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Original Article

Application of Allotransplantation of Fetal Liver-derived Stem-Cells for Treatment of Type 1 Diabetes: a Single-arm, Phase 3 Clinical Trial

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Abstract

Background: Stem-cell technology has been advocated as a potentially curative option for treatment of both type 1 and type 2 diabetes. In the current study, we aimed to assess the effectiveness of allotransplantation of fetal liver-derived cells for treatment of patients with type 1 diabetes.

Methods: For the purpose of the current study, 72 patients with recently diagnosed type 1 DM were recruited and fetal liver-derived cell suspension was administered by the means of intravenous injection. Anthropometric measurements and clinical data such as body mass index, duration of the disease, daily insulin requirement were recorded as well as some of laboratory indicators of favorable therapeutic response (hemoglobin A1c, c-peptide) before and after the intention at 0, 1, 3, 6 and 12 months following the intervention.

Results: Administration of fetal liver-derived fetal stem-cells resulted in significant changes in indicators of diabetes control in the patients. Required daily insulin dose and HbA1c showed significant changes, and c-peptide levels decreased significantly during the first three months of follow up period (P= 0.000) although they started the decrease after that point.

Conclusion: Stem-cell therapy resulted in significant changes in indicators of diabetes control and beta-cell function. More studies are required to demonstrate effectiveness of stem-cell therapy for type 1 diabetes.

Keywords: Stem cell, Type 1 diabetes, Therapy, Iran

Introduction

Type 1 diabetes is an endocrine disorder clinically presented by symptoms related to inadequate insulin secretion (1). Diabetes type 1 develops because of destruction of pancreas-islet beta cells due to an obscure immunologic mechanism (2). Currently, the only available treatment options for diabetes such as insulin therapy and antidiabetic agents do not offer cure and fall short of prevention from diabetes-related complications (3, 4).

Despite being effective in treatment of diabetes, insulin therapy still leads to considerable mortality and morbidity (5). Preservation of insulin secre-

tion, even to minor extents, may effectively prevent from short-term and long-term complications of diabetes (5-10). Therefore, recently, significant attempt has been made with the main objective of restoration of insulin secretion in diabetic patients. Stem cell therapy has recently been advocated as a potentially curative therapeutic option for different diseases including diabetes. This therapeutic intervention not only may lead to provision of a self-renewing source of glucose-responsive insulin-producing cells, but also can result in prevention and reversal of autoimmunity, thereby minimizing immune-mediated graft rejection (11-18).

Embryonic stem-cells have the highest potential for differentiation into insulin-secreting pancreatic beta-cells (19) and their application does not necessitate immunosuppressive treatment (20). However, their application is hindered by ethical concerns and legal limitations in many countries. It is demonstrated, however, that fetal stem cells are advantageous over their adult counterparts considering their superior homing and engraftment potency, better multipotential properties, and negligible immunoge-nicity (20). Moreover, ethical concern over the use of fetal stem cells is less intense in comparison with embryonic stem cells (21).

In the current study, we aimed to assess clinical outcomes and changes in the indicators of diabetes control following administration of the liver-derived fetal stem-cells for treatment of patients with type 1 diabetes. In one previous study, we reported the outcome of fetal liver-derived cell suspension allotransplantation for a cohort of patients with both type 1 and type 2 diabetes (20). This study was designed to assess the efficacy of stem-cell therapy exclusively in patients with type 1 diabetes.

Material and Methods

Patients

All study subjects were from Tehran (Iran) and were referred to Shariati Hospital of Tehran University of Medical Sciences in Tehran. The trial population included 72 patients (35 men and 37 women) aged between 5-39 and 17.52± 6.30 years (mean ±SD) with diabetes duration of 5.21 ± 4.62 month (mean ±SD). Diagnosis of type 1 diabetes was made based on clinical criteria for the disease and all patient fulfilled criteria set for type 1 diabetes according to the latest ADA guideline. Exclusion criteria were as follows: acute vascular inflammation, acute thrombosis, recent retinal hemorrhage, pulmonary hypertension, corpulmo-nale, bone marrow malignancy, end stage diseases, infections, and signs of refractory complications.

For the purpose of transplantation procedure, all patients were admitted to the hospital. Primary clinical examination and laboratory data (including FBS, HbA1c, fasting serum c-peptide, CBC, liver function tests, lipid profile tests and U/A) were collected and recorded on the first day of admission. These data were collected once again in the next follow up visits in the 1st, 3rd, 6th, and 12th mounts after the cells infusion. On each follow-up visit, a complete medical history was obtained and a thorough physical examination was performed.

With the approval of the Ethics Committee and after obtaining written informed consent from the participants, during the whole period of follow up, the subjects had access to a special 24-hour phone consultation service.

Cell preparation and transplantation

legally aborted human fetuses aged 6–12 weeks were used for obtaining fetal liver-derived hematopoietic stem cells (HSCs) after obtaining an informed consent from the parents (mother or both of the parents) (33). Karyotyping was done for each fetal sample in order to determine chromosomal abnormalities and to identify the sex of the donated fetus.

Whole fetal liver was stored in Hank's balanced salt solution without any added calcium and magnesium (HBSS, Sigma, USA) and they were dissociated and homogenized mechanically afterwards. The cell suspension was then filtered with the use of a nylon mesh; and then, isolated cells were cryopreserved with the using 5% dimethylsulfoxide (DMSO) in HBSS, (Wak Chemie, Germany) with a programmable freezer before being transferred to liquid nitrogen containers for long term storage. Samples were thawed at 37oC and cryoprotectant

was diluted by 5 milliliter normal saline before infusion. Total cell count in the prepared suspension was approximately 35-55×106 with twenty percent identified as hematopoietic (CD34+) stem cells.

Statistical analyses

The principal aim of the study was to examine the overtime variations (inclusion, 1, 3, 6 and 12 months) in HbA1C, required insulin daily dose, and c-peptide following allogenic transplantation of fetal-liver derived stem-cells. The data were averaged across 5 follow-up sessions (inclusion, 1, 3, 6 and 12 months) and the variables were initially tested for normality of distribution with the use of Shapiro Wilks "W" statistic. Subsequently, one-way repeated measure ANOVA was utilized to analyze the data. Mauchly's test was used for evaluation of sphericity as an important assumption of repeated-measures ANOVA. After a significant overall result, comparisons between follow-up times were made using tests of within-subjects effects (post hoc tests). In case of variables without normal distribution, the Friedman non-parametric test was used to compare the differences in the variables between the five periods.

Result

Safety outcomes

No acute adverse effects such as fever and other allergic reactions were seen on the day of transplantation or in the later months. There was no occurrence of death or any lymphoproliferative disease, malignancy, or infection after the procedure. One case of meningioma was detected however, which is described in details elsewhere (22). The results of laboratory and investigations including CBC, renal function test were all normal following the procedure.

Daily insulin dose

As regards daily insulin dose, the variations throughout the whole 12 months period were significant (Table 1). In the first month following the procedure, the required daily insulin dose was significantly lower in comparison with all 12 months of follow up period (P=0.000). Then, the required dose showed a significant increase throughout of the whole 12 months period of study (Fig. 1). At the end of 12 months period study, required insulin dose was higher than what was recorded at the beginning of the study, although with no statistically significant difference (P=0.09).

Glycated hemoglobin A1C (HbA1c)

As regards HbA1c, the variations throughout the whole 12 months period were significant (Table 1). HbA1c concentrations steadily increased in the first 6 months of the study, and then, the levels reduced to a level significantly higher in comparison with the baseline values (P=0.006) (Fig. 2).

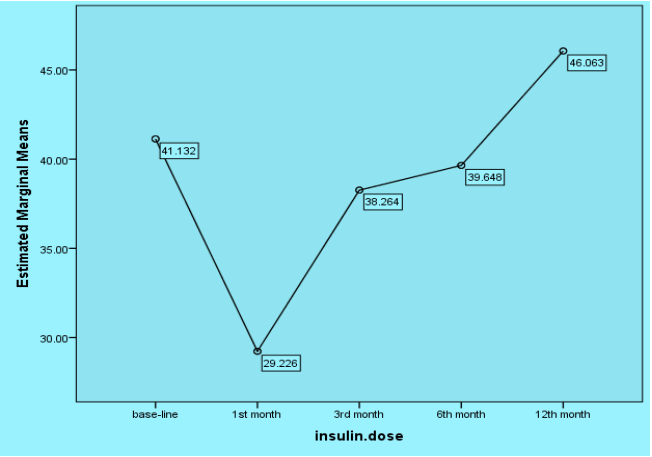

Fig. 1: Changes in daily insulin dose during the 12 months period of follow up

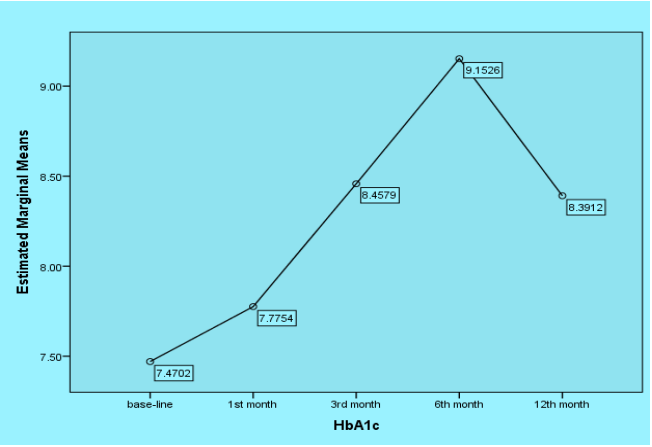

Fig. 2: Alternations in HbA1c levels during the 12 months period of follow up

Table 1: Indicators of therapeutic response in the patients

Time	Statistics		Screening	1st month	3rd month	6th month	12th month	*P*
HbA1c	Mean		7.47	7.77	8.45	9.15	8.39	0.00 €
`	Std. Deviation		2.01	1.38	1.68	1.88	2.22	
c-peptid	Percentiles	25th	0.62	0.05	0.05	0.01	0.05	0.000¥
		50th (Median)	0.10	0.31	0.37	0.05	0.05	
		75th	0.66	0.67	0.88	0.11	0.11	
insulin dose	Mean		41.13	29.22	38.26	39.64	46.06	0.00 €
	Std. Deviation		19.64	15.23	19.18	16.59	17.73	

€. Repeated ANOVA , ¥. Friedman test

C-peptide levels

As regards fasting c-peptide, the variations throughout the whole 12 months period were significant (Table 1). Although changes in fasting C-peptide levels during the whole period of the study was significant (*P*= 0.000), as their distribution was not normal and the analysis was non-parametric, it is not possible to pinpoint in which months the changes were significant. However, as it can be seen in the Fig. 3, C-peptide levels significantly increased during the first three months of the study (Fig. 3). However, C-peptide levels showed a significant decrease throughout the rest of the period.

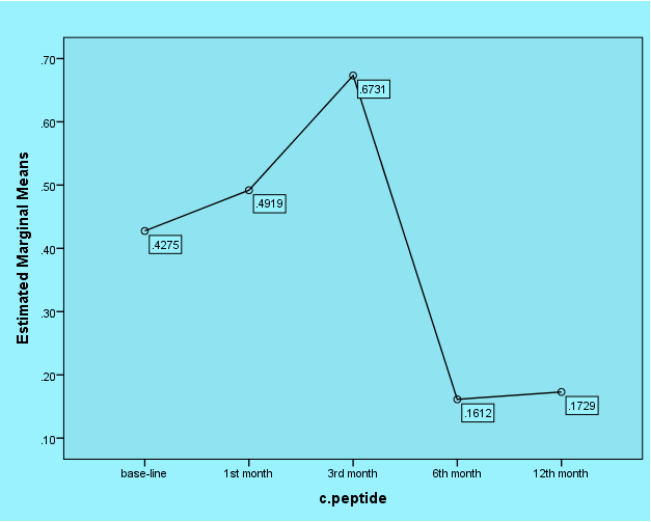

Fig. 3: Changes in baseline c-peptide levels of the patients during the 12 months period of follow up

Discussion

Result of our Phase 3 single-arm clinical trial showed that transplantation of fetal-liver derived stem-cells leads to significant changes in major indicators of type 1 diabetes control including : mean daily insulin dose, serum level of fasting C-peptide, and HbA1C concentrations in a group of 72 patients during a 12 months period of follow up.

Our results demonstrated significant changes in fasting C-peptide levels during the 12 months period of follow up. C-peptide levels significantly increased during the first three months of the study, and then, showed a progressive decline which continued until the end of the trial when the values returned to the baseline levels. This finding is quite similar to what Wang L et al. reported in their study. They reported a significant increase in C-peptide levels 90 days following the transplantation while these levels were similar to the baseline values at the other points of the trial (23). Measurement of stimulated C-peptide levels can provide a more reliable indicator for assessment of the preservation of beta-cells function, especially early in the history of diabetes and the levels are correlated with both superior glycemic control and decreased microvascular complications (24-26). However, in patients with type 1 diabetes, when fasting glucose levels are kept reasonably low, fasting C-peptide levels also decrease significantly (27). Therefore, as its application was

easier and less ethically challenging, we eventually decided to use fasting C-peptide levels.

Transplantation of hematopoietic stem cells need to be proceeded by temporary immune suppression so that to avoid rejection and graft-versus-host reaction (28). However, fetal stem cells do not elicit alloreactive lymphocyte proliferation, we did not use any immunosuppression protocol (29).

Although a few studies have investigated the effectiveness of stem-cell therapy for diabetes, our study, to our knowledge, is the first clinical trial designed to assess the therapeutic outcome of allogeneic transfusion of fetal stem cell for treatment of type 1 diabetes.

One major limitation of our study was that we included both patients with short and those with long history of diabetes. In terms of effectiveness stem-cell therapy for treatment of diabetes, patients can be categorized into two groups: patients with term diagnosed diabetes and those with long history of the disease. Clinical response was more significant in patients with short history of the disease when a considerable reserve of beta-cells still remains in the pancreas (28). However, in our study, we included both patients with short-term and those with long-term histories of diagnosed diabetes. It can be suggested that if we exclusively included patients with short history of diabetes, the therapeutic response would be more favorable.

Conclusion

Transplantation of fetal-liver derived stem-cells demonstrated significant changes in indicators of diabetes control in patients with type 1 diabetes. Further studies are warranted to shed more light on the effectiveness of fetal stem-cells in treatment of type 1 diabetes.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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