# Anti-Compliment Factor H Antibody Associated Hemolytic Uremic Syndrome in Children with Abbreviated Plasma Exchanges: A 12-Month Follow-up Study

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**Keywords.** anti- complement factor H (CFH) antibody, hemolytic uremic syndrome (HUS), immunosuppression, plasma exchange

**Introduction.** Anti-Compliment Factor H antibody hemolytic uremic syndrome (AFH-HUS) is a common cause of paediatric atypical HUS in India. We wanted to study the outcome of patients receiving less than recommended plasma exchange (PLEX) but adequate immunosuppression, with respect to hypertension, preservation of renal function and proteinuria.

**Methods.** A retrospective study was performed in 15 children admitted from 2016 to 2018 with AFH-HUS. Follow up details including physical examination, hematological parameters, renal function test and urine examination performed at 3, 6, and 12 months were noted. Risk stratification and staging for chronic kidney disease (CKD) were done according to the Kidney Disease Improving Global Outcomes (KDOQI) guidelines. Standard statistical tests which were appropriate were used.

**Results.** Mean age of our study cohort was 7.8 ± 1.9 years. 14 children had hypertension. Mean nadir hemoglobin was 5.8 ± 1.0 g/dL and platelet = 58 ± 37.7 × $10^9$ cells/L. Median anti factor H (AFH) level was 316 AU/mL (150 to 452). Hemodialysis was required in 7 children. Fourteen children received PLEX with a mean of 11 ± 6 cycles. Thirteen children received 6 cycles of intravenous cyclophosphamide. After six months, therapy was switched to mycophenolate mofetil in 4 children, steroids alone in 2 children and 9 children with azathioprine. On follow-up, risk of CKD reduced from 80% at 3 months to 26% at 12 months ($P$ = .01). Only 40% patients had CKD stage 2 at the end of 12 months (mean eGFR = 95.0 ± 19.4 mL/min/1.73m$^2$).

**Conclusion.** The adequate number of PLEX needed in AFH-HUS needs further studies. Till such reports come, PLEX in recommended strategies or lesser, if not available, with immunosuppression in AFH-HUS can decrease progression to CKD.

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## INTRODUCTION

Anti-Complement Factor H antibody associated haemolytic uremic syndrome (AFH-HUS) is the most common cause of atypical HUS among children in India.[1] They present characteristically with haemolytic anemia, thrombocytopenia and acute kidney injury (AKI). It occurs due to antibodies against Factor H of alternate complement

pathway that results in dysregulated activation of complement cascade.[2] Treatment of AFH-HUS is predominantly plasma exchange (PLEX) to remove circulating antibodies and immunosuppression to suppress their further production.[1] As with other causes of AKI, AFH-HUS can be prevented from progression to chronic kidney disease (CKD) by timely and appropriate intervention. Data on natural history of this disease is scarce, more so from developing countries where feasibility of 3 to 5 weeks of PLEX is almost questionable. A retrospective study from our centre revealed that the median plasma exchanges that children with HUS (Thrombotic microangiopathy [TMA], complement mediated) is 6 (IQR 4, 9), which is far less than recommended.[3] Therefore, we performed a retrospective study of AFH-HUS children admitted in our centre to look for evolution of their renal illness despite receiving fewer plasma exchanges.

## MATERIALS AND METHODS

We performed a retrospective study in which 15 children with AFH-HUS from 2016 to 2018 were included in the study. Relevant clinical information was documented along with appropriate investigations. All these children were confirmed for the presence of AFH antibody.

### Definitions Used

**Hypertension.** Blood pressure was measured and staged according to AAP guidelines.[4]

**Anemia.** Children were categorized for anemia according to the WHO classification taking ≤ 7g/dL as severe anemia.[5]

**Estimated Glomerular Filtration Rate (eGFR).** Serum creatinine was determined by enzymatic assay. eGFR was derived by using the bedside Schwartz formula which utilizes length/ height of the child in centimetre and serum creatinine values in mg/dL.[6]

Modified Schwartz Formula. Glomerular filtration rate was estimated using serum creatinine and height in cm.

eGFR = 0.413 × height (cm) / serum creatinine (mg/dL)

**Proteinuria.** Spot urine protein creatinine ratio (PCR) of ≥ 2 was taken as nephrotic range proteinuria and between 0.2 to < 2 as sub-nephrotic range proteinuria.

**Hematological Remission.** Defined by platelets > $100 \times 10^9$ /L, fragmented RBCs < 2% and LDH less than upper limit of normal for 2 consecutive days.

**Therapy Protocol.** Details of therapy received (number of plasma exchange cycles, drug used for immunosuppression) and their outcome were noted. Centrifugal therapeutic plasma exchange was offered as soon as we suspect HUS, with 1.5 times plasma volume exchanges per session using fresh frozen plasma as replacement fluid, every day and stopped as soon as haematological remission was achieved.

Immunosuppressants were offered as soon as we detect the high antibody titres. Methylprednisolone (30 mg/kg/dose) was given for initial 5 days, followed by oral steroids, prednisolone (2 mg/kg/d) for a month then tapering it every 3 weekly by 0.25 mg/kg till a dose of 0.25 mg/kg which was continued for 12 months and stopped. Intravenous cyclophosphamide (500 mg/$m^2$) was given monthly for 6 months. The choice of maintenance therapy between azathioprine (2 to 4 mg/kg/d) and mycophenolate mofetil (1000 mg/$m^2$) depended on the affordability and choice of patients and was given for 2 years.

This was similar to the consensus guideline of the Indian Society of Paediatric Nephrology, except that in our centre, plasma exchange was abruptly stopped and oral steroids were given at a higher dose.[7]

For hypertension, most patients were started on ß blocker labetalol. However, on follow up for those who were hypertensive, a switch to enalapril was done once their eGFR improved to more than 60 mL/min/1.73$m^2$.

**Follow-up Protocol.** These patients were followed according to the unit's protocol at timely intervals for 12 months (at 3, 6, and 12 months). Blood pressure in the non-dominant hand by oscillometric method, height in cm was documented and compliance to immunosuppressive drugs was checked at each visit. Hematological parameters, blood urea, serum creatinine, urine microscopy for hematuria and urine analysis for albuminuria by dipstick [further quantified by spot urine protein-creatinine ratio (PCR)] were also performed at each visit. Urine dipstick more than 2+ were quantified with corresponding spot urine PCR and any value more than or equal to 2 mg/mg was considered to be nephrotic range proteinuria.

CKD risk stratification was done according

to KDIGO (Kidney Disease Improving Global Outcomes) as low risk, moderate risk, high risk and very high risk according to albuminuria and eGFR categories.[8]

CKD staging was performed using KDOQI guidelines using eGFR values from stage 1 to stage 5.[9]

**Anti-complement factor H antibody analysis.** Anti-CFH antibody (IgG) testing was done by ELISA method using VIDITEST ELISA kit (Intra-Assay: CV < 6%, Inter-Assay < 10%, Detection limit: 0.6 AU/mL). IgG anti-CFH titre more than 26 AU/mL was considered to be significant as suggested by the manufacturer (MoBiTec- CE IVD certified kit).

Factor H, Factor I, Factor B, ADAMTS-13, and anti ADAMTS levels were not performed as per our Institute protocol.

## Statistical Analysis

Data were tabulated and analysed using SPSS software (version 20). Standard statistical tests were used for analysis. All quantitative data were analysed for their normal distribution using tests of normality (Kolmogrov test). Descriptive statistics was used for baseline comparison. For skewed data (age, creatinine, TLC) non-parametric test was used for comparison of medians. Trend of eGFR, CKD staging, albuminuria and hypertension was statistically tested using Cuzick's test for continuous variables or chi-square test for trend for categorical variables across follow-up at 3 months, 6 months, and 12 months. When a median value is presented, the interquartile range is presented in brackets.

## RESULTS

15 children were enrolled. Mean age was 7.8 ± 1.9 years with a male-female ratio of 4:1. Most common symptom at presentation was oliguria followed by pallor. Hypertension at presentation was noted in 96% children (14/15) requiring a mean of 3 anti-hypertensive drugs. Mean hemoglobin level at admission was 5.8 ± 1.0 g/dL with severe anemia being noted in 96% (14). Mean platelet count at admission was 58 ± 38.7 × $10^9$ cells/L. Mean serum creatinine at its peak was 442 ± 221 µmol/L and median eGFR at the time of hospitalisation was 10.6 (6.8 to 19) mL/min/1.73m$^2$. Hematuria and nephrotic range proteinuria were noted in 46.7% (7) and 66% children (10), respectively (Table 1).

**Table 1.** Baseline Characteristics of Patients

| Characteristics | Values |
|---|---|
| Age (years) | 7.8 ± 1.9 |
| Sex (n) | |
| Male | 12 |
| Female | 3 |
| Hypertension | 14 / 15 |
| Mean Hemoglobin (g/dL) | 5.8 ± 1.0 |
| Mean Platelet Count ($10^9$ cells/L) | 58 ± 38.7 |
| Mean Serum Creatinine (µmol/L) | 442 ± 221 |
| Hematuria (%) | 46.6 (n: 7 / 15) |
| Nephrotic range Proteinuria (%) | 66.6 (n: 10 / 15) |

Mean C3 level was 67.5 ± 19.7 mg/dL (normal values: 50 to 150 mg/dL) with only one having a low level of 48 mg/dL. While mean C4 level was 18.7 ± 7.9 mg/dL (normal values: 20 to 50 mg/dL), three patients had low C4 level. Median AFH level was 316 AU/mL (150 to 452) before the initiation of treatment.

Plasma exchange therapy was performed in 14 children with mean number of cycles 11 ± 6 cycles. There were no complications noted during these procedures. Supportive therapy in the form of hemodialysis was required in 46% children. All children received steroids while 13 children also received 6 cyclophosphamide pulses. Among maintenance immunosuppression, mycophenolate mofetil was used in 4 children, steroids alone in 2 children while 9 children were on azathioprine. All children attained remission.

The mean eGFR at 3 months was 80.7 ± 25.4 mL/min/1.73m$^2$, while at 6 months and 12 months it was 83.8 ± 21.5 and 95.0 ± 19.4 mL/min/1.73m$^2$. At 3 months, stage 3 CKD was noted in 20% while stage 2 was noted in 46.7% children. However, at 12 months follow up, only 6 children (40%) were noted to have stage 2 CKD with none in stage 3 (Table 2, Figure 1).

One who did not received PLEX but received steroids, cyclophosphamide and azathioprine at 12 month had an eGFR of 77 mL/min/1.73m$^2$. Another who received only PLEX but no immunosuppressants, as the AFH autoantibody reports came late, at 12 month had an eGFR of 88 mL/min/1.73m$^2$. Further, one who refused cyclophosphamide and received only PLEX, steroids and Azathioprine at 12 month had an eGFR of 87 mL/min/1.73m$^2$.

Risk stratification for CKD at each point of time was performed (Figure 2). Overall risk of

**Table 2.** CKD Parameters at Follow-up 3, 6, and 12 months

| Parameters | 3 months | 6 months | 12 months | *P* |
|---|---|---|---|---|
| eGFR (mean ± SD), mL/min/1.73 $m^2$ | 80.7 ± 25.4 | 83.8 ± 21.5 | 95.0 ± 19.4 | < .05 |
| CKD stage (%) | | | | |
| Stage 2 | 46.7 | 46.7 | 40 | > .05 |
| Stage 3 | 20 | 20 | | |
| Risk for CKD (%) | 80 | 53 | 26.6 | < .05 |
| Albuminuria (%) | 66.6 | 33.3 | 26.6 | < .05 |
| Hypertension [n (%)] | 7 (46.7) | 4 (26.6) | 3 (20) | > .05 |

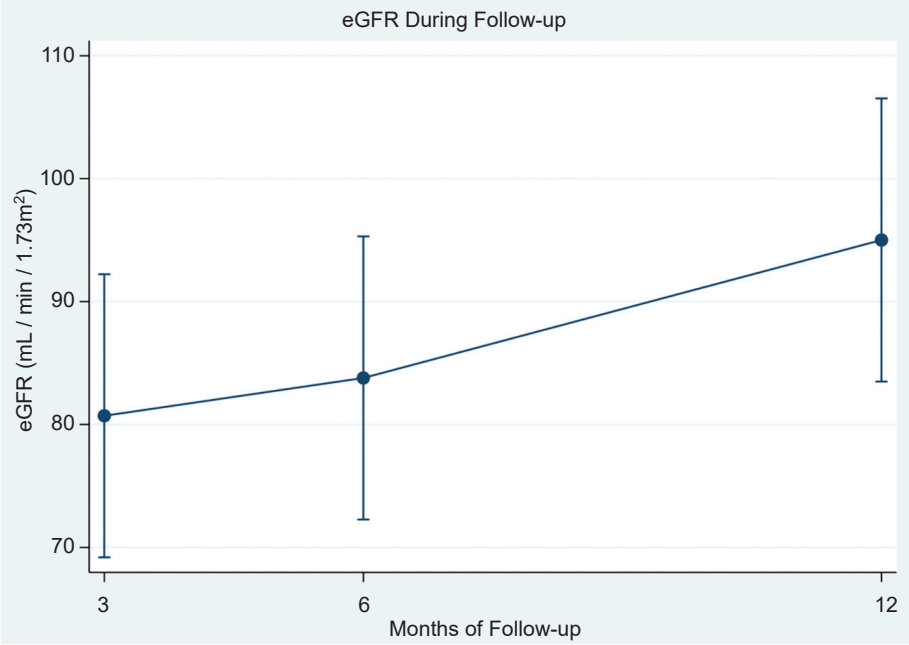


**Figure 1.** Trend of eGFR in children with AFH-HUS on follow up at 3, 6 and 12 months; respectively.

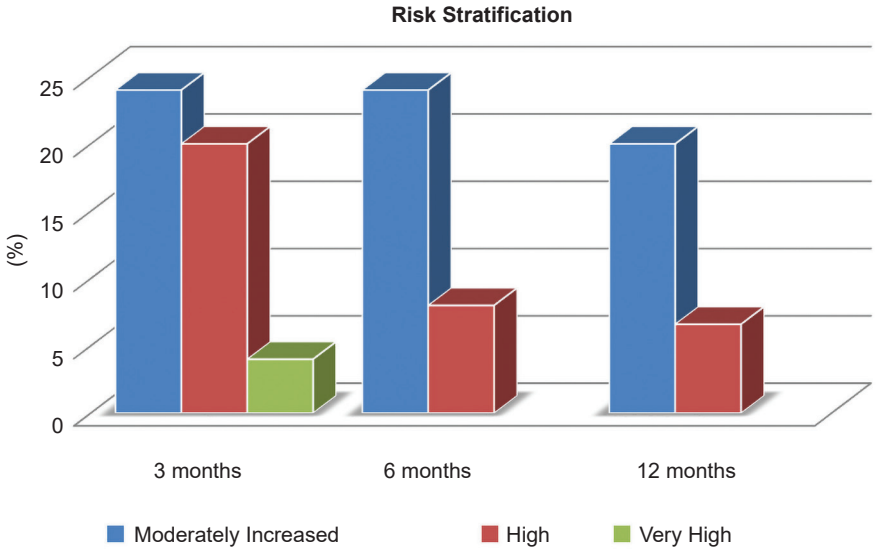


**Figure 2.** Risk stratification of children with AFH-HUS at 3 month, 6 month and 12 month interval on follow up.

developing CKD was 80, 53, and 26.6% at 3, 6, and 12 months; respectively. On assessing the trend of risk stratification at each point of time, there was a marked reduction in the proportion of children at risk during the course ($P < .05$, Figure 3).

Similarly the proportion of children with albuminuria was also on a decreasing trend through 3 months to 12 months of follow-up ($P < .05$).

After 12 months of follow-up, 40% children (6/15) had stage 2 CKD [median eGFR = 96.7 (78.3 to 109.6) mL/min/1.73m$^2$]. Out of these 6 patients, 3 did not received the combination of PLEX and immunosuppressant as outlined above. Of these 3, one also remain proteinuric. Hypertension, however, was present irrespective of treatment.

Only 3 children (20%) had hypertension requiring a median of 1 (1, 2) anti-hypertensive drug. They are currently continued on maintenance immunosuppression. There was no adverse effects noted due to immunosuppression in the reported children in terms of infections or other toxicity till the time this study was conducted.

## DISCUSSION

AFH-HUS is the most common form of atypical HUS in India. PLEX for 3 to 5 weeks and immunosuppression including steroids, cyclophosphamide, and mycophenolate mofetil are the only standard of care, especially in countries where eculizumab is not available. However, 3 to 5 weeks of PLEX may not be feasible for developing countries where affordability and technical expertise may not be widely available. Our retrospective study of children undergoing PLEX for HUS/TMA in fact showed only a median range of 6 procedures with a range of 1-22.[3] Thus, we performed this study to know their outcomes.

Age profile of children enrolled in our study was similar to previous studies suggesting that AFH-HUS is more prevalent in school going children.[1] A male dominant population was noted in our study with a sex ratio of 4:1. While this could be due to the fact that girl child in these regions are neglected, the possibility of disease being more common in boys cannot be ruled out. Baseline parameters like haemoglobin, platelet count and eGFR were similar to previous literature.[1,10] Hematuria was found to be in similar proportion of children as mentioned in previous studies, however children who had nephrotic range proteinuria were in larger proportion. While C3 levels were low in only one patient, three of our patients had low C4 levels. Low C4 level in AFH-HUS has been documented in literature.[11]

AFH-HUS apart from causing AKI is also known to persist and progress as CKD during the course of time. In a study by Durey *et al.* 39% patients showed a residual kidney disease with 27% reaching ESRD.[12] Noris *et al.* also reported 62% progression to ESRD among 8 children who were followed up for 36 months.[11] In our study 40% children had CKD stage 2 at the end of 12 months with none being dialysis dependant. Another multi-centric study by Puraswani M *et al.* had compared the outcomes of AFH-HUS treated in the last 6 years with PEX and immunosuppressant to those before, and showed that outcome has improved in present times. They, however stressed the need of long

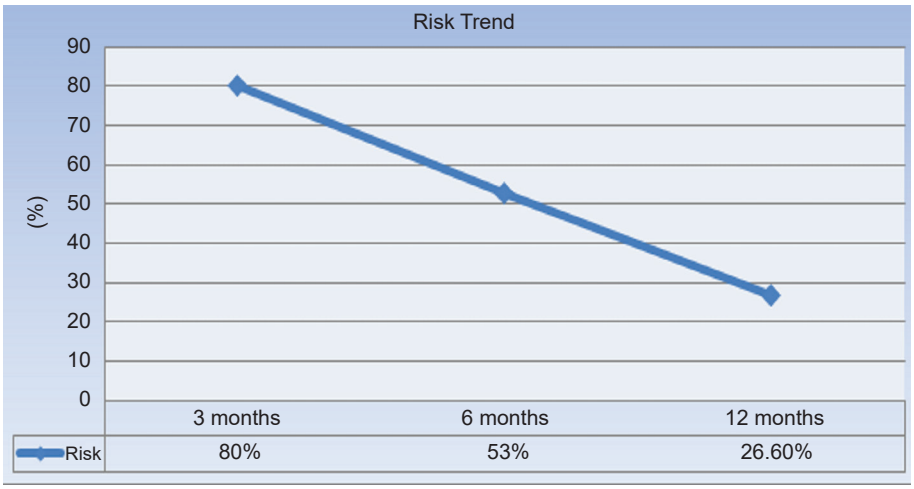


**Figure 3.** Proportion of children with AFH-HUS at risk for developing CKD at different time points (3, 6, and 12 months).

term follow up.[13]

Our study would be the first study to have a follow up data of AFH-HUS children in terms of risk stratification for CKD development. We noted that there was a significant decreasing trend in the proportion of children at risk for progressing to CKD during the follow-up. This throws a light on the fact that PLEX, even in less than recommended duration coupled with adequate immunosuppressant therapy can decrease the risk of progression to CKD. Interestingly, there was a decreasing trend in the proportion of children with albuminuria also, reiterating the point that albuminuria could be the major risk factor for CKD progression.

Most of our children received less number of PLEX cycles compared to standard protocol because of financial constraints. However they received PLEX till they attained haematological remission and adequate immunosuppression.

Literature suggests that outcome among children with aHUS varies depending predominantly on therapy provided. As per the international consensus guidelines, eculizumab is considered as the first line agent.[14] However, in developing countries where availability of eculizumab is still a matter of concern, plasma therapy is still considered as first line therapy.[7] On the other hand, PLEX is also a matter of concern due to its cost concerns and adverse events associated.[3] Adequate immunosuppression along with PLEX has showed a better outcome in aHUS.[1] Sana *et al*. also showed that children who received cyclophosphamide pulses had a normal renal function and decreased relapse rate.[15]

Along with these supporting evidences, interesting fact to be noted in our study is that, these children while on follow up had shown improvement in terms of renal function. This led us to suggest a hypothesis that abbreviated PLEX with adequate immunosuppression can help in treating disease and prevent sequalae. However, a larger sample size and longer follow up may be required.

Our study also had some limitations. It is a retrospective study with a very small sample size. Genetic studies were not performed in these children and hence particular genetic correlation for outcome could not be found. AFH titres during follow up which could not be done would have helped in supporting the fact of disease control, which was not done in our study. However, no child had clinical relapse during follow up which would be more important. Further, we had not done the work up of all the other complement proteins, which would have thrown a clearer picture.

## CONCLUSION

AFH-HUS children have the risk of progression from AKI to CKD. However appropriate therapy with PLEX (in recommended or even till haematological remission in resource limited settings) and immunosuppression can help in reducing the risk of progression and thereby making the kidney injury reversible. However, the used of abbreviated PLEX needs further controlled trials to be taken as a recommendation.

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## FUNDING

None.

## CONFLICT OF INTERESTS / COMPETING INTERESTS

None.

## ETHICS APPROVAL

The Departmental review board approved the study (Ref. No.DRB-196-20) Parental consent was taken in all and assent of children, wherever possible. Part of this paper was presented as an abstract in the "First Toronto Complement Conference", Toronto, Ontario, Canada on 6$^{th}$ to 7$^{th}$ March, 2020.

## AUTHOR'S CONTRIBUTION

KT conceptualized the idea, supervised the data collection and analysis, revised the manuscript critically for important intellectual content, approved the final version and agree to be accountable for all aspects of the work. SG collected the data, analysed it, made the first draft, approved the final version and agree to be accountable for all aspects of the work. LD supervised in collection of data, revised the manuscript critically for important intellectual content, approved the final version and agree to be accountable for all aspects of the work. AR, RR, RH supervised in interpretation of data, revised

the manuscript critically for important intellectual content, approved the final version and agree to be accountable for all aspects of the work.

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