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Clinical Transplantation

Short-term evaluation of autologous transplantation of bone marrow–derived mesenchymal stem cells in patients with cirrhosis: Egyptian study

Amin MA, Sabry D, Rashed LA, Aref WM, el-Ghobary MA, Farhan MS, Fouad HA, Youssef YA-A. Short-term evaluation of autologous transplantation of bone marrow–derived mesenchymal stem cells in patients with cirrhosis: Egyptian study.

Abstract: Background: Stem cell–based therapy has received attention as a possible alternative to organ transplantation. The aim of this study was to assess the safety and efficacy of autologous transplantation of bone marrow (BM)–derived stromal cells in post-HCV liver cirrhosis patients.
Methodology: 10×10^6 of isolated human bone marrow (HBM)-stromal cells in 10 mL normal saline were injected in the spleen of 20 patients with end-stage liver cirrhosis guided by the ultrasonography, and then patients were followed up on monthly basis for six months.
Results: A statistically significant decrease was detected in the total bilirubin, aspartate transaminase (AST), alanine transaminase (ALT) (p-value<0.01), prothrombin time (PT), and international normalized ratio (INR) levels (p-value<0.05), while a statistically significant increase in the albumin and PC (p-value<0.05) after follow-up.
Conclusion: This study suggested the safety, feasibility, and efficacy of the intrasplenic injection of autologous BM stromal cells in improving liver function in Egyptian patients with cirrhosis.

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Key words: Egypt – human – intrasplenic injection – liver cirrhosis patients – mesenchymal stem cells

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Liver transplantation has become a procedure with a relatively good five-yr survival, yet organ donation has many problems, including relative lack of donors, operative complication, risk of rejection and high cost (1). Furthermore, it is expected that over the next few years, there will be a fivefold increase in the need for liver transplantation; therefore, there is an urgent need to develop alternative strategies for the treatment of advanced liver disease (1). The bone marrow (BM) contains at least two populations of stem cells, hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), which provide a stromal support for HSCs. It has long been proposed that BM, a known source of stem cells, might be able to contribute to the repair of other organs (5, 6), and it was revealed that BM-MSCs have a potential therapeutic effect against the fibrotic process through their effect in minimizing collagen deposition in addition to their capacity to differentiate into hepatocytes. BM-MSCs transplantation either undifferentiated or differentiated can be used as a potential treatment for liver cirrhosis (7). Hepatocytes can be transplanted into the liver via intraportal or intrasplenic routes (8). Hepatocytes are well engrafted when injected into the splenic pulp as they are entrapped in the sinusoids and vascular

spaces (9). They can proliferate and replace approximately 40% of the splenic pulp and retain synthetic, metabolic and biliary transport functions (10). This study aimed at evaluating the safety and efficacy of transplantation of autologous BM stromal cells in patients with liver cirrhosis as a possible alternative to organ transplantation.

Patients and methods

After being approved by the local ethical committee, this prospective study was conducted on a group of 20 HCV-infected patients with end-stage liver cirrhosis (Child C) attending to the outpatient clinic of the internal medicine department in Kasr Al-Aini university hospital, with ages ranging between 40 and 60 yr old and with platelet count levels $>45 \times 10^3$. Patients with history of moderate to severe hepatic encephalopathy or variceal bleeding during the last two months and those with hepatic, portal, or splenic vein thrombosis as well as those with severe bleeding tendency, active untreated infectious diseases, or HCC were excluded from this study.

After a written informed consent was signed by all the patients, they were subjected to full clinical evaluation, laboratory investigations (including complete blood count, complete liver and kidney functions tests, HCV Ab and HBs Ag, and α fetoprotein), and conventional abdominal ultrasonographic examination. After BM aspiration, BM stromal cells were isolated, propagated, identified, and transplanted by ultrasonographic-guided intrasplenic injection. All the patients were followed up for six months by clinical assessment, laboratory tests (bilirubin, ALT, AST, PT, PC, and INR) that were assessed on the day of stem cell transplantation, and then at 4, 8, 12, 16, 20, and 24 wk after transplantation during clinical follow-up as well as by abdominal ultrasonography.

Safety was evaluated in terms of adverse events graded according to the Common Toxicity Criteria and laboratory test results. Efficacy was evaluated by physical examinations to determine the amount of ascites, as well as the evaluation of symptoms, was performed by one highly experienced physician.

Preparation of BM-derived stromal cells

BM (20 mL) was aspirated from the iliac crest under complete aseptic precautions and placed in heparinized tubes. Cell cultures were performed at the Medical Biochemistry and Molecular Biology Unit, Cairo University. Nucleated cells were isolated with a density gradient (Ficoll-Paque; GE HealthCare, Waukesha, WI, USA) and resuspended in culture medium (Dulbecco's modified Eagle's medium) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (10 000 μg/mL). FBS (Lonza, Boston, MA, USA) used in this study has non-immunogenic reaction and is relatively safe according to our research experience. Cells were incubated at 37°C in 5% CO_2 for 14 d; culture medium was changed every 2–3 d. When cells reached 80–90% confluence, cultures were washed twice with phosphate-buffered saline (PBS) and then the cells were trypsinized with 0.25% Trypsin in 1 mm EDTA for 5 min at 37°C. After centrifugation, cells were resuspended in medium and subcultured for 10 d, reaching an average count of 10×10^6. On the day of implantation, cells were trypsinized, collected, and 10×10^6 resuspended in 10 mL PBS, and transferred to the operating room in sterile tubes. Potential immunogenicity from FBS proteins was furthermore minimized by repeated copious irrigation of the cells pellet with PBS before final resuspension and transfer to the operating room (11).

BM stromal cells characterization

Unselected cultured BM stromal cells were characterized by their adhesiveness, fusiform shape, and by flow cytometry for MSC surface markers ($CD34^-$, $CD45^-$, $CD90^+$, and $CD105^+$). MSCs were identified by their capacity to differentiate into osteocytes and chondrocytes (12, 13).

Intrasplenic transplantation of BM-derived stromal cells

Under complete aseptic precautions, a mean of 10×10^6 of the isolated BM–derived stromal cells in 10 mL PBS were injected in the spleen to the patients under sonographic guidance, and without anesthesia, percutaneous intrasplenic injection of stem cells with guided ultrosonography is a much less complicated method for cell delivery in comparison with portal injection while some cells may be lost due to leakage. The patients were discharged after six h of observation.

Results

HBM-MSCs were identified morphologically as being characterized by their adhesiveness and fusiform shape in culture (Fig. 1; 14) and by fluorescence-activated cell sorting (FACs) analysis where the cells were subjected to fluorescence-activated cell sorting using a FACS and Cell Quest software (Becton Dickinson, San Jose, CA; Fig. 2; 15).

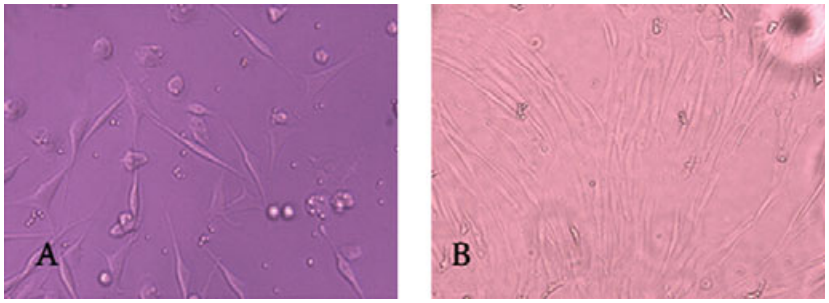

Fig. 1. Isolated and cultured undifferentiated bone marrow-derived stromal cells: (A) mesenchymal stem cells (MSCs) propagated for two d and (B) MSCs reached 80–90% confluence at 14 d. They were identified by their fusiform fibroblast like-structure (Original magnification × 100).

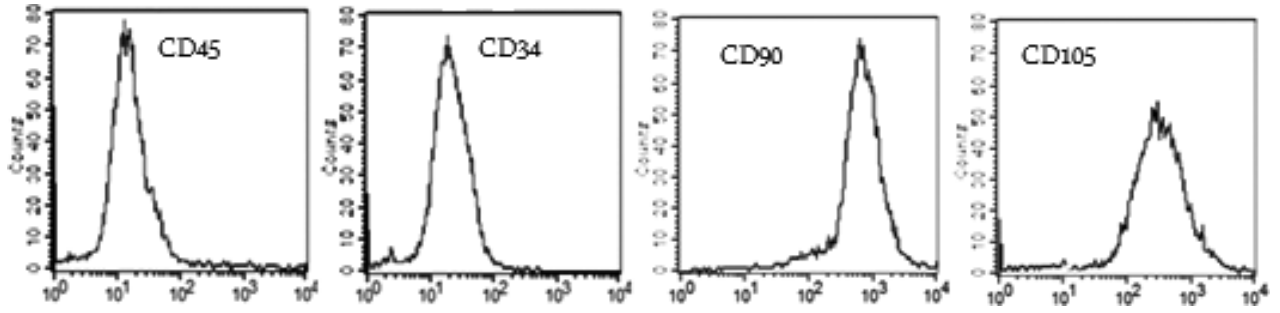

Fig. 2. Flow cytometric characterization analyses of bone marrow–derived mesenchymal stem cells. Cells were uniformly negative for CD45, CD34, and positive for CD90, and CD105.

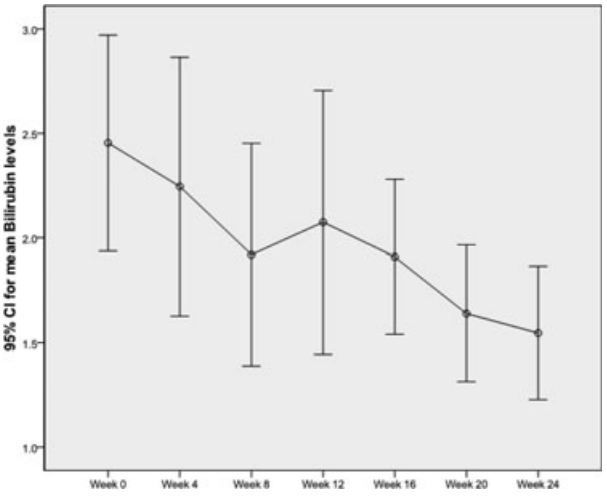

Fig. 3. Scatter plot of different biochemical parameters mean ± SD vs. times of patients follow-up at different weeks (Total bilirubin).

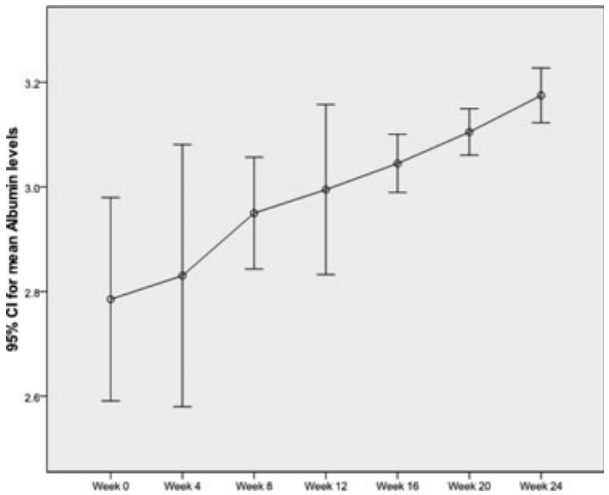

Fig. 4. Scatter plot of different biochemical parameters mean ± SD vs. times of patients follow-up at different weeks (Albumin).

This study was conducted on 20 patients, 14 males (70%) and six females (30%), with ages ranging between 42 and 60 yr, with a mean of 51.3 ± 6.2, with end-stage liver cirrhosis (Child C). Intrasplenic injection of BM-MSCs was illustrated in life video at additional file (1). Vital signs of the patients remained stable during the stem cell infusion; no side effects were reported during the follow-up period.

Follow-up abdominal ultrasonography showed no evidence of focal lesions in the liver in any of the patients. A significant decrease in the total bilirubin, AST, and ALT levels throughout the follow-up period was reported (p-value <0.01) and in the PT and INR levels as well (p-value <0.05), while a significant increase in the albumin and PC levels was reported at the end of study (p-value <0.05). Serum total bilirubin, albumin, ALT, AST, PT, PC, and INR follow-up biochemical assessment were illustrated at Figs. 3–9 respectively.

On clinical examination after BM-derived stromal cells injections, six patients showed improvement in the encephalopathy status, two patients

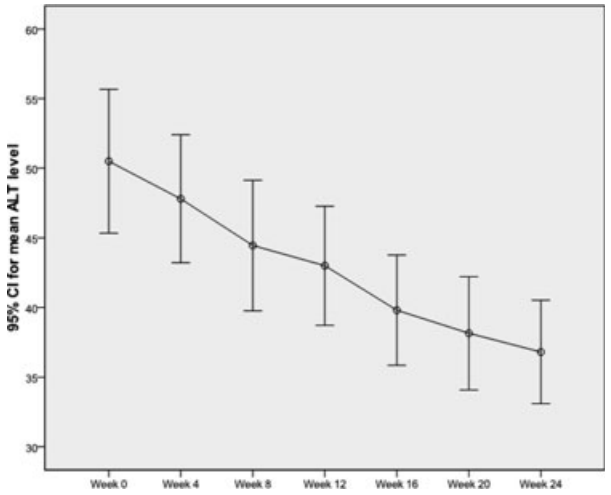

Fig. 5. Scatter plot of different biochemical parameters mean ± SD vs. times of patients follow-up at different weeks (ALT).

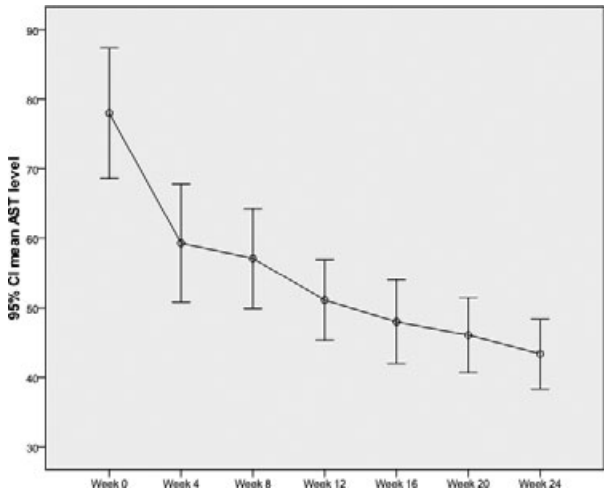

Fig. 6. Scatter plot of different biochemical parameters mean ± SD vs. times of patients follow-up at different weeks (AST).

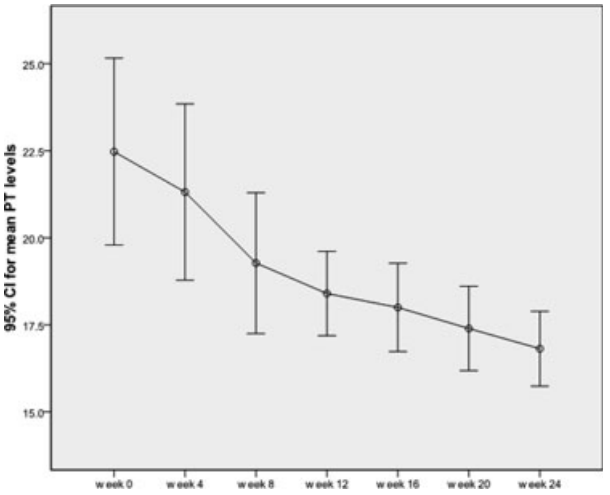

Fig. 7. Scatter plot of different biochemical parameters mean ± SD vs. times of patients follow-up at different weeks (PT).

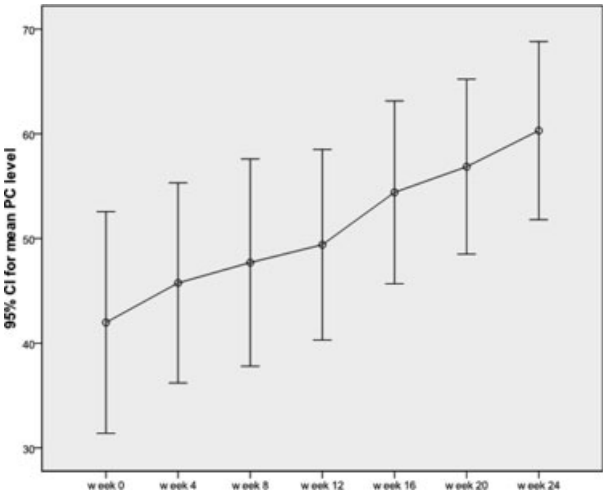

Fig. 8. Scatter plot of different biochemical parameters mean ± SD vs. times of patients follow-up at different weeks (PC).

showed no attacks of hematemesis and/or melena, 10 patients showed improvement of lower limb edema, while 10 patients showed gradual decrease in their ascitis grade (Table 1).

Discussion

The ideal strategy to treat liver injury is to generate new hepatocytes replacing the damaged cells without causing excessive ECM deposition. New findings in adult stem cell biology are transforming our understanding of tissue repair, raising hopes of successful regenerative hepatology (16).

The first demonstration of the existence of putative liver stem cells in the BM was reported by Petersen et al. (17), and showed that BM cells transplanted into lethally irradiated mice engrafted in the recipient's liver and differentiated into liver stem cells (oval cells) or mature hepatocytes. These *in vivo* results were confirmed in animal models and in patients who received BM transplantation for hematological disorders (18).

BM-derived MSCs have a significant impact on hepatic fibrogenesis through their ability of inhibiting activated HSC and reregulating the fibrogenic process. The interventions of MSCs include the following: (1) inhibit HSC proliferation; (2) stimulate HSC apoptosis; (3) inhibit ECM accumulation; (4) stimulate endogenous hepatocyte regeneration; and (5) hepatocyte-like differentiation (19).

In this study, it was reported that intrasplenic injection of *in vitro* expanded autologous MSCs is

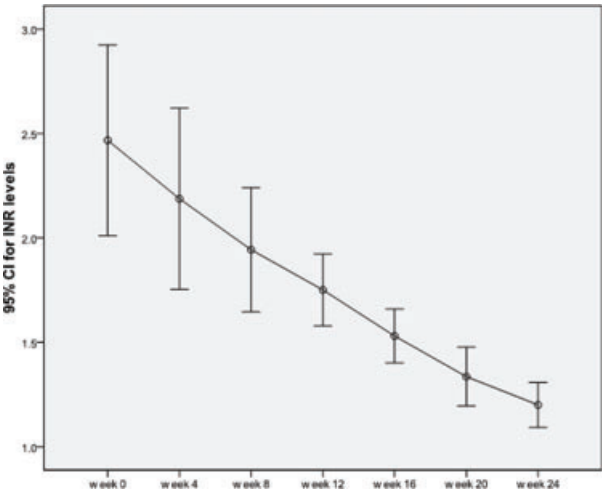

Fig. 9. Scatter plot of different biochemical parameters mean ± SD vs. times of patients follow-up at different weeks (INR).

Table 1. Clinical and abdominal ultrasonographic characteristics of patients at baseline

Item	Mean ± SD	Number	%
Age: (42–60 yr)	51.3 ± 6.3	20	100
Sex		Male = 14	30
		Female = 6	70
L. L. edema		16	80
Jaundice		10	50
Hematemesis and/or melena		2	10
Hepatomegaly		6	30
Splenomegaly		20	100
Ascites		16	80
Encephalopathy		6	30
P.V. diameter (mm)	14.25 ± 0.62	20	100

safe and feasible in patients with decompensated liver cirrhosis.

MSC-derived molecules directly inhibit hepatocellular death, enhance liver regeneration, and ultimately improve survival in rats undergoing D-galactosamine-induced fulminant hepatic failure. (20).

These validate the therapeutic benefits of MSC-derived molecules on liver disease and may create potential new avenues for the treatment of advanced liver disorders.

Terai et al. (21) conducted a clinical trial on nine patients with decompensated liver cirrhosis, where patients were infused with $5.2 \pm 0.63 \times 10^9$ autologous BM cells into the peripheral vein. At the 24th week after transplantation, significant improvements were observed. These improvements included total protein, serum albumin, Child-Pugh scores, and α-fetoprotein and proliferating cell nuclear antigen expression in liver biopsy tissues. Also BM stem cells are able to improve the residual liver function in patients with cirrhosis. (22).

Previous researches have shown that the fibrolytic effects of BM stem cells may be related to over-expression of matrix metalloproteinases (MMPs), especially MMP-9. Also, the migrated BM stem cells may lead to hepatic stellate cells apoptosis (23).

In this study, after six months of follow-up for the patients, improvement of liver function tests with decline of elevated bilirubin, liver enzymes, prothrombin time, elevation of serum albumin and prothrombin concentration was observed. Also 30% showed improvement of encephalopathy status, 10% showed no attacks of hematemesis and/or melena, 50% showed improvement of lower limb edema, and 50% showed gradual decrease in ascites.

The above findings may suggest that the beneficial effects of MSC transplantation are transient and that infusion of higher number of MSCs may make more beneficial effects. This is in agreement with the study of Mohamadnejad et al. (24), who performed two small-scale clinical studies. In their first trial, four patients with decompensated liver cirrhosis were infused 31.73×10^6 (mean) MSCs through a peripheral vein. At the end of follow-up (after 12 months), MELD scores of two patients improved by four and three degrees, the mean physical and mental component scales were more than doubled by the end of follow-up, computed tomography (CT) showed the increase in liver volume of three patients by the sixth month. However, the results of their second trial were not satisfactory. Four patients received 5.25×10^6 (mean) autologous BM-HSCs infusion through hepatic artery. Only slight improvements were observed in some patients. The results of their MSC transplantation were more promising than the study of HSC transplantation. They also indicated that hepatic artery delivery of stem cells was not a safe procedure.

Conclusion

Autologous BM-derived stromal cells transplantation through intrasplenic procedure is safe and feasible in the treatment of liver cirrhosis. However, the dose and frequency of this treatment are still to be defined.

Recommendation

Further studies with larger sample size with higher number of infused BM-derived stromal cells are recommended to investigate whether the improve-

ment conducted in this study was related to the stem cell transplantation only or not.

Authors' contributions

Mona A. Amin contributed to clinical selection and examination of patients suitable for MSCs transplantation and intrasplenic MSCs injection–guided ultrasonography. She approved the final manuscript. Wael M. Aref and Mohamed Ahmed el-Ghobary contributed to patients' follow-up clinical examinations after MSCs transplantation. Dina Sabry and Laila A. Rashed contributed to MSCs isolation, culture, propagation and characterization and biochemical parameters follow-up and assessment for patients after MSCs transplantation. They approved the final form of the manuscript. Marwa Salah Farhan and Hany Ahmed Fouad contributed to biochemical parameters follow-up and assessment for patients after MSCs transplantation. Youssef Abdel-Aziz Youssef contributed to sample collection, examination, bone marrow aspiration, data collection, and statistical analysis. All authors share equally in concept, design, and final approval of the work.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Video Clip S1. Video is showing intrasplenic injection of autologous BM-MSCs.