Correlation of Hepatitis C Viral Load With The Outcome of Stem Cell Therapy In Post Hepatitic C Cirrhotic Patients

Thesis submitted for partial fulfillment of the Master Degree in Tropical Medicine

Submitted by

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ABSTRACT

BACKGROUND The worldwide shortage of donor livers has prompted the search for alternative cell therapies for patients suffering from end-stage liver disease, including the transplantation of stem/progenitor cells. As outcome of stem cell therapy in post HCV end stage liver cirrhosis is variable and patients receiving stem cell therapy for non viral end stage liver cirrhosis had better outcome. This study was designed to determine the relation between HCV viral load and the outcome of stem cell therapy in patients with post HCV liver cirrhosis.

METHODS We report thirty post HCV liver cirrhosis (LC) patients that underwent bone marrow stem cell (BMSC) transplantation in the portal vein. Subjects were patients with post-HCV LC with abnormal liver functions. G-CSF was administered to suitable patients to increase their haematopoietic stem cells (HSCs) from the bone marrow**.** HSCs were isolated&seperated, CD34+ cells were isolated and injected in the patients. After stem cell therapy, liver function&HCV viral load were monitored for 6 months.

RESULTS There was significant improvement as regards PC and non-significant improvement as regards serum albumin and bilirubin levels . There was improvement in the hepatic functional reserve as assessed by the Child-Pugh score. There was significant improvement as regards performance score (PS). By the end of the study, 63.6% showed improvement and none of the patients showed deterioration of the degree of ascites. The improvement was independent on HCV viral load.Safety of the procedure was evidenced by the low incidence of complications encountered. 7 patients (23.3%) suffered attacks of haematemesis .

CONCLUSIONS HSCs therapy may be considered a novel treatment for patients with decompensated LC**.**

Key words: Hematopoietic stem cells, bone marrow stem cell transplantation, cell therapy.

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2-AAF	2-acetylaminofluorene
ABMI	Autologous bone marrow cell infusion
BM	Bone marrow
BMSCs	Bone marrow stem cells
BMT	Bone marrow transplantation
CK	Cytokeratin
CLD	Chronic liver diseases
CPT	Child Pugh Turcotte classification
ECM	Extracellular matrix
EB	Embryonal body
EPC	Endothelial progenitor cell
ERK-1 & 2	Extracellular signal-regulated kinases 1 and 2
ES cells	Embryonic stem cells
FAH	Fumaryl acetoacetate hydrolase
FAK	Focal adhesion kinase
HCV	Hepatitis C virus
HCC	Hepatocellular carcinoma
HE	Hepatic encephalopathy
HSCs	Haematopoietic stem cells
IL	Interleukin
INR	International normalized ratio
MAPC	Multipotent adult progenitor cells
MELD	Model for end-stage liver disease
MSCs	Mesenchymal stem cells
NTCB	2-nitro 4-trifluoro-methylbenzyol-1, 3 cyclohexanedion
OLT	Orthotopic liver transplantation
PHx	Partial hepatectomy
PKC	Protein kinase C
Pyk-2	Proline-rich tyrosine kinase 2
SDF-1	Stromal derived growth factor
TSSC	Tissue-specific stem cells
UCB	Umbilical cord blood
VCAM-1	Vascular endothelial cell adhesion molecule
VLA	Very late antigen

INTRODUCTION

Hepatitis C virus (HCV) infection is gaining increasing attention as a global health crisis. Egypt reports the highest prevalence of HCV worldwide, ranging from 6% to more than 40% among regions and demographic groups.**(Lehman and Wilson.,2009)**

Only 15-20% of people infected with HCV have an acute viral hepatitis syndrome, but the majority develop chronic hepatitis that is usually asymptomatic and undetected for many years. Over a course of 20-40 years, 20% of those with HCV-caused chronic hepatitis progress to cirrhosis, and a proportion of these (possibly 2-3% per year) die as a result of complications of cirrhosis or hepatocellular carcinoma**. (Strickland et al.,2002)**

End stage liver disease (ESLD) is a health problem worldwide. Liver transplantation is currently the only effective therapy, but its many drawbacks include a shortage of donors, operative damage, risk of rejection and in some cases recidivism of the pre-transplant disease. These factors account for the recent growing interest in regenerative medicine **(Lorenzini et al.,2008**).

New therapies have been actively searched for over several decades, primarily in the form of hepatocyte transplantation. 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. Very recent reports of unexpected plasticity in adult bone

marrow have raised hopes of stem cell therapy offering exciting therapeutic possibilities for patients with chronic liver disease. Both rodent and human embryonic stem cells, bone marrow stem cells, , umbilical cord blood cells, fetal liver progenitor cells, adult liver progenitor cells, and mature hepatocytes have been reported to be capable of self-renewal, giving rise to daughter hepatocytes both in vivo and in vitro. These cells can repopulate livers in animal models of liver injury and appear to be able to improve liver function**. (Bae ;2008**)

Hematopoietic stem cells (HSCs) and mesenchymal stem cell (MSCs) are two main subtypes of bone marrow stem cells. 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 has therapeutic effects of restoration of liver mass and function, alleviation of fibrosis and correction of inherited liver diseases. BMSCs can be delivered via intraportal vein, systemic infusion, intraperitoneal, intrahepatic, intrasplenic. The optimal stem cells delivery should be easy to perform, less invasive and traumatic, minimum side effects, and with high cells survival rate**.(Xuyq and Liu ;2008)**

BMSC transplantation can significantly improve the liver function of patients with terminal liver disease with good safety and effectiveness. (**Pan et al.,2008)**

Bhupinder and Maria Velez (2004) indicated that there is no correlation between any of the clinical and laboratory parameters and HCV viral loads & the severity of liver disease is independent of serum levels of hepatitis C virus. The precise mechanism by which hepatitis C virus damages the liver remains poorly understood. Until recently, a direct cytopathic effect of the virus was considered as the primary form of

liver injury caused by the virus. It has been suggested that the degree of liver damage is the result of a complicated interaction between virus and immune response of the host . Immune mediated liver damage is believed to be initiated by HCV-specific T cells and is enhanced by HCV-induced HLA-A, B and C and intracellular adhesion molecules . The results of **Bhupinder and Maria Velez (2004)** are important since they argue against a direct cytopathic effect of HCV and support the hypothesis that the pathogenesis of HCV-related liver damage is immune-mediated**.**

HCV RNA levels do not appear to differ significantly among patients with chronic active hepatitis with or without compensated cirrhosis. In contrast, HCV RNA levels seem to be significantly lower in patients with end-stage HCV-related liver cirrhosis. In these patients, high levels of replication are restored after liver transplantation, suggesting that low pretransplant viral loads are not due to the intrinsic characteristics of the infective viral strains, but rather to the severity of liver disease**.(Duvoux et al.,1999)**

The increase in HCV RNA concentration following the initial postanhepatic decline were dramatic, ranging from 1.4 log10 to 6.0 log10, and reached a steady state higher than the pretransplantation level in patients . These new steady states were between 1.4 and 450 times higher than the mean preanhepatic serum HCV RNA levels. Interestingly, patients with higher baseline viral loads reached the posttransplantation steady state faster**. (Kimberly et al.,2006)**

After stem cell transplantation, on comparing the biochemical parameters of patients grouped according to the etiology of their liver disease into viral and non-viral causes, it was observed that the mean serum albumin, bilirubin and INR levels were improving for the initial 3 months in post-viral cirrhotic patients group then started to decline once again, while in the non-viral group, this improvement continued

throughout the follow-up period. This difference was statistically significant as regards the serum albumin level, however it did not reach a statistical significance in the case of serum bilirubin and INR. This could be attributed to reactivation of the virus affecting the transplanted cells **(Azab.,2008**)

Piscaglia et al (2007) have shown that G-CSF facilitates hepatic regeneration by increasing the migration of BM-derived progenitors to the liver, as well as enhancing the endogenous oval cell reaction.

G-CSF has proven itself to be an anti-inflammatory immunomodulator. Animal, volunteer, and patient studies have all shown that G-CSF reduces inflammatory activity by inhibiting the production or activity of the main inflammatory mediators interleukin-1, tumor necrosis factor-alpha, and interferon gamma.(**Hartung ;1998**).

AIM OF OUR STUDY:

Main objective: as outcome of stem cell therapy in post HCV end stage liver cirrhosis is variable and patients receiving stem cell therapy for non viral end stage liver cirrhosis had better outcome, we aimed to determine the relation between HCV viral load and the outcome of stem cell therapy in patients with post HCV liver cirrhosis.

Sub-objective: Estimation of HCV viral load after taking G-CSF given to post viral cirrhotic patient before stem cell therapy as it may help to decrease viral load which in turn may improve outcome of stem cell therapy.