Clinical and laboratory outcomes of stem cell transplantation in patients with decompensated liver cirrhosis: single-arm pilot trial

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Received 20 March 2018
Accepted 20 April 2018

Journal of Current Medical Research and Practice
January-April 2019, 4:44–49

Introduction
The high prevalence of hepatitis C virus-associated liver disease has led to increasing number of Egyptian patients having end-stage liver disease and requiring liver transplantation. Because of several limitations of liver transplantation, new alternative treatment modalities are required for patients with liver cirrhosis. Many study results encourage the use of autologous bone marrow-derived mesenchymal stem cells (MSCs) for liver diseases.

Aim
The aim of this study was to assess the efficacy of using bone marrow-derived MSC on clinical manifestations, liver function, Child–Pugh score, and Model for End-stage Liver Disease score in patients with decompensated liver cirrhosis.

Patients and methods
This is a pilot single-arm prospective trial that included 13 patients with hepatitis C virus-associated liver cirrhosis who received transdifferentiated MSC via ultrasound-guided percutaneous portal vein infusion plus regular conventional treatment for patients with cirrhosis. Patients were followed up weekly for the first month and monthly for 6 months.

Results
There was significant improvement in some clinical manifestations, including frequency of attacks of hematemesis, ascites, and lower limb edema after MSC infusion compared with baseline data. Moreover significant increase was detected in mean serum albumin level as it began with 24.62 ± 3.93 and became 27.81 ± 4.14 ($P = 0.009$) after 6 months of follow-up. Moreover, the results showed improvement in Child–Pugh, but no changes were detected in Model for End-stage Liver Disease score after MSC infusion compared with baseline.

Conclusion
Our data show that transdifferentiated MSC injection may improve some clinical manifestations and some indices of liver function in patients with decompensated liver cirrhosis with successful tolerability.

Keywords:
mesenchymal stem cell, decompensated liver cirrhosis, clinical trial

J Curr Med Res Pract 4:44–49

2357-0121

Introduction

Liver cirrhosis irrespective of its cause is the endpoint of liver disease and leads to life-threatening complications [1].

The treatment modalities for decompensated cirrhosis are only symptomatic with transient improvement of quality of life. At present, liver transplantation (LT) is the only curative treatment for patients with decompensated cirrhosis [2]. The major limitation of LT is the shortage of donor organ, especially in Egypt, as living donor LT is the only option for patients with decompensated cirrhosis, beside other limitations including high cost and procedure-related complications [3].

During the recent years, the advancement in regenerative medicine including cellular therapy has opened new avenues in treating various disorders. In early 2000, studies suggested that multipotent stem cells may exist among bone marrow (BM) cells; these cells contribute to liver regeneration after injury [4,5].

This observation led investigators to examine the therapeutic potentials of BM stem cells in liver disorders. Several preclinical studies suggest that transplantation of BM stem cells reduces liver fibrosis [6–9]. Subsequent clinical trials in human suggested that infusion of different BM stem cells may transiently improve liver function in cirrhosis [10–13].

More specifically, mesenchymal stem cells (MSCs) have the potential for self-renewal and differentiation into multiple cell lineages including the hepatocytes lineage, so the efficacy and feasibility of bone marrow-derived mesenchymal stem cell (BM-MSC) therapy in patients with chronic liver diseases have been investigated in many clinical trials. These trials demonstrated that stem cell transplantation could significantly reverse hepatic failure with only limited adverse effects [14,15].

On the basis of these previous trials, the efficacy of using autologous BM-MSC transplantation in the treatment of patients with decompensated liver cirrhosis was assessed in this single-arm pilot trial.

Patients and methods

Study design and patients

This pilot trial included 13 patients (nine males and four females) with hepatitis C virus (HCV)-associated liver cirrhosis. Patients were recruited from Tropical Medicine and Gastroenterology Department and outpatient clinic, Al-Rajhi Hospital, Assiut University, from May 2016 to February 2017. An informed consent was taken from all patients who participated in this study. The study's protocol was approved by the Ethical Committee of Faculty of Medicine – Assiut University.

Inclusion criteria

The following were the inclusion criteria:

(1) Male or female patients aged from 20 to 70 years.
(2) Patients with decompensated liver cirrhosis according to clinical, biochemical, and radiological criteria of liver cirrhosis and have a WHO performance score less than or equal 2, Child B or C with scores ranging from 9–12, and Model for End-stage Liver Disease (MELD) score less than 20.

Exclusion criteria

The following were the exclusion criteria:

(1) Portal vein thrombosis.
(2) Hepatocellular carcinoma.
(3) Variceal bleeding within 1 month before the procedure.
(4) Vital organs failure.
(5) Pregnant or lactating women.
(6) Unable to give a written consent.

It was a single-arm pilot study in which the included patients received hepatocyte-like cell differentiated from autologous BM-MSC via ultrasound-guided percutaneous portal vein puncture plus regular conventional treatment, which included β-blockers, vitamin K, liver support as silymarin, and diuretics combination of furosemide and spironolactone.

The enrolled patients were subjected to the following:

(1) Full history and complete clinical examination.
(2) Laboratory investigations including serum total bilirubin, serum albumin, aspartate transaminase, alanine transaminase, prothrombin time, concentration and international normalized ratio (INR), complete blood count, serum urea and creatinine, and serum α-fetoprotein.
(3) Imaging including abdominal ultrasound and color Doppler for portal vein assessment.

Treatment protocol

(1) Under complete aseptic condition at operation room, 80–100 ml of BM was aspirated from the right posterior iliac crest and serial punctures from multiple sites were performed.
(2) The collected BM products were transferred to Tissue Culture and Molecular Biology Center (Stem Cell Unit) – Assiut University for MSCs isolation and preparation.
(3) Under aseptic conditions BM was layered over Ficoll-Hypaque and centrifuged for 20 min at 1800 rpm, and the harvested cell was diluted in normal saline.
(4) The cells were cultured in complete medium (Dulbecco's modified Eagle medium, supplemented with 1 cm of patient serum, 100 μl/ml penicillin/streptomycin, and 100 μl amphotericin), with the addition of 20 ng/ml fibroblast growth factor and 20 ng/ml hepatocyte growth factor for 5–7 days.
(5) Cell harvest: evaluation of hepatic lineage was done by using flow cytometry for immunophenotyping identification of MSCs and their differentiation into hepatocyte-like cells.
(6) Patients were admitted to hospital and infused with ~3 × 10^6–6 × 10^6 cells suspended in 10-ml normal saline through the ultrasound-guided percutaneous portal vein puncture.

Follow-up

The included patients were followed up monthly for 6 months. During the follow-up, the patients were observed for the following:

(1) Complete clinical evaluation and performance status score.
(2) Complete liver function tests.
(3) Prothrombin time and concentration and INR.
(4) Renal function tests.

(5) Complete blood count.
(6) Abdominal ultrasound and Doppler.
(7) Child and MELD score calculation.

Statistical analysis

Data entry and statistical analysis were processed by SPSS (Statistical Package for Social Science) version 19. The descriptive statistics were presented as mean ± SD for quantitative variables. All qualitative data were expressed by frequency (number) and percent. Comparisons between groups were done using independent sample *t* test and paired sample *t* test for normally distributed quantitative variables, while the non-parametric Mann Whitney test and Wilcoxon singed ranks test were used for abnormally distributed quantitative variables. In all tests, *P* values lower than 0.05 were considered statistically significant.

Results

The mean age (±SD) was 57.06 ± 4.29 years. Nine (69.23%) patients were males, whereas four (30.76%) patients were females.

Table 1 shows the evaluation of clinical findings of the studied patients before and after stem cell transplantation.

There was a statistically significant decline in the frequency of attacks of hematemesis after 1, 3, and 6 months of follow-up period after stem cell transplantation in comparison with baseline. There were no statistically significant changes in the frequency of attacks of hepatic encephalopathy throughout the follow-up period compared with the baseline data.

Regarding the ascites and lower limb edema, there were statistical significant differences observed only after 3 months of follow-up period in comparison with baseline.

Regarding bleeding tendency, there were no statistically significant changes observed before and after stem cell transplantation.

Table 2 shows the evaluation of liver function tests of the studied patients before and after stem cell transplantation.

The only statistical significant improvement was detected in the mean serum albumin level, as it began to increase 1 month after stem cell transplantation and maintained throughout the study period; however, there were no significant changes observed in other parameters of liver function tests.

Table 3 shows that the only statistical significant improvement in Child score was detected after 3 months of follow-up period compared with baseline data, where Child B and C were 53.8 and 46.15% of patients, respectively, before treatment and became 76.9 and 23.07% of patients, respectively, after 3 months of treatment ($P = 0.044$).

In contrast, there were no statistical significant changes in MELD score before and throughout the follow-up period after MSC infusion.

Discussion

Liver cirrhosis is a common final pathologic outcome of chronic liver diseases; it is considered one of the major causes of mortality worldwide [16]. Egypt has the highest prevalence of HCV infection in the world

Table 1 Baseline and follow-up clinical data of the studied patients before and after stem cell transplantation

	Before treatment [*n* (%)]	After 1 month [*n* (%)]	After 3 months [*n* (%)]	After 6 months [*n* (%)]	P^a	P^b	P^c
Hematemesis							
Yes	8 (61.5)	1 (7.6)	2 (15.3)	2 (15.3)	0.001*	0.005*	0.005*
No	5 (38.4)	12 (92.3)	11 (84.6)	11 (92.3)			
Hepatic encephalopathy							
Yes	7 (53.8)	5 (38.4)	6 (53.8)	4 (30.7)	0.180	0.494	0.110
No	6 (46.15)	8 (61.5)	7 (46.1)	9 (69.2)			
Bleeding tendency							
Yes	6 (46.15)	7 (53.8)	7 (53.8)	5 (38.4)	0.494	0.494	0.180
No	7 (53.8)	6 (46.15)	6 (46.15)	8 (61.5)			
Ascites							
Yes	9 (69.2)	9 (69.2)	2 (15.3)	5 (38.4)	0.402	0.005*	0.088
No	4 (30.7)	4 (30.7)	11 (84.6)	8 (61.5)			
Lower limb edema							
Yes	4 (30.7)	5 (38.4)	2 (15.3)	7 (53.8)	0.088	0.005*	0.110
No	9 (69.2)	8 (61.5)	11 (84.6)	6 (46.15)			

[a]1 month after treatment. [b]3 months after treatment. [c]6 months after treatment. *$P<0.05$, significant difference.

Table 2 Baseline and follow-up liver function tests of the studied patients before and after stem cell transplantation

	Before treatment	After 1 month	After 3 months	After 6 months	P^a	P^b	P^c
Total bilirubin (μmol/l)							
Mean±SD	46.60±33.61	39.25±28.47	33.73±23.60	34.16±18.19	0.586	0.136	0.163
Albumin (g/l)							
Mean±SD	24.62±3.93	28.96±4.18	29.84±4.64	27.81±4.14	0.001*	0.001*	0.009*
AST (U/l)							
Mean±SD	78.67±41.63	73.89±40.72	77.83±53.50	55.94±38.41	0.850	0.959	0.154
ALT (U/l)							
Mean±SD	68.22±44.82	60.11±35.38	65.44±55.41	61.67±36.03	0.938	0.965	0.557
PC							
Mean±SD	49.66±9.85	58.19±13.82	47.78±9.85	52.39±9.06	0.117	0.586	0.381
INR							
Mean±SD	1.54±0.22	1.42±0.16	1.61±0.24	1.54±0.15	0.052	0.513	0.698

ALT, alanine transaminase; AST, aspartate transaminase; INR, international normalized ratio; PC, prothrombin concentration. [a]1 month after treatment. [b]3 months after treatment. [c]6 months after treatment. *P<0.05, significance difference.

Table 3 Baseline and follow-up Child-Pugh score and Model for End-stage Liver Disease score in baseline and after 1, 3, and 6 months in the patients

	Baseline [*n* (%)]	After 1 month [*n* (%)]	After 3 months [*n* (%)]	After 6 months [*n* (%)]
Child-Pugh score				
Child B	7 (53.8)	7 (53.8)	10 (76.9)	5 (38.4)
Child C	6 (46.15)	6 (46.15)	3 (23.07)	8 (61.5)
P	-	0.494	0.044*	0.180
Model for End-stage Liver Disease score				
Mean±SD	15.33±3.50	14.00±3.07	13.94±1.86	14.67±2.70
P	-	0.317	0.271	0.611

*P<0.05, significance difference.

as the prevalence of HCV among the 15 – 59 year-age group is estimated to be 10% [17].

The high prevalence of HCV-associated chronic liver diseases in Egypt has led to increasing numbers of Egyptian patients having liver cirrhosis and its complications; it represents 64% of other etiologies of liver disease requiring LT [18].

Up to date, LT is the only curative treatment for decompensated cirrhosis, but it is a limited procedure owing to lack of donors, high cost, and also technical difficulties [2]. Therefore, cell-based therapy including stem cell therapy developed as a new therapeutic modality offers hope for those patients based on previous in-vivo and in-vitro studies using transplantation of bone marrow-derived hematopoietic cells or BM-MSCs.

Consequently, this pilot trial aimed to assess the efficacy of autologous BM-MSCs in patients with post-HCV decompensated liver cirrhosis using the portal vein as a route of cell delivery. This study is a part of a first clinical trial using stem cell transplantation in patients with decompensated cirrhosis in Upper Egypt.

The rationale for using the portal vein as a route for cell delivery in our study was based on the improvement of hepatic homing of the cells in the liver, and this fact is based on a preclinical model study, which reported that direct application of allogeneic hematopoietic stem cells to portal vein allows their persistent tolerance and entrapment in the liver [19].

Regarding the clinical manifestations, we observed significant improvement in the frequency of the attacks of hematemesis (variceal bleeding) 1 month after stem cell transplantation up to 6 months of follow-up period. This improvement in the frequency of the attacks of hematemesis may be related to good follow-up for all patients regarding patient compliance and variceal bleeding management, as we insisted on doing upper endoscopy and band ligation if needed on regular times.

Regarding the ascites and lower limb edema, in this study, we observed statistical significant changes only 3 months after stem cell transplantation. Although we observed individual improvement in the grades of ascites and lower limb edema after stem cell transplantation throughout the follow-up period, the statistically significant difference was detected only after 3 months of treatment. Our explanation regarding the improvement in ascites and in the lower limb edema probably related to the improvement of serum albumin, plus using the diuretics and salt restriction as a part of regular conventional treatment for those patients offers better results.

Regarding the liver function, our results showed statistically significant increase in mean serum albumin

level where it began with 24.62 ± 3.93 and became 27.81 ± 4.14 g/l (P = 0.009) after 6 months of follow-up period. Our results correlated with the results of Kharaziha *et al.* [13] who reported improvement in serum albumin level in eight patients with end-stage liver diseases after MSC injection over 24 weeks of their trial.

Our results showed no statistically significant changes in other indices of the liver function throughout the follow-up period after using MSCs infusion compared with baseline data. However, we noticed individual decrease in mean serum bilirubin level where it began with 46.60 ± 33.61 and became 34.16 ± 18.19 μmol/l after 6 months of follow-up. These findings are not in agreement with the results of Kharaziha *et al.* [13] who reported that serum bilirubin level and INR had lowered and the level of the prothrombin concentration had increased in their study. Analyzing our results regarding the liver function It may be explained by the therapeutic mechanisms of MSC in liver regeneration [20] together with regular conventional treatment for cirrhosis that including vitamin K and silymarin may be responsible for these results.

Regarding the Child–Pugh score, our data showed statistically significant decrease in the score after 3 months of follow-up period. These results probably related to the improvement in some of Child score parameters after stem cell therapy that was obviously detected after 3 months of follow-up period. These data correlated with results of Jang *et al.* [21] who conducted a clinical trial on 12 patients and reported significant improvements in Child score in 10 of the 11 patients after stem cell therapy.

We found no significant difference in MELD score, before and after stem cell therapy, and these results do not correlate with the results of Kharaziha *et al.* [13] who conducted a clinical trial on eight patients with end-stage liver disease and reported that MELD score had been decreased from 17.9 ± 5.6 to 10.7 ± 6.3.

This study had some limitations, including the small number of the patients included in the trial and being a single-armed study; however, it is a pilot study. Moreover, the follow-up period was short term in this trial. A randomized controlled trial with longer duration is underway based on the results of this trial and is expected to provide confirmative evidence of therapeutic effect of stem cell therapy.

Conclusion

Our results suggest that BM-MSCs may improve some liver function indices, some of clinical data, and Child score in patients with decompensated liver cirrhosis.

Recommendations

The following are some of the recommendations:

(1) Evaluation of stem cell therapy on large sample size population.
(2) Evaluation of long-term outcomes and safety of stem cell therapy.
(3) Possibility of repeated stem cell infusion and its efficacy.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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