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Original Article

Albuminuria in Diabetic Patients before and after Fetal Liver-Derived Cell Suspension Allotransplantion: a 24 Months Follow up Study

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Abstract

Background: Stem-cell therapy plays a preventive role for different complications of diabetes, including diabetes nephropathy. The current phase 3 single-arm clinical trial is designed to investigate the potential protective effect of allotransplantation of fetal liver-derived cell suspension in diabetic nephropathy.

Methods: Seventy-four diabetic patients with type 1 were selected according to the inclusion criteria defined and underwent the procedure of transplantation fetal liver-derived stem-cells. Patients were followed for a period on 24 months and indicators of diabetes and diabetes nephropathy (HbA1C, ACR, Cr and GFR) were monitored during the whole time. Statistical analysis was conducted with SPSS Vers.16 and one-way repeated measure ANOVA was used.

Results: The GFR values significantly increased during the 24 months period of follow up.

Conclusion: Stem-cell therapy can play a significant protective role in prevention from diabetic nephropathy.

Keywords: Stem-cell therapy, Diabetic nephropathy, Type 1 diabetes

Introduction

Diabetic nephropathy occurs in 20–40% of patients with diabetes and is the leading cause of chronic kidney disease and end-stage renal disease (1)**.**The key pathologic finding of high blood glucose levels for a duration of several years in diabetic nephropathy include glomerular hypertrophy, thickening of the glomerular and tubular basement membrane, tubule-interstitial fibrosis, low grade of renal inflammation and microalbuminuria (2).

High albumin levels in the urine can be an initial sign of development of diabetic nephropathy (3). Urinary albumin excretion can be affected by several factors including fasting blood glucose, glycated hemoglobin, and mean arterial blood pressure, and its defined as a urine albumin to urine creatinine ratio (ACR) of 30 to 300 mg/g creatinine, is thought to be a marker of endothelial dysfunction and inflammation, reflecting a systemic endothelial injury that affects multiple organs including the kidney (4).

Nowadays, therapies for diabetic nephropathy are limited to drugs that improve blood pressure or control blood glucose levels. In 1981, researchers for the first time isolated Stem cells (SCs) are characterized by several features, including pluripotency and self-renewal, and ability to regenerate virtually all adult tissues and organs. Since then, using stem cells for treatment of different diseases has opened up new horizons for researchers (5). The use of stem cell therapy in diabetes is an im-

portant subject that may influence diabetic complications and might prevent the development of microvascular complications, such as diabetic nephropathy and the following end-stage renal disease.

Several experimental studies using animal models of renal disease have clearly showed that Mesenchymal stem cell (MSc) therapy is capable of preserving renal parenchymal integrity from acute ischemic injury and improving kidney function from acute damage (6, 7).

Based on these evidences, and ability of stem cells to differentiate into insulin producing cells besides their immunomodulatory function (8-10), this interventional clinical study aimed to investigate the effect of fetal liver-derived cell suspension allotransplantation in the progression of diabetic nephropathy status especially in albuminuria using on diabetic patients.

Materials and Methods

The current study can be considered as a secondary study performed on the data of a phase 3 single-arm clinical trial carried out at the Endocrinology and Metabolism Research Institute of Tehran University of Medical Sciences with the objective of assessment of effectiveness of treatment with fetal-liver derived stem-cells in a cohort of 82 patients with type 1 diabetes. The detailed information of this clinical trial is published elsewhere (11).

Patients

This study was conducted in the Endocrinology and Metabolism Research Institute (EMRC) of Tehran University of Medical Sciences. After approval of the study protocol by the Ethics Committee of EMRI (ethical code number: E-0089) an informed consent was obtained from each patient or his/her parents before their enrollment in the study. Hereby, we are describing the results of the two-year follow-up after the transplantation (12).

Seventy-four diabetic patients with type 1 and type 2 were selected according to the following criteria: Age range between 10-40 years old, duration of the disease up to 20 years, blood glucose under 15 mmol/l (270 mg/dl).

All patients included in the study were subjected to full history taking, complete clinical examination (including blood pressure, temperature, plus rate, weight, height), ECG for exclusion of cardiovascular disease.

Laboratory investigations included fasting blood glucose, HbA1C, renal function tests (BUN, Cr), glomerular filtration rate (GFR):

Cockcroft- Gault Equation Creatinine clearance = {[140 – age (years)] ×weight (kg)}/ [72 × serum creatinine (mg/dl)] × (0.85 if female)

Albuminuria: albumin creatinine ratio in the first morning urine void (ACR): normoalbuminuria (ACR ≤30 mg/dL), microalbuminuria ACR 30–300 mg/dL, macroalbuminuria ACR >300 mg/dl.

Exclusion criteria were as follows: liver diseases, cardiovascular diseases, cancer, infections or inflammatory conditions, renal disease other than diabetic nephropathy, pregnancy, bone marrow malignancy, end stage diseases, and signs of refractory complications (12).

Stem cell preparation

Fetal liver-derived hematopoietic stem cells (HSCs) were isolated from legally and aborted human fetuses aged 6–12 weeks after obtaining an informed consent from the parents (mother or both of the parents). In order to determine chromosomal abnormalities and to identify the sex of the donated fetus, karyotyping was done for each fetal sample. Whole fetal liver was placed in Hank's balanced salt solution without calcium and magnesium (HBSS, Sigma, USA) and dissociated and homogenized mechanically. The cell suspension was filtered through nylon mesh to undergo transplantation; and then, isolated cells were cryopreserved using 5% dimethyl sulfoxide (DMSO) in HBSS, (WakChemie, Germany) with a programmable freezer, and were transferred to liquid nitrogen for long-term storage. Before transplantation, samples were thawed at 37 °C and cryoprotectant was diluted by 5-milliliter normal saline before infusion. Total cell count in the prepared suspension was approximately 35-55×10^6, twenty percent of which was recognized as hematopoietic (CD34+) stem cells. The suspension was checked before, during, and after processing for aerobic, an-

aerobic and fungal contamination as well as viral infections. Rubella, Herpes Simplex Virus, Cytomegalovirus, *Chlamydia*, *Mycoplasma homonis*, *Toxoplasma Gondii* and *Treponema pallidume* were checked using ELISA. DNA/RNA extraction and polymerase chain reaction (real-time PCR) were done for checking viral contamination (HBV, HCV, and HIV). After evaluating the results, cell samples were known qualified for the transplantation] (12).

Statistical analysis of the data of the present study was conducted with SPSS Version 16 (Chicago, IL, USA). Data are expressed as mean ± SD. *P* values <0.001 were considered significant. In order to investigate time effect on each response, one-way repeated measure ANOVA were used. Significant effect of time was followed by paired *t*-test while all *P* values were adjusted by bonferroni for multiple comparisons.

Results

To effect of fetal liver-derived cell suspension allotransplantation in the progression of diabetic patients for variables HbA1C, ACR, Crand GFR, patients were followed for duration of2 years.

Patients were measured and their characteristics recorded beforetreatmentand1, 3, 6, 12 and 24months after treatment. Demographic data and description of the percentage of people eat different levels and HbA1Cand Cr, ACR in Time Points (repeated measure ANOVA analysis) are shown in Table 1-5 respectively.

Table 1: Patient demographic characteristics (n=74)

Patient Characteristic		Frequency (%)
Age	<15	29 (39)
	15-30	42 (57)
	≥ 30	3 (4)
Gender	Female	38 (51)
	Male	36 (49)
Duration	< 10	64 (87)
	≥ 10	10 (13)

Table 2: Descriptive Statistics for ACR

	n	Minimum	Maximum	Mean	Std. Deviation	≤ 30 (%)	> 30, ≤ 300 (%)	> 300 (%)
Month 0	68	4.00	283.00	29.8207	38.91506	70	22	0
Month 1	64	3.90	342.00	30.7722	43.67065	61	24	1
Month 3	66	8.50	381.00	36.0627	49.57715	61	27	1
Month 6	70	4.10	530.00	34.8036	64.32766	68	26	1
Month 12	60	9.20	459.00	52.9165	66.80300	35	45	1
Month 24	62	6.30	472.00	38.7208	60.52194	57	26	1

Table 3: Descriptive Statistics for HgA1C

	N	Minimum	Maximum	Mean	Std. Deviation	≤ 6.5	> 6.5
Month 0	74	4.40	13.40	7.4351	1.92036	34	66
Month 1	72	4.50	11.60	8.0333	1.38198	18	80
Month 3	69	4.70	15.30	8.5377	1.72840	7	87
Month 6	71	4.90	13.30	9.0394	1.80170	5	91
Month 12	62	4.80	16.00	8.5629	2.32006	18	66
Month 24	63	3.90	13.90	7.8190	2.00152	26	60

As regards ACR values, in 3, 12 and 24 months after allotransplantation we did not detect any significant differences (*P*=0.414) (Fig. 1).

The GFR values significantly increased during the 24 months period of follow up. This increased started from the 12th months of follow up (*P*=0.000) (Fig. 2).

In terms of HbA1C value, we observed a significant increase in the 3rd and 6thmonths, which returned to normal levels at the end of the period (Fig. 3). It is noteworthy that we did not observe any significant difference between findings of male and female participants.

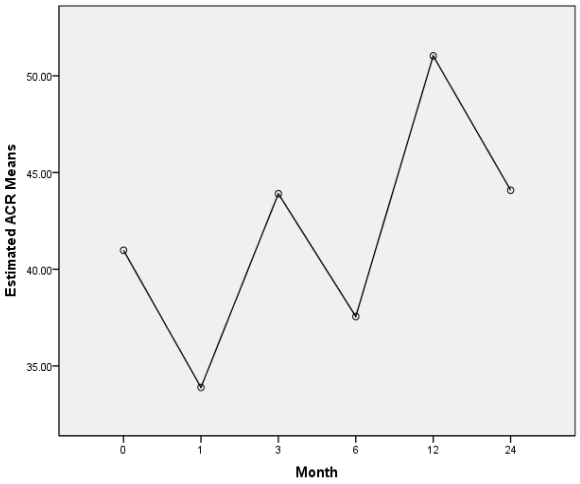

Fig.1: Changes in means of ACR

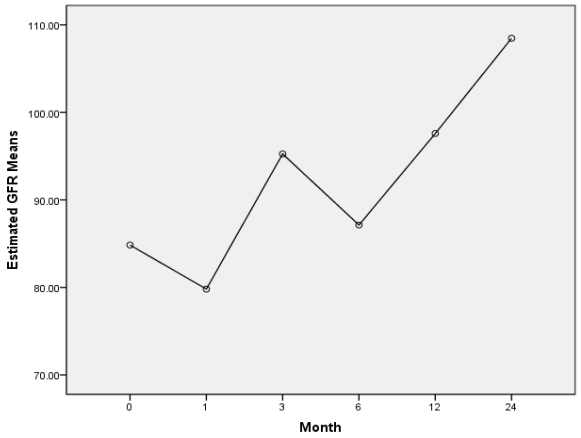

Fig.2: Changes in means of GFR

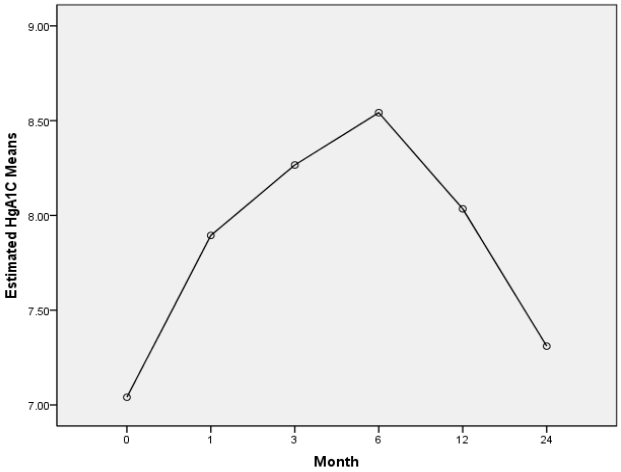

Fig.3: Changes in means of HbA1C

Table 4: Descriptive Statistics for GFR

	N	Minimum	Maximum	Mean	Std. Deviation
Month 0	71	40.57	156.53	86.6215	24.91816
Month 1	73	6.79	243.95	80.7060	30.48430
Month 3	66	38.75	307.12	98.6690	45.62752
Month 6	70	41.67	192.35	86.9733	24.25074
Month 12	60	43.75	158.24	97.0917	23.15878
Month 24	60	62.50	182.55	110.2849	24.20644

Discussion

Nephropathy ensues poor diabetes control and proteinuria and albuminuria are considered as indicators of diabetic nephropathy (13). Recently, it is demonstrated that stem-cell therapy can be effective in treatment of diabetes with significant changes in several diabetes control indicators such as HbA1C, required insulin dose, and blood c-peptide levels (14). The current study was carried out using the data from a phase 3 single-arm clinical trial to investigate the effect of treatment with stem-cells on indicators of progression or remission of diabetic nephropathy, namely: albumin-creatine ratio (ACR), blood creatinine levels (Cr), and Glomerular Filtration Rate (GFR).

Table 5: Repeated measures ANOVA

Response	Repeated measures		Pairwise Comparisons			
	Source	F (*P*)	Pairs of Months	Mean Difference (I-J)	Std. Error	Adjusted *P*
ACR	Month	0.92 (0.414)	0-1	7.087	7.831	1.000
	Month*Age	0.85 (0.509)	0-3	-2.925	8.781	1.000
	Month*Gender	2.96 (0.048)	0-6	3.421	10.936	1.000
	Month*Duration	0.61 (0.567)	0-12	-10.049	11.675	1.000
	Month*Level	3.22 (0.037)*	0-24	-3.107	10.773	1.000
A1C	Month	2.64 (0.027)*	0-1	-0.854	0.296	0.083
	Month*Age	1.14 (0.332)	0-3	-1.226	0.347	0.012*
	Month*Gender	0.13 (0.987)	0-6	-1.502	0.392	0.005*
	Month*Duration	1.36 (0.239)	0-12	-0.994	0.397	0.228
	Month*Level	0.65 (0.665)	0-24	-0.270	0.400	1.000
GFR	Month	5.62 (0.003)*	0-1	5.032	5.358	1.000
	Month*Age	0.36 (0.868)	0-3	-10.415	8.940	1.000
	Month*Gender	0.53 (0.620)	0-6	-2.287	3.705	1.000
	Month*Duration	1.01 (0.37)	0-12	-12.748	3.683	0.014*
			0-24	-23.632	5.049	0.000*

Our findings demonstrated that no significant alternations occurred in ACR levels in the whole duration of 24 months follow up. This finding indicates that stem-cell therapy might have played a protective role in terms as it was expected that the ACR would increase as the patients' age. Moreover, stem-cell therapy by itself causes some degree of nephropathy (15). Therefore, our finding as to finding which demonstrated no change in ACR is highly suggestive that stem-cell therapy can play a protective role in terms of development of diabetic nephropathy.

As mentioned, the deterioration of kidney function in diabetes is associated with poor diabetes control (16). In our study, although we intentionally decreased the daily insulin dose of the patient so that the induced hyperglycemia would stimulate proliferation of stem-cells into insulin-secreting beta-cells, no deterioration in kidney functions were detected during the 24 months period of study. Therefore, for this reason too, it may be suggested that our intervention played a protective role in terms of diabetic nephropathy.

GFR is significantly decreases in the final stages of diabetic nephropathy. On our study, we observed a significant increase in terms of GFR of our study subjects. Therefore, the increase in GFR we observed was due to the protective effect of stem-cell therapy in terms of diabetic nephropathy.

Stem-cell therapy has considerable positive effects in diabetic nephropathy in animal studies. Treatment with mesenchymal stem-cells can slightly ameliorate diabetic nephropathy (17). Moreover, treatment with bone marrow mesenchymal stem-cells led to in a significant reduction in albuminuria, glomerular hyalinosis, and mesangial expansion after in their 8 weeks period of follow up (18). Stem-cell therapy both results in antialbuminuric effects and prevents early podocyte phenotypic changes, which lead to glomerulosclerosis (19).All the mentioned studies were carried out on animal models and the follow up period was no longer than a few months. Therefore, our phase 3 single-arm clinical trial was the first one, which investigated the protective effect of stem-cell therapy in terms of diabetic nephropathy.

Conclusion

Stem-cell therapy can play a significant protective role in diabetic nephropathy.

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.

Acknowledgements

The authors declare that there is no conflict of interests.

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