

# **COVER PAGE**

**Title: Steroids in Fulminant Hepatitis A in the Pediatric Age Group**

**Clinical trial number:** NCT02375867

**Contents:** - Study Protocol - Statistical analysis plan – Results

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# STUDY PROTOCOL

## Background

Fulminant hepatic failure (FHF) (alternatively, acute liver failure [ALF]) is a rare but life-threatening critical illness that occurs most often in patients who do not have a preexisting liver disease. It is the clinical manifestation of liver cell death of a critical degree with insufficient hepatocellular regeneration and is characterized by hepatic encephalopathy and/or coagulopathy<sup>1</sup>. The mortality rate without liver transplantation (LT) is over 40% in patients with acetaminophen-induced FHF and 80% in those with nonacetaminophen-related FHF<sup>2</sup>. This 80% decreases to 30% when LT and artificial liver support system are available<sup>3</sup>. Hepatitis A virus (HAV) is considered to be the commonest cause of acute viral hepatitis worldwide. In acute infection with HAV, a cellular immune response attacking virus-infected cells is responsible for the clinical manifestations and the recovery mediated by CD8+ T cells and CD4+ T cells, in addition to B cell response producing immunoglobulin [Ig]M and IgG according to the time-course of the disease. HAV infection can cause FHF and death in about 0.2% of clinical cases<sup>4</sup>. Extensive destruction of infected hepatocytes by T cell-mediated lysis was supposed to be the cause of FHF<sup>5</sup>. Hepatitis A virus (HAV) is the most common cause of acute viral hepatitis worldwide. In acute HAV infection, a cellular immune response attacking virus-infected cells is responsible for the clinical manifestations and the recovery mediated by CD8+ T cells and CD4+ T cells, in addition to B cell response producing immunoglobulin [Ig]M and IgG according to the time-course of the disease. Although rare, HAV infection can cause FHF and death in approximately 0.2% of clinical cases<sup>4</sup>. Overwhelming elimination of infected hepatocytes by T cell-mediated lysis was supposed to be the cause of FHF<sup>5</sup>.

## **Material and methods**

### ***Study population:***

This study included 26 children with fulminant hepatitis A. They were recruited over a period of 4 years duration and divided into two groups. Group one is comprised of 18 children with fulminant HAV infection who received steroid therapy beside standard supportive measures (steroid group). The second group is comprised of 15 children with fulminant HAV infection who did not receive steroid therapy, but only standard supportive measures (non steroid group). All cases were recruited from the department of Pediatric Hepatology, Gastroenterology and Nutrition, National Liver Institute, Menofiya University. Those patients whose parents accepted to receive steroids were allocated in the steroids group; others were allocated in the control group. From each group a random sample was chosen using SPSS. An informed consent was signed by the parents of the steroid-treated patients before recruitment in the study which was approved by the Research Ethics Committee of National Liver Institute.

### ***Etiological diagnosis and group allocation***

Fulminant hepatitis A is defined by an acute onset of liver disease, due to HAV infection, with no known evidence of chronic liver disease, together with hepatic based coagulopathy (prothrombin time [PT]  $\geq 20$  seconds or international normalized ratio [INR]  $\geq 2.0$ ) that is not corrected by parenteral vitamin K and/or hepatic encephalopathy (must be present if the PT is 15–19.9 seconds or the INR is 1.5–1.9, but not if PT is  $\geq 20$  seconds or INR is  $\geq 2.0$ ) 5. After full investigations for possible etiologies, patients with proved etiology other than HAV or those with indeterminate FHF were not included.

### ***Serum viral markers***

Anti-HAV IgM was detected by enzyme linked immunosorbent assay (ELISA) (Dia Pro, Italy). Anti-hepatitis E virus IgM was tested by ELISA (Dia Pro, Italy). Hepatitis B surface antigen (HBsAg), anti-hepatitis B core IgM and IgG types, were tested for by ELISA kits (Sorin Biomedica Co, Spain). HBV-DNA was tested by COBAS AMPLICOR monitor test, version 2.0, Roche Molecular Systems, Inc., Branchburg, NJ, 08876 USA. Hepatitis C virus (HCV) antibody was tested by 4th generation ELISA (Innogenetics, Ghent-Belgium). Real-time polymerase chain reaction for HCV-RNA was performed using COBAS® Ampliprep/COBAS® TaqMan®, Roche Molecular Systems, Inc., Branchburg, NJ, 08876 USA.

### ***Treatment regimen and modifications***

Patients were initially assigned to receive prednisolone at a dose of 1 mg/kg/d or its equivalent dose of methylprednisolone (0.8 mg/kg/d) for those who cannot take oral prednisolone (as patients with encephalopathy or vomiting). In those with improved PT (while on steroid therapy) below 20 seconds in conscious patients or below 15 seconds in encephalopathic patients, the steroid dose was maintained for further two weeks then withdrawal started. In those who had deterioration of liver functions upon withdrawal, treatment was continued for a longer duration till satisfactory biochemical improvement was achieved. The treatment was scheduled to be discontinued if uncontrollable hyperglycemia, gastrointestinal bleeding, or brain edema develops.

### ***Outcome assessment***

The parameters of the primary response were improvement of PT followed by improvement of alanine transaminase and aspartate transaminase together with decreasing bilirubin during the period of observation. The overall clinical outcome (recovery or death) was

considered secondary response parameter. Treatment tolerability and side effects were also monitored.

### ***Statistical analysis plan (SAP)***

Quantitative data were presented as mean  $\pm$  standard deviation (SD) while qualitative data were presented as number and percentage. For both homogenously distributed and non homogenously distributed quantitative data, significance was tested by either independent samples *t*-test or by Mann-Whitney U test, respectively. Chi-square test or Fisher's exact test was used for the measurement of the qualitative data significance. Significance was set at *P* value  $< 0.05$ . A stepwise regression analysis was performed to examine independent factors associated with the outcome. Standardized regression coefficient ( $\beta$ ), 95% confidence interval (CI) and *P* values of each independent factor are presented. Statistical analysis was performed using SPSS, version 13 (SPSS Inc, Chicago, IL, USA).

## **Sponsor/Collaborators**

**Sponsor:** National Liver Institute, Egypt

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**Collaborators:** Quesna Central Hospital, Ministry Of Health, Egypt

## **Human Subjects Review:**

Board Status: Approved Approval Number: 00104/2015

Board Name: NLI IRB 00003413 FWA0000227

Board Affiliation: National Liver Institute, Menoufiya University IRB ,Shebin El-koum, EGYPT

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## Results

**Table 1:** Outcome of steroid therapy

Characteristics	Steroid (n = 18)	Non-Steroid (n = 15)	P-value
<b>Type of steroids</b>			
Methylprednisolone	10 (55.6)	--	
Prednisolone	8 (44.4)	--	
<b>Duration of steroids (days)</b>			
Minimum-Maximum (median)	5 – 50 (22)	--	
<b>Outcome</b>			
Living	15 (83.3)	4 (26.7)	0.001
Dead	3 (16.7)	11 (73.3)	

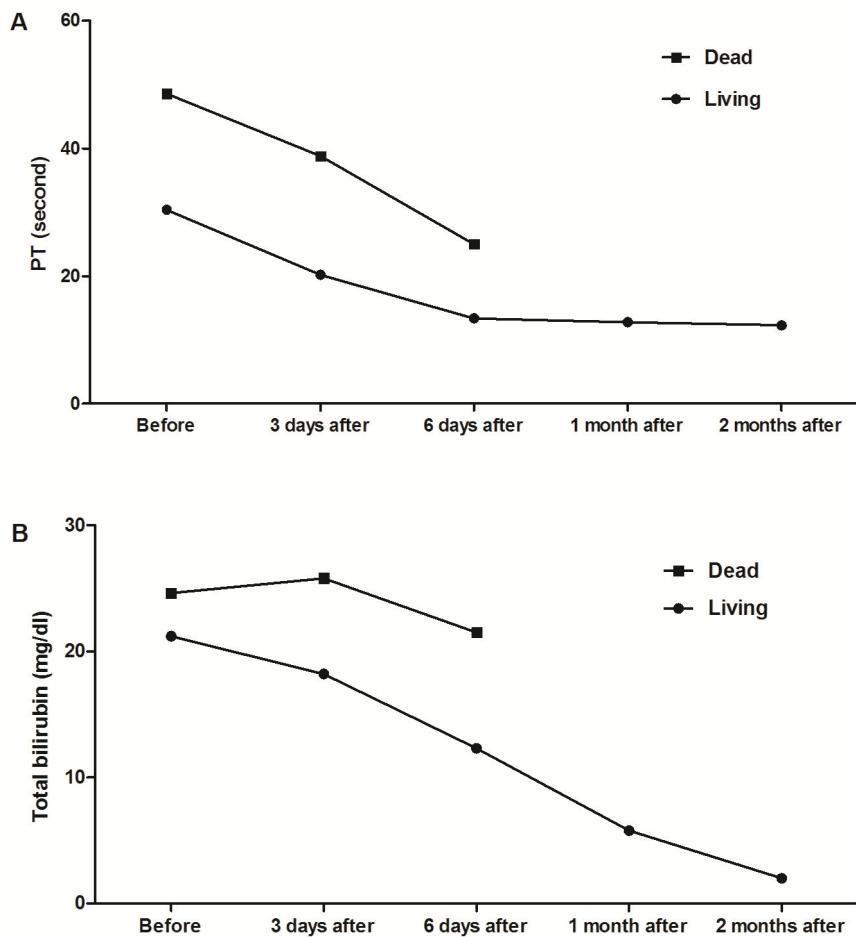
Number of living patients were significantly higher in the steroid group compared to non-steroid group

**Table 2:** Stepwise regression analysis for parameters with significance between living and dead patients

Characteristics	$\beta$	P-value	95% CI	
			Lower	Upper
Steroid therapy	0.571	0.001	0.268	0.865
Prothrombin time (seconds)	NI	--	--	--

$\beta$ : regression coefficient; CI: confidence interval; NI: not included

Steroid therapy was the only independent parameter associated with survival of patients



**Figure 1.** Serial changes of prothrombin time (A) and total bilirubin (B) in the steroid group.

PT: prothrombin time

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