Use of Granulocyte Colony-Stimulating Factor Among Patients of Chronic Liver Disease in a Tertiary Hospital in Nepal: A Pilot Study

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ABSTRACT

Introduction: Granulocyte colony stimulating factor improves short-term survival and clinical outcomes in alcoholic hepatitis, acute-on-chronic liver failure and decompensated chronic liver disease. Our study aimed to assess survival benefit and change in Child-Turcotte-Pugh and Model For End-Stage Liver Disease scores 30 days after Granulocyte colony stimulating factor therapy in chronic liver disease patients, irrespective of their mode of presentation.

Methods: This was a prospective observational study conducted in a university teaching hospital, where 25 patients with chronic liver disease were given 300 micrograms of Granulocyte colony stimulating factor subcutaneously 12 hourly plus standard medical therapy. We assessed survival until day 30. Child-Turcotte-Pugh and Model For End-Stage Liver Disease scores at enrolment and 30 days after treatment were compared.

Results: 21 of 25 patients treated with Granulocyte colony stimulating factor survived at day 30. Treatment with Granulocyte colony stimulating factor reduced Child-Turcotte-Pugh score from 10.33 ± 1.24 to 8.76 ± 1.79 ($p< 0.001$) at day 30 and Model For End-Stage Liver Disease score from 22.10 ± 4.67 to 16.38 ± 5.52 ($p < 0.001$) at day 30.

Conclusions: Granulocyte colony stimulating factor improves clinical outcome, Child-Turcotte-Pugh and Model For End-Stage Liver Disease scores in patients admitted with chronic liver disease for any cause. Further studies are needed to explore whether lower doses (total six doses) of Granulocyte colony stimulating factor are as effective as higher doses (total 10 doses).

Key words: Chronic Liver Disease; Granulocyte Colony Stimulating Factor

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INTRODUCTION

Chronic liver injury is defined as hepatic injury, inflammation and/or fibrosis occurring in the liver for more than six months. Chronic Liver Disease (CLD) can be non-cirrhotic or cirrhotic, and cirrhosis can again be classified as compensated and decompensated.[1] Compensated cirrhosis is defined by the absence of serious symptoms or events like distention of abdomen, haematemesis and jaundice or bouts of altered sensorium.[2] However, these compensated CLD patients may develop complications with progression of the disease and over time are said to have decompensated. Sometimes, with some known causes of acute decompensation, they deteriorate and end up as Acute on Chronic Liver Failure (ACLF), which has its own definition and criteria given by Asian Pacific Association for the Study of the Liver (APASL) or European Association for the Study of the Liver (EASL) or American Association for the Study of the Liver Disease (AASLD).[3] Once decompensation has developed, liver transplantation is the only option for survival. CLD has high in hospital mortality.[4-5] The mortality in certain subgroups is even higher.[6]

Studies have suggested that bone marrow–derived stem cells, stimulated by the glycoprotein Granulocyte Colony Stimulating Factor (G-CSF), might contribute to the regeneration after different kinds of liver injuries.[7] Recombinant G-CSF has been shown to increase the proliferative capacity of the hepatocytes.[8] G-CSF has been shown to mobilise CD34+ cells and increase hepatocyte growth factor, and to induce hepatic progenitor cell proliferation in patients with alcoholic steatohepatitis.[9]

CD34 antigen, present on early hematopoietic and vascular-associated tissue, is an important adhesion molecule with selective role in chemokine-dependent cell migration. Mobilisation of CD34+ hematopoietic stem cells has been shown to improve prognosis in other diseases like spinal cord injury and peripheral vascular disease too.[10-11] G-CSF has shown to improve short-term survival and clinical outcomes in Alcoholic Hepatitis[12], ACLF[13] and Decompensated CLD.[14]

Immune paralysis leads to sepsis and hence Hepato-Renal Syndrome (HRS) and Hepatic Encephalopathy (HE) in patients with cirrhosis.[15] G-CSF has been shown to improve immune function in patients of liver disease.[16] In a study G-CSF administration was shown to double the percentage of patients with ACLF who survived longer. It also showed significant improvement in CTP (Child-Turcotte-Pugh) scores, MELD (Model For End-Stage Liver Disease) scores, and SOFA (Sequential Organ Failure Assessment) scores and prevented the development of sepsis, HRS, and HE.[13] Therapy with G-CSF also showed significant reduction in hospital stay. GCSF therapy also improves both the chances of survival and the clinical outcome in patients with decompensated cirrhosis.[14]

Hence, in this pilot study we tried to see if there is any benefit of GCSF in overall chronic liver disease patients, in terms of mortality within one month of hospital admission and improvement in CTP and MELD scores, irrespective of their stage and presentation

METHODS

This prospective interventional pilot study was carried out at the Department of Gastroenterology, in a tertiary level hospital in Kathmandu, Nepal. First 25 patients meeting the criteria from January 2016 to December 2016 were studied. Written informed consent was taken from each patient or their relatives prior to enrolment in the study. Ethical approval was taken from the Institutional Review Board of the institute.

All patients aged 18 to 75 years admitted with the diagnosis of CLD were included irrespective of the mode of presentation - ACLF, alcoholic hepatitis, decompensated cirrhosis and compensated CLD (irrespective of their etiology). Patients aged <18 years and >75 years, those with hepatocellular carcinoma or portal vein thrombosis, splenomegaly of >15 cm, any concurrent evidence of sepsis, Grade 3 or 4 HE, pregnancy, hypersensitivity to

Table 1. Baseline characteristics of the patients

Variables	Value (n=25)
Age (yrs)	49.4 (30-72)
Sex	Males : 18 Females : 7
Risk factor for CLD	Alcohol : 22 Alcohol and viral : 2 Others : 1
Prior diagnosis of CLD	No : 20 Yes : 5
Ascites	18 out of 25
Encephalopathy	4 out of 25
Hematemesis or melena	None : 21 Melena only : 4
Hemoglobin (mg/dl)	9.87 (5 - 12.6)
TLC (x 10^3 /cumm)	8.99 (0.84 - 20)
Platelets (x 10^3 /cumm)	142.84 (30 - 332)
Total bilirubin (µmol/L)	197.88 (8.0 - 530)
Direct bilirubin (µmol/L)	117.66 (2.0 - 377)
ALT (IU/L)	77.99 (15 - 235)
AST (IU/L)	164.46 (25 - 408)
ALP (IU/L)	211.84 (53 - 751)
GGT (IU/L)	186.53 (41 - 514)
Albumin	26.52 (20 - 35)
Creatinine (µmol/L)	74.93 (34 - 197)
Sodium (mEq/L)	131.34 (115 - 144)
Potassium (mEq/L)	3.72 (2.7 - 4.6)
INR	2.15 (1.48 - 5.00)
CTP	10.52 (7 - 13)
MELD	23.28 (11 - 35)

G-CSF and those who didn't give consent were excluded from the study.

All the patients enrolled, in addition to standard medical treatment as advised by the treating physician, received GCSF injection 300 micro grams subcutaneously twice daily for three days, total six doses. Base line characteristics and lab parameters were recorded. CTP score and MELD scores were calculated at the baseline and at the end of 30 days from the completion of G-CSF therapy.

Table 2. CTP and MELD scores at enrolment and Day 30 (n=21)

	Mean	SD	Co-rrelation	Sig.
Pair 1				
CTP score at enrollment	10.3333	1.23828	0.467	33
CTP score at day 30	8.7619	1.78619		
Pair 2				
MELD score at enrollment	22.0952	4.67873	0.571	0.007
MELD at Day 30	16.3810	5.52699		

Descriptive statistics were expressed as mean (range) or number (%). Comparison of CTP and MELD scores prior to and after GCSF was done using paired samples t-tests for those patients who survived till the completion of the study. A two-tailed $p < 0.05$ was considered statistically significant with 95% CI. Independent samples t-test was used to analyse baseline quantitative data. Statistical analysis was performed using SPSS v 16.

RESULTS

164 patients were screened before reaching the first 25 cases that met the inclusion criteria and gave consent. The patients were followed up till one month after enrolment. Their CTP and MELD scores were calculated at enrolment and at the end of one month.

The baseline characteristics of the patients are summarised in table 1. Patients received GCSF at fixed dose of 300 µg subcutaneously twice a day for three days. No significant adverse effects requiring dose alteration or stoppage was seen. A few patients developed minor adverse effects like diarrhoea, fever and vomiting after first dose but responded to symptomatic treatment.

Of the 25 patients treated with GCSF, three patients died during treatment in the hospital and one died after discharge but prior to one-month follow up. Of the three patients that died in the hospital in our

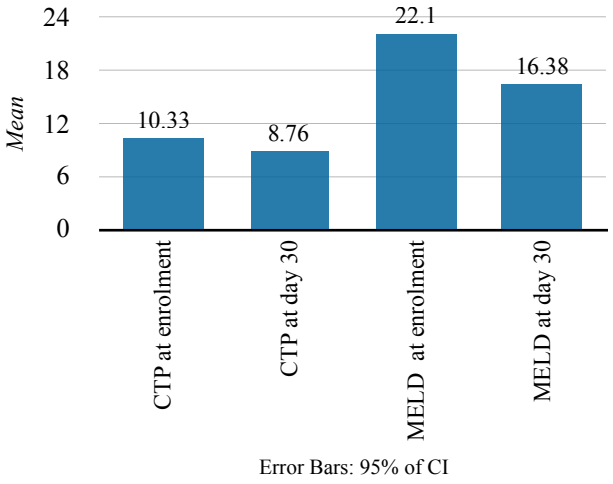

Figure 1. Bar diagram showing changes in mean CTP and MELD scores at 30 days

study, one had sepsis, one had HE with AKI and the last one had severe sepsis. Hence, the in-hospital mortality of patients admitted with Chronic liver disease treated with GCSF was 12% in this pilot study and the one month mortality (irrespective of presentation) was 16%. Treatment with G-CSF showed statistically significant reductions in CTP and MELD scores as shown in Table 2 and 3 and bar diagram (figure 1).

DISCUSSION

Treatment with GCSF, along with standard treatment, showed significant improvement in clinical outcome as evidenced by improvements in CTP and MELD scores at one month. It also showed good results in terms of decreased in-hospital morbidity and decreased mortality rates at one month.

G-CSF has been used in patients with promising results in CLD as well as in animal models of ACLF.[17-19] Similar promising results were seen in studies in ACLF, alcoholic hepatitis and decompensated CLD. They support our data on the benefits of G-CSF in patients with CLD. First clinical use of G-CSF for possible liver regeneration in advanced liver disease was done by Garg et al.[13] Duan et al. documented improved three month survival in Hepatitis B-virus associated ACLF patients.[20] GCSF, with darbopoietin-α, has been shown to improve clinical outcomes in decompensated cirrhosis. [21]

G-CSF induces both neutrophil production and release from the bone marrow.[22-23] Although the exact mechanism of the benefits of G-CSF therapy is not clear, it leads to a significant increase in the CD34 cell population in the liver tissue after four weeks of G-CSF administration.[13] However, we could not document the increase in CD34 cell in the peripheral blood or the liver tissue. G-CSF may mediate liver regeneration through the use of Hepatocyte Growth Factor (HGF) which is known to promote cell survival and regeneration of tissues. It suppresses chronic inflammation and fibrosis.[24]

Although the mechanisms are not clearly understood yet, G-CSF therapy has shown significant benefit in reducing short-term mortality rates. With the improvement of CTP and MELD scores and reduction in the rates of complications like sepsis, renal impairment or HE, G-CSF has shown therapeutic benefit in all cases of CLD.

Table 3. Paired samples test

	Paired differences					t	df	Sig
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the difference				
				Lower	Upper			
CTP score at enrollment - CTP at Day 30	1.57143	1.63007	0.35571	0.82943	2.31343	4.418	20	<0.001
MELD score at enrollment - MELD at Day 30	5.71429	4.78689	1.04458	3.53532	7.89325	5.470	20	<0.001

G-CSF is safe for use in these patients with very few minor adverse reactions. In our study, only four out of 25 patients developed complications like fever, vomiting and diarrhoea. However, none of the complications were severe enough to change therapy or stop G-CSF. None of the patients developed the dreaded complication of splenic infarction. Beside daily clinical evaluation, USG abdomen was used to evaluate the increase in spleen size in patients with spleen size >13.5 cm.

Unlike other studies with 5 µg/kg/dose of G-CSF for a total of 10 doses, we used a total of six doses of 300 µg G-CSF subcutaneously. The shorter duration of G-CSF therapy has been tried in patients with hepatitis B virus-associated ACLF in China.[20] Some significant limitations of our study include the lack of liver biopsy and assessment of CD34+ cells. A double-blind placebo-controlled trial would have been a better study design.

CONCLUSIONS

This study showed improvement with GCSF in CLD in terms of CTP and MELD scores and also showed lower in-hospital mortality rates in comparison to mortality rates in older studies without GCSF. Further studies are needed to quantify the survival benefit in these patients, specially compensated CLD alone. Longer follow up studies are needed to see if the benefit is long term. Further studies (head-to-head randomised trials) are needed for comparison of the shorter duration of therapy (total six doses) and longer duration of therapy (total ≥ 10 doses).

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