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Original Article

Insulin Independence after Fetal Liver-Derived Cell Suspension Allotransplantation in Patients with Type 1 Diabetes: A Pilot Study

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Abstract

Background: Cell-based treatments are currently being actively received great attention among scientists and clinicians for a variety of diseases as well as diabetes .The aim of this study was to investigate the effect of allotransplantation of fetal liver-derived cell suspension in patients with type 1 diabetes.

Methods: Patients with type 1 diabetes (n=16) aged 6-30 years-old were included in the study. Fetal liver-derived cell suspension was transplanted by the means of intravenous injection patient.

Results: In most of patient, blood glucose levels gradually decreased within the first day of infusion. Insulin independence occurred in 3 patients out of the 16 (18.7%) for 4 to 24 months. They showed increasing levels of serum c-peptide along with decreasing of levels of HbA1c level. In other patients, no significant changes in parameters of diabetes control were observed.

Conclusion: Findings of this study indicated that transplantation of fetal stem cells could, although not permanently, be an effective therapeutic intervention in patients with type 1 diabetes. To demonstrate effectiveness of stem-cell therapy for treatment of diabetes, more clinical trials with stricter inclusion criteria, modified protocols, and larger number of patients and are necessary as well as long periods of follow up.

Keywords: Stem cell, Type 1 diabetes, Allotransplantation, Fetal Liver-Derived Cell Suspension, Cell Therapy

Introduction

Type 1 Diabetes (T1D) is considered as an autoimmune disease, which targets the β cells of pancreases and lead to seriously decreasing of insulin been secreted by the β cells (1). Although substantial progress has been made in elucidation of the pathogenesis of the disease, there is no effective cure available hitherto for diabetes (2). The increasing urge to develop new and novel methods for cure of diabetes has led to the emerging of the cell-based therapeutics approaches. The capacity of different types of stem cells for renewal, differentiation into insulin producing cells (3-11) and modulation of immune system (12-17) has highlighted their potential as a novel treatment in medicine. With the goal of preserving β cell function and restoring tolerance, some cell-based clinical trials have been conducted in T1D patients up until now (18, 19). Currently, a significant contro-

versy exists in the issue of which type of stem cells would be the best choice for implementation in T1D.

While recent trials of Autologous Hematopoietic Stem Cell Transplantation (AHST) have shown promising results in a small number of new-onset T1D patients with preservation of C-peptide levels and prolonged insulin independence in many patients (20). Results of 3-5 years of follow up of patients with similar condition have revealed that AHST had no advantage over the conventional insulin therapy (21, 22). Furthermore, the effect of autologous cord blood infusion on beta-cell and immune function investigated in new onset T1D patients, although the result did not show any notable changes in the natural course of metabolic parameters of diabetes (23, 24), they did show some changes in frequency of regulatory T cells (Treg) after the infusion (24).

Human fetal liver-derived Mesenchymal Stem Cells (hfMSC) are multipotent stem cells with greater self-renewal and differentiation capacity than their adult counterparts (25-27). It has been shown that proliferative capacity of hfMSC is higher than that of adult MSC (aMSC) in ex-vivo (27). It is noteworthy that fetal tissue cells are also less vulnerable to rejection as these cells are less antigenic; a feature, which is attributed to their expressing HLA-G for immune tolerance during pregnancy (28) .It, is demonstrated that fetal mesenchymal stem cells do not prompt alloreactive T-cell proliferative responses (27). Inhibitory Effects of fMSC on T-Lymphocyte Proliferation are Long Lasting as well (28). So that, It can be conclude that transplantation of fetal-derived stem cells (fSCs) theoretically offers the advantages of being immune-privileged(26, 28, 29) as well as having great ability to self-renewal and differentiation (25-27).

Previously, we described the effect of fetal liver-derived cell suspension allotransplantation on patients with both type of diabetes (30). The heterogeneity of patients was known account for the negative results. In next step, we enrolled a limited number of patients with T1D in this open-label, single-arm clinical trial in which we aimed to investigate the effect of fetal liver-derived cell suspension allotransplantation on patients with T1D.

Material and Methods

The study protocol was approved by the Endocrinology and Metabolism Research Center (EMRC) of Tehran University of Medical Sciences ethical code number: E-0089 and IRCT number: 138811071414N10). According to the Declaration of Helsinki, an informed consent was obtained from each patient or his/her parents before their enrollment in the study.

Patient selection

Patients with T1D (N=16) comprised of 11 male and 5 female were enrolled to the study after fulfilling its criteria. The inclusion criteria were patient of both sexes, aged between 6-30 years old, with diagnose of T1D confirmed by measurement of serum levels of anti-glutamic acid decarboxylase (anti-GAD) antibodies, duration of diabetes up to 5 years and blood glucose level under 15mmol/l (270/mg/dl). Anti-GAD was considered positive when ≥0.08 (31).

Similar to our previous study (30), the exclusion criteria were as follow: acute vascular inflammation or thrombosis, recent retinal hemorrhage, pulmonary hypertension, corpulmonel, bone marrow malignancy, end stage diseases, any infection, and signs of refractory complications.

Intervention & Assessments

All patients received fetal liver derived cell suspension from human legally aborted early fetus aged 6 -12 weeks (30, 32). At the day of the cell infusion, each patient was hospitalized for 24 hours. Clinical and laboratory examination (FBS, HbA1c, serum C-peptide) (total area under the curve of serum C-peptide) performed just before intervention and next 5 follow-up sessions (30). Total area under the curve of serum C-peptide was measured by the mixed-meal tolerance test (during fasting and at 30, 60, 90, and 120 minutes) (33). Patients were followed up for 2 years. The follow up sessions were at 1st, 3rd, 6th, 12th and 24th month after the transplantation.

Data analysis

Because of the small sample size, the variables were non-normally distributed. In order to analyze the collected data, we used nonparametric test (Mann-Whitney) and considered the median as central measure. All statistical tests were carried out by SPSS software version: 19.0 (Chicago, IL, USA) and level of significance was set at 0.05.

Results

Sixteen patients of both sexes (11 men and 5 women), aged 6 to 30 years (14.87± 6.39; mean ±SD), and with the diagnosis of T1D diabetes during the past previous 5 years were enrolled in the study based on the inclusion-exclusion criteria. The diabetes duration was ranged from 2 to 48 months (12.13± 11.08; mean ±SD) and mean ±SD of serum levels of anti-GAD antibodies was 1.33±0.42 IU/ml (Min=0.1, Max=1.9) at the screening time (base-line). Demographic characteristic data of each participant are described in Table 1.

Most patients experienced mild fever 6 hour after receiving the cell suspension, which resolved spontaneously without treatment. There was also a remarkable decrease in the blood sugar within 24 hours following the infusion. In the first week, daily insulin dose of all patients was intentionally decreased for stimulation of stem-cells according to the study protocol. The " insulin free" period was defined as times without any need for insulin injection or any other anti-diabetes agents based on normal blood glucose levels as daily self-glucose monitoring indicated as well as favorable HbA1c levels (HbA1c<5.7) in the follow-up sessions. The patients were divided into two groups based on experiencing or not experiencing the insulin free periods following the cell infusion.

Difference between the mentioned two groups in base line variables are shown in Table 2. As demonstrated, mean age (P=0.025) and serum c-peptide (P =0.014) was significantly higher in responded group than non-responded. Moreover, daily insulin dose was significantly lower in responded group in comparison with non-responded group (P=0.004). Follow up variables in both groups are demonstrated in Table 3. Figure 1 show that in the months following infusion of cell suspension to the participants, mean HbA1c and daily insulin dose remained lower in "responded" group in comparison with the "non-responded" group.

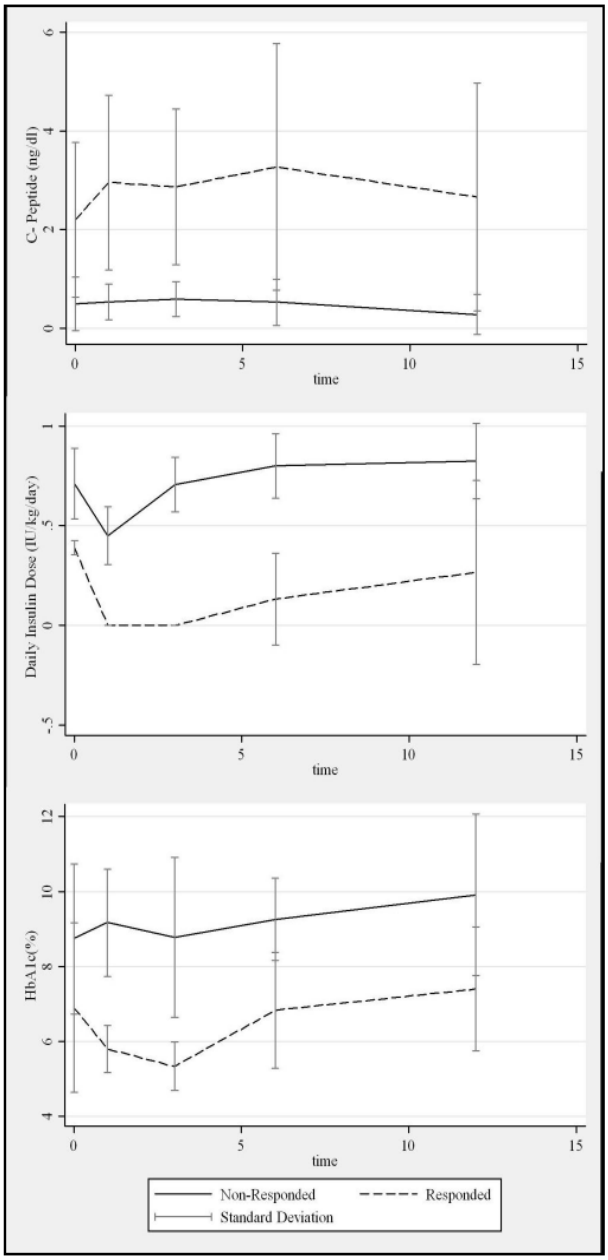

Fig. 1: Trend of changes in c-peptides, HbA1c and daily insulin in patients

Table1: Demographic characteristic data

Code	Insulin free period (month)	age (year)	Diabetes Duration (month)	Anti-Gad Abs ‡ (IU/ml)	Hx of DKA	Insulin Dose at Base-line (IU/kg/day)	Insulin Dose at 12th month (IU/kg/day)	BMI at Base-line (kg/m^2)	BMI at 12th month (kg/m^2)
1/m	4	20	9	0.1	No	0.36	0.8	21	21.8
2/f	0	12	3	1.1	No	0.64	1.2	26	25.7
3/m	0	6	12	1.5	No	0.73	0.7	16.79	16.7
4/f	0	14	8	1.3	Yes	0.76	0.7	20.5	21.4
5/f	0	10	6	1.1	No	0.95	1.1	18.78	16.86
6/m	0	10	10	1.5	No	0.61	0.8	19.46	20.62
7/m	0	30	12	1.9	No	0.87	0.7	21.79	21.45
8/m	16	25	2	1.7	No	0.43	0	20.09	20.01
9/m	0	13	7	1.1	No	0.7	0.82	19.97	18.73
10/m	0	12	8	1.7	No	0.57	0.8	18.17	16.66
11/m	24	20	12	1.1	No	0.38	0	19.67	19.67
12/m	0	16	3	1.5	No	0.48	0.6	20.52	20.67
13/f	0	11	12	1.5	No	0.59	0.8	19.06	19.47
14/f	0	15	48	1.1	No	1	1.1	21.8	21.5
15/m	0	17	18	1.7	No	0.9	0.7	19.6	20.1
16/m	0	7	24	1.5	No	0.45	0.7	18.7	17.3

‡Glutamic acid decarboxylase, *body mass index: calculated as weight in kilograms divided by height in meters squared.

Table 2: Difference between Non-responded and respond groups in base-line variables

Variables	Non-responded (N=13) Descriptive Statistics				Responded (N=3) Descriptive Statistics				*P*-value**
	Min	Max	Mean	SD	Min	Max	Mean	SD	
age (year)	6.0	30.0	13.31	5.96	20.0	25.0	21.67	2.89	0.025*
Diabetes Duration (month)	3.0	48.0	13.15	11.96	2.0	12.0	7.67	5.13	0.521
Anti-GAD Ab (U/ml)	1.1	1.9	1.42	0.27	0.1	1.7	0.97	0.81	0.439
HbA1c Base-line (%)	6.1	12.3	8.75	2.00	5.4	9.5	6.90	2.26	0.189
C-Peptide Base-line (ng/dl)	0.1	2.0	0.50	0.54	1.0	4.0	2.20	1.57	0.014*
Insulin Dose Base-line (IU/kg)	0.5	1.0	0.71	0.18	0.4	0.4	0.39	0.04	0.004*
BMI(kg/m^2)	17	26	20.09	2.25	20	21	20.25	0.68	0.521

**Independent-Mann-witney U test, * Significancy level set at 0.05

Table 3: Diabetes control variables in patients during the 1st year of follow up

Code	HbA1c (%)					C-Peptide (ng/dl)					Daily Insulin Dose (IU/kg/day)				
	Base-line	1st month	3rd month	6th month	12th month	Base-line	1st month	3rd month	6th month	12th month	Base-line	1st month	3rd month	6th month	12th month
1/m	5.8	6.5	6	8.4	9.3	0.98	1.42	1.51	1.08	0.5	0.36	0	0	0.4	0.8
2/f	6.4	8.8	8.8	10.7	8.7	0.79	0.91	1.1	0.48	0.3	0.64	0.5	0.7	1	1.2
3/m	10.1	11.2	10	10.1	9.1	0.08	0.02	0.05	0.05	0.01	0.73	0.5	0.73	0.6	0.7
4/f	6.9	10.1	8.5	8.7	9.7	0.79	0.92	0.78	0.89	0.4	0.76	0.5	0.76	0.8	0.7
5/f	10.7	10.8	8.9	10.3	9.9	0.3	0.3	0.16	0.16	0.1	0.95	0.6	0.95	1	1.1
6/m	12.3	9.5	8.1	10	10.6	0.4	0.8	0.7	0.59	0.6	0.61	0.3	0.61	0.7	0.8
7/m	8.4	10.2	7.6	8.2	8.2	0.09	0.06	0.07	0.07	0.07	0.87	0.6	0.87	0.9	0.7
8/m	9.5	5.3	4.7	5.3	6.3	3.97	4.89	4.6	5.99	5.1	0.43	0	0	0	0
9/m	6.1	7.6	7.9	9.3	10.1	0.96	0.83	0.6	0.71	0.3	0.7	0.4	0.7	0.8	0.82
10/m	9.4	10.7	9.2	10	10.8	0.16	0.47	0.5	0.27	0.1	0.57	0.5	0.57	0.66	0.8
11/m	5.4	5.6	5.3	6.8	6.6	1.66	2.58	2.5	2.75	2.4	0.38	0	0	0	0
12/m	6.5	7.4	7.1	8.8	9.4	0.24	0.65	0.4	0.18	0.1	0.48	0.5	0.56	0.65	0.6
13/f	11.5	8.9	8.5	10.6	11.1	2.01	1.07	1.1	1.57	1.5	0.59	0.5	0.65	1	0.8
14/f	9	9.6	15.3	8.1	16	0.1	0.1	0.7	0.9	0.01	1	0.16	0.9	1	1.1
15/m	7.9	7.3	7	7.3	7.5	0.1	0.2	0.9	0.05	0.01	0.9	0.6	0.7	0.7	0.7
16/m	8.5	7.2	7.3	8.3	7.7	0.5	0.6	0.7	1	0.2	0.45	0.2	0.5	0.6	0.7

Moreover, serum c-peptide levels were higher in the "responded group" in comparison with the "non-responded" one in all follow up sessions besides the base-line levels. Figure 1 shows that during the first six months, the amount of serum C-peptide have an incremental trend in respond group. Slope of the changes during the first three months is sharper. In the 4th month of follow up, the 1st insulin free patient became insulin dependent after an upper respiratory (probably viral) tract infection (ID number= 1). Two out of 16 patients remained consistently insulin-free for the whole period of the 1st year of follow-up. On 16th and 24th month of follow up, two patients (with ID numbers of 8 and 11) became insulin dependent again respectively. Unlike the previously mentioned case, there was no report of any infections in the 3 last months of their insulin free period. Diabetes control indicators of all patients during the 1st year of follow up are described in Table 3.
Finally yet importantly, after two years follow up, no life threatening side effect was seen in the patients. However, in one patient (with the ID number of 14) multiple and recurrent hypoglycemic attacks following receiving even the smallest dose of insulin were seen. Self-monitoring blood glucose showed normal levels of blood glucose in all time of 24 h except morning fasting time when it was mildly elevated but still it was lower than 160 mg /dl.

Discussion

Cell-based treatments are increasingly being popular among scientists and clinicians for a variety of diseases as well as diabetes (34, 35). In the current clinical trial, the effect of allotransplantation of fetal-derived stem cells without immunoablation for treatment of T1D patients was investigated. Among the 16 participants enrolled, three experienced insulin free periods between 4 to 24 months. In 13 patients, who were defined as non-respondents, no changes in daily-required insulin dose were observed following the treatment. It is noteworthy that different theories are proposed as being the underlying mechanism of therapeutic effect of stem cells such as their capability of differentiation into insulin-producing cells, stim-ulation of the remaining islets, and immunomodulation (36-38). It can be postulated however, that a combination of the mentioned mechanisms are responsible (36-39). The transient observed response of the patient to the FSC infusion might explain by the fact that although fSCs are immune privileged, this is transient. The allogeneic infused cells finally recognized and rejected by the immune system of the host (40).
Most patients, including those in the non-respond group, showed remarkable decrease in blood sugar levels in the first day after cell therapy. This can be explained by the fact that stem cell transplantation can promote the secretion of a variety of cytokines and growth factors that have both paracrine and autocrine activities (38, 41). Transplanted cells may be mobilized to injured pancreas region and interact with the local microenvironments to secret factors helping pancreatic functional recovery (38, 41).Therefore, the paracrine function of transplanted cells rather than cell transdifferentiation may play a role in the hyperglycemic reversal happened immediately after the infusion in our participants (42, 43).
The various responses to the fetal-derived cell infusion treatment observed in the present study may be due to the different functional beta cell storage in the patients. The level of c-peptide was significantly greater in the responded group than the non-responded group at base line (Table 2). Also within follow-up months, c-peptide level increased in responded patients along with decrease in HbA1c levels during the insulin free periods (Table 3). These changes were not seen in the other group.
Moreover, three or more years after diagnosis of T1D, detectable levels of c-peptide are found in approximately 30% of patients (44). Mean duration of diabetes were significantly lower in the responded group than non-responded group. However, we did not define tightly limited duration of diabetes as inclusion criteria like the other cell-based clinical trials been conducted on T1D patients and reported number of success (20, 22, 33, 45).

On the other hand, in the absence of any history of DKA, the newly diagnosed T1D patients are more likely to have an appropriate preservation of β cells, which increase the likelihood of cell therapy success (20, 33, 46). In support of this fact, none of patient in responded group had history of DKA. Moreover, in the current study, in approximately 60% of patients in the responded group, the diagnosis of diabetes was made before presentation of classic overt diabetes symptoms (47). This might indicate that they possessed a larger preservation of functional β cells. In support of the aforementioned explanation, all patients in non-responded group showed symptoms of overt diabetes at the time of diagnosis. These findings indicate the feasibility of screening family members and relatives of patients with T1D patients to commence therapeutic intervention while the reservation of pancreatic beta cells is still sufficient (48). In these individuals, close follow-up leads to diagnosis of diabetes in the very early stages (48) which in turn may leads to enhance the success of the cell therapy intervention.

It might be speculated that both deference in serum levels of c-peptide and response to cell therapy can be defined by of T1D patients in terms of their age of diabetes onset, sex, the status of immune system, and genetic background (13, 20, 49, 50). In the current study, all in the respondent group were male and adult (at the time of diagnosis of diabetes). It is demonstrated that β Cell destruction occurs more slowly in adults in comparison with children. Moreover, this destruction is more pronounce in males compared to the female counterparts (49, 50). These differences in sex and age might be predictive of the better response to cell therapy in male adult patients as they are expected to possess larger β-cell mass reserves and lower anti-Gad antibodies at the time of diagnosis (13, 20, 33, 49-51).

As described in another article published by our team, no serious safety concern attributable to stem-cell therapy is reported and the procedure can be considered as safe with (30, 52) with no life threatening side effects in our current patients. However, some minor limitations to the use of stem-cell therapy do exist. The first notable study investigating the application of HSC for treatment of diabetes was carried out in a clinical trial conducted from 2003 – 2006 (33). In this study, high-dose immunosuppression was administered prior to the procedure in newly diagnosed patients with T1D (33, 53). Its findings demonstrated remarkable results in terms of the number of insulin-free patients, preservation of β cell function, and duration of the insulin free period (20, 33). In contrast to immunosuppression administered by previous study, in our study, 4-24 months of insulin-free periods were observed without any immunosuppressive drug administered.

Considering the transient therapeutic response and recurrence of the disease, it might be more effective if the infusion is administered repeatedly for a certain period. Furthermore, concurrent administration of other therapeutic interventions such as that was administered in a group of polish patients (45) (consisted of plasmapheresis, Infection prophylaxis and Acarbose) enhance therapeutic effectiveness of the intervention. It is necessary to recruit a larger group of patients need to be included in cell therapy protocols to more accurately assess the effectiveness of the intervention. Findings of our studies, cumulatively, indicate that (21, 22, 46) the outcome of stem-cell therapy is highly dependent of several factors such as: onset diabetes in adulthood versus childhood, timing of the diagnosis, commencement of insulin therapy in very early stages of diabetes (prior to onset of clinical symptoms), no history of DKA, shorter duration of diagnosed diabetes, and male gender.

Conclusion

Considering the high and increasing prevalence of diabetes in the world and lack of an effective curative treatment method, it is necessary to develop novel techniques for treatment of diabetes. Findings of the current study demonstrated that fetal stem cell transplantation could be considered as a potentially effective curative option for treatment of diabetes. It is noteworthy however, that further studies and larger clinical trials are needed to

demonstrate effectiveness of stem-cell therapy for treatment of 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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