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RESEARCH ARTICLE

AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN SUB-TERMINAL LIVER DISEASE PATIENTS.

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Abstract

Back ground and objective: The ultimate goal of this work is to evaluate the effect of autologous hematopoietic stem cells transplantation as alternative therapeutic option in patients with sub terminal liver diseases. The work divided into 3 parts: Mobilization of CD34⁺ HSCs to peripheral blood, Leukapheresis, Intravenous infusion of autologous CD34⁺ HSCs to the patient.

Patients and Method: 30 patients with HCV induced liver cirrhosis divided into 3 groups: **Group I:** consists of 10 patients with liver cirrhosis selected as Child C with scoring range from (14-21) this group was injected with G-CSF for 5 consecutive days to mobilize stem cells from the bone marrow to peripheral blood. **Group II:** consists of 10 patients with liver cirrhosis selected as Child C with scoring range from (16-23). This group was injected with granulocytes colony stimulating factor (G-CSF) 5 days before collection followed by leukapheresis and peripheral vein infusion of autologous hematopoietic stem cells CD34⁺ on day 6. **Group III:** control group consists of 10 cirrhotic patients maintained on supportive medical therapy.

Results: The synthetic functions of liver in both groups I and II improved as evidenced by liver function tests (serum albumin, PT / INR& serum bilirubin) and MELD score after the procedure.

Conclusion: Stem cell therapy is expected to be a last chance for patients suffering from end stage liver disease ESLD and waiting for donor livers. Consequently, decrease the need for orthotopic liver transplantation especially in the early stages of liver disease.

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Introduction:-

Liver cirrhosis results from progressive, extensive fibrosis and impaired hepatocytes regeneration and is associated with many serious systemic complications resulting from both liver failure and portal hypertension and it is mainly caused by viral hepatitis and alcohol abuse (Margini et al., 2014).

Orthotopic liver transplantation (OLT) is the only curative remedy for cirrhotic patients, but it is coupled with paucity of donor organs available for liver transplantation as well as high financial burden and surgical

complication, all that limitation paved the way for finding alternative therapeutic options for this potentially life-threatening condition (**Sharma et al., 2015**).

Advances in liver regenerative therapy through cell transplantation as a non-invasive treatment for cirrhosis will overcome these restrictions. Clinical trials have included hematopoietic stem cell (HSC) mobilization by administration of granulocyte colony-stimulating factor (G-CSF), infusion of autologous bone marrow cells and administration of autologous mesenchymal stem cells (MSC) derived from bone marrow or umbilical cord. Several reported randomized controlled studies highlighted the effectiveness of these ground breaking approaches (**Margini et al., 2014**).

The intriguing capability of blood-derived stem cells that differentiate into multiple cell lineages raises the exciting opportunity of using these cells for tissue repair when the intrinsic pool of tissue stem cells is overwhelmed (**Zhang et al., 2012**).

Adult stem cells are found in human bone marrow, blood, eye, brain and skeletal muscle. They do not appear to have the same capabilities as embryonic stem cells. However, under laboratory conditions, they were manipulated to form other cells types. It might be eventually possible, to direct these cells to function in other areas of the body and to replenish body tissue that have been damaged or diseased with healthy cells (**Grove et al., 2004**).

Cytokine administration to induce mobilization of bone marrow stem cells (BMSC) could be a potential approach to improve engraftment in the damaged liver. G-CSF was used in liver regeneration because of its ability to increase the number and enhance homing of circulating BMSCs to the liver in order to promote repair in the cirrhotic liver as suggested by (**Jinet et al., 2010 ; Mizunaga et al., 2012**).

The peripheral blood CD34⁺ HSC count under steady state condition is very low (<0.05 of white blood cells). G-CSF can mobilize large number 15-35 fold of CD34⁺ HSCs from the bone marrow pool into circulation following 4-5 days of administration (**Al Tayebet et al., 2015**).

The feasibility and safety of mobilizing BMSC following G-CSF administration was reported in study by (**Gaia et al., 2006**) on eight patients with ESLD. Additionally, they demonstrated improved model for end-stage liver disease (MELD) scores and did not find any development of hepatocellular carcinoma (HCC) up to eight months after G-CSF administration. A favorable effect of G-CSF administration on survival and clinical parameters in patients with liver failure was also reported by (**Spahar et al., 2008**), they showed that G-CSF mobilizes CD34⁺ cells, increases hepatocyte growth factor (HGF) and induces hepatic progenitor cells to proliferate within 7 days of administration in patients with alcoholic steatohepatitis.

In addition, **Lorenzini et al., 2012** demonstrated the safety of BMSCs mobilization and collection through leukapheresis in patients with liver cirrhosis, even though no improvement of liver function tests occurred.

On the other hand, G-CSF administration could be associated with increased risk of splenomegaly in healthy donors mentioned by (**Picardi et al., 2003**) or even rupture, as reported by (**Falzetti et al., 1999**).

Hematopoietic stem cells are the most widely studied example of adult SC sources, they sustain formation of blood and immune system cells throughout normal life. These cells are capable of differentiating into many types of other tissues, including skeletal and cardiac muscle, endothelium and a variety of epithelia including neuronal cells, pneumocytes and hepatocytes (**Zhang and Huang, 2012**).

The ultimate goal of this study is to evaluate the clinical effect of cellular therapy using autologous G-CSF mobilized CD34⁺ HSCs in cirrhotic patients.

Patients and Methods:-

The study was conducted on 30 patients with HCV induced liver cirrhosis, they were recruited from patients admitted to Gastroenterology Department in Theodor Bilharz Research Institute.

Inclusion criteria:-

1. Male or female aged from 30 to 65 years.

2. Chronic hepatic failure due to hepatitis C or hepatitis B virus
3. Child C liver cirrhosis
4. Model of End stage Liver Diseases (MELD) score >12

Exclusion criteria:-

1. Patients aged below 30 or over 65 years
2. Active bleeding
3. Hepatocellular carcinoma
4. Hepatic coma of any grade
5. History of autoimmune disease
6. Presence of any type of malignancy

They were divided into 3 groups:

Group I:-

Consists of 10 patients with liver cirrhosis on top of HCV they were selected as Child C with scoring range from (14-21) this group was injected with G-CSF for 5 consecutive days to mobilize stem cells from the bone marrow to peripheral blood, their age range between 40-60 years with mean (47 ± 3.4) They were 7 males and 3 females.

Group II:-

Consists of 10 patients with liver cirrhosis on top of HCV they were selected as Child C with scoring range from (16-23) according to Child Pugh grading, this group was injected with granulocytes colony stimulating factor (G-CSF) 5 days before collection followed by leukapheresis and autologous CD34⁺ stem cells infusion through peripheral vein on day 6. Their age range between 42-61 years with mean (48 ± 7.4) they were 6 males and 4 females.

Group III:-

Control group consists of 10 cirrhotic patients maintained on ordinary liver supportive therapy including albumin, fresh plasma and vitamin K. They matched with group I & II in age, sex and Child scoring their age range between 39 and 62 years with mean (45 ± 4.1) they were 6 males and 4 females.

All patients were subjected to clinical examination, blood tests (CBC and prothrombin time) chemistry tests including (ALT, AST, Alkaline phosphatase, Albumin & Bilirubin) and imaging investigations as ultrasound and endoscopy. The study protocol was approved by the Institutional Review Board (IRB TBRI). All patients gave their written informed consent for the study.

1. Stem cell mobilization was achieved in Group I and Group II by subcutaneous injection of G-CSF in the dose of 300 mcg/mL for 5 days before leukapheresis to increase the number of circulating CD34⁺HSC in their peripheral blood.
2. Leukapheresis was carried out on Group II patients on the 6th day of G-CSF administration using Apheresis System (Cobe® Spectra, Terumo BCT Inc., Lakewood, Co, USA).
3. Flowcytometric assessment of the CD34⁺ HSCs using (Coulter® Epics® XL™, USA) equipment and monoclonal anti-CD 34 FITC (Miltney biotech, Germany) before undergoing apheresis and subsequently on an aliquot taken from apheresis product after completion of stem cells harvest protocol, the average count was 1×10^6 - 2×10^8 cells.

The patients included in this study were followed up every 2 weeks for the first month then regularly every month for 6 months. During the follow up the patients were assessed for the following clinical data (ascites, edema, hepatic encephalopathy and jaundice), liver enzymes (AST and ALT), albumin, bilirubin, prothrombin time, concentration & INR, creatinine and MELD score.

Statistical analysis:-

The Statistical Package for Social Sciences (SPSS) for Windows (version 18) computer program was used for statistical analysis.

Results:-

The study was conducted on 30 patients with HCV induced liver cirrhosis divided into 3 groups

Group 1:-

Ten patients were injected with G-CSF for 5 consecutive days to mobilize stem cells from the bone marrow to peripheral blood.

Group II:-

Ten patients received cellular therapy in the form of autologous G-CSF mobilized HSC CD34⁺ transplantation.

Group III:-

Ten cirrhotic patients maintained on medical liver supportive therapy served as control group.

No major complication or side effect was noticed during the procedure of stem cells mobilization and transplantation.

Regarding Clinical data:-

The majority of patients in all groups showed major hepatic manifestation including lower limb edema, ascites and encephalopathy. However, there is no significant difference between the three groups in frequency of advanced hepatic diseases at the base line and these manifestations improved during the follow up period for both **group I** and **group II**. Two patients from **group I** showed complete resolution of ascites and remained ascites free at 6 months. Edema and encephalopathy showed improvement in both **groups I & II**.

Regarding laboratory data:-

At base line patients of all studied groups had low prothrombin concentration (PC), low serum albumin level, liver enzymes were moderately elevated, bilirubin levels were high and platelet count was low. Model of End stage Liver Disease (MELD) scores were high in all groups. There was no significant difference between all groups concerning albumin, liver enzymes, PC, INR, platelets, creatinine and MELD score at the baseline.

There was significant higher serum albumin level in **group I** and **group II** compared to control group during the follow up period at 3 and 6 month. Conversely, no difference was noticed in comparing **group I** and **group II** during the follow up.

Moreover, during the follow up period, there was a statistically significant increase in serum albumin comparing the baseline level with 3 and 6 months follow up after SCT (p 0.02) and (p 0.01) respectively, in **group I**. Similarly, in **group II** there was a statistically significant improvement in serum albumin level during the follow up period (p 0.03) and (p 0.04). This is in contrast to the non-significant difference in serum albumin in control group during the follow up period (**Table 1**).

Table 1:- Change in Albumin level in studied groups

Albumin	Baseline	After 3 months	After 6 months	P1 value	P2 value
Group I N=10 Mean \pm SE	2.07 \pm 0.16	2.7 \pm 0.12	2.6 \pm 0.13	0.03*	0.04*
Group II N=10 Mean \pm SE	2.09 \pm 0.09	2.47 \pm 0.09	2.48 \pm 0.1	0.02*	0.01*
Control N=10 Mean \pm SE	1.95 \pm 0.05	1.98 \pm 0.06	1.97 \pm 0.07	0.6	0.7
^a P value	0.4	0.001**	0.001**		
^b P value	0.1	0.001**	0.001**		
^c P value	0.9	0.1	0.4		

P1: comparison after 3 months. **P2:** comparison after 6 month, while ^aP: comparison of group1 ^{vs} control, ^bP: comparison of group2 ^{vs} control, and ^cP: comparison of group1 ^{vs} group2.

*P value = 0.05 significant; **P value = 0.01 highly significant.

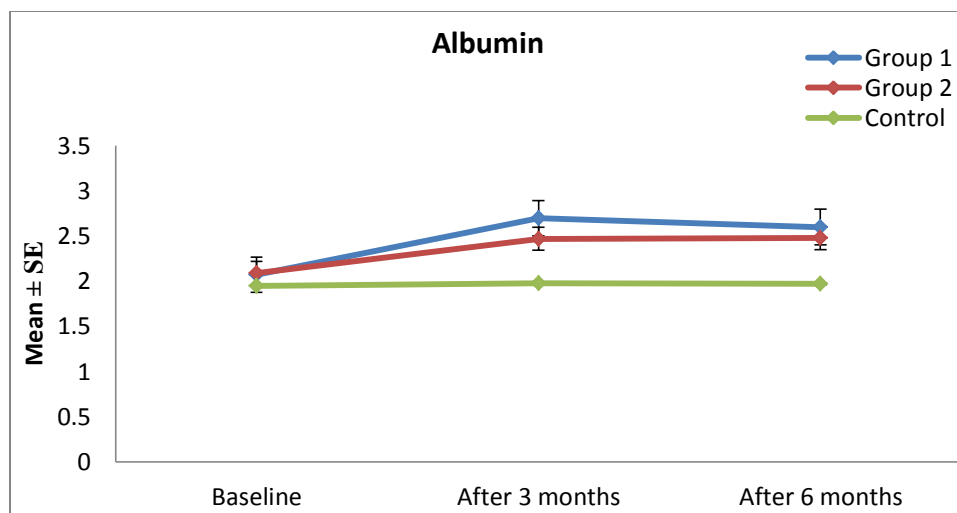


Fig 1:- Change in Albumin level in studied groups

Regarding serum ALT level there was no significant difference noticed on comparing **group I** and **group II** with the control group during the follow up period. However, there was mild significant improvement in serum ALT level on comparing **group II** and **group I** at 6 months (p 0.03).

Also, there was statistically significant decrease in ALT level during the follow up period obtained in **group I** after 3, 6 months follow up (p0.01) and (p 0.04), respectively. On the contrary no significant change was noticed in **group II** and control group (**Table 2**).

Table 2:- Change in ALT level in studied groups

ALT	Baseline	After 3 months	After 6 months	P1 value	P2 value
Group I N=10 Mean ± SE	52.0±2.4	45.4±2.1	46.8±1.3	0.01*	0.04*
Group II N=10 Mean ± SE	53.9±1.52	48.7±2.6	52.0±1.9	0.1	0.4
Control N=10 Mean ± SE	51.0±3.7	50.0±3.4	51.8±3.5	0.6	0.7
^a P value	0.6	0.2	0.2		
^b P value	0.8	0.7	0.9		
^c P value	0.5	0.3	0.03*		

P1: comparison after 3 months. **P2:** comparison after 6 month, while ^aP: comparison of group1 ^{vs} control, ^bP: comparison of group2 ^{vs} control, and ^cP: comparison of group1 ^{vs} group2.

*P value = 0.05 significant; **P value = 0.01 highly significant.

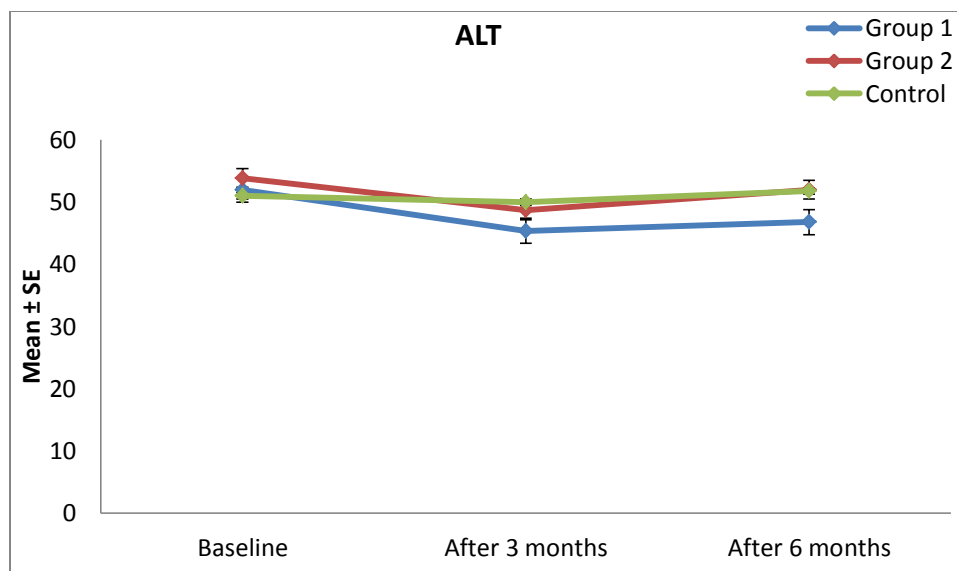


Fig 2:- Change in ALT level in studied groups

In addition, there was statistically significant decrease in serum bilirubin level on comparing both groups I and II with control group during the follow up period at 3, 6 months (**Table 3**)

Also, there was statistically significant decrease in serum bilirubin level in comparing the baseline and 3 months and 6 months follow up (p 0.001) and (p 0.01) respectively in **group I** and also there was significant decrease in serum bilirubin level obtained after 3 and 6 months follow up (p 0.001) and (p 0.01), respectively in **group II**. On the other hand there was no change during the follow up in control group.

Table 3:- Change in bilirubin level in studied groups

Bilirubin	Baseline	After 3 months	After 6 months	P1 value	P2 value
Group I N=10	2.3±0.2	1.6±0.2	1.6±0.2	0.001**	0.01*
Mean ± SE					
Group II N=10	3.0±0.4	2.0±0.2	2.1±0.2	0.001**	0.01*
Mean ± SE					
Control N=10	2.8±0.3	2.8±0.3	2.7±0.2	0.6	0.7
Mean ± SE					
^a P value	0.1	0.003*	0.002*		
^b P value	0.5	0.03**	0.05**		
^c P value	0.09	0.2	0.1		

P1: comparison after 3 months. **P2:** comparison after 6 month, while ^aP: comparison of group I ^{vs} control, ^bP: comparison of group II ^{vs} control, and ^cP: comparison of group I ^{vs} group II.

*P value = 0.05 significant; **P value = 0.01 highly significant.

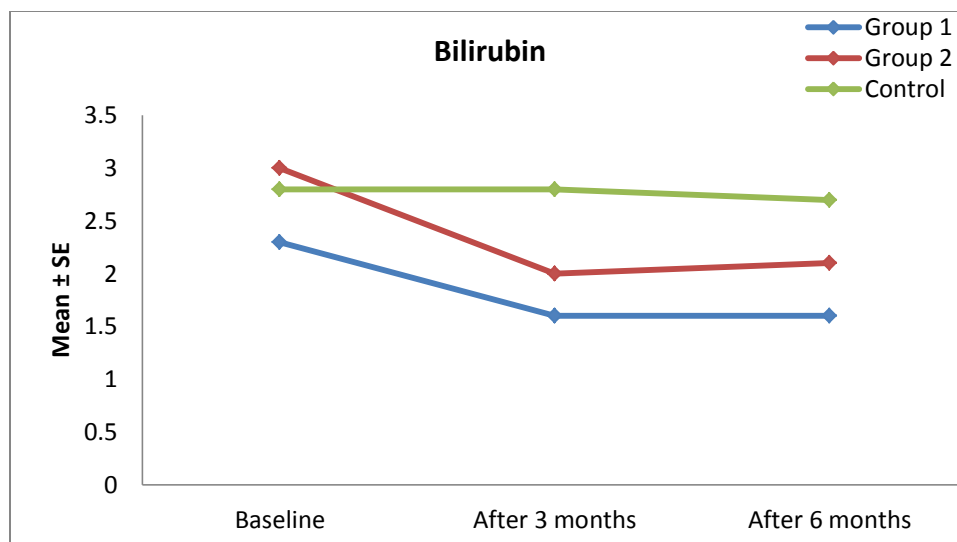


Fig 3:- Change in bilirubin level in studied groups

Regarding INR level there was significant improvement in **group I** compared to control group after 3 and 6 months (p0.03) and (p 0.02)

There was an improvement in INR levels during the follow up period in group **I** at 3, 6 months (p 0.001) and (p 0.01) compared to the baseline, respectively. Also, follow up in **group II** showed significant improvement (p 0.001) and (p 0.001) during the follow up at 3, 6 months, respectively.

Table 4:- Change in INR level in studied groups

INR	Baseline	After 3 months	After 6 months	P1 value	P2 value
Group I N=10 Mean ± SE	1.7±0.1	1.4±0.1	1.5±0.1	0.001**	0.001**
Group II N=10 Mean ± SE	2.1±0.2	1.7±0.1	1.7±0.1	0.001**	0.01*
Control N=10 Mean ± SE	1.9±0.2	1.8±0.2	1.9±0.1	0.3	0.9
^a P value	0.4	0.03*	0.02*		
^b P value	0.5	0.4	0.3		
^c P value	0.1	0.1	0.08		

P1: comparison after 3 months. **P2:** comparison after 6 month, while ^aP: comparison of group1 ^{vs} control, ^bP: comparison of group2 ^{vs} control, and ^cP: comparison of group1 ^{vs} group2.

*P value = 0.05 significant; **P value = 0.01 highly significant.

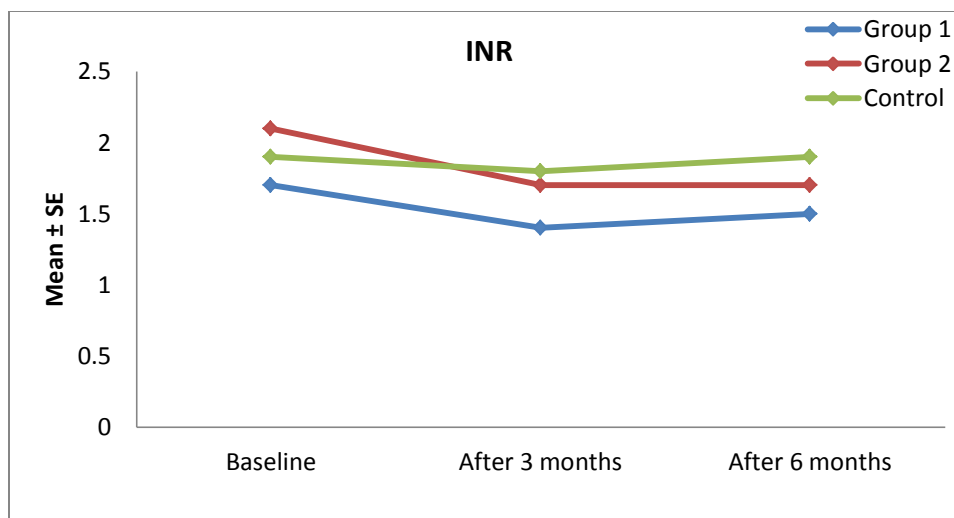


Fig 4:- Change in INR level in studied groups

Regarding MELD score there was a significant improvement in **group I** compared with the control group after 3 months (P 0.003). Furthermore, there was a significant difference between **group I** and **group II** after 6 months.

Moreover, during the follow up period there was a significant improvement in MELD score in **group I** (p 0.001) and **group II** (p 0.001).

Table 5:-Change in MELD level in studied groups

MELD	Baseline	After 3 months	After 6 months	P1 value	P2 value
group I N=10 Mean ± SE	16.7±0.7	12.4±0.9	12.8±1.0	0.001**	0.001**
Group II N=10 Mean ± SE	18.8±0.8	14.9±1.0	15.6±0.9	0.001**	0.001**
Control N=10 Mean ± SE	17.4±1.2	17.1±1.0	15.7±1.7	0.43	0.36
^a P value	0.6	0.003*	0.1		
^b P value	0.3	0.1	0.9		
^c P value	0.07	0.08	0.05*		

P1: comparison after 3 months. P2: comparison after 6 month, while ^aP: comparison of group1 ^{vs} control, ^bP: comparison of group2 ^{vs} control, and ^cP: comparison of group1 ^{vs} group2.

*P value = 0.05 significant; **P value = 0.01 highly significant.

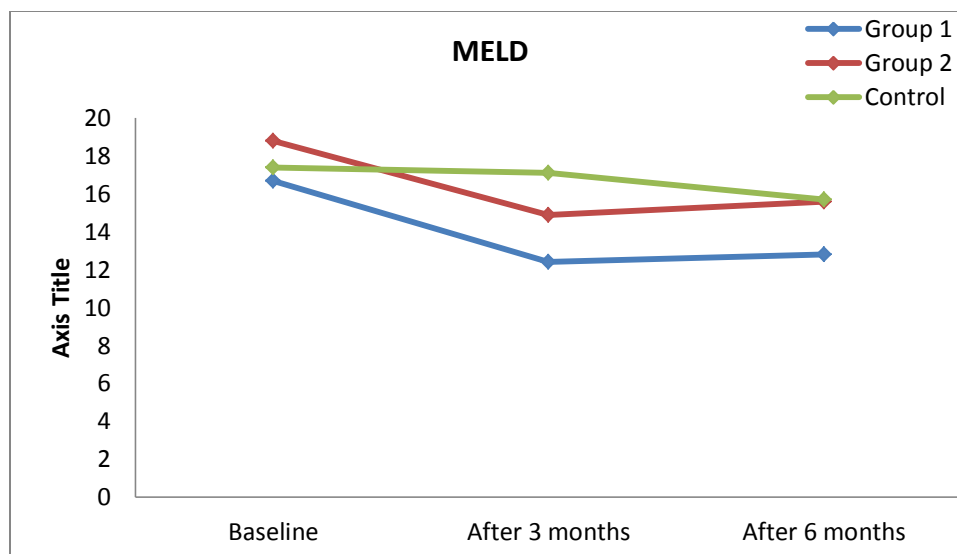


Fig 5:- Change in MELD level in studied groups

Indeed, there is a statistically significant decrease in serum creatinine level comparing the baseline and 3 months after SCT (p 0.03) in **group II**.

Table 6:- Change in serum creatinine level in studied groups

Creatinine	Baseline	After 3 months	After 6 months	P1 value	P2 value
Group I N=10 Mean \pm SE	0.9 \pm 0.1	0.8 \pm 0.1	0.8 \pm 0.1	0.2	0.5
Group II N=10 Mean \pm SE	0.9 \pm 0.1	0.7 \pm 0.1	0.9 \pm 0.1	0.03*	0.4
Control N=10 Mean \pm SE	0.8 \pm 0.1	0.7 \pm 0.1	0.8 \pm 0.1	0.08	0.8
^a P value	0.7	0.8	0.9		
^b P value	0.4	0.8	0.6		
^c P value	0.6	0.7	0.7		

P1: comparison after 3 months. P2: comparison after 6 month, while ^aP: comparison of group1 ^{vs} control, ^bP: comparison of group2 ^{vs} control, and ^cP: comparison of group1 ^{vs} group2.

*P value = 0.05 significant; **P value = 0.01 highly significant.

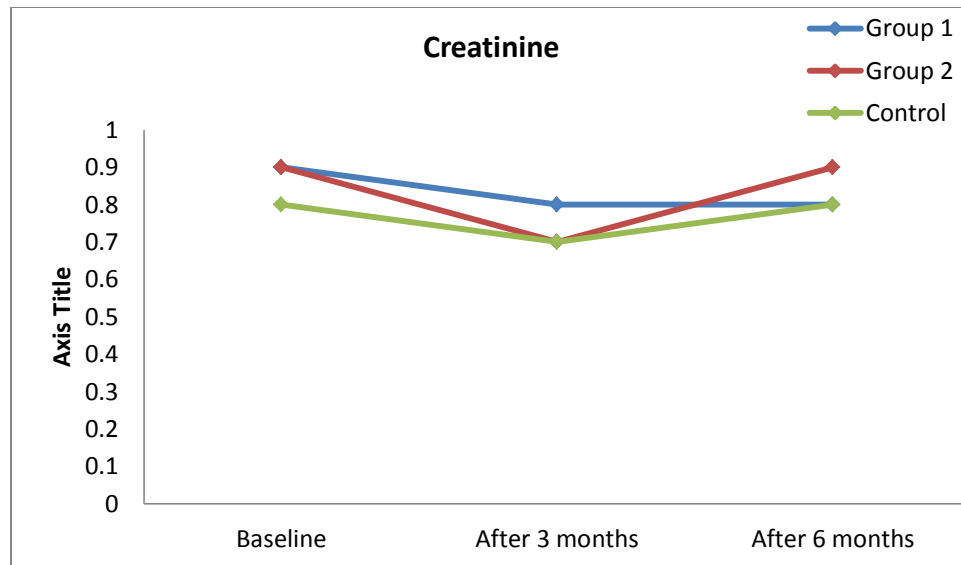


Fig 6:- Change in serum creatinine level in studied groups

Discussion:-

Hepatic cirrhosis is defined as the histological development of regenerative nodules surrounded by fibrous bands in response to chronic liver injury, which leads to portal hypertension and ESLD. The majority of patients with hepatic cirrhosis die from life-threatening complications at early age (Margini *et al.*, 2014).

Orthotopic liver transplantation had been the most effective treatment for patients with hepatic cirrhosis. Since liver transplantation is critically limited by the shortage of available donor livers, searching for an effective alternative therapy has attracted great interest in preclinical studies. The encouraging advances in stem cell research have paved the way towards the treatment of the end-stage of chronic liver disease (Saito *et al.*, 2013; Palanisamy *et al.*, 2015).

Cell therapy in the setting of ESLD or cirrhosis due to HCV infection one must take into account the depletion of parenchymal cells, architectural distortions, vascular reorganization, inflammation and excessive extracellular matrix deposition and myofibroblast activation and proliferation. This distorted liver microenvironment is naturally challenging for the engraftment and proliferation of transplanted cells (Nicolas *et al.*, 2017).

An impressive number of cell types are investigated as sources of liver regeneration: hepatocytes, liver progenitor cells (LPCs), mesenchymal stem cells (MSCs) originated from bone marrow, umbilical cord blood, adipose tissue, hematopoietic stem cells (HSCs), induced pluripotent stem cells (iPSCs) and bone marrow mononuclear cells (BM-MNCs) (Shiota and Itaba, 2017).

Autologous adult stem cells have the least number of obstacles for clinical application, their potential interventions on cirrhosis are especially illustrated in terms of the cellular and molecular mechanisms of hepatic fibrogenesis (Panminerva Med. 2010)

Stem cells have an inherent ability to migrate that is as important as their capacity for self-renewal and differentiation enabling them to maintain tissue homeostasis and mediate repair and regeneration. The migration of stem cells from circulation into damaged or pathological tissues is the most crucial step. Biological signals released from the injured area and corresponding receptors expressed on cell surface of stem cells are critical determinants in this step (De lucas *et al.*, 2017)

Stem cell therapy may contribute to the improvement of liver function. Although, the mechanisms involved are not yet fully understood. One hypothesis is that genomic plasticity, in response to the microenvironment, causes the trans-differentiation of stem cells into functional hepatocytes (Jang *et al.*, 2004). Another mechanism is presumably related to the cell fusion of BMSCs and hepatocytes. It had been proposed that stem cells may exert paracrine effects on endogenous hepatocytes to increase their ability to regenerate, through the release of proliferative cytokines and

the production of matrix metalloproteinase-9 or by enhancing angiogenesis through the release of vascular endothelial growth factors (Anthony DF and Shiels PG. 2013)

Hematopoietic stem cells (HSCs) can be obtained by BM aspiration or from peripheral collection through leukapheresis after granulocyte-colony stimulating factor (G-CSF) administration. Previous studies hypothesized that infusion of CD34⁺ HSCs may help to improve liver function in patients with decompensated liver cirrhosis (Al Tayeb *et al.*, 2015).

The key aspect of the present study was to assess and evaluate the therapeutic effect of autologous transplantation of G-CSF mobilized peripheral blood CD34⁺ HSCs into cirrhotic patients in improving liver function tests and quality of life as possible alternative for OLT.

This investigation was conducted on a total of 30 patients with chronic hepatic failure due to hepatitis C virus (HCV) infection. The degree of hepatic affection was determined according to Child Pugh scoring. All patients were Child C liver cirrhosis, MELD score >12. In (**Group I**), G-CSF was injected for 5 consecutive days to mobilize stem cells from the bone marrow to peripheral blood. Additionally, (**Group II**) patients were peripherally infused with autologous HSC CD34⁺. HSCs mobilization was induced using G-CSF followed by leukapheresis collection. Both groups were compared to the control group (**Group III**) which was maintained on supportive medical therapy.

In the current study, no complication or specific side effect related to mobilization of CD34⁺ HSCs was observed in any group this goes with agreement with Sharma *et al.*, 2015, who assessed the therapeutic effect of mobilized autologous CD34⁺ HSCs infusion in patients with non-viral decompensated liver cirrhosis, there was no complications including splenic rupture during mobilization procedure in any patients enrolled in their study.

All patients included in this study were re-evaluated clinically after 3 and 6 months after mobilization of CD34⁺ cells, improvement in quality of life was observed, almost all symptoms of liver failure improved such as ascites, jaundice, lower limb edema and there is marvelous decrease in ascites in one patient in **group I** during the follow up, this goes with the trial of (Al Tayeb *et al.*, 2015) who noticed improvement in quality of life in all of their studied patients especially over the first 2 months. Similarly, another study by (Salama *et al.*, 2010) noticed improvement in ascites, hepatic coma and hematemesis which occurred as result of stem cells transplantation.

The laboratory finding before and after mobilization of CD34⁺ HSCs in **Group I&II** showed statistically significant improvement in serum albumin, serum bilirubin, INR and MELD score. In addition, there was significant decrease in ALT level in **group I** and improvement of serum creatinine level in **group II** during the follow up. This could be explained by improved liver synthetic function.

Statistical comparison between both groups and control group after 3 & 6 months follow up, showed partial improvement of liver function tests elicited in **group I** and **II** compared to control, as there was statistically significant improvement in serum albumin and bilirubin levels.

In addition, statistically significant improvement in INR and MELD score was noticed in **group I** compared to the control group.

Conversely, no significant difference could be elicited between **group I** and **group II** except for mild significant improvement in MELD score and serum ALT level in **group I** who had undergone mobilization of stem cells using G-CSF.

The data obtained from this study conformed with observations from clinical trial on 20 patients with liver cirrhosis on top of HCV by (Al Tayeb *et al.*, 2015) who implanted their patients with autologous stem cell infused through hepatic artery after mobilization with G-CSF and separation by leukapheresis, they displayed statistically significant improvement in quality of life, serum albumin, total bilirubin, liver enzymes and Child Pugh score over the first 2-3 months after procedure.

Likewise, intravenous or intrahepatic infusion of autologous CD133⁺/ CD34⁺ HSCs had a supportive role in the treatment of ESLD (Salama *et al.*, 2014). Similarly, repeated combined intravenous / intraportal infusion of

autologous BM CD34⁺/CD133⁺HSCs had a beneficial effect on liver functions with minimal adverse events and more lasting clinical efficacy after repeated infusion (Zekri *et al.*, 2015).

Another support was offered by (Levicar *et al.*, 2008), who infused adult stem cells CD34⁺ into portal vein or hepatic artery on five patients with chronic liver disease. They recorded improvement in serum albumin and bilirubin level. They explained this improvement in liver function by release of vascular endothelial growth factor by stem cells, thereby increasing blood supply to the cells and thus helping to repair damage tissue.

In agreement with (Terai *et al.*, 2006) who performed clinical trial on 9 patients with liver cirrhosis. These patients were infused with autologous bone marrow stem cells into peripheral vein, they displayed statistically significant improvement in total protein, serum albumin and Child Pugh score.

Perusing the same subject (Gordon *et al.*, 2006) evaluated the therapeutic effect of intrahepatic injection of CD34⁺ HSCs in five patients with liver cirrhosis, their results showed improvement in serum albumin and decrease in serum bilirubin in four and three patients respectively. Nakamura *et al.*, 2004 studied indeed, the effect and safety of intrahepatic transplantation of autologous granulocyte colony stimulating factor (G-CSF) mobilized PB CD34⁺HSCs in ten patients with decompensated liver cirrhosis, they noticed significant increase in serum albumin in patients who received middle and high doses of CD34⁺ HSCs.

On the other hand, (Mohamadnejad *et al.*, 2007) performed two small scale clinical studies using HSCs or MSCs. They found marginal improvement in liver function in patients who received autologous bone marrow derived HSCs and highlighted the results of MSCs transplantation as more promising and claimed that hepatic artery delivery of stem cells was not safe.

Two broad strategies have been employed to enhance liver regeneration: (i) administration of granulocyte stimulating factor (G-CSF) to enhance endogenous stem cell regeneration pathways and (ii) infusions of exogenous stem cells to drive regeneration.

G-CSF has been used in liver regeneration because of its ability to increase the number of circulating BMSCs and to promote repair in the cirrhotic liver (Garg *et al.*, 2012) also (Jin *et al.*, 2010) suggested that G-CSF may also enhance MNC homing to the liver.

Several studies had shown that G-CSF may be effective in facilitating liver repair, although it remains unclear whether G-CSF acts by mobilizing bone marrow (BM) cells or by acting locally within the liver microenvironment by facilitating endogenous repair pathways (Gaia *et al.*, 2006). It has been reported that oval cells express the receptor for G-CSF and that G-CSF administration significantly increased oval cell proliferation and migration in this model (Spahar *et al.*, 2008).

The feasibility and safety of mobilizing BM derived cells following G-CSF administration was demonstrated by (Gaia *et al.*, 2006) in eight patients with ESLD. Additionally, they reported improved MELD scores but did not find any signs of hepatocellular carcinoma development or increase in alpha-fetoprotein up to eight months after G-CSF administration.

A favorable effect of G-CSF administration on survival and clinical parameters in patients with liver failure has also been reported in other studies. Lorenzini *et al* (2008) demonstrated the safety of BMSC mobilization and collection through leukapheresis in patients with cirrhosis, even though no improvement of liver function tests occurred. On other hand G-CSF administration can be also associated with the risk of spleen or even rupture, as reported by (Falzetti *et al.*, 1999) in a healthy donor.

Hypothesis that BMSCs act through the delivery of specific substances (cytokines and growth factors), rather than through transdifferentiation or cell fusion, suggests that improvement in liver function might be temporary. This hypothesis is supported by the results of the majority of the clinical trials: the improvement in laboratory data and MELD scores did not persist longer than three-six months regardless of the type of BMSCs infused, the route of delivery or the etiology of the disease (Margini *et al.*, 2014).

Conclusion:-

Hematopoietic stem cells have shown great promise in treatment of chronic liver disease because of their capacity for self-renewal and differentiation which enable them to maintain tissue homeostasis and mediate repair and regeneration. Administration of G-CSF and peripheral collection of CD34⁺ HSCs is a well-tolerated procedure with no adverse effects. Besides, it has clear impact on improving hepatic function tests such as serum albumin, bilirubin, INR and MELD score in cirrhotic patients.

Stem cell therapies are expected to open avenues toward better outcomes for ESLD patients who wait for OLT. Consequently, the demand for donor organs would decrease especially in the early disease stages. However, further investigations are required to establish the dose, frequency and route of administration.

Reference:-

1. **Sharma M, Rao P, Sasikala M, Kuncharam M, Reddy C, Gokak V, Raju B, Singh J, Nag P and Reddy D (2015);** Autologous mobilized peripheral blood CD34⁺ cell infusion in non-viral decompensated liver cirrhosis. *World J Gastroenterol* 2015; (23):7264-7271.
2. **Margini C, Vukotic R, Brodosi L, Bernardi M and Andreone P (2014).** Bone marrow derived stem cells for the treatment of end-stage liver disease; *World J Gastroenterol.* (27): 9098-9105
3. **Zhang Z, Lin H, Shi M, Xu R, Fu J, Lv J, Chen L, Lv S, Li Y and Yu S (2012).** Human umbilical cord mesenchymal stem cells improve liver function and ascites in decompensated liver cirrhosis patients. *J Gastroenterol Hepatol.* ; 27Suppl 2:112–120.
4. **Grove J, Bruscia E and Krause D (2004)** Plasticity of bone marrow-derived stem cells. *Stem Cells* 22:487–500.
5. **Mizunaga Y, Terai S, Yamamoto N, Uchida K, Yamasaki T, Nishina H, Fujita Y, Shinoda K, Hamamoto Y and Sakaida I (2012)** Granulocyte colony-stimulating factor and interleukin-1 β are important cytokines in repair of the cirrhotic liver after bone marrow cell infusion: comparison of humans and model mice. *Cell Transplant.* 21:2363-2375.
6. **Jin S, Meng X, Sun X, Han M, Liu B, Wang X, Sun L, Huang Q, Zhao R and Ban X (2010)** Granulocyte colony-stimulating factor enhances bone marrow mononuclear cell homing to the liver in a mouse model of acute hepatic injury. *Dig Dis Sci.* 55:2805-2813.
7. **Al Tayeb H, El Dorry A, Amer N, Mowafy N, Zimaity M, Bayoumy E and Saleh S (2015).** Autologous stem cells transplantation in Egyptian patients with liver cirrhosis on top of hepatitis C virus. *Int J Stem Cells,* 8: 118-132.
8. **Gaia S, Smedile A, Omedè P, Olivero A, Sanavio F, Balzola F, Ottobrelli A, Abate ML, Marzano A, Rizzetto M (2006)** Feasibility and safety of G-CSF administration to induce bone marrow-derived cells mobilization in patients with end stage liver disease. *J Hepatol.* 45:13-19.
9. **Spahr L, Lambert JF, Rubbia-Brandt L, Chalandon Y, Frossard JL, Giostra E, Hadengue A (2008)** Granulocyte-colony stimulating factor induces proliferation of hepatic progenitors in alcoholic steatohepatitis: a randomized trial. *Hepatology.* 48:221-229
10. **Lorenzini S, Isidori A, Catani L, Gramenzi A, Talarico S, Bonifazi F, Giudice V, Conte R, Baccarani M, Bernardi M (2008)** Stem cell mobilization and collection in patients with liver cirrhosis. *Aliment Pharmacol Ther.* 27:932-939.
11. **Picardi M, De Rosa G, Selleri C, Scarpato N, Soscia E, Martinelli V, Ciancia R and Rotoli B (2003)** Spleen enlargement following recombinant human granulocyte colony-stimulating factor administration for peripheral blood stem cell mobilization. *Haematologica.* 88:794-800.
12. **Falzetti F, Aversa F, Minelli O and Tabilio A (1999)** Spontaneous rupture of spleen during peripheral blood stem-cell mobilization in a healthy donor. *Lancet.* 353:555
13. **Zhang Mand Huang B (2012)** the multi-differentiation potential of peripheral blood mononuclear cells. *Stem Cell Res.*, 30; 3(6):48.
14. **Saito T, Tomita K, Haga H, Okumoto K, Ueno Y (2013)** . Bone marrow cell-based regenerative therapy for liver cirrhosis. *World J Methodol.* 3:65-69.
15. **Palanisamy AP, Taber DJ, Sutter AG, Nadig SN, Dowden JE, McGillicuddy JW, Baliga PK and Chavin KD (2015)** Clinical outcomes and costs associated with in-hospital biliary complications after liver transplantation: a cross-sectional analysis. *J Gastrointest Surg.* 19:282-289.

18. **Nicolas C, Hickey R, Chen H, Mao S, Manuela Lopera Higuaita M, Wang Y and Nyberg S (2017).** Liver Regenerative Medicine: From Hepatocyte Transplantation to Bioartificial Livers and Bioengineered Grafts. *Stem Cells*, 35: 42–50
19. **Shiota G and Itaba N (2017).** Progress in stem cell-based therapy for liver disease *Hepatology Research*, 47: 127–141.
20. **Panminerva medica Article (2010)** · Literature Review 52(2):149-65 ·
21. **De Lucas, Perez LM and Galvez BG (2017)** Importance and regulation of adult stem cell migration; *J cell Mol Med* doi:1111/jcmm13422
22. **Anthony DF and Shiels PG (2013)** Exploiting paracrine mechanisms of tissue regeneration to repair damaged organs, *Transplantation Research*; 20: 2 (1):10
23. **Salama H, Zekri A, Bahnassy A, Medhat E, Halim H, Ahmed O, Mohamed G Al Alim S, and Sherif G (2010)** Autologous CD34⁺ and CD133⁺ stem cells transplantation in patients with end stage liver disease: *World J Gastroenterol*. 16(42): 5297–5305.
24. **Zekri A, Salama H, Medhat E, Musa S, Abdel-Haleem H, Ahmed O, Abdel Hafez H, Lotfy M, Zachariah K and Bahnassy A (2015).** The impact of repeated autologous infusion of hematopoietic stem cells in patients with liver insufficiency. *Stem Cell Research & Therapy*, 6:118-125.
25. **Salama H, Zekri A, Medhat E, Abd- Al Alim S, Ahmed O, Bahnassy A, Lotfy M, Ahmed R and Musa S (2014).** Peripheral vein infusion of autologous mesenchymal stem cells in Egyptian HCV-positive patients with end-stage liver disease. *Stem Cell Research & Therapy*, 5:70-80.
26. **Levicar N, Pai M, Habib N, Tait P, Jiao L, Marley S, Davis J, Dazzi F, Samadja C, Jensen S, Nicholls J, Apperley J, and Gordon M (2008)** Long term clinical results of autologous infusion of mobilized adult bone marrow derived CD34⁺ cells in patients with chronic liver diseases. *Cell Prolif*. 41 (Suppl. 1), 115-125.
27. **Terai S, Ishikawa T, Omori K, Aoyama K, Marumoto Y, Urata Y, Yokoyama Y, Uchida K, Yamasaki T and Fujii Y (2006)** Improved liver function in patients with liver cirrhosis after autologous bone marrow cell infusion therapy. *Stem Cells*. 24:2292–2298.
28. **Gordon M, Levicar N, Pai M, Bachellier P, Dimarakis I, Al-Allaf F, M'Hamdi H, Thalji T, Welsh J and Marley S (2006)** Characterization and clinical application of human CD34⁺ stem/progenitor cell populations mobilized into the blood by granulocyte colony-stimulating factor. *Stem Cells*. 24 (7) 1822-30
29. **Nakamura K, Nonaka H, Saito H, Tanaka M and Miyajima A (2004)** Hepatocytes proliferation and tissue remodeling is impaired after liver injury in oncostatin M receptor knockout mice. *Hepatology*, 39:635-644.
30. **Mohamadnejad M, Namiri M, Bagheri M, Hashemi S, Ghanaati H, Zare Mehrjardi N, Kazemi Ashtiani S, Malekzadeh R and Baharvand H (2007)** Phase I human trial of autologous bone marrow-hematopoietic stem cell transplantation in patients with decompensated cirrhosis. *World J Gastroenterol*. 13:3359-3363
31. **Garg V, Garg H, Khan A, Trehanpati N, Kumar A, Sharma B, Sakhuja Pand Sarin S. (2013)** Granulocyte colony-stimulating factor mobilizes CD34⁺ cells and improves survival of patients with acute-on-chronic liver failure. *World J Methodol*. 3(4): 65–69.
32. **Gaia S, Smedile A, Omedè P, Olivero A, Sanavio F, Balzola F, Ottobrelli A, Abate M, Marzano A, Rizzetto M and Tarella C (2006)** Feasibility and safety of G-CSF administration to induce bone marrow derived cells mobilization in patients with end stage liver disease. *J Hepatol*; 45:13–19.
33. **Jin S, Meng X, Sun X, Han M, Liu B, Wang X, Sun L, Huang Q, Zhao R and Ban X (2010)** Granulocyte colony-stimulating factor enhances bone marrow mononuclear cell homing to the liver in a mouse model of acute hepatic injury. *Dig Dis Sci*. 55:2805-2813.
34. **Lorenzini S, Isidori A, Catani L, Gramenzi A, Talarico S, Bonifazi F, Giudice V, Conte R, Baccarani M and Bernardi M (2008)** Stem cell mobilization and collection in patients with liver cirrhosis. *Aliment Pharmacol Ther*. 27:932-939
35. **Falzetti F, Aversa F, Minelli O and Tabilio A (1999)** Spontaneous rupture of spleen during peripheral blood stem-cell mobilization in a healthy donor. *Lancet*. 353:555
36. **Sakaida I, Terai S, Yamamoto N, Aoyama K, Ishikawa T, Nishina H and Okita K (2004)** Transplantation of bone marrow cells reduces CCl₄-induced liver fibrosis in mice. *Hepatology*. 40:1304-1311
37. **Jang Y, Collector M, Baylin S, Diehl A and Sharkis S (2004)** Hematopoietic stem cells convert into liver cells within days without fusion. *Nat Cell Biol*. 6:532-539.