Autologus stem cell therapy in End stage liver disease patients through portal vein infusion

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Abstract: End stage liver disease is considered as a serious health problem. Liver transplantation is almost the only curative treatment. the limited number of the donors and the post operative complications are major obstacles. **Aim:** This study was done to demonstrate the probability of Autologous haematopoietic stem cell transplantation into end stage liver disease to improve function & Quality of life. **Methods**: Group of patients with End stage liver disease will be enrolled in the study and the patients will be identified by coded numbers to maintain the privacy. Patients will be randomized into one of three groups:*Group1:*patient who received autologous CD 34 stem cell by portal vein infusion. *Group 2:* patients will take portal vein infusion of bone marrow mononuclear cells (which include stem cells) *Group 3:* patients who served as a control and those received regular supportive treatment. An informed consent will be taken from all participants. **Results:** serum bilirubin was improved in group 1 and group 2 with significant decrease in its level by the time while in group 3 the serum bilirubin was significant increase in its level by the time. **T**he mean baseline Prothrombin activity value in our studied population started to improve one month after the procedure, and continued to improve until the 6th month. This improvement was statistically significant. The serum albumin was maintained in patients of group 1, 2 without intravenous administration of human albumin or plasma. While in control group serum albumin levels were decreasing throughout follow up period in spite of administration of intravenous albumin and fresh plasma. There was borderline improvement in AST serum level in both groups (1, 2) while in group 3 there was significant decrease in its serum level by the time. There was significant improvement in ALT serum level in both groups (1, 2) while in group 3 there was significant decrease in its serum level by the time**. Conclusion:** Stem cell transplantation have a beneficial effect on synthetic function of the liver and possibly improve survival and quality of life of patients with end stage liver disease.Since it is based on autologous bone marrow cells, this therapy will not be limited by a shortage of organs and will be cheaper than whole liver transplantation. Hepatocyte transplantation may not be able to reverse portal hypertension, or the development of HCC, but improvement in liver physiology and patient survival could be possible and could play a significant role in the management of patients with liver failure.

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Introduction

Egypt probably has the highest prevalence of hepatitis **C** virus in the world averaging from 12 to 18% in the entire population **(Frank *et al*., 2000). HCV** is apparently transmitted hepatotrophic virus that persists in the majority of those infected **(El-Zayadi *et al*., 2005).** Liver has a fantastic regenerative capacity but, following chronic liver damage, this begins to fail and then fibrosis and eventually cirrhosis develops **(Fausto, 2004).** The only curative treatment for advanced liver cirrhosis is liver transplant. Although liver transplant has become a procedure with a relatively good 5 year survival, organ donation has not kept up with demand which has result in an increasing number of mortality.Various alternation to transplantation has been evaluated such as split liver and relatively donor liver transplant these procedure are still limited by donor capacity, the high costs and the long life immunosuppressive treatment that they are required **(Di Campii *et al*., 2003).**

There is still urgent need to develop alternative strategies for the treatment of advanced liver disease and numerically cirrhosis is the most important target. The alternative solution of particular interest is so called "Regenerative medicine" based on the therapeutic potential of stem cells **(Piscaglia *et al*., 2008).** These stem cells have remarkable potential to develop into many cell type in the body during early life and growth. In addition in many tissues they serve as sort of internal repair system, dividing without limit to replenish other cells as long as person still alive **(Bethesda, 2009)**.

Bone marrow stem cells have been intensively investigeted as potential source of liver stem cell and as a mean to regenerate the cirrhotic liver **(Willenbring *et al*., 2004)**. Scientists primarly used with two kinds of stem cells "embryonic stem cells and somatic or adult stem cell**" (Bethesda, 2009)**.

Most of adult stem cells lineage restricted (multipotent and generally reflected by their tissue origin:mesenchymal stem cell, adipose-derived stem cell, endothelial stem cell, dental pulp stem cell, etc). **(Barrilleaux *et al* 2006 and Gimble *et al*, 2007).**

Nowadays the most promising source for stem cell- based therapies is represented by the intra portal or intra hepatic infusion of freshly isolated or invitro expanded haemopoietic stem cells**. (Mallet *et al*., 2005).** Another appealing option is represented by the administration of mobilizing \proliferating agents, such as G-CSF, that is able to both enhance the heamopoietic stem cell mobilization into the portal vein and facilitate the endogenous liver stem cell activation **(Piscaglia *et al*., 2010)**. Bone marrow stem cell transplantation improved the residual liver function in cirrhotic patient into recently published studies, through CD34 heamopoietic stem cell intrahepatic injection **(Gordon *et al* 2006 and Terai *et al*., 2006).** Salama and others concluded that autologous stem cell transplantation may be safely administered and appears to offer some therapeutic benefit to patient with viral hepatic end stage liver dtsease **(Salama *et al*.,** 2010).

Patient and methods

This study was conducted in Clinical Pathology department and tropical medicine and infectious diseases department – Faculty of medicine, Tanta University.

Studied groups:

Group of patients with end stage liver disease will be enrolled in the study and the patients will be identified by coded numbers to maintain the privacy.

Patients will be randomized into one of three groups:

Group 1: including 6 patients with end stage liver disease who received autologous CD 34 stem cell by portal vein infusion.

Group 2: including 8 patients with end stage liver disease will take portal vein infusion of bone marrow mononuclear cells (which include stem cells).

Group3: including 10 patients with end stage liver disease who served as a control and those received regular supportive treatment including (albumin transfusion, fresh plasma and vitamin K).

A daily portal vein injection of distilled water was given for all patients while receiving their usual supportive measures before and after injection. And an informed consent will be taken from all participants.

Inclusion criteria

- Male or female patients aged from 40 to 60 years with evidence of liver decompensation having:
  - Ascites.
  - Low prothrombin time less than 50%.
  - Low serum albumin less than 2.5 g/dl.
- Some have past history of hepatic encephalopathy.
- patients unlikely to receive a liver transplant and has the ability to give a written consent

Exclusion criteria

- Pregnant or lactating women, recent gastrointestinal bleeding (one month), hepatocellular carcinoma, spontaneous bacterial peritonitis, an evidence of human immunodeficiency virus, any life threatening infection, unable to give a written consent, having history of hypersensitivity to G-CSF and they included in any other clinical trial within the previous month.

Data Collection methods, instruments used, measurements

The study tools will include the following**:**

Detailed medical history and complete clinical examination, with special emphasis on the presence markers of liver cell failure e.g. ascites, jaundice, and Lower Limb edema**.** Abdominal ultrasound scanning to evaluate liver cirrhosis and exclude focal lesions. Laboratory investigations included liver biochemical profile [serum bilirubin and albumin, prothrombin activity, INR, alanine transaminase (ALT) and aspartate transaminase (AST)]; blood urea and serum creatinine levels; blood sugar (fasting and two hours post-prandial); complete blood analysis, CHILD score will be calculated to assess the degree of hepatic decompensation for every patient

1) Group I and Group II will be submitted to bone marrow aspiration

a- From days one to three, patients received a daily SC injection of granulocyte colony stimulating factor (G-CSF at 300 µg/vials) for three days to increase the number of circulating hematopoietic cells. Blood samples were then taken for hematology, biochemical analysis, and coagulation profile on day one and three. Data regarding symptoms and adverse events were collected and recorded. Patients were admitted to the hospital and underwent BM aspiration on day four.

b- BM stem cell aspiration: before aspiration of B.M, It was used heparin (5000 IU=1ml) in the operating room to coat the syringes (20ml), each syringe was coated with 1ml heparin, B.M. biopsy needle (Islam needle) coated with heparin, 3ml heparin was add to each tubes(50 ml conical tubes).

c-Separation of human bone marrow mononuclear cells (BM MNCs):

The aspirated B.M. was diluted at a ratio of 2:1 with clinical buffer and then filtered thus can be separated using ficoll – paque. The mononuclear cells including **SC** and thrombocytes) were carefully transferred to a new conical tube. Cell pellet were washed by clinical buffer, Cell pellet was resuspended in appropriate amount of clinical buffer (final volume up to 1^{08} total cells/ 300 µl of clinical buffer).

d- Immune-magnetic purification of CD34 stem cell population:

Magnetic labeling:

Cells were kept cold and all the solutions used were at room temperature, working fast was important to prevent capping of anti bodies on the cell surface and non - specific cell labelling. Cells were passed through 30µm nylon mesh (pre-separation filter) to remove cell clumps which might clog the column. Cell number was determined by using heamocytometer.. Cell pellet was resuspended in 2 ml clinical buffer. CD_{34} Microbeads (150µm) were added to the cell suspension. Mixing well for the cell suspension and refrigerated for 30 minutes. Cells were washed with clinical buffer and then suspended in 500µl buffer.

Magnetic separation:

Column (MS column) was placed in the magnetic field of Mini MACS separator..Cell suspension was applied into the column. The Unlabeled cells that passed through were collected and the column was washed with Clinical buffer..Total effluent was collected and this was the unlabeled cell fraction. The column was removed from the Mini MACS separator and placed on a suitable collection tube. Clinical buffer (1ml) was pipetted onto the column. The magnetically labelled cells were immediately flushed out by firmly pushing the plunger into the column. The purity of the cells was determined by flowcytometry.

Cells were suspended in 10 ml clinical buffer and divided in 10 syringes; each one of them contained 1 ml.

Patients were admitted to hospital and infused, while fasting, with the purified human stem cells into the portal vein.

Table(1): base line ultrasounographic feature of studied groupsn(%)

	Groups						Chi-Square	
	Group I		Group II		Group III			
	N	%	N	%	N	%	X^2	P-value
Liver size **Shrunken** **enlarged**	4 2	66.67 33.33	5 3	62.50 37.50	6 4	60 40	0.152	0.926
Spleen **spleenectomy** **spleenomegly**	0 6	0 100	0 8	0 100	0 10	0 100	-	-
No Ascitis	0	0.00	2	25.00	2	20.00	1.905	0.928
Mild	1	16.67	1	12.50	1	10.00		
Moderate	2	33.33	3	37.50	3	30.00		
Sever	3	50.00	2	25.00	4	40.00		

Follow up of hepatic coma and bleeding tendency

Table (2): Regress of hepatic coma after1st month, 2nd month, 3rd month and 6th month

Attack of encephalopathy	Groups						Chi-Square	
	Group I		Group II		Group III			
	N	%	N	%	N	%	X^2	P-value
Attack of encephalopathy pretreatment	4	66.67	4	50.00	4	40.00	0.476	0.788
Attack of encephalopathy 1st month	2	33.33	2	20.00	6	60.00	2.286	0.319
Attack of encephalopathy 2nd month	2	33.33	3	37.50	4	40.00	0.152	0.927
Attack of encephalopathy 3rd month	0	0.00	2	20.00	6	60.00	4.142	0.126
Attack of encephalopathy 6th month	0	0.00	0	000.00	7	70.00	9.351	0.009*

2-Bleeding tendency

Table (3): Regress of bleeding tendenccies after1st month, 2nd month, 3rd month and 6 th month.

Bleeding tendencies (haematemesis or melena)	Groups						Chi-Square	
	Group I		Group II		Group III			
	N	%	N	%	N	%	X^2	P-value
Bleeding tendenccies pretreatment	4	66.67	4	50.00	3	30.00	1.391	0.499
Bleeding tendenccies 1st month	0	0.00	0	0.00	4	40.00	4.747	0.093
Bleeding tendenccies 2nd month	6	100.00	3	37.50	6	60.00	3.616	0.164
Bleeding tendenccies 3rd month	2	33.33	2	20.00	7	70.00	4.100	0.129
Bleeding tendenccies 6th month	0	0.00	2	20.00	6	60.00	4.142	0.126

biochemical changes in the studied groups after and before treatment

1- Billirubin

2-

Table(4): changes of billirubin in the studied groups before and after treatment

billirubin				Paired t-test	
		Mean	SD	t	P-value
Group I	Pretreatment	2.875	1.132		
	After 1st month	2.670	1.073	0.000	1.000
	After 2nd month	2.525	0.991	2.215	0.050*
	After 3rd month	2.625	1.014	0.862	0.452
	After 6th month	2.325	1.072	3.091	0.042*
Group II	Pretreatment	3.300	1.982		
	After 1st month	3.333	1.972	-0.852	0.433
	After 2nd month	3.210	1.974	2.986	0.0481*
	After 3rd month	3.117	2.035	3.337	0.034*
	After 6th month	3.083	2.013	2.339	0.031*
Group III	Pretreatment	3.286	1.870		
	After 1st month	3.071	1.879	2.785	0.032*
	After 2nd month	3.300	2.148	3.596	0.012 *
	After 3rd month	3.643	1.902	-3.047	0.023*
	After 6th month	3.886	1.892	-5.862	0.001*

Table(5): Changes of albumin in the studied groups before and after treatmentm

Albumin				Paired t-test	
		Mean	SD	t	P-value
Group I	Pretreatment	2.850	0.265		
	After 1st month	3.025	0.263	-2.782	0.046*
	After 2nd month	3.125	0.299	-5.745	0.010*
	After 3rd month	3.150	0.100	-3.286	0.046*
	After 6th month	3.400	0.337	-2.494	0.044 *
Group II	Pretreatment	3.100	0.460		
	After 1st month	3.083	0.264	0.183	0.862
	After 2nd month	3.203	0.293	3.113	0.0475*
	After 3rd month	3.350	0.283	3.170	0.048*
	After 6th month	3.400	0.395	3.57	0.035*
Group III	Pretreatment	3.043	0.346		
	After 1st month	3.114	0.157	-0.801	0.454
	After 2nd month	3.086	0.279	-0.311	0.766
	After 3rd month	2.943	0.223	4.734	0.014 *
	After 6th month	2.814	0.508	4.563	0.0194*

Post-treatment follow up of the patients

All patients in all groups 1 and 2 and 3 followed up clinically at first month, and regularly every month for 6 months. During the follow up, patients were observed for the following: serum bilirubin, albumin, prothrombin activity, ALT, AST, estimation of the degree of ascites, Child-Pugh score, hepatic encephalopathy, hematemesis and any unexpected complications.

Table(6): Changes of prothrombin activity in the studied groups before and after treatment.

Pa				Paired t-test	
		Mean	SD	t	P-value
Group I	Pretreatment	44.650	2.588		
	After 1st month	46.550	3.078	3.105	0.045 *
	After 2nd month	48.010	2.872	4.319	0. 015 *
	After 3rd month	50.500	3.572	6.735	<0.001**
	After 6th month	55.125	5.006	9.109	<0.001**
Group II	Pretreatment	41.767	2.365		
	After 1st month	50.333	2.338	5.073	0.01*
	After 2nd month	52.600	2.346	8.086	<0.001**
	After 3rd month	54.867	2.354	11.122	<0.001**
	After 6th month	56.000	2.972	15.257	<0.001**
Group III	Pretreatment	45.929	9.338		
	After 1st month	44.000	9.522	-1.758	0.129
	After 2nd month	42.457	6.537	-5.879	<0.001**
	After 3rd month	40.443	6.497	6.678	<0.001**
	After 6th month	38.000	5.151	10.477	<0.001**

Table(7): changes of AST in the studied groups before and after treatment.

AST				Paired t-test	
		Mean	SD	t	P-value
Group I	Pretreatment	56.000	13.191		
	After 1st month	45.750	3.304	11.947	<0.001**
	After 2nd month	46.500	13.026	10.919	<0.001**
	After 3rd month	39.000	17.776	12.228	<0.001**
	After 6th month	37.000	15.513	20.739	<0.001**
Group II	Pretreatment	55.000	4.050		
	After 1st month	51.833	13.906	4.031	0.0221*
	After 2nd month	53.833	22.551	-1.320	0.244
	After 3rd month	46.833	19.722	8.960	<0.001**
	After 6th month	41.167	19.146	12.533	<0.001**
Group III	Pretreatment	47.286	27.299		
	After 1st month	48.143	25.700	0.691	0.515
	After 2nd month	44.714	23.915	5.993	0.011 **
	After 3rd month	53.429	26.570	8.858	<0.001**
	After 6th month	58.714	29.534	7.170	<0.001**

Results

This study included 24 patients with Child C class liver cirrhosis. They were selected from the inpatients of tropical medicine and infectious disease department faculty of medicine tanta university. They were classified into three groups.

1) Group I: six patients (4 males and 2 female) their age ranged from 52-60 years with a mean value of 56.750± 3.403years, (table 5).

2) Group 2: eight patients (5 males and 3 females) their age ranged from 45-60 years with a mean value of 54.333± 5.888 years, (table 5).

3) Control group: ten patients (7 males and 3 females) their age ranged from 46-60years with a mean value of 52.714± 5.469 years, (table 5).

Base line ultrasonographic features of the studied group

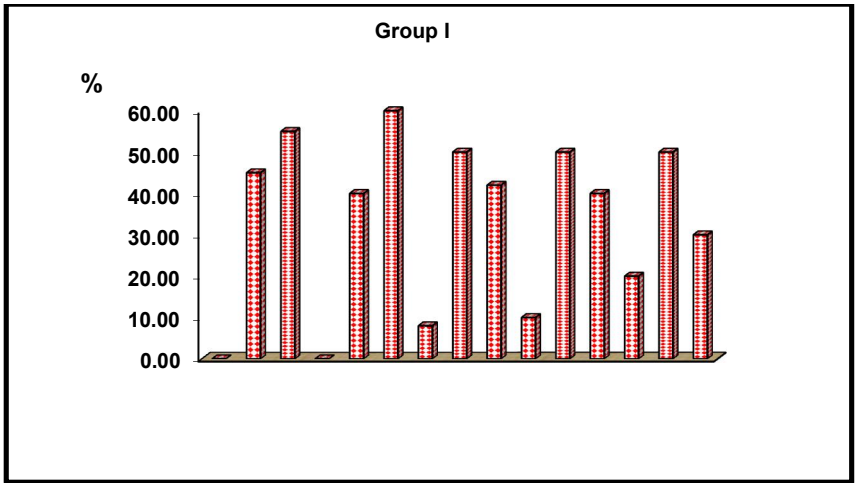
no statistical significant differences between three groups as regard liver size spleen and ascitis.

Table (8): Changes of ALT in the studied groups before and after treatment.

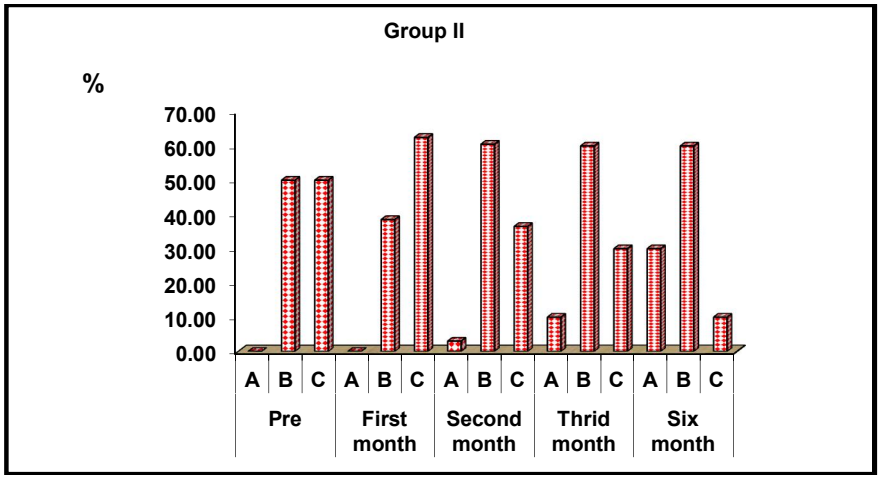
ALT				Paired t-test	
		Mean	SD	t	P-value
Group I	Pretreatment	33.750	6.946		
	After 1st month	30.750	15.283	3.454	0.041*
	After 2nd month	35.250	19.242	4.471	0.038*
	After 3rd month	32.500	22.338	2.97	0.048*
	After 6th month	30.000	12.447	3.474	0.034*
Group II	Pretreatment	35.500	11.095		
	After 1st month	32.167	17.577	4.504	0.022*
	After 2nd month	36.000	17.607	2.854	0.049*
	After 3rd month	34.500	9.894	3.44	0.034*
	After 6th month	31.333	12.612	2.980	0.047*
Group III	Pretreatment	38.143	9.459		
	After 1st month	40.571	8.182	3.844	0.031*
	After 2nd month	41.714	7.931	4.846	0.020*
	After 3rd month	39.714	11.870	2.972	0.0475*
	After 6th month	46.143	16.567	13.108	<0.001**

5- Child Pugh score:

(A)



(B)



(C)

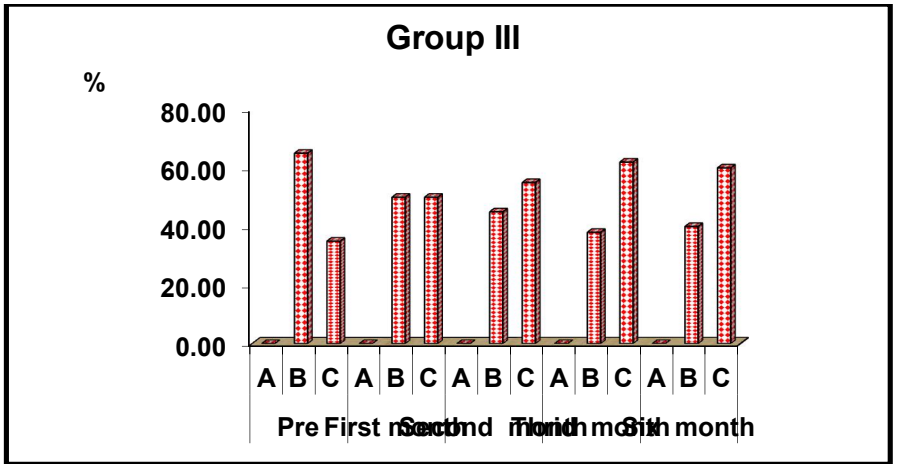


Figure: Child Pugh scores in the group1, 2and3 per:pretreatmen;1st Month;2nd month;3thmonth;sixthmonth after treatment

Discussion

Stem cells have recently shown promise in cell therapy because they have the capacity for self-renewal and multilineage differentiation, and are applicable to human diseases. The diseased liver may recruit migratory stem cells, particularly from the bone marrow, to generate hepatocyte-like cells either by transdifferentiation or cell fusion. Transplantation of BMSCs can restore liver mass and function, alleviate fibrosis, and correct inherited liver diseases. Therefore, it can significantly improve the liver function of patients with terminal liver disease, with good safety and effectiveness BMSCs can be delivered *via* the intraportal vein, systemic infusion, or *via* intraperitoneal, intrahepatic, and intrasplenic routes, (*Pai et al., 2008)*.

The current study was designed to evaluate the effects of autologous mononuclear and CD34+ hematopoietic stem cell intrahepatic infusion via portal vein, and to assess the outcome of stem cell therapy, in patients with end stage liver disaese. This was the basis for conducting a randomized study on patients with end stage liver disease. They were selected from in patients of the topical medicine department- tanta hospital- tanta University.

It was found a significant improvements in the serum albumin levels of the transplanted groups compared to the control group for the six month of study.

The mean serum bilirubin level showed an improvement in the transplanted patients, starting from the second month after stem cell therapy in group 2 and at 2ndand 6th month in group 1. The serum bilirubin was improved in group 1 and group 2 with significant decrease in its level by the time while in group 3 the serum bilirubin was significant increase in its level by the time.

The mean baseline Prothrombin activity value in our studied population started to improve one month after the procedure, and continued to improve until the 6th month. This improvement was statistically significant. The serum albumin was maintained in patients of group 1, 2 without intravenous administration of human albumin or plasma. While in control group serum albumin levels were decreasing throughout follow up period in spite of administration of intravenous albumin and fresh plasma.

There was borderline improvement in AST serum level in both groups (1, 2) while in group 3 there was significant decrease in its serum level by the time.

There was significant improvement in ALT serum level in both groups (1, 2) while in group 3 there was significant decrease in its serum level by the time.

At the start of the study, the percent of patients in group 1and 2 with history of hepatic encephalopathy was 66.7 % and50% respectively, the percent became 33.33 %, 33.33%, 0.00 % and 0.00% after 1, 2, 3, and 6 month respectively in group1. (maximum improvement occurred after 3 month) and 20%, 37.50%, 20.00%and 0.00% after 1, 2, 3, and 6 month respectively in group2. (maximum improvement occurred after 6 month).

Results of this study agree with *Salama et al.(2010)*who conducted a study on 48 end-stage liver cirrhosis who attended the Hepatology Clinic in Kaser El-Aini School of Medicine, Cairo University, Patients were randomized into one of two groups: Group 1: patients who received granulocyte colony stimulating factor (G-CSF) (Neupogen, Roche) for five days, followed by autologous CD34+ and CD133+ stem cell infusion in the portal vein; Group 2: patients who served as a control and those received regular liver treatment and demonstrated that there were borderline significant improvements in the serum albumin levels of the transplanted group compared to the control group at the end of the 6 mo study, and there was an improvement in PA that started in the 2nd month after HSCs transplantation and reached its maximum after 6 mo., demonstrated a highly significant improvement in the transplanted group as regards the recurrence of hepatic encephalopathy From the data presented in the their study, which included the largest cohort of HCV-associated end stage liver disease patients to date, They conclude that autologous stem cell transplantation may be safely administered and appears to offer some therapeutic benefit to patients with viral hepatic end-stage liver disease.*(Salama et al., 2010).* While our study disagree with study done by *Austin and Lagasse (2003)* found only a small number of BMSCs actually differentiated into hepatocytes, the emergence of bone marrowderived hepatocytes was rather slow, with the first cells appearing 2 months after BMT but expansion of these hepatocytes created a significant mass of donor-derived hepatocytes visualized by P-galactosidase staining. The mechanism by which BMSCs became functional hepatocytes in this system may be cell-cell fusion. And *Am Esch et al (2005.* reported that portal administration of autologous CD133+BMCs accelerated liver regeneration and is a novel therapy to support hepatic resection. While *Orit Kollet(2003)* reported that adult stem cell treatments currently being used in human patients. In case of liver damage stress on the body can trigger adult stem cells to change into specialized cells that migrate to the damaged area and help repair the injury. For example, a damaged liver can send signals to bone marrow stem cells which respond by creating liver cells for the

damaged liver. And *Young (2004*) reported that this type of stem cell technique could eventually be used to treat chronic diseases such as diabetes, cirrhosis of the liver, heart disease and cancer. Bone marrow stem cells, when exposed to damaged liver tissue, can quickly convert into healthy liver cells and help repair the damaged organ, in mouse-tissue cultures, scientists found that stem cells, in the presence of cells from damaged liver tissue, developed into liver cells in as little as seven hours. So **r**esult of the present study show that stem cell have a beneficial effect on synthetic function of the liver and possibly improve quality of life of patient with end stage liver disease.

Conclusion

Bone marrow aspiration was chosen as a source of stem cells to treat end stage liver disease that had not been responded to conventional and other advanced Treatments.

Stem cell transplantation have a beneficial effect on synthetic function of the liver and possibly improve survival and quality of life of patients with end stage liver disease.Since it is based on autologous bone marrow cells, this therapy will not be limited by a shortage of organs and will be cheaper than whole liver transplantation.Hepatocyte transplantation may not be able to reverse portal hypertension, or the development of HCC, but improvement in liver physiology and patient survival could be possible and could play a significant role in the management of patients with liver failure.

References:

1. Am Esch, J.S. 2nd; Knoefel, W.T.; Klein, M. (2005): Portal application of autologous CD133+ bone marrow cells to the liver: A novel concept to support hepatic regeneration. STEM CELLS., 23:463–470.
2. Austin, T.W. and Lagasse, E. (2003): Hepatic regeneration from hematopoietic stem cells. Mech Dev., 120: 131-135.
3. Barrilleaux, B.; Phinnes, D.G.; Prock op, D.J. and O[f]connor KC. (2006): "Review:ex vivo engineerivg of living tissues with adult stem cells". Tissve Eng., 12 (11): 3007-3019.
4. Bethesda, M.D. (2009): stem ell Bassics: introduction in stem cell information. National Institutes of Health U.S Department of health & human services.
5. Di Campli, C.; Nestola, M.and Piscaglia, A.C. (2003): Cell-based therapy for liver diseases. European Review for Medical and Pharmacological Sciences., 7(2):41-44.
6. El-Zayadi, A.R.; Badran, H.M.; Barakat, EM.F.; Attia, M.E.D.; Shawky, S.; Hiohamed, M.K.; Selim, O. and Saeid, A. (2005); Hepatocellular carcinoma in Egypt: a single center study over a decade, World J. Gastroenterol II., (33) 5193 -5198.
7. Fausto, N. Liver (2004): regeneration and repair: hepatocytes, progenitor cells, and stem cells. Hepatology, 39:1477-87.
8. Frank, C.; Mohamed, M.K.; Strickland, G'.T.; Lavanchy, L.; Arthur, R.R.; Magder, L.S.; ElKhoby, T.; Abdel-Wahab, K.; Afy Ohn, E.S.; Anwar, W.; Sallam, L.(2000): ITie role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet., 355:887—891.
9. Gordon, M.Y.; Levicar, N.; Pal, M.; Bacheilier, P.; Dimarakis 1; Al-Allaf, F.; M'Hamdi, H.; Thalji, T.; Welsh, J.P.and Marley, S.B. (2006); Characterization and clinical application of human CD34+ stem/progenitor cell populations mobilized into the blood by granulocyte colony-stimulating factor. Stem Cells., 24:1822-1830.
10. Kollet, O.(2003): "HGF, SDF-1, and MMP-9 are involved in stress-induced human CD34+ stem cell recruitment to the liver". Journal of Clinical Investigation., 112 (2):160-169.
11. Mallet, V.Q.and Gilgenkrantz, H. (2005); Mobilizing stem cells to repair liver after surgery: dream or reality? Journal of Hepatology., 43(5):7 54- 756.
12. Pai, M.; Zacharoulis, D.and Milicevic, M.N. (2008). Autologous infusion ofexpanded mobilized adult bone marrow-derived CD34+ cells intopatients with alcoholic liver cirrhosis. Am J Gastroenterol., 103:1952–8.
13. Piscaglia, A.C.; Di Campli, C.; Pola, P.and Gasbarrini, A.(2002): When biology bursts into the clinic: stem cells and their potential. European Review for Medical and Pharmacological Sciences., 5(5-6):151-154.
14. Piscaglia, A.C.; Novi, M, t.; Campanale, M.and Gasbarrini, A. (2008): Stem cell-based therapy in gastroenterology and hepatology. Minimally Invasive Therapy and Allied Technologies., 17(2): 100-1 18.
15. Piscaglia, A.C.; Campanate, M.; Gasbarrini, A. and Gasbarrini, G. (2010): Stem Cell-Based Therapies for Liver Diseases: State of the Art and New Perspectives Stem Cells Int., 2010: 259461 U.
16. Salama, H.; Zekri, A.R.; Bahnassy, A.; Medhat, E.; Halim, H.; Ahmed, O.; Mohamed; Alim, S. and Sherif, G.(2010): Autologous CD34 and CDJ33 stem cells transplantation in patients with end stage liver disease World J Gastroenterol. November 14., 16(42): 5297-5305.
17. Willenbring, H.; Bailey, A.S.and Foster, M.(2004): Myelomoncytic cells are sufficient for therapeutic cell fusion in liver. Nat Med, 10: 744-8.
18. Young, Y.(2004): Hematopiotic stem cells convert into liver cells within days without fusion, "Nature Cell Biology, June 1, 2004".