Diagnosis and Management of Atypical Hemolytic Uremic Syndrome In Children: Single Centre Experience

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Abstract Atypical hemolytic uremic syndrome (aHUS) although rare is the commonest cause of acute renal failure (ARF) in children and has poor prognosis. We present single centre experience of aHUS. Thirty six children (29 males, 7 females) with mean age, 7.9 years presented with ARF, 2 children also had tonic-clonic type convulsions. Their hematology examination revealed hemolytic anemia with s. creatinine (SCr), 5.54 mg/dl. Acute HUS was observed in 75 %, acute on chronic HUS in 19.4 % and patchy cortical necrosis (PCN) in 5.6 % biopsies. Mean 5.4 plasma exchanges (PE) were carried out. Supportive management of anti-hypertensives and prednisone was also given. Recovery end points were establishment of urine output, improvement of SCr and hematological profile. Hematology and renal function profile improved variably in all children, 5.6 % died, relapse was observed in 80.5 % over mean 70 days; 13.9 % children are doing well over mean follow-up of 268.8 days. Thus poor prognosis was observed in 86.1 % children. Children with acute on chronic HUS and PCN did not recover. Six children who recovered had acute HUS. aHUS in Indian children occurs at an older age of around 8 years and chronic/irreversible

changes on histopathology examination are harbingers of poor prognosis. PE is life-saving however further research for developing strategies to improve long-term survival is needed.

Keywords Hemolytic uremic syndrome · Thrombotic thrombocytopenic purpura · Plasma exchange · Microangiopathic hemolytic anemia

Abbreviations

ARF	Acute renal failure
aHUS	Atypical hemolytic uremic syndrome
CAPD	Continuous ambulatory peritoneal dialysis
CBC	Complete blood counts
CRF	Chronic renal failure
FFP	Fresh frozen plasma
Hb	Hemoglobin
HUS	Hemolytic uremic syndrome
IF	Immunofluorescence
LDH	Lactic dehydrogenase
MAHA	Microangiopathic hemolytic anemia
PE	Plasma exchange
SCr	Serum creatinine
TLC	Total leucocyte count
VWF	Von Willebrand factor

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Introduction

Hemolytic uremic syndrome (HUS) is rare with an overall incidence of 2 cases/100,000 persons/year and 6 per 100,000/year in children below 5 years of age [1]. Most common affection in children is HUS secondary to infection by certain strains of *Escherichia coli* (serotypes

O157:H7, O111:H8, O103:H2, O123, O26, and others) or *Shigella dysenteriae*, manifesting as bloody diarrhea (D+ HUS). About 10 % HUS are not caused by bacterial infections and are classified as atypical HUS (aHUS) which have poorer prognosis with mortality of up to 25 % in acute phase and about 50 % progress to chronic renal failure (CRF) [2, 3]. HUS is characterized by the triad of microangiopathic hemolytic anemia (MAHA), thrombocytopenia and acute renal failure (ARF). Low serum C3 with normal C4 levels and elevated activated products C3b, C3c and C3d along with deposition of C3 in glomeruli and arterioles have been reported, reflecting complement activation and consumption in this disease [4, 5]. Therapeutic plasma exchange (PE) has been recommended for all types of HUS [6].

We present our experience of PE in pediatric patients with aHUS.

Materials and Methods

This was a retrospective analysis of 36 pediatric patients who had been treated in our institute for acute/acute on chronic HUS between 2005 and '10. All the children in age group 1–14 years who had presented with MAHA with ARF were included in the study. Those patients who were not willing for biopsy/PE were excluded from the study. Their clinical presentation was noted. Lab investigations included complete blood counts (CBC), peripheral smear study, platelet counts, serum lactic dehydrogenase (LDH) and reticulocyte counts for evidence of hemolysis, and s. creatinine and blood urea, for evaluating renal function status at the time of presentation. Urinalysis was also performed periodically as and when the patients could pass urine. Renal biopsies were performed to evaluate the cause of ARF. Tissues were subjected to 3 μ thick paraffin sections for light microscopy (LM) and stained by hematoxylin and eosin, Periodic Acid Schiff, Jone's silver methanamine, and Gomori's trichrome stains. For direct immunofluorescence (IF) studies, anti-human IgA, IgG, IgM, C1q, C3, albumin and fibrinogen antisera (Dako, USA) were used on 3 μ thick frozen sections. Findings were graded as trace, +1, +2, +3 and +4 and intensity of $\geq$ +2 was considered as positive.

In event of reversible injuries, they were treated with steroids (Prednisone, 40 mg/day) and PE (Cobe Spectra version 7, Gambro, China). PE was performed on alternate days till clinical recovery was observed. In patients with irreversible injuries noted in the form of patchy cortical necrosis continuous ambulatory peritoneal dialysis (CAPD) was offered.

PE was carried out under low spin to remove the antibodies/insulting agents. In every cycle approximately 60 % (1–1.5 l) total body plasma volume was removed and 90 %

of the removed plasma was replaced by crystalloids (60 %) in the form of normal saline or dextrose saline, and colloids (40 %) in the form of fresh frozen plasma (FFP) +20 % human albumin in 2:1 proportion. FFP was used to supplement factors lost. Injection calcium gluconate, 20 ml diluted in 100 ml dextrose saline was simultaneously administered to prevent citrate toxicity. Crystalloid: colloid replacement was maintained in the proportion of 60:40 %. Patients were monitored for hemodynamic stability, citrate toxicity and allergic reactions during PE. Supportive management with anti-hypertensives was also given.

End points of recovery were establishment of urine output, improvement of SCr and hematological profile. Prednisone was tapered to maintenance dose of 10 mg/day with recovery.

There were 36 (29 males, 7 females) children with mean age, 7.9 ± 3.1 years (range: 2–14 years). All of them presented with ARF with fever, edema, oliguria/hematuria, joint pains and hypertension. These symptoms were noted for mean 15.9 ± 5.4 days (range: 6–23 days) before presentation. Two children also had tonic-clonic type of convulsions. None of them had any episode of diarrhea or gastrointestinal infection.

Mean hemoglobin (Hb) was 7.1 ± 2.3 gm/dl, total leucocyte count, $13.6 \pm 2.3 \times 10^3$ cells/ μ l, platelet count, $0.82 \pm 0.65 \times 10^5$ / μ l, s. LDH, 1475.9 ± 1035.8 U/l (normal range: 90–190 U/l), SCr, 5.54 ± 2.23 mg/dl and blood urea, 194.9 ± 60.77 mg/dl before PE. All the patients had MAHA in their peripheral blood smears. Urine examination showed reddish brown colored samples with albumin, +2 (range: trace to +4) and microscopy revealed plenty of crenated and/or dysmorphic RBCs/high power field.

Acute HUS was observed in 75 % ($n = 27$), acute on chronic HUS in 19.4 % ($n = 7$) and acute patchy cortical necrosis in 5.6 % ($n = 2$) renal biopsies. In acute HUS there was moderate mesangial prominence with variable mesangiolysis and ectatically dilated bloodless capillaries or occasionally congested capillaries lined by membranes of normal thickness. There was moderate to marked tubular necrosis, interstitial edema and diffuse infiltration with few mononuclear cells. Medium caliber arteries showed variable mucointimal proliferation with subintimal fibrin deposition and partial luminal obliteration, and small caliber arteries showed segmental subintimal hyalinosis. In biopsies with acute on chronic HUS there were associated changes of duplication and wrinkling/thickening of glomerular capillaries and focal tubular atrophy with interstitial fibrosis in addition to acute changes mentioned previously (Fig. 1). On IF studies there was fine granular fluorescence (+1/+4) across most of the glomerular capillaries and 30–45 % mesangial regions on staining with anti-human fibrinogen, and (trace/+3) across 60–80 %

Fig. 1 Microphotograph showing one glomerulus on the *left* and a medium caliber artery on its *right* along with part of two glomeruli with surrounding tubules. Glomeruli show mesangiolytic changes with wrinkled and duplicated capillary membranes and artery shows marked mucointimal proliferation with subintimal focal fibrinoid necrosis characteristic of acute on chronic hemolytic uremic syndrome; Periodic Acid Schiff Stain, $\times 100$

mesangial regions and 40–60 % capillaries of all glomeruli on staining with anti-human IgM and C3 antisera in all biopsies. No other significant immune deposits were observed.

Mean 5.4 ± 1.5 (range: 2–8) PE were carried out.

Statistical Analysis

Student's paired *t* test for equal variances was carried out on all parameters studied to test the efficacy of PE; *P* value of <0.05 was considered as statistically significant.

Results

Recovery

All PE procedures were uneventful. Patients started improving clinically after PE. Hb improved to 8.9 ± 2.2 gm/dl, total leucocyte count to $8.13 \pm 2.49 \times 10^3$ cells/ μ l, platelet count

to $1.61 \pm 1.58 \times 10^5$ / μ l, s. LDH to 371.5 ± 269.5 U/l, SCr to 3.16 ± 1.86 mg/dl and blood urea to 78.3 ± 35.98 mg/dl after PE. (Table 1). Urine out put was established to mean 1.5 ± 0.52 (0.9–2.3) l/day following PE with decrease in urine albumin with a mean of +1 (range: nil to +3) and decreased intensity of red discoloration as well as hematuria. The improvement was found to be statistically significant in all parameters as mentioned in Table 1.

Over a mean follow-up of 268.8 ± 152.7 days (range: 168–456) days, 5 (13.9 %) children (all males) are doing well with mean SCr of 0.88 ± 0.2 mg/dl (range: 0.62–1.1 mg/dl) with unremarkable hematological profile and are under close observation.

Relapse

Out of 36 children, 5.6 % ($n = 2$) died in spite of PE and supportive management, relapse was observed in 80.5 % ($n = 29$) children over a mean of 70 ± 91.2 days (range: 61–350 days). All of them are on CAPD. Thus long-term prognosis was poor in 86.1 % children.

Both children who died were 2 and 3 years respectively and had presented with acute aHUS. All the children with acute on chronic HUS and patchy cortical necrosis on histology examination did not recover. The mean age of 5 children who recovered, was 9 ± 3.1 years and all had histological findings of acute HUS. They were subjected to mean 6 ± 1.79 PE (range: 4–8) procedures after which their renal and hematological findings had started improving and currently are under close monitoring with prednisone, 10 mg/day and other supportive management.

Discussion

HUS is the most common cause of ARF in children, with aHUS affecting slightly older children with mean age 4.6 years as compared to D+ HUS typically affecting children around 2 years [7, 8]. The term HUS was first used by Gasser and co-workers in 1955 when they found acute fatal syndrome in children that featured hemolytic anemia,

Table 1 Comparison of parameters pre and post-plasma exchange

Parameter ^a	Pre-plasma exchange	Post-plasma exchange	<i>P</i> value
Urine out-put (litres/day)	<100	1.5 ± 0.52 (0.9–2.3)	<0.0001
Hemoglobin (gm/dl)	7.1 ± 2.3 (4.0–12.4)	8.9 ± 2.2 (4.8–13.2)	0.001
Total leucocyte count ($n \times 10^3$ / μ l)	13.6 ± 2.91 (7.5–23.2)	8.13 ± 2.49 (4.8–14.3)	<0.0001
Platelet count ($n \times 10^5$ / μ l)	0.82 ± 0.65 (0.3–1.6)	1.61 ± 1.58 (0.7–5.39)	0.01
S. Lactic dehydrogenase (U/l)	1475.9 ± 1035.8 (380–4850)	371.5 ± 269.5 (190–546)	0.0001
Serum creatinine (mg/dl)	5.54 ± 2.23 (1.03–9.8)	3.16 ± 1.86 (0.53–6.8)	0.0001
Blood urea (mg/dl)	194.9 ± 60.77 (92.5–306)	78.33 ± 35.98 (16–136)	<0.0001

^a All parameters are in mean values with ranges in parenthesis

thrombocytopenia and severe renal failure [9]. More research was carried out and subsequently its different types were identified, role of complement anomalies and association with renal and other systemic diseases were found [8].

It is believed that the formation of occlusive platelet aggregates in the terminal arterioles and capillaries leads to ischaemic organ dysfunction, platelet consumption and shearing of erythrocytes. Abnormalities of plasma von Willebrand factor (VWF) have been linked to TTP and there is an excess of unusually large VWF multimers [10–12]. Recent studies suggest that deficiency of a VWF-cleaving protease (termed ADAMTS13) may be responsible for the presence of these multimers. Multiple mutations of the ADAMTS13 gene can result in ADAMTS13 deficiency and cause congenital TTP, while acquired TTP may be linked to autoantibodies neutralizing ADAMTS13 protease activity [13, 14]. PE has to date, been the only established mainstay of therapy for the past 20 years. This hypothesis explains why PE may be so effective; it removes all the antibodies against cleaving proteases and replaces fresh proteases [15].

Our study shows that mean age of our patients was 7.9 years, which differs from other studies stating mean age of 4 years and interestingly all of them were aHUS. Two children who had tonic-clonic convulsions could be cases of TTP. Majority of our patients were males; although no gender predilection has been recorded in literature our observation could reflect the tendency to care and save male children in our society.

Regarding PE procedures, our study correlates with that of other workers that PE is life-saving procedure for acute HUS [6, 15–31]. We planned our own PE treatment protocol after reviewing other studies where it was found that FFP was useful to replenish the depleted plasma factors and we did not encounter any serious allergic reactions to FFP or hemodynamic instability [16].

The limitation of our study was that we considered only PE as the mainstay of management as advised in literature. Endoxan was not used in our patients due to lower platelet counts. We also could not carry out genetic studies however there was no familial incidence in any of our patients. Perhaps early admission could have improved the prognosis. More research is required in understanding this enigmatic disease to improve the quality and survival of children suffering from this disease.

Conclusion

To our knowledge this is the first study on diagnosis and management of aHUS in Indian children. We have observed that manifestation of aHUS in Indian children

occurs at an older age of around 8 years and chronic/irreversible changes on histopathology examination are harbingers of poor prognosis. PE is definitely life-saving to tide over the emergencies however further research for developing strategies to cure HUS/TTP or at least improve and preserve renal functions are needed.

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