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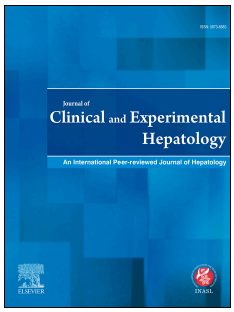

Severe Acute Hepatitis of unknown etiology presenting as pediatric acute liver failure: Analysis of likely etiology, clinical course and outcome

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Case Series

Severe Acute Hepatitis of unknown etiology presenting as pediatric acute liver failure: Analysis of likely etiology, clinical course and outcome

Short Running Title: Severe Acute Hepatitis

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Abbreviations:

ALF:	Acute Liver Failure
CMV:	Cytomegalovirus
DNA:	Deoxyribonucleic acid
EBV:	Epstein Barr Virus
GI:	Gastrointestinal
HAV:	Hepatitis A virus
HE:	Hepatic Encephalopathy
HHV:	Human Herpes Virus
ICP:	Intracranial pressure
IgM:	Immunoglobumin M
INR:	International normalized ratio
LT:	Liver Transplantation
MIS-C:	Multisystem inflammatory syndrome
NLS:	Native Liver Survival
PCR:	Polymerase chain reaction
SARS-COV2:	Severe acute respiratory syndrome- coronavirus-2
SPSS:	Statistical Package for Social Sciences

Abstract: Adenovirus, adeno-associated virus, and severe acute respiratory syndrome-coronavirus-2 (SARS-COV2) have been recently implicated as probable causative agents of severe acute hepatitis of unknown etiology reported from most of Europe. High mortality and liver transplantation (LT) rates have been observed in those presenting with acute liver failure (ALF). Such cases have not been reported from the Indian subcontinent. We analyzed the etiologies, clinical course, and in-hospital outcomes of cases of severe acute hepatitis with ALF presenting to us between May and October 2022. A total of 178 children presented with severe acute hepatitis of known/ unknown etiology including 28 presenting as ALF. Eight of them fulfilled the definition of severe acute hepatitis of unknown etiology presenting as ALF. Adenovirus was not associated with cases of ALF in these children. SARS-COV2 antibodies were detected in 6 (75%) of them. Children with severe acute hepatitis of unknown etiology presenting as ALF were young (median age 4 years), had hyper-acute presentation with a predominance of gastrointestinal symptoms, and a fulminant course with worse outcomes (native liver survival 25%). Expedited evaluation of these children for LT would be the key to management.

Keywords: Liver failure; liver failure, acute; Severe Acute hepatitis; Indeterminate ALF; pediatric acute liver failure; adenovirus; SARS-COV2; viral hepatitis

Introduction: A sudden surge of severe acute hepatitis of unknown etiology has been reported from around the world with over 1000 cases reported from 35 countries since March 2022 [1]. Adenovirus has been the most frequently detected pathogen in these cases accounting for as many as 52% of cases in Europe [1-2]. Adeno-associated virus (AAV2) has been detected in the blood and liver tissue in a proportion of these cases. However, AAV2 is non-pathogenic by itself and requires a helper virus such as adenovirus or herpesvirus for their replication and survival. Other postulated theories for the surge in cases include a novel pathogen, a novel severe acute respiratory syndrome – coronavirus 2 (SARS-COV2) variant, a variant of adenovirus, and environmental exposure to a drug/toxin [2]. Cases of severe acute hepatitis have only been reported from one center in India previously [2]. Cases developing acute liver failure (ALF) had a fulminant course and poor outcomes with only 25% native liver survival (NLS) in a recent study from London [3]. We describe the workup, clinical course, and outcome of children with severe acute hepatitis of unknown etiology presenting as ALF.

Methods: This is a collection of cases presenting with severe acute hepatitis over 6 months (May to October 2022) at a tertiary care pediatric hepatology and liver transplant referral center in India. Ethical approval for data retrieval from the hospital information system was obtained (IEC/2023/98/MA13). Severe acute hepatitis (with ALF) was defined as per the recent World Health Organization (WHO) definition to include children up to 16 years of age presenting with severe acute hepatitis (serum transaminases >500 IU/L) of unknown etiology (hepatitis A-E viruses excluded) and presence of ALF (International normalized ratio [INR] > 2) [4]. Data from children with known etiology of ALF during the same period were also collected for comparison. All cases of severe acute hepatitis (with ALF) underwent diagnostic evaluation for hepatotropic viruses (hepatitis A, B, C, and E), autoimmune hepatitis (immunoglobulin G and autoimmune markers), fulminant Wilson disease

(ceruloplasmin, 24 hours urinary copper), and rigorous history of drug intake. Patients, where no etiology could be found on first-line investigations, were labeled as having severe acute hepatitis of unknown etiology and subjected to second-line tests. The second-line tests included immunoglobumin M (IgM) cytomegalovirus (CMV), IgM Epstein Barr virus (EBV), parvovirus deoxyribonucleic acid (DNA) polymerase chain reaction (PCR) and IgM, adenovirus DNA PCR in blood and stool, SARS-COV2 PCR from the respiratory sample, SARS-COV2 IgG antibody, IgM dengue, and human herpes virus (HHV-6) DNA PCR. The clinical course and outcomes of those presenting with severe acute hepatitis and ALF have been described and compared to those with hepatitis A virus (HAV) induced ALF which is the commonest cause of ALF in India [5]. NLS was defined as survival with the native liver on day 28 of the presentation. Mann Whitney U test was used to compare the quantitative variables while the Fisher Exact test was used to compare categorical variables. All analysis was done using Statistical Package for Social Sciences (SPSS), IBM SPSS statistics version 22, IBM corporation, Armonk, NY.

Results: A total of 178 children fulfilled the definition of severe acute hepatitis during May-October 2022, of whom 28 presented as ALF. Although the number of cases of acute hepatitis has increased in 2022 compared to 2020-21, the proportion of severe acute hepatitis of unknown etiology among ALF has remained constant over the years **(Supplementary table 1)**. Of the 28 patients with ALF, 18 (64.3%) were HAV-induced ALF, while 8 (28.6%) had an unknown etiology. **Table 1** describes the clinical presentation and outcome of the 8 children with severe acute hepatitis of unknown etiology presenting as ALF. The median age of presentation was 4 years (range: 1.5 – 15 years); 6 (75%) were males. All viral markers were negative in these cases except a girl with detectable IgM parvovirus antibody and negative Parvovirus PCR. Six (75%) of these children (5 unvaccinated) had SARS-COV2 IgG antibodies. Adenovirus DNA PCR was negative in all 8 cases. All patients had a

hyperacute presentation with median jaundice to hepatic encephalopathy (HE) interval being 2 days. Five (62.5%) children had gastrointestinal (GI) symptoms (diarrhea, vomiting, abdominal pain) with the median duration of these symptoms being 3 days (range 1-10 days). All except 2 children (Patients 2 and 8) either had grade 3 or higher grades of HE at presentation or progressed to grade 3/ grade 4 HE during hospitalization. All of them had clinical evidence of raised intracranial pressure, increased optic nerve sheath diameter, high ammonia; all of them required mechanical ventilation and intracranial pressure (ICP) lowering therapies. All six patients fulfilled King's College criteria for liver transplantation (LT), and were started on high volume therapeutic plasma exchange awaiting preparation for LT. Patients with very high ammonia not responding to medical therapy were additionally started on continuous renal replacement therapy. The 2 children (Patients 2 and 8) who did not progress beyond grade 2 HE survived with their native liver; while all those with grade 3 or 4 HE died (4, 50%) or received LT (2, 25%). Most had a fulminant course with the median time from admission to death/LT being 2.5 days (range 1 – 14 days). Explant of the 2 patients and post-mortem biopsy of the 4 patients showed massive hepatic necrosis and large areas of parenchymal collapse. Compared to HAV-induced ALF, these children were younger (4 years vs 13 years), had higher INR (6.31 vs 2.9), had a greater prevalence of GI symptoms (62.5% vs 11.1%) and worse outcomes (NLS: 25% vs 50%) **[Table 2].**

Discussion: Children with severe acute hepatitis of unknown etiology presenting as ALF were young, had a hyperacute presentation with a predominance of GI symptoms, and a fulminant course with poor NLS. Adenovirus was not detected in the cases of severe acute hepatitis in Indian children. Adenovirus F41 is the commonest virus implicated in these cases reported from Europe and Japan [1,6-7]. Although adenovirus was not detected by PCR in any of our cases, the predilection to very young children and high prevalence of GI symptoms (62.5%) points to a possible novel variant of this virus in our cases. The presence of high

titres of SARS-COV2 antibodies in 6 cases (5 unvaccinated), points to the possibility of a recent or past SARS-COV2 infection in them. However, similar high seroprevalence (45.9% - 81.8%) have also been reported from population based pediatric studies in India during the same period [8-9]. None of the cases with SARS-COV2 IgG antibody positivity fulfilled the definition of multisystem inflammatory syndrome (MIS-C). SARS-COV2 alone or in association with another virus has been postulated to cause acute hepatitis, although the association remains unproven to date [7]. Moreover, few recent papers have described severe autoimmune hepatitis following SARS-COV2 vaccination [10-11]. SARS-COV2 virus or antibody against it was present in 16% of European cases, 8% of cases from the United States, and 8% of cases from Japan [6-7]. Morita et al. demonstrated widespread CD8+ T-cell activation (predominant Th1 type) in a patient presenting with severe acute hepatitis following SARS-COV2 infection suggesting the possible role of superantigen-mediated T-cell immune dysregulation [7]. Yet, there is a lack of any convincing evidence whether SARS-COV2 alone or as co-infection with adenovirus can lead to severe acute hepatitis.

The worrying factor is the poor outcome in these children with only 2 children (25%) surviving with their native liver. Two children received living donor LT and are doing well. Poor outcomes have been reported previously among cases of severe acute hepatitis presenting as ALF [3]. Deep et al. reported NLS of only 25% [3]. All their patients had advanced HE and signs of raised ICP. Adenovirus was not detected in the hepatocytes of the explants specimens. Massive hepatocyte necrosis was seen in their explants. In light of poor NLS, and fulminant course (median admission to LT/death time: 2.5 days), these children require expedited donor and recipient workup for LT, and bridging therapies while awaiting LT. The limitation of this study is it is a single-center retrospective study leading to a small sample size. Genetic sequencing to identify novel viruses/ microbes or novel variants might have a role in identification of the possible etiology of this outbreak. We conclude that

children with severe acute hepatitis of unknown etiology are young with predominance of GI symptoms, and have a rapidly progressive liver failure with low native liver survival as compared to HAV-induced ALF. Those with grade 3 and 4 HE and with high ammonia have poor outcomes. Use of bridging therapies and expedited evaluation for LT would be the key to survival in these cases.

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Conflict of Interest: Bikrant Bihari Lal, Vikrant Sood, Ekta Gupta, Reshu Agarwal, Rajeev Khanna, and Seema Alam declare no financial or non-financial conflict of interest

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Table 1: Clinical presentation, course, and outcome of children presenting with severe acute hepatitis of unknown etiology (with acute liver failure) from May to October 2022

Sl No	Age (years) / Gender	Jaundice to HE interval (Days)	Grade of HE	Other Symptoms	Bilirubin (total/ direct) (mg/dl)	Peak INR	AST (IU/L)	ALT (IU/L)	Ammonia (µg/dl)	Adenovirus PCR	SARS-COV2 Ab S/CO ratio	IgG/ AI markers	HVP/ CRRT	KCH criteria for LT	Outcome	Duration of ICU stay (Days)
1	14/F	10	2 →3	--	29.8/ 19.8	2.44	656	588	144	-ve	5.28	18.2/ ANA, ASMA, LKM1, SLA -ve	HVP x 3 sessions	3	LT	8
2	1.5/M	HE prior to jaundice	1 → 2	GI symptoms	1/0.5	3.8	8158	5317	154	-ve	3.05	6.8/ ANA, ASMA, LKM1, SLA –ve	No	2	Survived	7
3	3/M	HE prior to jaundice	2 → 3	GI symptoms	3.3/2	5.92	6976	5442	1785	-ve	3.24	12.8/ ND	HVP x 1 session	3	Death	1
4	15/M	8	4	Previous Acute hepatitis	13.6/7.6	6.7	3377	3540	346	-ve	2.26	23.3/ ANA, ASMA, LKM1, SLA –ve	HVP x 3 sessions	3	Death	3
5	1.5/M	1	2 → 3	GI symptoms	19.4/7.6	6.8	636	900	256	-ve	2.8	12.2/ ANA, ASMA, LKM1, SLA –ve	No	3	Death	2
6	4/M	1	3	GI symptoms	2.8/1.9	11.5	7800	7775	943	-ve	-ve	5.89/ ANA, ASMA, LKM1, SLA –ve	CRRT	2	Death	1
7	4/M	7	3	GI	23.8/	7.2	3341	1966	146	-ve	-ve	12.2/	HVP x 3	3	LT	14

				symptoms	13							ANA, ASMA, LKM1, SLA -ve	sessions, CRRT			
8	8/F	2	1	--	5.8/3.8	4.78	3198	2881	121	-ve	2.93	14.3/ ANA 1:20 +ve, ASMA, LKM1, SLA –ve		3	Survived	11

Abbreviations: HE: Hepatic encephalopathy; INR: International normalized ratio; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; PCR: Polymerase chain reaction; SARS-COV2 Ab S/CO: Severe acute respiratory syndrome coronavirus 2 antibody (sample: cut-off ratio); IgG: Immunoglobulin G; AI markers: autoimmune markers; HVP: High volume plasma exchange; CRRT: Continuous renal replacement therapy; ICU: Intensive care unit; F: female; M: male; GI: gastro-intestinal; ANA: anti nuclear antibody; ASMA: anti smooth muscle antibody; LKM1: anti liver-kidney-microsomal antibody; SLA: antibody against soluble liver antigen; LT: Liver transplantation; KCH criteria: King's College Hospital criteria for Liver transplantation

Table 2: Comparison of clinical presentation, biochemical parameters and outcome between indeterminate and hepatitis A virus related acute liver failure

Parameters	**Indeterminate ALF (n = 8) Median (IQR)/ n (%)**	**Hepatitis A induced ALF (n = 18) Median (IQR)/ n (%)**	**p value (non parametric)**
Age (Years)	***4 (1.5 – 14)***	***13 (9.8 – 15.3)***	***0.021***
Grade 3-4 hepatic encephalopathy	4 (50%)	11 (61.1%)	1.000
Jaundice to encephalopathy interval (days)	2 (1 – 10)	2.5 (2 – 6)	0.883
Gastrointestinal symptoms	**5 (62.5%)**	**2 (11.1%)**	**0.037**
Serum Bilirubin (mg/dl)	9.7 (2.8 – 23.8)	8.1 (5.8 – 20.2)	0.657
Serum Albumin (g/dl)	3 (2.9 – 3.4)	3.1 (3 – 3.3)	0.745
INR	***6.31 (3.8 – 7.2)***	***2.9 (2.1 – 4.5)***	***0.029***
AST (IU/L)	3359 (636 – 7800)	2288 (920 – 5063)	0.357
ALT (IU/L)	4981 (900 – 5442)	2872 (958-4850)	0.790
Ammonia (µg/dL)	205 (144 -943)	320 (222- 646)	0.615
Lactate (mmol/l)	3.8 (1.7 – 6.6)	4.4 (2.3 – 20.5)	0.604
Platelet count (x 10^9/ cu mm)	284 (208 – 481)	307 (160 – 402)	0.885
Serum creatinine (mg/dl)	0.3 (0.1 – 1)	0.7 (0.4 – 1)	0.221
Procalcitonin (ng/ml)	1.6 (0.4 – 75.6)	2.3 (1.3 – 12.7)	0.539
ICU stay (days)	5 (1-11)	7 (2-14)	0.381
Outcome			
Native Liver Survival	***2 (25%)***	***9 (50%)***	
Death	***4 (50%)***	***9 (50%)***	***0.041***
Liver Transplantation	***2 (25%)***	***0***	

Abbreviations: ALF: Acute liver failure; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ICU: Intensive Care unit; INR: International normalized ratio; IQR: Interquartile range

Credit Authorship Contribution Statement:

Bikrant Bihari Lal: Conceptualization; Data Curation; Formal analysis; Methodology; Visualization; Writing - original draft; Writing - review & editing

Vikrant Sood: Study design; Methodology; Writing - review & editing;

Ekta Gupta: Study design; Investigation; Data Curation; Writing - review & editing

Reshu Agarwal: Study design; Investigation; Data Curation; Writing - review & editing

Rajeev Khanna: Study design; Data Curation; Writing - review & editing

Seema Alam: Study design; Data Curation; Analysis; Writing - review & editing