according to the reported case numbers (Table I). Sex distribution (Table I) and clinical findings (Table II) were similar; however, the median age was greater from the previously reported international HUS series[5,7-11]. The number of stool specimens (n=29) sent to the Refik Saydam Hıfzıssıhha Center and time elapsed before the stool specimens were taken highlighted that the information report distributed by the Turkish Ministry of Health did not achieve the goal. From these, three different STEC types were identified from only four patients' stool specimens. *E. coli* O104:H4 was identified in two patients from the outbreak zone. However, the outbreak remained limited both to children and the outbreak zone. That is, different age and sex distribution of the German outbreak occurring with *E. coli* O104:H4 did not present both in the outbreak zone and outside of the outbreak zone[3]. From 70 patients, only one had a history of travel to Germany, and he was reported from outside the outbreak zone. Urban inhabitation of the HUS cases as an outbreak revealed that social, cultural, economical, and environmental factors in Turkey resemble those in industrialized countries[5, 7-11].

The rate of patients with CNS involvement, severe disease, poor prognosis, and mortality was not unusual[5, 7-11]. Fifty-one (72.9%) patients required dialysis. The most frequent modality was PD. Twenty patients were treated with plasma exchange. Only half of them had CNS involvement. That means some patients without CNS involvement were treated with plasma exchange at the initiative of the local doctor.

Lapeyraque et al.[12] published the success of eculizumab in CNS involvement in three patients with STEC-HUS that did not respond to plasma exchange in May 2011 during the German outbreak. After this report, 193 patients were treated in combination with plasma exchange and eculizumab (PE-Ecu). Those were severely ill patients according to the patients treated with best supportive care (n=57) and plasma exchange (n=241), and as such, a direct comparison of the three treatment groups was not possible. At the study endpoint, the median creatinine was higher in the PE-Ecu group. Need for dialysis and total hospital mortality did not differ significantly between the plasma exchange and PE-Ecu groups. With the limitations of the database, results do not support the notion that PE-Ecu in comparison to plasma exchange offers a marked short-term benefit in the treatment of STEC-HUS over plasma exchange alone[13]. Of the four patients requiring eculizumab in our database, two had CNS involvement resistant to plasma exchange, and CNS symptoms subsided very quickly as reported previously[12, 14]. However, three of these patients had some renal sequelae three months after discharge, which is in accordance with the endpoint results of the German database[13]. The duration of prodrome was found to be a significant predictor of poor outcome; elevated polymorphonuclear leukocyte count during admission was found to be a significant predictor of severe disease and poor outcome, as reported previously[5].

In conclusion, an increased number of D+HUS cases throughout Turkey in 2011 attracted attention. With increasing numbers of D+HUS, identification and surveillance of STEC is mandatory in a systemic base, as in Germany[15]. The Turkish Ministry of Health and pediatric nephrology centers should work together to determine the definitive causative agent for limitation of outbreaks in the future.

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