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Original Article

The Effect of Fetal Liver-derived Cell Suspension Allotransplantation on Patients with Wolfram Syndrome: the First Year of Follow-up

Ensieh NASLI ESFAHANI [1], ***Maryam GHODSI*** [1], ***Ali TOOTEE*** [1], ***Camelia RAMBOD*** [1], ***Bagher LARIJANI*** [2], ****Akbar SOLTANI*** [3]

1. *Diabetes Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran*
2. *Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences Institute ,Tehran University of Medical Sciences, Tehran, Iran*
3. *Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences Institute, EBM Group, Tehran University of Medical Sciences, Tehran, Iran*

***Corresponding Author:** Email: soltania@tums.ac.ir

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Abstract

Background: Cell therapy has emerged as a promising curative intervention for several diseases including diabetes and Wolfram Syndrome (WS). The current study aimed to assess the effectiveness of clinical application of fetal-liver derived stem cells for treatment of patients with WS.

Methods: Six patients with WS aged 23-34 (mean: 29.50, SD: 4.76) were recruited for the current phase 3 single-arm clinical trial. The participants underwent fetal liver-derived hematopoietic stem cell transplantation. In order to evaluate the effectiveness of transplantation, glycemic control indexes were measured at regular follow-up sessions.

Results: One patient (out of six) experienced a 6 months insulin-free period with acceptable HbA1c levels. In another patient with history of recurrent hypoglycemic attacks, the frequency of bout of attacks remarkably decreased. There was no significant change in other patients.

Conclusion: Stem-cell therapy may represent a new method for treatment of patients with Wolfram Syndrome.

Keywords: Stem Cell, Allotransplantation, Wolfram Syndrome, Diabetes

Introduction

Wolfram syndrome (WS), also known as DIDMOAD (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy and Deafness), was first described in 1938 (1).This neurodegenerative disease is an autosomal recessive pattern and prevalence of it is estimated to be 1/770,000 in normal population (and 1 /150 in patients with diabetes) (2, 3). Clinically, insulin-dependent diabetes mellitus with optic nerve atrophy is a sufficient criterion for the diagnosis of WS (4).Other manifestations of WS also include deafness, hydronephrosis, neurologic and anterior pituitary dysfunction (2, 5). In recent decade, there emerged some hope of finding a curative treatment for incurable diseases such as diabetes is most likely using stem-cell research (6).

Previously, we reported the effect of fetal liver-derived cell suspension allotransplantation for treatment of patients with both type 1 and type

2 diabetes (7). In the second stage of our study, a larger cohort of exclusively type 1 diabetic patients, were recruited for a phase 3 single-arm clinical trial (unpublished data). Among the recruited patients, there were a small group of patients with the WS. Considering the potential of stem- cell therapy for curative treatment of diabetes and neurodegenerative disease, we aimed to describe the effect of fetal liver-derived cell suspension allotransplantation on patients with WS for the first time in the world.

Materials and Methods

Ethics Committee of Endocrinology and Metabolism Research Center (EMRC) of Tehran University of Medical Sciences approved the study protocol (ethical code number: E-0089 and IRCT number: 138811071414N10). Before the enrollment, each patient or his/her guardian signed an informed consent form.

Hereby, we are describing the results of the effect of fetal liver-derived cell suspension allotransplanttation on patients with DIDMOAD syndrome for the first time.

Patient Selection

Six patients with WS (insulin-dependent diabetes mellitus and optic nerve atrophy as major diagnosis criterion (4) were selected according to following criteria: Aged between 10-60 yr old, duration of the diabetes up to 20 years, blood glucose under 15 mmol/l (270 mg/dl) (7). Exclusion criteria were as followed: acute vascular inflammation, acute thrombosis, recent retinal hemorrhage, pulmonary hypertension, Cor pulmonale, bone marrow malignancy, end stage diseases, infections, and signs of refractory complications (7).

Interventions & assessments

All patients received fetal liver derived cell suspension from human legally aborted early fetus aged 6 -12 weeks (7, 8). Clinical and laboratory examination (FBS, HbA1c, fasting serum C-peptide, Urine Analysis, urine Albumin-creatinine Ratio) performed just before the intervention and the next 5 follow-up sessions. According to the protocol, follow-up visits were set to be performed on the 1st, 3th, 6th, 12th and 24th week after the cell infusion.

Analysis

Non- parametric methods (Wilcoxon's signed rank test, Friedman's test) were used for the assessment of changes in in diabetes control indicators (HbA1c, C-peptide, FBS, BMI) during follow-up sessions. All statistical tests were carried out by SPSS software version: 21.0, (Chicago, IL, USA) and significance level was set at 0.05. We use STATA software (version 11) to draw a schematic diagram about the relationship between different diabetes control indicators in the patients during follow-up period.

Results

Six patients with WS aged 23-34 (mean: 29.50, SD: 4.76) year old were recruited to the study. The patients had been diagnosed with diabetes for 10-26 (mean: 16.83, SD: 6.940) years. Summary of clinical features with relevant laboratory variables and demographic data of the patients are shown in Table 1. In Fig. 1 and 2, schematic diagram of changes in diabetes control parameters of patients are shown during follow-up sessions. Detailed description of the changes is available in detail in Table 2.

As demonstrated in Fig. 1, after stem cell transplantation one patient (patient number 3) experienced a 6 month insulin-free period with normal HbA1c levels. The aforementioned patient was a 27 year old woman with 10 years duration of diabetes (Table 1). In this patient, serum C-peptide level rapidly increased during the first three months after the transplantation while the required daily insulin dose decreased (Fig. 2). Patient remained insulin free with acceptable levels of HbA1c up to the 6th month of follow-up. In the month 6, however, 2 IU NPH insulin started for her on the grounds of the increasing levels of FBS. At the end of the 1st year of follow-up, the patient who needed 1 IU/kg /day insulin before the intervention was receiving 0.26 IU/kg /day insulin while HbA1c level was 7.1% (Table 2).

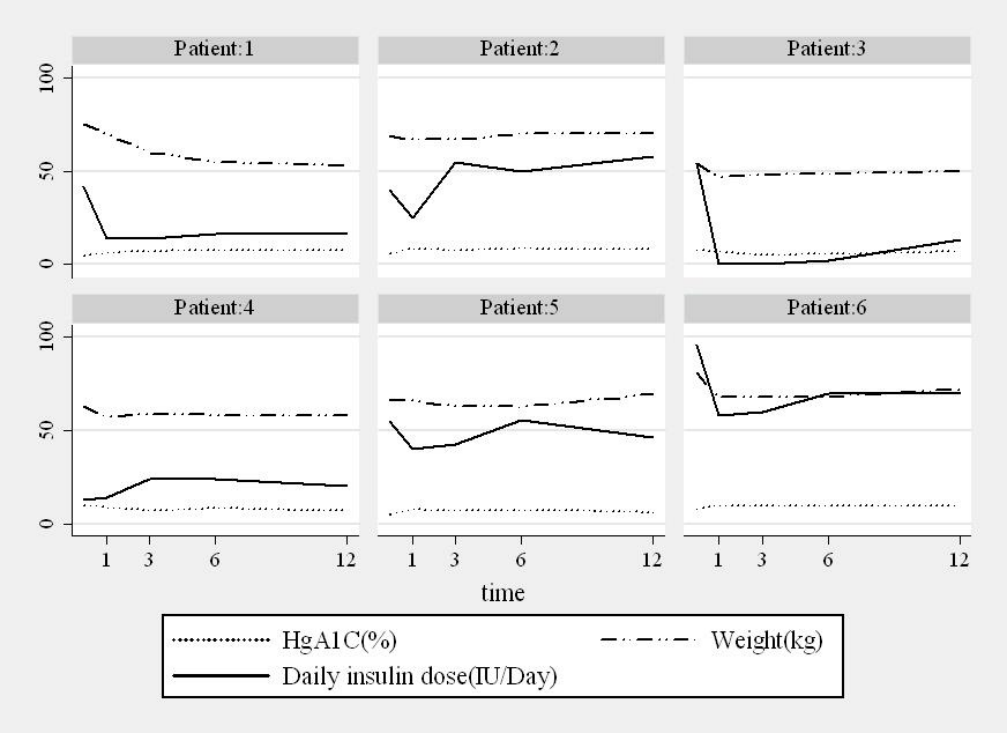

Fig 1: Changes in amount of daily insulin (IU) along with HbA1c and body weight in patients during follow-up

Table 1: Summary of clinical features with relevant laboratory variables of the subjects with Wolfram syndrome

Clinical variable	Patient 1	Patient2	Patient 3	Patient 4	Patient 5	Patient 6
Age (yr)	23	26	27	33	34	34
Sex (M:F)	m	m	f	m	m	m
Consanguinity (T1DM)	(-)	(+)	(-)	(-)	(-)	(+)
Diabetes mellitus duration (age of onset) (years)	12 (11)	18 (8)	10 (17)	10 (23)	24 (10)	26 (8)
Central diabetes insipidus (age of onset) (years)	(-)	(-)	(-)	(-)	(+)	(-)
Optic atrophy (age of detection) (years)	(+)	(+)	(+)	(+)	(+)	(+)
Diabetes retinopathy	(+)	(+)	(+)	(-)	(?)	(+)
Deafness (age of detection) (years)	(-)	(-)	(-)	(+)	(±)	(-)
Hydronephrosis	(-)	(-)	(+)	(+)	(+)	(-)
Nephrogenic diabetes insipidus	(-)	(-)	(-)	(-)	(+)	(-)
Seizures	(+)	(-)	(-)	(-)	(+)	(-)
Other features	Depression, neuropathy (lower extremities)	hypothyroidism, cataract surgery and intraocular lens (IOL) , Lasik surgery	hypothyroidism, motor neuropathy (lower extremities)	Neurogenic bladder (Atonic), mild MVP, Glaucoma, Anxiety Disorder, repeated attack of hypoglycemic unawareness /severe hypoglycemia	hypothyroidism, Neurogenic bladder (Atonic), Anxiety Disorder	History of 5 times Lasik surgery , Depression

(+): Present, (-): absent.

As demonstrated in Table 2 and Fig. 1, there were no significant changes in diabetes control indicators in any of the other participants. All patients received diabetes diabetic diet and lost weight in the first 3 months of the procedure. Prior to the intervention, HbA1c level of patients was 6.90±2.02 % (mean±SD) and BMI was 25.10±3.90 kg/m^2. Patients received 13-96 IU insulin (50.00± 27.01; mean±SD). Taking into account the patient's weight, average daily insulin intake per kilogram of body weight was set between 0.21-1.19 (0.72±0.35; mean±SD). Friedman's test showed that there was no significant changes in the mean level of HbA1c, C-peptide, required insulin dose and body weight in patients with baseline time during follow-up sessions. In patient number 4 who suffered from repeated attacks of hypoglycemic accompanied with the loss of conscious, number of attacks decreased (Table 1). However, required insulin dose increased. In the other patients, symptoms and compilations neither increased nor decreased.

Table 2: Changes in parameters of diabetes control in patients during time

	Follow-up	HbA1c (%)	C-peptide (ng/dl)	Insulin Dose (IU)	Weight (kg)		Follow-up	HbA1c (%)	C-peptide (ng/dl)	Insulin Dose (IU)	Weight (kg)
P 1	Baseline	4.6	0.1	42.00	65.00	**P 4**	Baseline	10	0.07	13.00	63.00
	1st month	6.6	0.4	14.00	60.00		1st month	8.7	0.16	14.00	57.00
	3rd month	7.1	0.62	14.00	56.00		3rd month	7.3	0.05	24.00	59.00
	6th month	7.80	0.2	14.00	53.00		6th month	8.4	0.05	24.00	58.00
	12th month	7.3	0.2	16.00	53.00		12th month	7.1	0.05	20.00	58.00
P 2	Baseline	6.10	0.10	40.00	69.00	**P 5**	Baseline	5.10	0.40	55.00	66.50
	1st month	8.4	0.1	25.00	67.00		1st month	7.8	0.05	40.00	65.80
	3rd month	7.4	0.01	55.00	67.00		3rd month	7.20	0.05	42.00	63.00
	6th month	8.9	0.01	50.00	70.00		6th month	7.50	0.05	56.00	62.60
	12th month	7.9	0.05	58.00	70.00		12th month	6.00	0.05	46.00	69.40
P 3	Baseline	7.5	0.5	54.00	54.00	**P 6**	Baseline	8.10	0.10	96.00	81.00
	1st month	7	1.4	0.00	51.00		1st month	9.60	0.05	58.00	68.00
	3rd month	4.8	2.3	0.00	50.00		3rd month	10.00	0.05	60.00	68.00
	6th month	5.6	1.9	2.00	49.00		6th month	10.20	0.05	70.00	68.00
	12th month	7.1	1	13.00	50.00		12th month	10.00	0.05	70.00	72.00

P: Patient

Discussion

Cell therapy is demonstrated to be a promising option for treatment of several diseases including diabetes (9). The underlying responsible mechanisms of stem-cell therapy are hypothesized to be engraftment and promotion of β-cell regeneration through enhanced neo-vascularization and im-

munomodulatory effects (10-14). Considering the potential of stem cells to differentiate into insulin producing beta cells (6, 7, 9, 15-18) and modulation of immune system (19-21), we conducted a phase 3 single arm clinical trial for the assessment of the effectiveness of fetal liver-derived cell suspension allotransplantation on patients with WS for the first time in the world. As mentioned, 1 out of the 6 patients (patient number 3) experienced a 6 months period requiring no insulin any anti diabetes agents with excellent levels of HbA1c (Table 2). Moreover, in another patient (number 4), a favorable glycemic control was achieved.

Diabetes occurs when pancreatic beta-cells no longer function properly or have been destroyed. Inflammation and cell stress play important roles in diabetes as they are the cause of death of insulin-secreting cells (22). In type 1 diabetes, destruction of beta-cells is the result of an autoimmune response against pancreatic beta-cells. There are also several rare forms of diabetes, including WS, caused by mutations in genes that may play important roles in beta-cell survival (23). Affected patients usually develop insulin-dependent diabetes and optic atrophy in early childhood, and diabetes insipidus as teenagers or young adults (4). The precise mechanism of severe insulin-dependent diabetes in WS is not well-elucidated; however, general consensus is that immunologic factors are not involved (24).

The findings of the recent research indicate that endoplasmic reticulum (ER) can be a potential target for WS, type 1 and type 2 diabetes, atherosclerosis, and neurodegenerative diseases (25). It has been demonstrated that cell stress plays an influential role in WS (22). Wolframin is a transmembrane glycoprotein in endoplasmic reticulum membrane of pancreatic beta cells and neurons which maintains cellular homeostasis (2, 26, 27). Alteration of the WFS 1 gene, one of genes play role in development of WS, is believed to lead to chronic ER stress which results in apoptosis of pancreatic beta cells, neuroendocrine cells, and neuronal cells (28). Moreover, recent studies have suggested that mutation in the WFS1 leads to impaired acidification of insulin secretory granules (29, 30).Together, the aforementioned processes hypothetically result in the constellation of symptoms described in the WS (24).

In all cell-based researches has been performed on patient with diabetes (20, 31-33) hitherto, complete response (independence from receiving insulin) was only achieved in the presence of an immunosuppression method. Voltareli et al. for the first time investigated the effect of autologous nonmyeloablative hematopoietic stem cell transplantation on newly diagnosed type 1 diabetes patients (34) and they reported some clinical success in the management of the disease (34-37). Stem cells can also be genetically manipulated to upregulate certain trophic factors secretion, which is believed to heal injured pancreatic cells and control hyperglycemia (38, 39). In the field of hereditary disorders, a number of studies are carried out with the use of embryonic stem cells for gene engineering and gene therapy (40). In a study on rhesus monkey, direct differentiation of embryonic stem cells into pancreatic cell phenotypes has been reported (41). Besides, embryonic stem cells (ESC) can be differentiated into insulin-producing cells (42). Transplanted cells may be mobilized to injured pancreas region and interact with the local microenvironments to secret factors helping pancreatic functional recovery such as (IGF1, VEGF, HGF) (38, 39). Bioactive agents may inhibit pancreatic beta cell apoptosis, promote cell survival and induce endogenous progenitor cell proliferation (43). Isolating and delivering such factors at high concentration may result in more significant results (38). The mentioned findings can explain the underlying mechanism of the insulin-free period in the patient number 3. A mixture of these mechanisms is also possible to occur. The patients did not genetically assessed before and after the cell infusion but special blood sample of them were saved in standard condition and exist to further genetic researches.

Findings of one study demonstrated that the prevalence of severe hypoglycemia was approximately 37% in patients with WS compared to only 8% in a cohort with type 1 autoimmune diabetes (44). In our study patient number 4 had frequent attacks of hypoglycemia with and without una-

wareness before cell transplantation. The attacks, however, decreased after the cell infusion despite the increasing required daily insulin dose (Fig. 2). It should be mentioned, however, that as the patients had the twenty-four hours access to a physician during the whole period of the trial, this clinical achievement cannot be solely attributed to the transplanted stem cells.

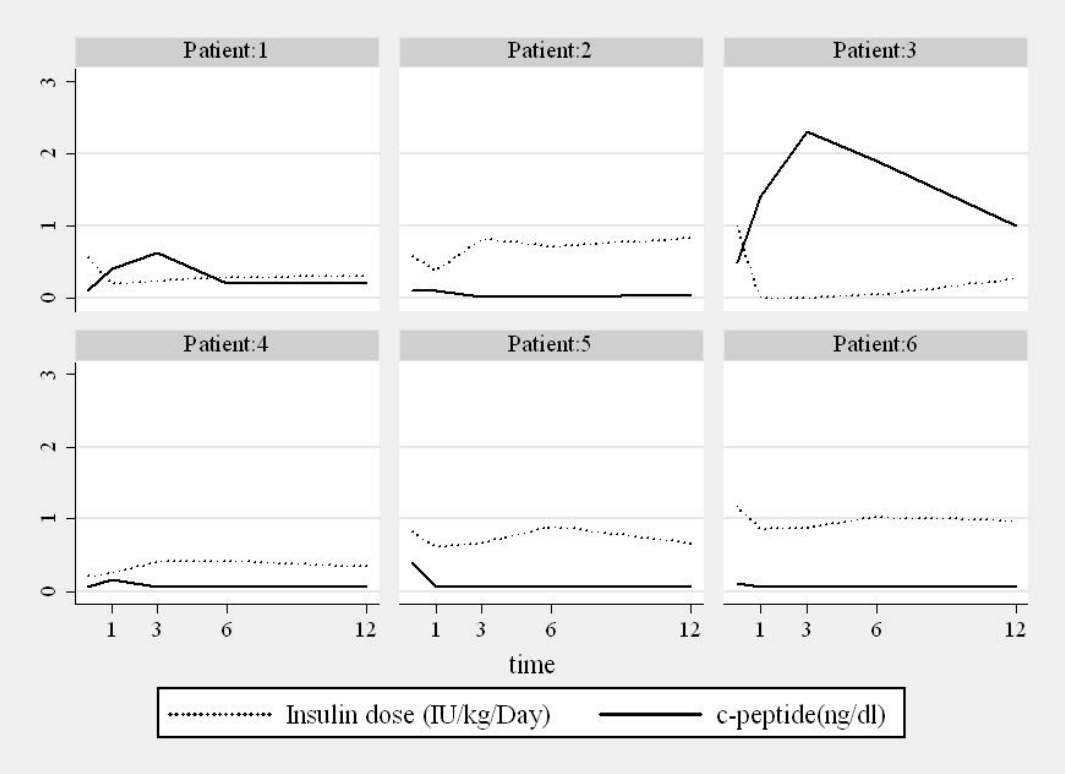

Fig. 2: Changes in serum C-peptide level and required insulin daily dose (IU/kg/day) during follow-up

Conclusion

Stem-cell therapy can result in favorable therapeutic outcomes in different types of diabetes including Wolfram Syndrome. More studies need to be carried out in this field to demonstrate effectiveness of stem-cell therapy for treatment of diabetes and its related syndromes.

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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