Phase I Trial: Mesenchymal Stem Cells Transplantation in End Stage Liver Disease

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Abstract: Background**,** End-stage liver disease and in particular human liver cirrhosis represents a worldwide health problem. Currently, liver transplant is the only effective treatment, but it is affected by many problems including relative lack of donors, operative damage, risk of rejection and high costs. Stem cell therapy is very attractive in this setting because it has the potential to help tissue regeneration while providing minimally invasive procedures and few complications. The aim of this study was to evaluate the effect of autologous transplantation of bone marrow derived mesenchymal stem cells in cirrhotic patients following chronic hepatitis C virus infection. Methods, Twelve patients with Child C liver cirrhosis, Model of End Stage Liver Disease (MELD) score > 12 were included. They divided into 2 groups according to method of MSCs injection, 1st group was injected intrasplenic and 2nd group was injected through the peripheral blood. First group patient's ages ranged from 32 to 69 years, mean value was 48.50 ±11.09, they were 4 males (67%) and 2 females (33%). Second group patient's ages ranged from 43 to 59 years, mean value was 50.83 ±6.88, they were 5 males (83%) and 1 female (17%). Fifty ml bone marrow was aspirated from the iliac bone for separation of MSCs. Surface expression of CD271 and CD34 were analyzed using flowcytometry. Finally approximately 10 million MSCs/ 5ml saline were infused intrasplenic or peripherally in one session. There was highly statistical significant difference between CD271 before and after culture, p value was <0.01. Results, Monthly Follow up of patients for 6 months revealed partial improvement of liver function tests with decline of elevated bilirubin and liver enzymes and elevation of prothrombin concentration and serum albumin levels. There was statistically significant difference between total bilirubin, direct bilirubin, MELD score and creatinine level before and after MSCs injection in both groups, p value was <0.05. Conclusion, MSCs are the most potent component of bone marrow cells in its ability to differentiate into hepatocytes thus, MSC transplantation can be used as a potential treatment for liver cirrhosis. The dose, frequency and route of administration of this treatment are still to be defined. [Journal of American Science. 2010;6(12):135-144]. (ISSN: 1545-1003).

Keywords: End-stage liver disease; liver cirrhosis; liver transplant; autologous transplantation; bone marrow; mesenchymal; stem cell

1. Introduction

Cirrhosis represents a late stage of progressive hepatic fibrosis characterized by distortion of hepatic architecture and formation of regenerative nodules. This results in many clinical manifestations including ascitis, variceal hemorrhage and encephalopathy (1).

Cirrhosis is the terminal outcome of viral hepatitis (particularly hepatitis C) *in our country* and alcoholic liver diseases. Other less frequent causes include some parasitic infections such as shistosomiasis, some metabolic disorders, toxic chemicals and unknown conditions. Egypt has the highest world wide prevalence of Hepatitis C reaching 20% in some areas. This is apparently due to past parenteral antischistosomal therapy. HCV infection was found to be a major cause of liver cirrhosis in Egypt (2).

Although liver cirrhosis carries a poor prognosis, the only very dated treatment for advanced liver cirrhosis is liver transplantation (3). Liver transplantation has become a procedure with a relatively good 5-year survival. Yet, organ donation has not kept up with the demand because of many problems, including relative lack of donors, operative complication, risk of rejection and high cost (4). Furthermore, it is expected that over the next few years there will be a 5- fold increase in the need for liver transplantation. For all these reasons, there is an urgent need to develop alternative strategies for the treatment of advanced liver disease (4).

Owing to the ability of stem cells to repopulate and differentiate at the engrafted site, stem cell-based therapy has received attention as a possible alternative to organ transplantation (5).

Bone marrow is a reservoir of various stem cells including hematopoietic (HSCs) and non-hematopoietic stem cells variously referred to as mesencymal stem cells or marrow stromal cells (MSCs) (6).While MSCs have been shown to be capable of mesodermal and neuro-ectodermal differentiation, they have the potential of endodermal differentiation; their differentiation into functional hepatocyte-like cells has also been demonstrated in vivo (7) and in vitro by continuous exposure to cytokine cocktail (8).

Both HSCs and MSCs have the ability to trans-differentiate to hepatocytes, but MSCs are the most potent component of bone marrow cells in hepatic differentiation. Thus, bone marrow stem cell transplantation, particularly MSC transplantation can be a potential treatment for liver cirrhosis (9).

Aim of work:

The aim of this study was to evaluate the effect of transplantation of autologous bone marrow derived mesenchymal stem cells into cirrhotic patients in improving liver function tests and patient's quality of life as a possible alternative to organ transplantation.

2. Subjects and Methods

Subjects & Methods:

Subjects:

The present study included 12 patients with chronic hepatic failure due to hepatitis C virus infection. According to modified Child Pugh scoring all our patients were Child's C liver cirrhosis, (MELD) score was > 12. They divided into 2 groups according to method of MSCs injection, the 1st group was injected intrasplenic and the 2nd group was injected through the peripheral blood. These patients were selected among cases referred from the medical department in Kasr EL- Aini hospitals; a written informed consent was taken from all patients.

Selection of the patients will be based on
Inclusion Criteria:

- Age 20-70 years
- Chronic hepatic failure due to hepatitis C or hepatitis B virus infection
- Child C liver cirrhosis
- Model of End –Stage Liver Disease (MELD) score > 12

Exclusion criteria:

History of moderate to severe hepatic encephalopathy or variceal bleeding during the last 2 months before enrolment ,presence of hepatic, portal or splenic vein thrombosis on Doppler ultrasonography, history of autoimmune diseases ,presence of active untreated infectious diseases, severe respiratory or cardiac diseases, presence of any types of malignancy, use of hepatotoxic drugs within the last 6 month before enrolment, severe bleeding and life threaten bleeding disorder.

The diagnosis was based on detailed history taking**,** complete clinical examination with special emphasis on abdominal examination**,** laboratory investigations including complete blood picture, Liver function tests, prothrombin time and concentration, alpha fetoprotein, kidney function tests, HCV Ab and HBs Ag using 4th generation ELISA technique, HCV (RNA) by RT-PCR and HBV (DNA) by PCR, radiological investigations including abdominal ultrasonography, doppler and duplex study of portal system.

Methods:

1. *Sampling*:

Fifty ml bone marrow was aspirated from the iliac bone after local anesthesia and placed in sterile tubes contains preservative free heparin.

2. *Mononuclear cells (MNCs) isolation:*

The bone marrow aspirate was diluted with phosphate buffer saline containing 2mM EDTA (PBS/EDTA buffer). MNCs were separated by density gradient centrifugation.

3. *Mesenchymal stem cells (MSCs) separation:*

The MNCs were plated in Dulbecco`s modified Eagle's medium (DMEM) and were cultured at 37˚C in 5% CO_2. After one day, non adherent cells were removed and adherent cells were cultured in presence of mesenchymal media for 3 weeks. After reaching 80% confluence the mesenchymal stem cells (MSCs) were harvested by incubation with trypsin / EDTA and counted on hemocytometer then flowcytometric analysis of surface expression of MSCs using anti CD271 and antiCD34 monoclonal antibodies was done. Finally, in a single session ten million MSCs in 5ml saline were injected percutaneously through the splenic vein under computerized tommograpy guidence.

4. Follow up:

Follow up of patients for 1-6 months by clinical assessment and laboratory work was done.

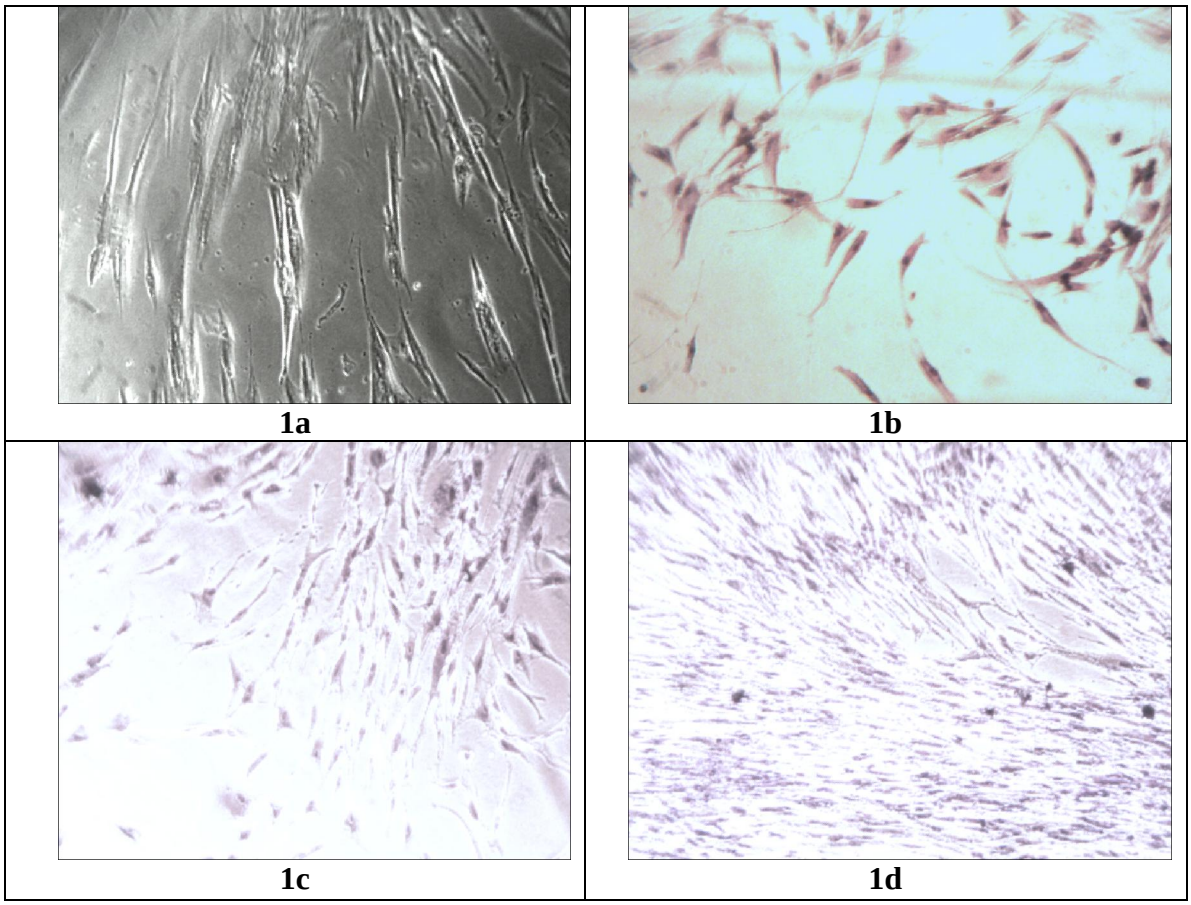

Figure (1): Photography of MSCs culture; 1a unstained MSCs 20% confluence, 1b stained MSCs 20% confluence, 1c stained MSCs 50% confluence and 1d stained MSCs 90% confluence.

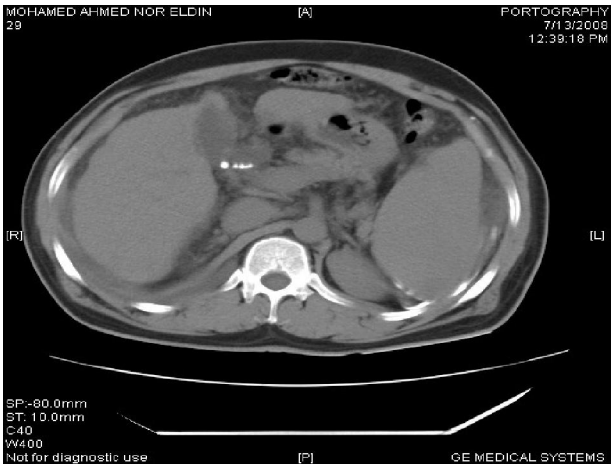

Figure (2): CT photography taken after MSCs injection into the spleen

Statistical analysis:

Quantitative values are expressed as mean ± S.D, and were compared using Student's *t*-test. Qualitative data were compared using Qui Square test. A *p* value <0.05 was considered a significant. A *p* value <0.01 was considered as highly significant. Pearson's correlation coefficients for the different variables were calculated. The SPSS statistical package was used

3- Results:

First group (injected intrasplenic) patient's ages ranged from 32 to 69 years, mean value was 48.50 ±11.09, they were 4 males (67%) and 2 females (33%). Second group (injected through PB) patient's ages ranged from 43 to 59 years, mean value was 50.83 ±6.88, they were 5 males (83%) and 1 female (17%).

- On clinical examination before MSCs injection:

All patients of 1st and 2nd group had clinical evidence of decompensated liver cirrhosis. Three

patients (50%) of 1st group and 2 patients (33%) of 2nd group had a past history of at least one attack of encephalopathy, 5 patients (83%) of 1st group and 4 patients (67%) of 2nd group presented with jaundice, 2 patients (33%) of both groups had a history of hematemesis and/or melena, 5 patients (83%) of 1st group and 4 patients (67%) of 2nd group had lower limb edema during their initial assessment and 3 patients (50%) of both groups had mild to moderate ascitis. Splenomegaly was found in all patients.

- On clinical examination after MSCs injection:

In both groups of patients, only 1 patient (17%) show improvement of encephalopathic manifestation and ascitis, 3 patients (50%) show marvelous decline of jaundice, 2 patients (33%) show improvement of lower limb edema, however non of patients show improvement of bleeding manifestation (Table 1).

Statistical comparison between the 2 groups as regards clinical data before and after MSCs injection: On comparing the 2 groups regarding clinical data before and after MSCs injection there was no statistical significant difference, p value >0.05.

In both groups there was statistically significant difference between total bilirubin, direct bilirubin, MELD score, creatinine level before and after MSCs injection, p value was <0.05, also there was a highly statistically significant difference between CD271 before and after culture, p value was <0.01. However, comparison revealed statistically non significant difference as regards other laboratory data before and after injection, p value was >0.05, table-2 & table-3.

Correlation of CD271 expression after MSCs culture with clinical & fold change of laboratory data of patients before and after injection:

There was no statistical significant correlation between CD271 expression after culture with age, sex, clinical data and fold change of laboratory data before and after culture in both groups.

Statistical comparison between the 2 groups as regards laboratory data before and after MSCs injection:

On comparing the 2 groups regarding laboratory data before and after MSCs injection there was no statistical significant difference, p value >0.05.

Table (1): Clinical data of all patients before and after MSCs injection

Items	**Patients** (No. 12)			
	Group 1 (No. 6)		**Group 2** (No. 6)	
Age (years)				
• **Range**	32-60		43-59	
• **Mean ± SD**	48.50 ± 11.09		50.83 ± 6.88	
Sex				
• **Male (No ;%)**	4 ; 67 %		5; 83%	
• **Female (No ;%)**	2 ; 33 %		1; 17%	
Clinical data	**Before**	**After**	**Before**	**After**
Encephalitis (No ;%)				
• **Present**	3 ; 50 %	2 ; 33 %	2 ; 33 %	1 ; 17 %
• **Absent**	3 ; 50 %	4 ; 67 %	4 ; 67 %	5 ; 83 %
Jaundice (No ;%)				
• **Present**	5; 83 %	2; 33 %	4; 67 %	1; 17%
• **Absent**	1; 17 %	4; 67 %	2; 33 %	5; 83 %
Hematemesis and/or melena (No ;%)				
• **Present**	2; 33 %	2; 33 %	2; 33 %	2; 33 %
• **Absent**	4; 67 %	4; 67 %	4; 67 %	4; 67 %
LL edema (No ;%)				
• **Present**	5 ; 83 %	3 ; 50 %	4 ; 67 %	2 ; 33 %
• **Absent**	1; 17 %	3; 50 %	2; 33 %	4; 67 %
Ascitis (No ;%)				
• **Present**	3; 50 %	2; 33 %	3; 50 %	2; 33 %
• **Absent**	3; 50 %	4; 67 %	3; 50 %	4; 67%

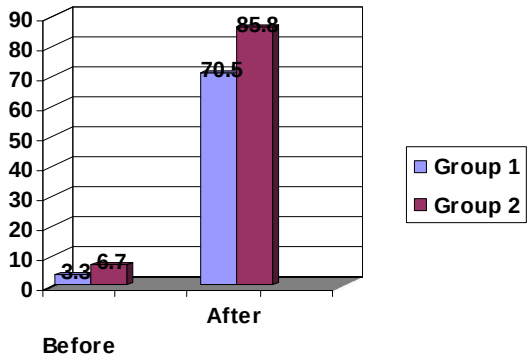

Figure (3): Comparison of mean value of CD271 % before and after MSCs culture in the 2 groups

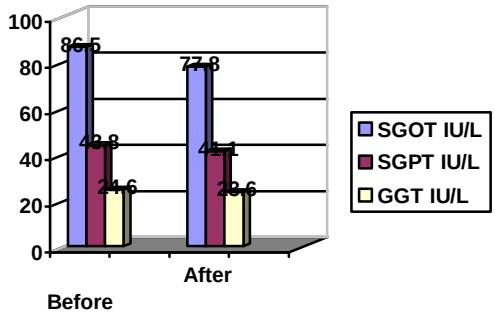

Figure (4): Comparison of mean value of SGOT, SGPT and GGT before and after MSCs injection in group 1

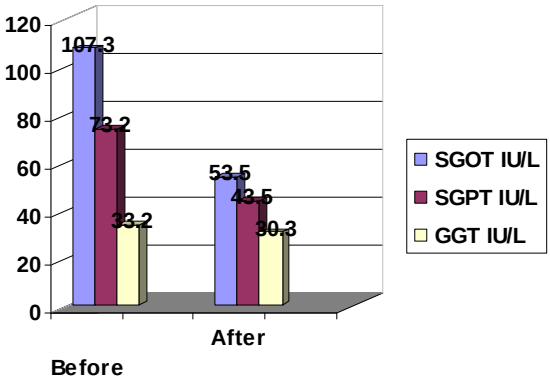

Figure (5): Comparison of mean value of SGOT, SGPT and GGT before and after MSCs injection in group 2

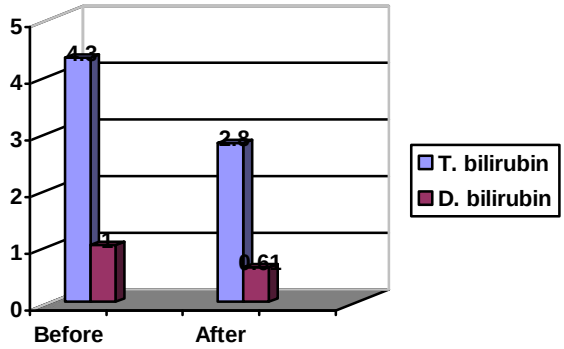

Figure (6): Comparison of mean value of total and direct bilirubin before and after MSCs injection in group 1

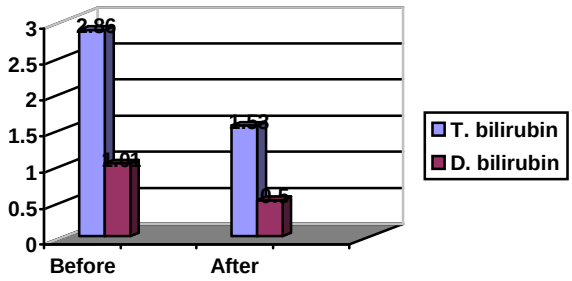

Figure (7): Comparison of mean value of total and direct bilirubin before and after MSCs injection in group 2

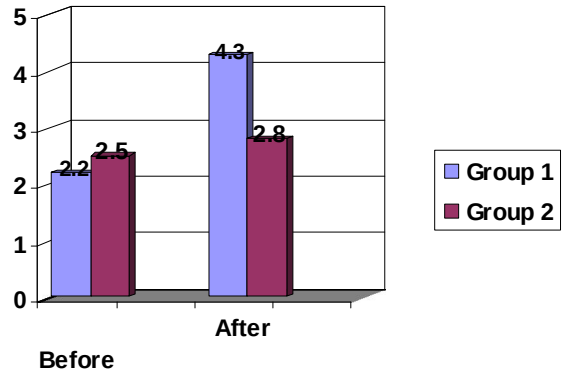

Figure (8): Comparison of mean value of albumin before and after MSCs injection in the 2 groups

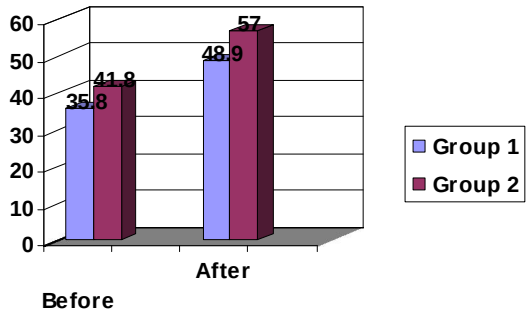

Figure (9): Comparison of mean value of prothrombin concentration before and after MSCs injection in the 2 groups

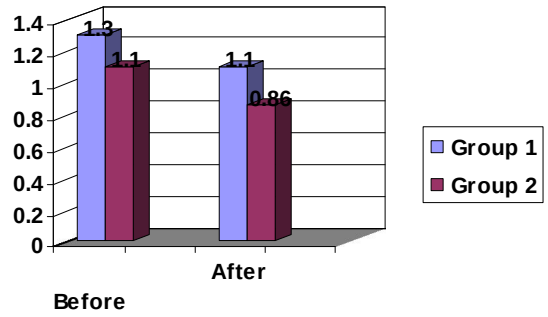

Figure (11): Comparison of mean value of creatinine before and after MSCs injection in the 2 groups

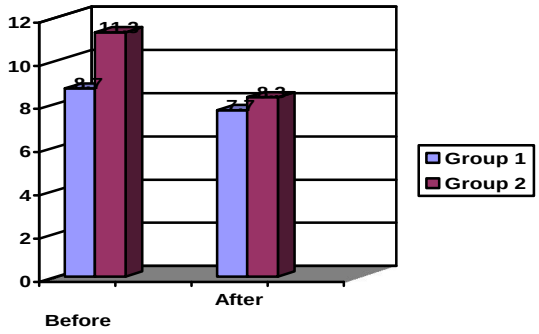

Figure (10): Comparison of mean value of alpha fetoprotein (AFP) before and after MSCs injection in the 2 groups

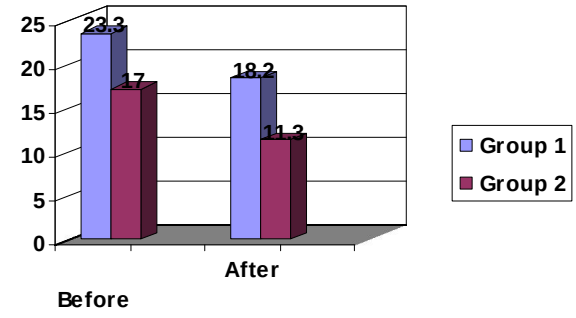

Figure (12): Comparison of mean value of MELD score before and after MSCs injection in the 2 groups

Table (2): Laboratory and Statistical comparison of laboratory data before and after MSCs injection

Items	Patients (No. 12)					
	Group 1 (No. 6)			Group 2 (No. 6)		
	Before	After	P value	Before	After	P value
Hb (g/dl) • **Range** • **Mean ± SD**	8.00- 13.40 10.76 ± 1.83	7.10- 13.90 10.63 ± 2.46	0.873 NS	10.90- 12.30 11.73 ± 0.45	11.00-12.90 11.71 ± 0.76	0.56 NS
TLC (x10³ / cmm) • **Range** • **Mean ± SD**	2.00– 6.20 3.56 ± 1.59	2.50– 7.70 3.76 ± 1.98	0.684 NS	3.00-8.30 5.34 ± 2.10	2.90-8.50 5.00 ± 2.21	0.93 NS
Platelets (x10³ / cmm) • **Range** • **Mean ± SD**	44.00 – 296.00 92.83 ± 99.70	36.00 – 176.00 85.00 ± 50.19	0.769 NS	34.00-179.00 94.16 ± 48.79	48.00-160.00 93.00 ± 44.93	0.96 NS
SGOT (u/L) • **Range** • **Mean ± SD**	12.00- 179.00 77.83 ± 58.12	33.00- 234.00 86.50 ± 78.98	0.811 NS	57.00-157.00 107.33 ± 46.02	37.00-79.00 53.50 ± 16.37	0.02 S
SGPT (u/L) • **Range** • **Mean ± SD**	19.00– 83.00 43.83 ± 23.13	23.00– 77.00 41.16 ± 23.03	0.898 NS	39.00-138.00 73.17 ± 35.80	23.00– 77.00 43.50 ± 11.76	0.08 NS

Alkaline phosphatase (u/L) • **Range** • **Mean ± SD**	52.00– 162.00 89.83 ± 40.48	53.00– 160.00 103.00 ± 38.06	0.243 NS	53.00– 160.00 103.00 ± 38.06	32.00-60.00 103.00 ± 38.06	0.69 NS
Total bilirubin (mg/dl) • **Range** • **Mean ± SD**	2.00- 6.00 4.30 ± 1.73	1.30- 4.30 2.79 ± 1.21	0.02 **S**	1.50-4.00 2.86 ± 0.86	0.90-1.95 1.53 ± 0.39	0.01 **HS**
Direct bilirubin (mg/dl) • **Range** • **Mean ± SD**	0.30- 1.60 1.00 ± 0.46	0.20- 1.00 0.61 ± 0.38	0.04 **S**	0.30-1.50 1.01 ± 0.42	0.20- 0.80 0.50 ± 0.22	0.02 **S**
GGT (u/L) • **Range** • **Mean ± SD**	14.00– 32.00 24.66 ± 6.86	12.00– 39.00 23.66 ± 9.95	0.658 NS	19.00-41.00 33.17 ± 8.73	20.00-40.00 30.33 ± 8.26	0.57 NS
Alphafeto protein (Iu/ml) • **Range** • **Mean ± SD**	2.70– 33.00 8.71 ± 11.98	2.16– 30.00 7.71 ± 10.95	0.167 NS	9.00-15.00 11.33 ± 2.25	5.00-15.00 8.33 ± 3.98	0.13 NS
Albumin (g/dl) • **Range** • **Mean ± SD**	2.10- 2.40 2.20 ± 0.12	1.90- 2.90 2.43 ± 0.39	0.128 NS	2.20-2.80 2.48 ± 0.28	2.4-3.00 2.76 ± 0.28	0.11 NS
Prothrombin conc. (%) • **Range** • **Mean ± SD**	31.90- 43.00 35.81 ± 4.89	36.60- 73.00 48.93 ± 13.06	0.087 NS	22.00-67.00 41.83 ± 16.52	40.00-75.00 57.00 ± 11.30	0.09 NS
MELD score • **Range** • **Mean ± SD**	19.00- 35.00 23.33 ± 5.95	15.00- 27.00 18.16 ± 4.49	0.01 **HS**	13.00-22.00 17.00 ± 3.41	8.00-14.00 11.33 ± 2.16	0.01 **HS**
Creatinine (mg/dl) • **Range** • **Mean ± SD**	0.50- 2.50 1.26 ± 0.73	0.30- 2.30 1.10 ± 0.74	0.01 **HS**	0.90-1.20 1.10 ± 0.11	0.70-1.00 0.86 ± 0.09	0.01 **HS**

Table (3): Statistical comparison of CD271% of all patients before and after MSCs culture

Items	**Patients** (No. 12)					
	Group 1 (No. 6)			**Group 2** (No. 6)		
	Before	**After**	**P value**	**Before**	**After**	**P value**
CD271 (%) • **Range** • **Mean ± SD**	2.00- 5.00 3.33 ± 1.21	46.00- 96.00 70.50 ± 20.83	0.01 **HS**	4.00-9.00 6.67 ± 2.07	83.00-94.00 85.83 ± 5.30	0.01 **HS**

4. Discussion:

End-stage liver disease, and in particular human liver cirrhosis, represents a worldwide health problem. Cirrhosis is the terminal outcome of viral hepatitis (particularly hepatitis C) and alcoholic liver diseases. Egypt has the highest prevalence of Hepatitis C Virus (HCV) in the world, up to 20% in some areas. HCV infection is a major cause of liver cirrhosis in Egypt (2). Currently, liver transplant is the only effective treatment, but it is affected by many problems, including relative lack of donors, operative damage, risk of rejection, and high costs. Stem cell therapy is very attractive in this setting because it has the potential to help tissue regeneration while providing minimally invasive procedures and few complications (4).

The bone marrow (BM) contains at least two populations of stem cells, haematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), which provide stromal support for HSCs. It also contains many other haematopoietic cell types involved in immune surveillance, inflammatory responses and pathogen removal. It has long been proposed that bone marrow, a known source of stem cells, might be able to contribute to the repair of other organs (10).

Both HSCs and MSCs have the ability to trans-differentiate to hepatocytes, but MSCs are the most potent component of bone marrow cells in hepatic differentiation (9). MSCs are present in low numbers in BM and have a capacity to differentiate into a wide range of mesenchymal tissue types, including cartilage, bone, muscle, stroma, fat, tendon, and other connective tissues. Their differentiation

into functional hepatocyte-like cells has also been demonstrated in vivo (7) and in vitro by continuous exposure to cytokine cocktail (8).This term more recently has been applied to plastic adherent fibroblastic cells that are isolated from the bone marrow and other tissues that show mesenchymal multipotency. Unlike HSC, once isolated, these mesenchymal stromal cells can be grown in culture for many population doublings and now have been shown also to have a much broader potential, including neural differentiation. In some studies, the surface phenotype of an MSC has been investigated. They are negative for markers that include CD34, CD45, and CD14 and positive for CD166, CD105, CD29, and CD44 (11).

Upon liver injury, the typical repair process involves two distinct phases: a regenerative phase, in which injured liver cells are replaced with regenerated hepatocytes; and a phase known as fibroplasias or fibrosis, in which connective tissue replaces normal parenchymal tissue. Although initially beneficial, the repair process becomes pathogenic when it is not controlled appropriately. Extensive accumulation of ECM components can ultimately lead to cirrhosis and liver failure (12). Moreover, fibronectin, a component of ECM, has been proved to promote the MSC-induced cytoprotection following transplant for liver disease (13). The ideal strategy to treat liver injury is to generate new hepatocytes replacing damaged cells without causing excessive ECM deposition.

The first demonstration of the existence of putative liver stem cells in the bone marrow was reported by Petersen et al. (14). They showed that bone marrow 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 bone marrow transplantation for hematological disorders (15).

Hepatic stellate cells (HSCs) are the main ECM-producing cells in the injured liver. When a liver injury occurs (e.g., viral hepatitis), HSCs proliferate and undergo a dramatic phenotypical alteration, which is characterized by the acquisition of a proliferative, contractile, migratory, fibrogenic and inflammatory phenotype. Activated HSCs secrete large amount of ECM proteins, including collagen (I, III, and IV), fibronectin, undulin, elastin, laminin, hyaluronan, and proteoglycans (16). The accumulating interstitial ECM constituents that collectively form the hepatic scar replace the low-density type IV collagen with the normal subendothelial space of Disse. These interstitial fibril-forming collagens (especially types I and III collagens) become distributed primarily in the connective septa surrounding the regenerative hepatic nodules. A cirrhotic liver may contain up to six times more collagen and proteoglycan than a healthy organ (17). In addition to the resident HSCs, periportal fibroblasts, bone marrow-derived fibrogenic cells, epithelial-mesenchymal transition, and possibly circulating fibrocytes can contribute to the fibrogenesis in the liver. MSC have a significant impact on hepatic fibrogenesis through their ability of inhibiting activated HSC and re-regulating the fibrogenic process. The interventions of mesenchymal stem cells (MSC) include: (1) inhibit HSC proliferation; (2) stimulate HSC apoptosis; (3) inhibit ECM accumulation; (4) stimulate endogenous hepatocyte regeneration; and (5) hepatocyte-like differentiation (18).

MSC paracrine-mediated hepatic regeneration from endogenous liver stem cells may also contribute to the hepatocyte replication and recovery of hepatic function (19).

The aim of this study was to evaluate the effect of transplantation of autologous bone marrow derived mesenchymal stem cells into cirrhotic patients in improving liver function tests and patient's quality of life as a possible alternative to organ transplantation. The present study included 12 patients with chronic hepatic failure due to hepatitis C virus infection. The degree of hepatic affection was determined according to modified Child Pugh scoring. All our patients were Child's C liver cirrhosis, MELD score was > 12. They divided into 2 groups according to method of MSCs injection, the 1st group was injected intrasplenic and the 2nd group was injected through the peripheral blood. These patients were selected among cases referred from department of Internal medicine in Kasr EL Eini hospitals in Cairo University; a written informed consent was taken from patients. Bone marrow was aspirated from the iliac bone for separation of MSCs then 10 million MSCs in 5 ml saline were infused intrasplenic or peripherally in one session.

After 1-6 months follow up of patient's we observed that 1 patient (17%) show improvement of encephalopathic manifestation and ascitis, 3 patients (50%) show marvelous decline of jaundice, 2 patients (33%) show improvement of lower limb edema, however non of patients show improvement of bleeding manifestation. Partial improvement of liver function tests with decline of elevated bilirubin and liver enzymes and elevation of prothrombin concentration and serum albumin levels was noticed in both groups. There was statistical significant difference between total bilirubin, direct bilirubin, MELD score, creatinine level before and after injection in both groups, p value <0.05. Also there

was highly statistical significant difference between CD271 before and after culture, p value <0.01. However, comparison revealed no statistical significant difference as regards other laboratory data before and after injection of our patients following laboratory culturing of MSC, p value >0.05. On comparing the 2 groups regarding laboratory data before and after MSCs injection there was no statistical significant difference, p value >0.05 that might be explained by small number of patients, so large scale population study is required. There are only a handful of clinical trials in the field of regenerative cell therapy specifically in the field of hepatology, all of which are small-scale, uncontrolled safety and feasibility studies.

Our results were more or less in consistent with Terai et al. (20) who implemented a clinical trial on nine patients with decompensated liver cirrhosis. These patients were infused with $5.2 \pm 0.63 \times 109$ autologous bone marrow cells into the peripheral vein. At 24 weeks 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 Gordon et al. (21) evaluated the effects of CD34+ hematopoietic stem cell intrahepatic injection and whole bone marrow peripheral infusion in five patients with liver cirrhosis. Their results showed a decrease in serum bilirubin and an improvement in serum albumin in three and four of the five patients, respectively, with the disappearance of ascites observed in one patient. They concluded that bone marrow stem cells are able to improve the residual liver function in cirrhotic patients.

More recently, Mohamadnejad et al. (22) performed two small scaled clinical studies, in their first trial, four patients with decompensated liver cirrhosis were infused 31.73×106 (mean) MSCs through a peripheral vein. At the end of follow-up (after 12 months), MELD scores of two patients improved by four points and by three points. The mean physical and mental component scales were more than doubled by the end of follow-up. Computed tomography (CT) showed the increase of liver volumes of three patients by the sixth month. However, the results of their second trial were not satisfactory. Four patients received 5.25×106 (mean) autologous bone marrow-hematopoietic stem cells infused through hepatic artery. Only marginal improvements were observed in some patients. The results of their MSC transplantation were more promising than the study of hematopoietic stem cell transplantation. They also indicated that hepatic artery delivery of stem cells was not a safe procedure. Because of the lack of reliable means of identifying transplanted stem cells in the human body, they recommended caution during the evaluation of the clinical outcomes.

Kuo et al. (23), Xu and Liu (24) and Dai et al. (25) described some parameters governing the success of using MSCs and characteristics of various delivery approaches in their recent papers. We can conclude from our results and others that MSCs are the most potent component of bone marrow cells in hepatic differentiation thus, bone marrow stem cell transplantation, particularly MSC transplantation can be a potential treatment for liver cirrhosis. Also from our results there was no difference in clinical and laboratory improvement regarding the route of administration. However, the dose, frequency and route of administration of this treatment are still to be defined.

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