RESEARCH ARTICLE

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Assessment of Short-Term Effects of Cell Transplantation in Cirrhosis DUE to HCV

Akezhan Astrakhan, Samat Saparbaev, Ainur Amanzholkyzy, Aigerim Iskakova, Gulzhanat Nurlanova*, Meirambek Kurmangazin

Abstract

Background and Objectives: Recent studies have highlighted the potential of fetal hepatic stem cells in regenerative treatments for liver diseases. This study aimed to evaluate the short-term effects of fetal stem cell transplantation in patients with liver cirrhosis resulting from chronic hepatitis C. **Materials and Methods:** Thirty patients with liver cirrhosis of all Child-Turcotte-Pugh classes due to chronic hepatitis C, aged 18 to 65 years, were selected for this study. A single intravenous dose of 1 ml containing 6*106 fetal hepatic stem cells, diluted in 20.0 ml of 0.9% sodium chloride solution, was administered. The efficacy of the treatment was assessed by measuring levels of ALT, AST, total and direct bilirubin, gamma-glutamyltranspeptidase, alkaline phosphatase, total protein, and albumin before and after cell therapy. **Results:** Post-treatment, a significant reduction was noted in the Child-Pugh score from 8 [6-9] to 7 [6-8] ($p<0.001$) and the MELD index from 11 [7-15] to 10 [7-14] ($p=0.004$). Skin itching decreased from 36.7% to 10%. Complaints of weakness increased significantly from 3.3% to 23.3% after 30 days of therapy ($p=0.014$), and the incidence of reduced appetite increased from 20% to 46.7% ($p=0.021$). No statistical differences were observed in the frequency of nosebleeds (86.7% initially vs. 90% at day 30, $p=0.655$) or drowsiness (63.3% initially vs. 76.7% at day 30, $p=0.157$). Significant reductions were noted in ALT levels by 35% and total bilirubin by 44%. The lack of significant changes in indicators of hepatic-cell insufficiency, particularly the protein-forming function as reflected in total protein and albumin levels, is likely due to the extent of liver tissue damage and thus a delayed recovery. **Conclusion:** The findings of this study affirm the clinical efficacy and promise of fetal hepatic stem cell therapy as part of a comprehensive treatment regimen for patients with liver cirrhosis.

Keywords: Cell therapy- fetal stem cells- liver cirrhosis- hepatitis C

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Introduction

Chronic infection caused by the hepatitis C virus (HCV) is a leading cause of morbidity and mortality related to liver damage worldwide and predisposes individuals to liver fibrosis and end-stage liver complications. Liver fibrosis, characterized by the excessive accumulation of extracellular matrix proteins, including collagen, is seen as a wound healing response to chronic liver damage. Staging of fibrosis is crucial for the treatment and prognosis of patients with chronic hepatitis C (CHC), and the number of such patients is expected to increase over the next decades, presenting a significant healthcare challenge [1].

Scientific discoveries over the last decade have altered our understanding of the mechanisms of liver fibrosis. Removing or mitigating the causative agent, such as controlling or treating the viral infection, has shown that liver fibrosis can be reversible. However, reversal often occurs too slowly or infrequently to prevent life-threatening complications, especially with progressing fibrosis. Consequently, there is a significant unmet medical need for antifibrotic therapies to prevent the progression of liver diseases and the development of hepatocellular carcinoma (HCC). Although many antifibrotic candidates have demonstrated promising effects in animal models, their antifibrotic effects in clinical trials have been limited or absent. Thus, no approved therapy for liver fibrosis currently exists [2, 3]. Liver transplantation remains the only curative treatment for patients in the terminal stages of liver disease. However, a shortage of donor organs is a major limitation, and therefore alternative strategies are urgently needed [4].

One treatment method, hepatocyte transplantation, has been proposed as an alternative therapeutic approach for patients with liver diseases to address this urgent and unmet medical need. This cell replacement approach does not replace orthotopic liver transplantation but rather complements it, especially for patients who do not require a complete liver replacement [5]. For the therapy of cirrhosis, mature hepatocytes, liver progenitor cells,

*West Kazakhstan Marat Ospanov Medical University, Aktobe, Kazakhstan. *For Correspondence: gul_88_25@mail.ru*

mesenchymal stem cells, and induced pluripotent stem cells have been used. However, the long-term survival and ability to proliferate continuously in vivo of these cells are unsatisfactory, whereas fetal liver stem cells, given their unique superiority, may be the best candidates for stem cell transplantation techniques. Recent studies have shown that fetal stem hepatocytes can be used as regenerative treatment for liver diseases [6].

The pathogenesis of liver fibrosis/cirrhosis is complex, complicating treatment. Endogenous mesenchymal stromal cells play a key role in the pathogenesis of liver fibrosis. Paradoxically, exogenous mesenchymal stem cells have also been used in clinical trials for liver cirrhosis, and their effectiveness has been observed in most completed clinical trials [7]. Research indicates that fetal stem hepatocytes have significant potential in treating liver cirrhosis due to their ability to regenerate liver tissue and modulate the pathological processes inherent in cirrhosis. Fetal stem hepatocytes can restore liver function through the regeneration of liver tissue and replacement of damaged hepatocytes. This is enabled by their ability to differentiate into mature hepatocytes, restoring the functional structure of the liver. According to the literature, fetal stem hepatocytes also possess immunomodulatory properties, which help reduce liver inflammation associated with cirrhosis and stimulate the formation of new blood vessels, potentially improving blood supply and oxygenation of liver tissue, thereby facilitating its recovery [8, 9].

To assess the repopulation ability of transplanted cells, it is important to define their specific characteristics and to study the mechanisms by which transplanted donor cells replace tissue mass in the liver microenvironment, using well-established models of cell transplantation.

The aim of the study was to evaluate the short-term effects of fetal stem hepatocyte transplantation in patients with liver cirrhosis resulting from chronic viral hepatitis C.

Materials and Methods

Thirty patients with liver cirrhosis of all Child-Turcotte-Pugh classes, resulting from chronic viral hepatitis C and spanning both genders and ages 18 to 65, were selected for therapy. According to data from the Aktobe Hepatological Center at City Clinic No7 in Aktobe Region as of December 2023, 718 patients were under dynamic observation with a diagnosis of "Chronic Viral Hepatitis C," among whom 84 had Grade 4 fibrosis according to fibroscan results.

Diagnosis was established based on clinical-epidemiological and laboratory data, verified by detecting serological markers of HCV: total antibodies of classes G and M to hepatitis C virus (HCV) using enzyme-linked immunosorbent assay (ELISA). Hepatitis C virus RNA was identified by polymerase chain reaction (PCR). Liver fibroscanning was performed using a "Fibroscan 502" device, serial number F01486, manufactured in France in 2013 and put into operation the same year. From the time of registration, all patients received antiviral therapy according to the clinical protocol "Chronic Viral Hepatitis C in Adults" of the Ministry of Health of the Republic of Kazakhstan №118 from October 23, 2020, using a sofosbuvir + daclatasvir regimen for 12/24 weeks. In addition to immediate antiviral therapy, patients received supportive treatment (ursodeoxycholic acid at a dose of 15 mg/kg, lactulose, aldaron, etc.) according to the clinical protocol "Cirrhosis of the Liver in Adults" № 135 from April 29, 2021.

Exclusion Criteria

1. Liver cirrhosis of other etiologies (due to HBV, HBV with delta agent, alcoholic, drug-induced liver cirrhosis, congenital cirrhosis, and secondary biliary cirrhosis).
2. Infectious diseases (HIV/AIDS, tuberculosis).
3. Mental status disorders.
4. Benign and malignant neoplasms.
5. Alcohol and/or drug dependency.
6. Pregnancy or lactation.
7. Patients younger than 18 years or older than 65 years.

The transplantation was conducted according to the protocol recommended by the expert council of the Republican Health Development Center of the Ministry of Health and Social Development of the Republic of Kazakhstan from September 30, 2015, protocol №10, "Transplantation of Fetal Stem Cells" within the framework of state health insurance and social medical insurance, by a clinician licensed in surgery and transplantology. Hospitalization for fetal cell therapy was carried out in the cell therapy department of West Kazakhstan Marat Ospanov Medical University.

We adhered to the ethical guidelines adopted by the International Committee for the Study of Human Tissue Transplantation (Canada, 1994) and the Convention on Human Rights and Biomedicine (Strasbourg, 1996). Approval was obtained from the local ethical committee of West Kazakhstan Marat Ospanov Medical University, protocol №10 dated December 28, 2022. The biomaterial for cell technology, obtained from abortive material (fetus) at 17-22 weeks of gestation, was used in laboratory conditions. Fetal stem hepatocytes were sourced from the cell bank laboratory of the medical center of West Kazakhstan Marat Ospanov Medical University, Aktobe, in the transplantation and cell therapy department.

The thawing of cryopreserved fetal stem hepatocytes was conducted in a water bath equipped with a heating element and temperature regulator. Tubes of 10.0 ml were thawed at a temperature of 37°C over 5.0 minutes. Following thawing, the concentration of surviving cells was determined by adding an equal volume of 0.2% trypan blue, which stains dead cells blue. The stained suspension was loaded into a Goryaev counting chamber. The total percentage of viable cells after thawing should be no less than 70% of the total cell count. Fetal stem hepatocyte transplantation was administered once intravenously in the form of 1 ml of suspension containing 6*106 cells diluted in 20.0 ml of 0.9% sodium chloride solution.

While the patient lay in a horizontal position on their back, after the operative field was treated three times with 96°-20.0 ml alcohol, a peripheral intravenous catheter was placed. Subsequently, an infusion of human fetal cells of the corresponding blood group and Rh factor

was administered at a rate of 40-50 drops per minute via a perfusor. The average transfusion time of the cells was 30-40 minutes.

Post-treatment observation was conducted 30 days after therapy by a specialist licensed in clinical practice for infectious diseases and therapy based at the Aktobe Hepatological Center (City Clinic No7).

To assess the activity of cytolysis and cholestasis syndrome and liver insufficiency, levels of ALT, AST, total and direct bilirubin, gamma-glutamyl transpeptidase, alkaline phosphatase, total protein, and albumin were determined before and after 30 days of cell therapy.

Data collection, accumulation, and systematization of research results were conducted using the MS Excel database. Statistical analysis was performed using STATISTICA 10 software. Quantitative indicators were assessed for conformity to normal distribution using the Shapiro-Wilk test (for sample sizes less than 50) or the Kolmogorov-Smirnov test (for sample sizes greater than 50). Quantitative indicators with normal distribution were described using mean arithmetic values (M) and standard deviations (SD), and 95% confidence intervals (95% CI).

In cases of non-normal distribution, quantitative data were described using the median (Me) and lower and upper quartiles (Q1 – Q3). Categorical data were described indicating absolute values and percentage shares. Comparison of binary indicators characterizing two related populations was performed using the McNemar test. For comparison of normally distributed quantitative indicators calculated for two related samples, the paired Student's t-test was used. For comparison of quantitative indicators with a distribution differing from normal in two related groups, the Wilcoxon test was used. The analysis protocol was approved by the local ethics committee of Marat Ospanov West Kazakhstan Medical University.

Results

Stem cells have made remarkable progress in clinical trials for the treatment of liver cirrhosis or fibrosis. Common indicators used to assess the efficacy of stem cells include serum ALT levels, serum bilirubin, the Child-Pugh score, and the MELD score (Figure 1).

Overall, the group showed a positive response to a single cell transplantation. Notably, there was a decrease in the Child-Pugh score from 8 [6-9] to 7 [6-8], $p<0.001$. More than half of these 30 patients experienced a decrease in the Child-Pugh score that resulted in a transition to a different class: the proportion of patients with liver cirrhosis due to chronic hepatitis C virus infection classified as Child-Pugh class A was 33.3% (n=10), class B – 53.3% (n=16), class C – 13.3% (n=4). One month after the transplantation of fetal stem hepatocytes, the distribution was as follows: class A according to Child-Pugh – 46.7% (n=14), class B – 50.0% (n=15), class C – 3.3% (n=1). The difference in scores was statistically significant, p=0.008. The MELD score significantly decreased on day 30 post-transplantation of fetal stem hepatocytes, from 11 [7-15] to 10 [7-14], p=0.004. The therapy positively affected the dynamics of skin itching, as evidenced by a decrease in patients reporting this symptom: from 36.7% (n=11) to 10% (n=3).

It should be noted that during the therapy, the proportion of patients reporting weakness and reduced appetite significantly increased. Prior to therapy, only 3.3% (n=1) of patients reported weakness, which increased to 23.3% (n=7) by day 30 of therapy, p=0.014. The proportion of patients with reduced appetite increased from 20% (n=6) to 46.7% (n=14), p=0.021. During the study, no statistically significant changes were observed in the frequency of nosebleeds (86.7% (n=26) pre-transplantation, 90% (n=27) on day 30 post-transplantation, p=0.655), or drowsiness (63.3% (n=19) pre-transplantation, 76.7% (n=23), p=0.157). In search of predictors of response to cell therapy, a retrospective analysis of clinical and laboratory indicators was conducted in subgroups of patients differing in treatment outcomes. The dynamics of biochemical indicators after a single transplantation were characterized by decreased activity of liver enzymes – AST and ALT, alkaline phosphatase, and levels of total and direct bilirubin (Table 1).

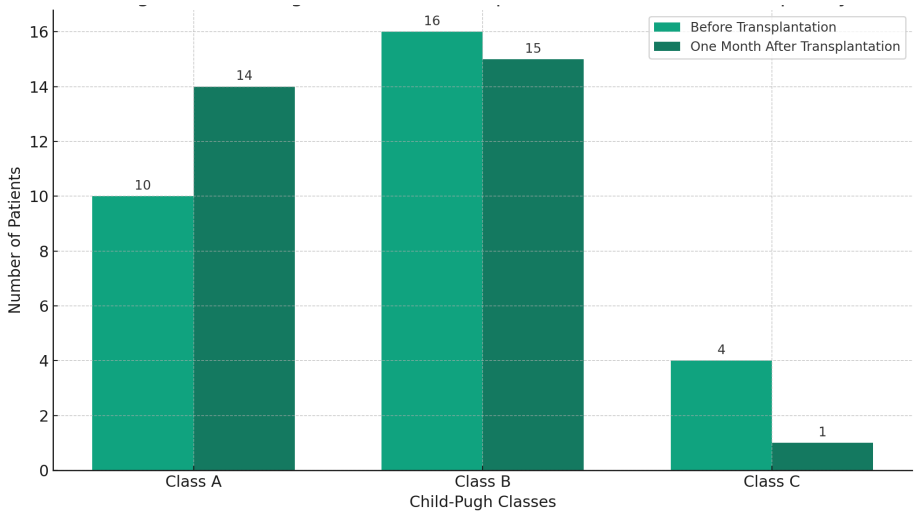

Figure 1. Comparison of Child-Pugh Classes before and after Transplantation in Patients with Liver Cirrhosis due to HCV.

Table 1. Changes in Liver Function Tests in Patients Undergoing Cell Therapy for Liver Cirrhosis 30 Days Post-Procedure

Indicator	Before therapy		After 1 Procedure		p
	M ± SD/ Me	95% CI/ $Q_1 – Q_3$	M ± SD/ Me	95% CI/ $Q_1 – Q_3$	
ALT (U/L)	37	17 – 73	24	14 – 57	0,020*
AST (U/L)	41	23 – 77	43	22 – 58	0,518
Total Bilirubin (μmol/L)	34	15 – 47	19	11 – 38	0,006*
Direct Bilirubin (μmol/L)	19	7 – 31	11	4 – 23	< 0,001*
GGT (U/L)	46	39 – 70	55	44 – 94	0,524
Alkaline Phosphatase (U/L)	88	76 – 104	94	76 – 127	0,977
Total Protein	63 ± 10	60 – 67	64 ± 8	61 – 67	0,563
Albumin (g/L)	31 ± 6	29 – 34	32 ± 5	30 – 34	0,302

$p < 0.05$ indicates statistical significance; Measurements are presented as the median (Q1 - Q3) for skewed data and mean ± SD for normally distributed data.

As shown by the data presented in Table 1, patients exhibited significant positive dynamics in both the improvement of liver function and the assessment of their subjective well-being. According to the evaluation of clinical-laboratory indicators, a month after cell therapy, a reliable decrease in signs of cytolysis and cholestasis was observed. For instance, the level of ALT one month post-transplantation significantly decreased by 35%, and total bilirubin by 44%. The absence of statistically significant dynamics in indicators of hepatic-cell insufficiency, particularly in protein-forming functions such as total protein and albumin content, appears to be related to the depth of liver tissue damage and, consequently, delayed recovery.

Discussion

The reduction in skin itching after hepatocyte transplantation may be linked to the restoration of liver function, as hepatocytes participate in the metabolism and excretion of toxins from the body. Studies have shown that hepatocyte transplantation can improve survival and metabolic function in animals with acute and chronic liver failure, which may lead to a reduction in symptoms such as itching. These conclusions are based on experiments in which hepatocyte transplantation led to improvements in neurobiological status and amino acid balance in animal models of liver failure [10].

The use of fetal stem cells in the treatment of liver cirrhosis represents a cutting-edge area of medical science that explores the potential for regenerating damaged liver tissues. However, there are certain risks and side effects associated with this type of therapy. Primarily, as noted by Bieback and Brinkmann, stem cells can provoke an immune response due to their perceived foreignness by the body, which can lead to inflammation and overall worsening of well-being. Moreover, stem cells can induce a cytokine storm, resulting in the massive release of inflammatory mediators causing systemic and sometimes severe side effects [11]. These side effects, including increased complaints of weakness and decreased appetite as noted in our data, may affect the quality of life of patients. According to research by Trounson and McDonald, the safety and efficacy of stem cells are still under intense scrutiny in clinical trials. While stem cells show promising results in tissue and organ restoration, further studies are necessary to determine the long-term safety and potential risks of this type of therapy [12].

In the study by Shi M. et al., treatment with fetal stem hepatocytes led to improved liver function, as evidenced by reductions in ALT, AST, ALP, and GGT levels over a 48-week observation period [13]. This may be due to the improvement of liver function and partial blockade or reversal of liver cirrhosis through various mechanisms, which can be divided into three aspects: migration of stem cells to the liver and transdifferentiation into hepatocytes, secretion of angiogenesis-related cytokines to stimulate neovascularization enhancing liver regeneration, and activation of proliferative signaling pathways in the host's body [14].

The discussion of the cell therapy results shown in Table 1 indicates significant improvement in the laboratory indicators of liver function in patients with cirrhosis 30 days after the procedure. In particular, the observed reduction in ALT levels and total bilirubin is especially significant, indicating a decrease in hepatic cytolysis and cholestasis respectively.

The decrease in ALT levels by 35% is an indicator of reduced cytolytic activity associated with the destruction of hepatocytes. This change may be a sign of the beginning of liver tissue recovery or the reduction of the inflammatory process in the liver. These results are consistent with studies showing that stem cells can modulate the immune response and facilitate hepatocyte regeneration [15]. Specifically, the reduction in ALT and bilirubin levels post-therapy may reflect improved cellular integrity and biliary function. The significant reduction in total bilirubin by 44% and direct bilirubin confirms a reduction in cholestasis. These changes demonstrate the potential of mesenchymal stem cells in liver regeneration and inflammation modulation, which is supported by meta-analysis results indicating improvements in such indicators as bilirubin and albumin levels, as well as a reduction in the MELD score in patients receiving cell therapy [16].

The stability of total protein and albumin levels, despite

their important role in assessing liver protein-forming function, may indicate the ongoing impact of cirrhosis on liver structure, requiring a lengthy period for recovery. This underscores the importance of further research into the long-term effectiveness and safety of stem cells in treating liver diseases [17]. However, it should be noted that in the study by Shi M., while analyzing survival, there was no obvious difference between the group treated with fetal stem hepatocytes and the control group over a 13-month observation period, with overall survival being significantly higher in the main group only after 13–75 months of observation. This means that the effectiveness of treatment with fetal stem hepatocytes in decompensated liver cirrhosis is evident only after 13 months [14]. As emphasized by Knoepfler, the stem cell market can sometimes be misleading with promises of quick and guaranteed results, which is particularly relevant in the context of treating serious conditions such as liver cirrhosis. Patients and clinicians must be informed about the scientific evidence and limitations of current treatment methods [18]. We expect that questions regarding their effectiveness will be resolved in the near future through further observation of patients.

In conclusion, the results of our study affirm the presence of clear clinical efficacy and the promise of cell therapy using fetal stem hepatocytes in the comprehensive treatment of patients with liver cirrhosis. This approach can be considered as one of the methods preventing further progression of the disease, and in cases of decompensated forms of liver cirrhosis—allowing time until liver transplantation. However, further research involving controlled, randomized clinical trials with extended prospective observation is necessary to verify these hypotheses.

Author Contribution Statement

All authors participated in designing the study. All authors except AAR participated in data collection. AAR and GNN analyzed the data. GNN and AAR prepared the first draft of the manuscript. All authors provided critical comments to the first draft. All authors approved the final version of the manuscript.

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This research received no external funding.

Ethical Declaration

The study protocol corresponded to the Helsinki Declaration of 1975 and was approved by the local ethical committee of the West Kazakhstan Marat Ospanov Medical University, protocol №10 of December 28, 2022.

Conflict of Interest

The authors declare no conflict of interests.

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