Original Paper

Efficacy and Safety of Autologous Bone Marrow Mesenchymal Stem Cell Transplantation in Patients with Diabetic Retinopathy

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Key Words

Diabetic retinopathy • Mesenchymal stem cell • Stem cell transplantation • Best corrected visual acuity • Macular thickness

Abstract

Background/Aims: The treatment options for diabetic retinopathy (DR) are limited. Mesenchymal stem cells (MSCs) are a promising treatment option for diabetes and its complications. In this pilot clinical trial, we evaluated the safety and efficacy of intravenous autologous bone marrow MSCs (ABMSC) for the treatment of DR. **Methods:** In total, 34 eyes with non-proliferative or proliferative DR (NPDR, n = 19; PDR, n = 15) from 17 patients were analyzed. Treatment involved one intravenous infusion of $3 \times 10^6/\text{kg}$ ABMSCs. The patients' vital signs were monitored, along with immune and allergic reactions. Treatment efficacy was evaluated via measurements of the following parameters at baseline, and at 1, 3, and 6 months after treatment: the levels of fasting blood glucose (FBG), Hemoglobin A1C (HbA_{1c}), interleukin-6 (IL-6), and hypersensitive C-reactive protein (CRP); best corrected visual acuity (BCVA); and central macular and subfield thickness (via optical computed tomography). **Results:** ABMSC infusion led to a significant decrease in FBG and CRP levels ($P < 0.05$). There were no significant differences in HbA_{1c} or IL-6 levels. Sub-group analysis revealed that only eyes in the NPDR group had the macular thickness reductions and a significant improvement in BCVA from baseline ($P = 0.006$ at 3 months and 0.027 at 6 months), while those in the PDR group did not. There were no acute reactions during the treatment or severe adverse

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events during the follow-up period. **Conclusion:** ABSMCs are a potentially safe and effective treatment option for DR, and the optimum therapeutic window appears to be during the NPDR stage.

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Introduction

Diabetic retinopathy (DR), one of the main complications of diabetes mellitus, has become the leading cause of blindness in developed countries [1]. While its pathogenesis remains to be elucidated, the most widely-accepted theory is that diabetes-associated chronic low-grade inflammation and retinal endothelial cell loss lead to DR [2, 3]. This suggests that the control of inflammation may be an effective way to treat DR.

The classification and grading of retinopathy is based on the severity of vascular lesions [4, 5]. Generally, DR is classified into two stages based on the occurrence of abnormal neovascularization: non-proliferative diabetic retinopathy (NPDR) and advanced, proliferative diabetic retinopathy (PDR); these stages determine the treatment strategy. DR cannot be cured, regardless of its stage, but some preventive and control measures have been developed. First, sufficient blood glucose, pressure, and lipid control have been evaluated for the prevention and retardation of DR [6, 7]; however, existing lesions cannot be reversed. Second, intravitreal injections of anti-vascular endothelial growth factor (VEGF) agents show promise in treating diabetic macular edema and neovascularization; however, these are associated with large costs and the need for repeated injections [8]. Third, laser photocoagulation surgery, which is the standard technique for treating diabetic retinopathy, can improve retinal oxygen supply and indirectly treats neovascularization; however, it can reduce visual acuity and visual field due to its destructive effect on the retina [9, 10]. Fourth, vitrectomy forms an important part of the treatment strategies; however, it can only be applied to high-risk PDR, and it has some risks and complications similar to other types of surgeries [11, 12]. In view of the large socio-economic burden and treatment limitations, there is an urgent need to develop effective measures for the treatment of DR.

Mesenchymal stem cells (MSCs) have been broadly investigated due to their multi-directional differentiation potential, potent immune modulation ability, and ability to support nutrition by releasing a broad range of trophic factors [13-15]. Cai et al. showed that transplantation of umbilical cord tissue derived-MSCs was safe and associated with moderate improvement in metabolic measures in patients with type 1 diabetes [16]. Closing and healing of non-healing diabetic ulcers have been achieved through the use of autologous MSCs by Vojtaššák et al [17]. Researchers agree that MSCs can play a role in the treatment of diabetes and its associated complications due to their immune regulation, blood glucose control, and vascular repair abilities, which offset the pathogenesis of DR. MSCs have not yet been used in DR patients. However, they have been shown to improve the integrity of the blood-retinal barrier and visual function in rats with diabetes through the differentiation of photoreceptor and glial-like cells, and reductions in blood glucose levels [18, 19]. They have also been shown to maintain the original vascular structure through reductions in apoptotic vascular cells and suppression of abnormal neovascularization in retinal disease [20, 21]. MSCs can be obtained from a variety of tissues. Autologous bone marrow MSCs (ABMSCs) are more convenient to obtain compared with other tissue-derived stem cells; moreover, they reduce immunological rejection risk and do not need to be type matched.

Therefore, we performed a prospective clinical trial using intravenous ABMSC to treat 10 patients with severe NPDR and seven with non-high-risk PDR. The aim of this study was to evaluate the efficacy and safety of ABMSC transplantation in DR by assessing the changes in visual function, macular edema, and retinal vascular leakage after treatment.

Materials and Methods

Study design

This was a 6-month, non-randomized, open-label, prospective, no-placebo, single-center study conducted from April 2013 to December 2016. It was approved by The First Affiliated Hospital Ethics Committee of Third Military Medical University, Chong Qing, China (No. ChiCTR-ONC-16008055; chictr.org.cn). All patients provided informed consent before treatment. The study protocol adhered to the tenets of the Declaration of Helsinki.

Inclusion criteria

1. Aged 18–70 years.
2. Type 2 diabetes with HbA1C $\leq 10\%$ (86 mmol/mol), counseled by an endocrinologist on intensive drug treatment with self-monitoring of blood glucose, participated in exercise (2–3 km three times a week), and had a healthy diet and blood glucose control.
3. At least one eye with visual impairment secondary to severe NPDR or non-high-risk PDR.
4. Ability to adhere to the study visiting schedule and protocol requirements.

Exclusion criteria

1. Presence of other diseases with the potential to influence the interpretation of research results (severe cataract, glaucoma, macular hole, retinal detachment, or choroidal neovascularization).
2. Affected eye had a prior history of intraocular surgery.
3. Evidence of active infection or abscesses before MSC infusion.
4. Stroke, coronary heart disease, or renal insufficiency requiring dialysis or kidney transplantation, as well as other systemic chronic diseases that are not suitable for clinical trials.
5. Blood clotting functional abnormalities, or currently taking anticoagulant drugs.
6. Severe hypertension (systolic pressure > 140 mmHg or diastolic pressure > 90 mmHg) after taking antihypertensive drugs.
7. Psychiatric, addictive, or any other disorder that compromised the ability to provide true informed consent for participation in the study.

Bone marrow-derived MSC

Bone marrow-derived MSCs for the study were obtained from patients with DR according to the International Society for Cellular Therapy's standardized and approved protocol. During routine surgical procedures, 50 mL bone marrow was aspirated from both iliac crests, separated using a quadruple blood collection bag (Terumo Medical Corporation, Changchun, China), and immediately transported to the Good Manufacturing Practice laboratory of the Biological Therapy Center (Southwest Hospital, China). The marrow was then diluted with PBS (pH 7.3, Hyclone™, GE Healthcare, Logan, UT, USA) and loaded over a Ficoll solution (GE Healthcare, Uppsala, Sweden) with a density of 1.073 g/mL, and centrifuged (J-26XP, Beckman Coulter, Brea, CA, USA) at 2,000 *g* for 30 min. The upper layer containing the plasma and platelets was drawn off, leaving the layer of mononuclear cells, which was transferred to a centrifuge tube and washed twice with PBS. The precipitate was re-suspended in MSC culture medium, with harvesting at passage three. Final product release was based on the following criteria: sterile, absence of endotoxin, mycoplasma, hepatitis B, hepatitis C, and syphilis, surface marker expression ($\geq 95\%$ of cells CD44+/CD73+/CD90+/CD105+, $\leq 0.5\%$ of cells CD11b+/CD19+/CD34+/CD45+/HLA-DR+), and cell viability $\geq 95\%$ (Fig. 1).

Treatment

All patients received one intravenous (dorsal hand vein) infusion of ABMSCs, of which the total number for each patient was 3×10^6 /kg [22–25], sequentially infused within 30 min. Before transplantation, close monitoring of vital signs was performed including measurements of body temperature, blood glucose, blood pressure, pulse, blood oxygen saturation, and an electrocardiogram. If these evaluations were normal, patients received 5 mg of dexamethasone sodium. Monitoring continued during and up to 2 h after MSC infusion. During the infusion, criteria for stopping the infusion included development of tachypnea, fever, local pain, and headache. After the infusion, potential immune rejection and allergic reactions were also monitored.

Examination

We evaluated efficacy and safety parameters at baseline, and at 1, 3, and 6 months after infusion of ABMSC.

Efficacy assessments included measurements of fasting blood glucose (FBG) (Glucose determination kit (hexokinase method), Beckman Coulter, China), Hemoglobin A1C (HbA1C) (Tosoh Automated Glycohemoglobin Analyzer HLC-723G8, Japan), interleukin-6 (Elecys IL-6 assay kit, Roche Diagnostics, Germany), and hypersensitive C-reactive protein (CRP) (Ultrasensitive CRP kit, Orion Diagnostica, Finland), as well as ophthalmologic examination. Best corrected visual acuity (BCVA) measurements were performed according to a standardized refraction protocol using the Early Treatment Diabetic Retinopathy Study letters. Third-generation optical computed tomography (OCT) evaluation (Topcon LLC, Tokyo, Japan) was performed in all patients, involving six linear 6.00-mm scans obtained at intervals of 30° and centered on the fovea region. By using the OCT software “retinal thickness/volume” analysis protocol, with the center on the patient’s fovea, three concentric circles with the diameters 1,000 μm , 1,000–3,000 μm , and 3,000–6,000 μm were set. Two straight lines perpendicular to each other with an intersection at the fovea were then set. The whole macula was separated into nine regions: the average thickness of the nine regions represented the central macular thickness (CMT) and the central circle represented the central subfield thickness (CST) (Fig. 2).

Safety assessments included monitoring of all adverse events and assessment of biochemical parameters (liver function test, renal function test, and myocardial enzyme spectrum).

Fig. 1. Immunophenotype of autologous bone marrow mesenchymal stromal cells (MSCs) after expansion. MSCs are labeled with monoclonal antibodies against CD90, CD44, CD105, and CD73, and negative cocktail (CD34, CD11b, CD19, CD45, and HLA-DR) at third passages, then analyzed by flow cytometry.

Fig. 2. Study chart, OCT examination range, and macular edema proportions. A: Completion of 6-month evaluations, with patients divided into PDR and NPDR groups for analysis. B: Schematic diagram of OCT examination range. With the center on the patient’s fovea, three concentric circles with 1 mm, 3 mm, and 6 mm diameters are set. The middle circle represents the CST and all of the nine regions represent the CMT. C: Percentage of eyes with OCT-measured macular edema defined as CMT $\geq$ 300 μm or CST $\geq$ 250 μm . CMT = central macular thickness; CRVO = central retinal vein occlusion; CST = central subfield thickness; PDR = proliferative diabetic retinopathy; NPDR = non-proliferative diabetic retinopathy; OCT = optical coherence tomography.

Statistical analysis

Statistical calculations were performed using SPSS version 18.0 (IBM Inc., NY, USA) and Microsoft Excel software 14.4.8 (Microsoft Inc., Redmond, WA, USA). All quantitative variables are presented as mean $\pm$ standard deviation or median and interquartile range. The repeated-measures data of the patients' general condition were calculated using a mixed-effects linear model due to missing values. One-way repeated-measures analysis of variance (ANOVA) was used to analyze optic data assuming that the BCVA, CMT, and CST changes from baseline followed a normal distribution. Pairwise comparisons at each time point used the least significant difference when there was a significant difference between before and after MSC transportation calculated by the mixed-effects linear model or one-way repeated-measures ANOVA. An independent t-test was performed to detect the difference in change from baseline between the two patient groups at the same follow-up time point, when their baseline value had been confirmed to have adequate power to detect this assumed difference by independent t-test. Tests yielding $P < 0.05$ were considered statistically significant.

Results

Patient characteristics

We enrolled 17 participants (34 eyes) including 19 eyes at the severe non-proliferative stage (participants no. 1–8, and 10 = binoculus, and participant no. 9 = right eye) and 15 eyes at the non-high-risk proliferative stage of DR (participants no. 11–17 = binoculus, and participant no. 9 = left eye). They were aged between 37 and 67 years of age, with an average age of 53 years. Nine patients had a duration of diabetes exceeding 10 years and eight a duration of less than 10 years, while seven had hypertension. Eight eyes with non-high-risk PDR were treated with laser photocoagulation, while eyes with severe NPDR did not receive laser photocoagulation. Participant no. 6 was excluded from the subsequent eye examination due to central retinal vein occlusion at 1 month, but the patient's general condition was observed until 6 months. In participant no. 1, the monitoring of changes in IL-6 and C-reactive protein (CRP) failed due to practical reasons. There were nine missing values out of the total 248 observed values of general condition during follow-up (Fig. 2; Table 1). There was no significant change in blood pressure or systemic diabetes control over the study period.

We were not able to expand enough cells as study design demand for each patient because of its rare proportion in bone marrow and only the third-passage cells expanded *in vitro* are used for transplantation. The average number of infusion-MSC is $2.84 \times 10^6/\text{kg}$ and there is no significance difference between NPDR and PDR groups (2.85 vs. $2.82 \times 10^6/\text{kg}$, $P = 0.742$) (Table 1).

For the analysis of BCVA, CMT, and CST, the eyes were divided into NPDR (19 eyes) and PDR groups (15 eyes). Both groups were not well-matched in terms of baseline characteristics (Table 1) due to differences in disease severity. Between the two groups, there were statistically significant differences in BCVA (66.94 ± 12.86 in the NPDR group and 48.93 ± 17.31 in the PDR group) and CST $\geq 250 \mu\text{m}$ ($352 \pm 122.58 \mu\text{m}$ in the NPDR group and $580.44 \pm 135.16 \mu\text{m}$ in the PDR group). However, there was no significant difference in CMT $\geq 300 \mu\text{m}$ between the two groups ($342.36 \pm 46.43 \mu\text{m}$ in the NPDR group and $388.87 \pm 68.62 \mu\text{m}$ in the PDR group).

Therapeutic efficacy

Metabolic Function and Immune Monitoring

After MSC infusion, FBG levels decreased significantly at 1 and 3 months (mixed-effects linear model, $P = 0.033$); by 21.4%, 23.4%, and 14.0% from $9.09 \pm 3.08 \text{ mmol/L}$ to $7.14 \pm 2.53 \text{ mmol/L}$, $6.96 \pm 2.70 \text{ mmol/L}$, and $7.82 \pm 3.01 \text{ mmol/L}$ at 1, 3, and 6 months, respectively ($P = 0.007$ at 1 month, $P = 0.013$ at 3 months, and $P = 0.154$ at 6 months; Fig. 3A). HbA_{1c} showed a decreasing trend during the follow-up period, but without statistical difference. Correspondingly, the level decreased by 5.6% from $6.95 (52 \text{ mmol/mol}) \pm 1.20\%$ to 6.56

Table 1. Patients' baseline characteristics. ^aexcluded from eye examination, but body examination data included. ^bright eye in severe non-proliferative stage while left eye in non-high risk proliferative stage. ^cleft eye accepted laser therapy while right eye did not. * missing value. BM-MSC = bone marrow mesenchymal stromal cell; CMT = central macular thickness; CRP = C-reactive protein; CST = central subfield thickness; FBG = fasting blood glucose, HbA_{1c} = hemoglobin A1c; IL-6 = interleukin-6; PDR = proliferative diabetic retinopathy; NPDR = non-proliferative diabetic retinopathy; T2D = type 2 diabetes

Patient No.	Sex	Age (years)	T2D duration (years)	BCVA (ETDRS letters) OD/OS	CMT (μm) OD/OS	CST (μm) OD/OS	HbA _{1c} (%) (mmol/mol)	FBG (mol/L)	hs-CRP (mg/L)	IL-6 (ng/L)	Infusion BM-MSC (×10 ⁶ /kg)	Laser therapy
1	M	58	2	82/69	311.2/318	240/339	9.2 (77)	4.58	*	*	2.21	NO
2	M	50	10	72/77	323.2/374.5	259/319	6.8 (51)	8.89	1.3	14.11	2.75	NO
3	M	67	2	68/80	203.7/235.9	228/241	5.7 (39)	5.29	0.65	5.67	2.89	NO
4	M	58	14	68/68	318/226.9	228/331	7.9 (63)	8.71	0.65	*	2.94	NO
5	F	37	4	64/64	325.3/296.4	262/257	7.8 (62)	7.56	5.25	5.75	2.98	NO
6 ^a	M	57	10	46/26	481/219.4	770/855	6.3 (45)	10.73	0.67	5.39	3	NO
7	F	56	3	40/36	360.4/322.7	667/409	8.6 (70)	10.87	1.2	6.18	3.01	NO
8	F	45	1	65/67	293.4/289.5	229/233	8.9 (74)	5.17	2	5.59	2.97	NO
9 ^b	M	51	2	73/59	310/345.5	312/560	6.1 (43)	7.53	4.1	3.35	2.87	YES/NO ^c
10	M	55	3	85/60	294.3/459.9	262/455	7.2 (55)	10.85	3.2	3.94	2.92	NO
11	M	60	8	25/68	320.3/291.9	210/240	6.3 (45)	15.51	10.6	*	2.52	YES
12	F	47	10	57/55	433.7/506.8	480/663	6.9 (52)	13.99	3	11.72	2.97	NO
13	M	64	10	68/71	273/266.3	353/245	6.4 (46)	8.89	0.66	4.87	2.77	YES
14	M	45	10	47/48	249.9/231.7	155/157	6.3 (45)	11.99	8.82	6.5	2.95	NO
15	M	50	15	16/18	451.9/421.1	764/748	7.2 (55)	9.52	3.09	5.82	2.78	YES
16	F	48	5	47/53	282.4/385	183/644	5 (31)	9	0.24	11.1	2.83	NO
17	F	51	10	59/43	314.3/321.2	496/516	5.5 (37)	5.42	0.62	6.09	2.89	YES
mean	11/6	52.88	7.15	58.5	323.71	365.16	6.95 (52)	9.09	2.89	6.86	2.84	4/13

(48 mmol/mol) $\pm$ 1.03% at the end point (Fig. 3B). Although there was a 119.3% increase in IL-6 serum levels from 6.86 $\pm$ 3.13 mg/L to 15.05 $\pm$ 16.59 mg/L at 3 months, the difference was not significant (Fig. 3C). Considerable reductions in CRP serum level after transplantation were observed at 1, 3, and 6 months (mixed-effects linear model, $P=0.018$) with 67% (0.949 $\pm$ 0.86 ng/L vs. 2.878 $\pm$ 3.06 ng/L, $P=0.004$), 59.40% (1.169 $\pm$ 1.58 ng/L vs. 2.878 $\pm$ 3.06 ng/L, $P=0.012$), and 55.40% (1.283 $\pm$ 0.92 ng/L vs. 2.878 $\pm$ 3.06 ng/L, $P=0.021$) reductions from baseline, respectively (Fig. 3D).

Visual Outcomes

The mean BCVA gains of all eyes showed a substantial increasing trend from baseline (repeated-measures ANOVA, $P=0.002$) with 1.22, 3.41, and 3.56 letters gained at 1, 3, and 6 months, respectively ($P=0.032$, 3 months vs. baseline, $P=0.018$, 6 months vs. baseline; Fig. 4A). In the NPDR group, the mean change from baseline was 2.53, 5.18, and 4.12 letters versus -0.27, 1.40, and 2.93 letters in the PDR group at 1, 3, and 6 months, respectively. Moreover, BCVA in the NPDR group increased significantly during the follow-up period (repeated-measures ANOVA, $P=0.001$), especially at 3 months ($P=0.006$) and 6 months ($P=0.027$), while there was no significant increase in the PDR group (Fig. 5A, Table 2).

The proportions of eyes that gained five letters or more from baseline showed a similar trend to the mean BCVA gain. For all eyes, the proportions were 25.00%, 46.88%, and 40.63% at 1, 3 and 6 months, respectively. In the NPDR group, the proportions were 23.53%, 58.82, and 35.29%, versus 26.67%, 33.33%, and 46.67% in the PDR group at 1, 3, and 6 months,

Fig. 3. Metabolic function and immune monitoring (n = 34 patients). A: FBG levels significantly decrease from baseline (mixed-effects linear model, $P=0.033$), and during follow-up ($P=0.007$, 1 month vs. baseline, $P=0.013$, 3 months vs. baseline). B: HbA_{1c} levels do not change over time compared with baseline. C: Serum levels of IL-6 do not significantly change after transplantation. D: Serum CRP levels significantly decrease from baseline (mixed-effects linear model, $P=0.018$), and during follow-up ($P=0.004$, 1 month vs. baseline; $P=0.012$, 3 months vs. baseline; $P=0.021$, 6 months vs. baseline). ** $P<0.01$, * $P<0.05$; CRP = C-reactive protein; FBG = fasting blood glucose; HbA_{1c} = hemoglobin A1c; IL-6 = interleukin-6.

respectively. When eyes that gained five letters or more and those that were maintained within five letters were combined, the proportions did not decrease; 84.38%, 90.63%, and 90.63% in all eyes, and 94.12%, 94.12%, and 100.00% in the NPDR group versus 73.33%, 86.67%, and 80.00% in the PDR group at 1, 3, and 6 months, respectively (Fig. 4B, Fig. 5, Table 2).

Macular Anatomic Outcomes

The proportions of eyes included in the analysis when OCT-measured macular edema was defined as CMT ≥ 300 μm and CST ≥ 250 μm were 59.38% (19/32) and 62.50% (20/32), respectively [26, 27]. Neither CMT nor CST changed significantly during the follow-up (Fig. 4C, D), but when the data was divided into the two groups (NPDR and PDR) and then analyzed, the results differed.

The proportions of eyes included in the analysis when the standard of CMT ≥ 300 μm was used were 60% (9/15) and 58.8% (10/17) in the PDR and NPDR groups, respectively

Fig. 4. Visual and anatomic macular outcomes (n = 32 eyes). A: Best-corrected visual acuity (BCVA) significantly increases from baseline (repeated-measures ANOVA, $P = 0.002$), and during follow-up ($P = 0.032$, 3 months vs. baseline, $P = 0.018$, 6 months vs. baseline). B: Respective proportion of eyes that gained ≥ 5 , stayed within ± 4 , and lost ≥ 5 Early Treatment Diabetic Retinopathy Study (ETDRS) letters in 1, 3, and 6 months, compared with baseline. C, D: Mean change in CMT and CST from baseline (repeated-measures ANOVA, $P > 0.05$). * $P < 0.05$ comparison between baseline and follow-up period. CMT = central macular thickness; CST = central subfield thickness; ETDRS = Early Treatment Diabetic Retinopathy Study.

Fig. 5. Visual and anatomic macular outcome from baseline to 6 months in the PDR and NPDR groups. A: Mean $\pm$ standard deviation change in best-corrected visual acuity (BCVA) from baseline to 6 months. In the NPDR group, it significantly increases from baseline (repeated-measures ANOVA, $P = 0.001$), and during follow-up ($P = 0.006$, 3 months vs. baseline, $P = 0.027$, 6 months vs. Baseline). It did not change in the PDR group over time compared with baseline. B: Respective proportion of eyes that gained ≥ 5 , stayed within ± 4 and lost ≥ 5 ETDRS letters at 1, 3, and 6 months compared with baseline. C, D: Mean change in CMT and CST from baseline to 6 months in the NPDR and PDR groups (repeated-measures ANOVA $P > 0.05$). * $P < 0.05$ and ** $P < 0.01$ comparison between baseline and the follow-up period. $\Delta P < 0.05$ comparison of changes from baseline and the baseline value between the two groups. CMT = central macular thickness; CST = central subfield thickness; ETDRS = Early Treatment Diabetic Retinopathy Study; PDR = proliferative diabetic retinopathy; NPDR = non-proliferative diabetic retinopathy.

(Fig 2C). Baseline values in the PDR group ranged from 314.3 to 506.8 μm with a mean of $388.9 \pm 68.6 \mu\text{m}$, and from 310.4 to 459.9 μm in the NPDR group with a mean of $342.4 \pm 46.4 \mu\text{m}$ ($P = 0.099$). After transplantation, the NPDR group achieved substantial CMT reductions with a 17.39 μm decrease at endpoint from baseline. However, the PDR group showed an 8.76- μm increase at endpoint from baseline. There was no significant difference in CMT change over time between groups, but at 3 months, CMT decreased significantly in the NPDR group compared with the PDR group (-10.96 vs. $21.24 \mu\text{m}$, $P = 0.033$) (Fig. 5C).

When $\text{CST} \geq 250 \mu\text{m}$ was used to define the central macular edema, 60% (9/15) and 64.7 (1/17) of eyes were included in analysis in the PDR and NPDR groups (Fig 2C), respectively (baseline 580.4 ± 135.2 vs. 352 ± 122.6 , $P = 0.001$). Similar to the change in CMT, there was a decreasing trend in CST in the NPDR group, with the maximum change (36.3 μm ; from $352 \pm 122.6 \mu\text{m}$ to $315.7 \pm 65.3 \mu\text{m}$) recorded at 3 months. In the PDR group, CST increased from $580.4 \pm 135.2 \mu\text{m}$ to $610 \pm 133 \mu\text{m}$ at 6 months. However, there were no significant differences between the two groups (Fig. 5D).

Both CMT and CST in the eyes not defined as OCT-measured macular edema remained stable during the follow-up.

Safety

There were no acute infusion-related reactions including fever, nausea and vomiting, chills, and headaches observed during or after ABMSC injection in all 17 recipients. No severe adverse events, such as malignant tumors and immunological rejection, were observed during the follow-up period. Renal, liver function, and myocardial enzyme tests were used to assess the impact of ABMSCs on the body's vital organs. Patient no. 17 showed an increase in creatine kinase (CK) with time after transplantation, but showed no other dysfunctions during the follow-up. All other patients showed steady fluctuations within the normal range or slightly higher than the upper limit in the blood biochemical index. Moreover, patients no. 9 and 14 had abnormal CK and creatinine levels, respectively, which continuously decreased after infusion (Tables 3 & 4).

Table 2. Change in visual acuity throughout follow-up for eyes with NPDR and PDR. NPDR = non-proliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy

	Baseline	NPDR (n = 17)			Baseline	PDR (n = 15)		
		1 m	3 m	6 m		1 m	3 m	6 m
Median (IQR)	68 (64–73)	74 (67–77)	75 (70–78)	74 (66–79)	53 (45–59)	50 (42.5–62.5)	52 (41–65)	55 (41.5–66.5)
Mean (SD)	66.9 (12.9)	69.5 (14.3)	72.1 (13.3)	71.1 (14.0)	8.9 (17.3)	48.7 (18.3)	50.3 (18.0)	51.9 (17.3)
Change in visual acuity from baseline								
≥ 5 -letter gain, NO (%)	-	23.53%	58.82%	35.29%	-	26.67%	33.33%	46.67%
Within ± 4 letters, NO (%)	-	70.59%	35.29%	64.71%	-	46.67%	53.33%	33.33%
≥ 5 -letter loss, NO (%)	-	5.88%	5.88%	0%	-	26.67%	13.33%	20.00%

Table 3. Monitoring of liver function. * data within the normal range. ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase.

Patient No.	ALT (0–42 IU/L)				AST (0–42 IU/L)				ALP (34–114 IU/L)			
	Baseline	1 month	3 months	6 months	Baseline	1 month	3 months	6 months	Baseline	1 month	3 months	6 months
1	*	*	*	*	*	*	*	*	*	*	*	*
2	*	*	*	*	46	46	44	*	*	130	130	*
3	*	*	*	*	*	*	*	*	*	*	*	*
4	*	*	*	*	*	*	*	*	*	*	116	*
5	*	*	*	*	*	*	*	*	*	*	*	*
6	*	*	*	*	*	*	*	*	122	161	154	143
7	*	*	*	*	*	*	*	*	*	*	*	*
8	*	*	*	*	*	*	*	*	*	*	*	*
9	45	47	*	53.6	*	*	*	90.6	*	*	*	*
10	*	49	*	*	*	*	*	*	*	*	*	*
11	*	*	*	*	*	*	*	*	*	*	*	*
12	*	*	*	*	*	*	*	*	*	*	*	*
13	*	*	*	*	*	*	*	*	*	*	*	*
14	43	*	*	48	*	*	*	*	*	*	*	*
15	*	*	*	*	*	*	*	*	*	*	*	*
16	*	*	*	*	*	*	45	*	118	*	173	*
17	*	*	*	*	*	*	*	*	*	*	*	*

Table 4. Monitoring of renal function and myocardial enzymes. * data within the normal range. CK = creatine kinase; Cr = creatinine; LDH = lactate dehydrogenase.

Patient No.	LDH (114–240 IU/L)				CK (0–190 IU/L)				Cr (40–97 μ mol/L)			
	Baseline	1 month	3 months	6 months	Baseline	1 month	3 months	6 months	Baseline	1 month	3 months	6 months
1	*	*	*	*	*	*	*	*	*	*	99	103
2	289.7	*	259.3	*	203	*	*	*	*	*	*	*
3	*	*	*	*	*	*	*	*	*	*	*	*
4	*	*	*	*	*	*	*	*	*	*	*	*
5	*	*	*	*	*	*	*	*	*	*	*	41
6	*	*	294.6	281.5	*	*	*	193	*	*	*	117
7	*	292.7	293.6	295.1	175	290.2	247.2	263.1	43	*	*	*
8	*	*	*	*	*	*	*	*	*	*	41	*
9	*	*	*	274.5	599.4	265.5	315.6	215	*	*	*	48
10	246.2	*	*	245.2	130.2	*	*	*	130.2	140	170	154.6
11	*	*	*	*	*	*	*	*	100	107	*	101
12	*	241	*	*	*	202.7	*	*	*	*	*	*
13	*	*	*	*	*	*	*	*	*	*	*	107
14	*	*	*	*	*	*	*	*	148	150	138	115
15	*	*	*	*	*	*	*	*	*	*	*	*
16	299.8	249.5	258.6	255.3	*	*	*	*	*	*	*	*
17	*	*	*	*	196.1	236.6	337.9	429.7	*	*	*	*

Discussion

Effective diabetes management, and repair and regeneration of retinal vessels and neuron cells are two indispensable therapeutic strategies for DR. Most DR clinical trials conducted to date have focused on improving local circulation and metabolism, while ignoring the improvement in general condition [8, 26, 28]. Moreover, previous trials investigating MSC for patients with DR did not report retinal changes, although most showed an improvement in glucose metabolism [16, 29, 30]. Herein, we describe the outcomes of ABMSC transplantation in patients with DR, including changes in glucose metabolism, immune function, visual function, and retina microcirculation.

We enrolled 17 participants (34 eyes) with either non-high-risk PDR or severe NPDR. In contrast with mild and moderate NPDR, both non-high-risk PDR and severe NPDR stages show obvious signs in the retina, which are conducive to observe the progress and outcome of the disease after transplantation. Although intraocular preventions are necessary for these two stages, re-examination is acceptable within 2 to 4 months without treatment, while at the high-risk PDR stage, panretinal photocoagulation is recommended to reduce the risk of severe visual loss [31]. Therefore, we believed that patients in either stage of DR are suitable for this study; moreover, the differences in treatment effectiveness between the two stages could be observed.

MSCs can sense and control inflammation when in an inflammatory environment; the cells constitutively produce IL-6 that induces the differentiation of M0 macrophages towards an anti-inflammatory cell type that shares properties with M2 macrophages [32]. Therefore, we selected IL-6 to assess the function of MSCs after transplantation. In support of previous research [30], IL-6 levels increased with time, accompanied by CRP reductions. CRP is a nonspecific sensitive index reflecting the state of inflammation; its level is positively correlated with the development of DR [33]. With improvements in inflammation, recovery of β -cell function may be achieved [34]. This may explain why improvements in glucose metabolism were observed in the present study.

More useful information regarding functional and anatomic retinal outcomes can be obtained when the data are divided into PDR and NPDR groups. When the data were analyzed together, there was no obvious change in CMT and CST, only an improvement in visual acuity after transplantation. When these data were analyzed separately, macular thickness reductions and significant BCVA gains were observed in the NPDR group, while only slight BCVA gains and macular thickening were recorded in then PDR group. Therefore, it appears that eyes with NPDR have better outcomes than those with PDR after MSC transplantation.

Inflammation can be detected in experimental models of DR [35, 36] and in the aqueous humor of patients with diabetic macular edema [37]; pro-inflammatory cytokines associated with vascular damage and neuronal cell loss may contribute to the pathogenesis

of DR. Due to vascular damage, increased retinal vascular permeability occurring at any stage of retinopathy may result in retinal thickening (edema) [31]. Some studies suggest that damaged tissue with inflammation can specifically attract mesenchymal stem cells [38]. Regarding chronic low-grade retinal inflammation [28] and the presence of the blood retinal barrier, we believe that MSCs are attracted less towards the retina than other parts of body, such as the pancreas. Therefore, it is not possible for them to differentiate into vascular and neuronal cells in order to repair the damaged ones with insufficient cell numbers; this may explain why there was no improvement in vascular leakage in the FFA (fundus fluorescein angiography) test in the present study (data not shown). Although cell differentiation did not appear to be successful in our study, we demonstrated that ABMSCs are anti-inflammatory due to macular thickness reductions controlling inflammation. Regarding the improvements in BCVA, as well as electrophysiological function (data not shown), MSC-derived neurotrophic factor [13] and control of inflammation may be the main explanations. The variations in baseline BCVA and macular thickness could be the major cause for the differences in outcomes between the two groups. In addition, the fact that 57% of PDR patients had undergone destructive laser therapy may also be an attributable factor; the same amount of infusion-cells could improve visual acuity and decrease macular edema to a greater extent in the NPDR group. Therefore, the severe NPDR stage and forward stages may be the best therapeutic window for cell transplantation.

Although several studies obtained impaired stem cells from individuals with diabetes [3, 39], we successfully harvested sufficient cells for transplantation and demonstrated their effectiveness. Therefore, we believe that ABMSCs derived from patients with diabetes can be suitable seed cells for DR.

The gradual loss of infusion-cells and re-damage by hyperglycemia comprised the turning point 3 months after transplantation in some indexes. This may explain why HbA_{1c} levels, used to assess average levels of glycaemia over the previous 3 months [40], did not change substantially. Therefore, infusion times, cell concentration, and the use of modified MSCs [38] may help to improve the efficiency of the transplantation. In the present study, we chose intravenous infusion for safety; however, this can result in the loss of a large amount of cells due to the pulmonary first-pass effect [41]. Therefore, local arterial catheterization injection when the retinal and pancreatic inflammation level is relatively high may be a better choice.

A major limitation of this pilot study was the lack of a placebo group, which may generate bias in the outcome measurements. For practical reasons, it was difficult to design a control cohort of patients with severe NPDR and non-high-risk PDR that developed quickly in a short time and that may have required laser therapy or anti-VEGF therapy at any point [10]. Other limitations include the relatively small sample size and the short duration of follow-up. Long-term safety assessments are required in cases of MSC tumorigenesis. Inflammatory cytokines and chemokines from the aqueous humor of patients should be investigated in further clinical trials in order to assess changes in intraocular inflammation.

To the best of our knowledge, the present study is the first clinical trial to evaluate the efficacy and safety of ABMSCs harvested *in vitro* for the treatment of patients with DR by intravenous infusion. We successfully harvested sufficient cells for transplantation and demonstrated their effectiveness. The severe non-proliferative stage appears to be a better therapeutic window than the non-high-risk proliferative stage. Stem cell transplantation is a promising therapy for diabetes and its complications. We believe that optimization of the transplantation strategy, such as appropriate selection of therapeutic window and infusion times, may enhance the therapeutic effects of ABMSC or other stem cells in future large-scale trials. The safety of the treatment was demonstrated through observations of a number of indicators over a 6-month follow-up.

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