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Umbilical cord-derived mesenchymal stem cell implantation in patients with optic atrophy

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Abstract

Background: Optic nerve cells can be irreversibly damaged by common various causes. Unfortunately optic nerve and retinal ganglion cells have no regenerative capacity and undergo apoptosis in case of damage. In this study, our aim is to investigate the safety and efficacy of suprachoroidal umbilical cord-derived MSCs (UC-MSCs) implantation in patients with optic atrophy.

Methods: This study enrolled 29 eyes of 23 patients with optic atrophy who were followed in the ophthalmology department of our hospital. BCVA, anterior segment, fundus examination, color photography, and optical coherence tomography (OCT) were carried out at each visit. Fundus fluorescein angiography and visual field examination were performed at the end of the first, third, sixth months, and 1 year follow-up.

Results: After suprachoroidal UC-MSCs implantation there were statistically significant improvements in BCVA and VF results during 12 months follow-up ($p < 0.05$). When we evaluate the results of VF tests, the mean deviation (MD) value at baseline was -26.11 ± 8.36 (range -14.18 to -34.41). At the end of the first year it improved to -25.01 ± 8.73 (range -12.56 to -34.41) which was statistically significant ($p < 0.05$). When we evaluate the mean RNFL thickness measurements at baseline and at 12 month follow-up the results were $81.8 \pm 24.9\,\mu m$ and $76.6 \pm 22.6\,\mu m$, respectively. There was not a significant difference between the mean values ($p > 0.05$).

Conclusion: Stem cell treatment with suprachoroidal implantation of UCMSCs seems to be safe and effective in the treatment for optic nerve diseases that currently have no curative treatment options

Keywords

Optic neuropathy, neuro ophthalmology, optic neuritis, open angle glaucoma, glaucoma, orbital trauma, orbital disease, neuro-ophthalmic disease, pediatric ophthalmology

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Introduction

Optic neuropathy is a general definition used for many disorders that can lead to optic nerve damage which usually manifests as variable degrees of visual dysfunction, visual field (VF) defect, dyschromatopsia, abnormal papillary response and a gray or pale optic disc. Optic nerve fibers consist of ganglion cell axons located in the inner layer of the retina. Retinal ganglion cell damage occurs in the case of optic neuropathy by retrograde degeneration.[1,2]

Optic nerve cells can be irreversibly damaged by common various causes such as glaucoma, ischemia, trauma, infection or inflammation. Compressive, toxic, nutritional causes and hereditary neurodegenerative disorders can also be responsible for optic neuropathy. Although the clinical course of the patients may show some differences from each other, the end point is retinal ganglion cell damage and optic atrophy. Unfortunately optic nerve and retinal ganglion cells have no regenerative capacity and undergo apoptosis in case of damage.[3,4]

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Researchers are investigating various strategies to stimulate neuronal survival and axonal outgrowth. Stem cell therapies are promising and have great potential for the treatment of untreatable diseases including optic nerve diseases. Experimental studies showed neuroprotective and regenerative properties of stem cells in optic nerve pathologies.[5]

Mesenchymal stem cells (MSCs) are thought to be a good alternative for regenerative therapy of optic nerve diseases. These cells are capable of differentiating into other cell types. They also have paracrine effects by secreting neurotrophic factors which can induce new functional neural synaptic connectons.[6,7]

Adult tissues like blood, skin, dental pulp, bone marrow, adipose tissue, human placenta, and umbilical cord-derived tissues can be used as a source of MSCs. Umbilical cord is widely used as a rich and ethically acceptable source of stem cells and is known to have some advantages like having a higher proliferative potential and quality than the other sources of MSCs.[8–10]

In this study, our aim was to investigate the safety and efficacy of suprachoroidal umbilical cord-derived MSC (UC-MSC) implantation in patients with optic atrophy. To the best of our knowledge, this is the first study in the literature using UC-MSCs as a source to treat optic nerve diseases.

Methods

Study design and setting

This prospective, interventional, single center, clinical study enrolled 29 eyes of 23 patients with optic atrophy who were followed in the ophthalmology department of our hospital. Aims of this study were to evaluate the safety and efficacy of stem cell implantation. The patients were recruited to the study during 2018; operated and followed-up between 01.01.2019 and 01.04.2020. The study was performed in accordance with the Declaration of Helsinki, after obtaining the approval of the Ethics Committee of the University (Number: 2017/480, Date: 13.10.2017) and the approval by the Review Board of Stem Cell Applications of the Ministry of Health (Registration number: 56733164/203) according to the regulations in our country. Written informed consent was obtained from all participants of the study.

Patients

Patients with at least 1 year follow-up with a diagnosis of optic atrophy due to various causes were evaluated for stem cell therapy. All patients had no visual acuity and VF improvement during the follow up period before recruiting the study and considered as stable or progressive. The patients were investigated for eligibility according to the inclusion and exclusion criteria.

The inclusion criteria of the study were:

*18 years and older age

*Clinical diagnosis of optic atrophy confirmed by ancillary tests

*Having best corrected central visual acuity (BCVA) of $<20/50$

*Having abnormal VF (MD value worse than −10.0)

*Receiving medical therapy for an optic nerve disease and being stable or progressive on that treatment

The exclusion criteria of the study were:

*Having previous ocular surgery other than cataract extraction

*Presence of ocular media opacities that would affect ocular imaging or VF evaluation,

*Having coexisting ocular disease

*Having systemic or neurological disease that would affect the results

*Having the habit of smoking

*Using toxic drugs to the optic nerve

A single vitreoretinal surgeon (A.O.) performed all surgical procedures and ophthalmic evaluations. Visual acuity was recorded with a Snellen chart at a distance of 3 m. Optical coherence tomography (OCT) and retinal nerve fiber analysis (RNFL) was performed using the Optovue (Optovue Inc USA). VF examination was performed by Humphrey visual field analyzer device (Carl Zeiss Meditec AG Germany). Program 30–2 of the Humphrey Field Analyser (Carl Zeiss Meditec, Jena, Germany) was used for VF testing of each eye.

The stem cell preparation

Umbilical cord was disinfected and cut into pieces of 1 to 2 mm^2. The pieces were transferred to 75 cm^2 culture flasks in Dulbecco's Modified Eagle's Medium-low glucose (DMEM-LG) containing a concentration of 10% Human Serum (HS) and of 1% Penicillin/Streptomycin, and it was cultured at 37°C with 5% density of CO_2. Culture medium was changed with fresh medium once every 3 days and waited for 70% confluency. Culture-expanded cells at the third passage were examined for surface protein expression by using flow cytometry.

The UC-MSCs were positive for CD-73, CD-90, and CD-105, and negative for CD-34, CD-45, and HLA-DR. No evidence of bacterial or fungal contamination was observed in the cells which were tested before releasing.

Cell viability evaluated by Trypan Blue dye exclusion test, and it was >90.0% ± 0.5 before cell transplantation. A concentration of 5×10^6 cells in isotonic solution containing 1% human serum albumin were transferred in vials with the temperature controlled bag within 12 h, and the product was used within 24 h.

Surgical technique

All operations were carried out with local anesthesia. We performed a surgical technique defined as the Limoli retinal restoration technique (LRRT), described by Limoli et al.[11] The technique was also used by our group in our previous studies.[12,13] Each eye received 5 million UC-MSCs during the surgery.

Postoperative follow-up

Patients were hospitalized for 1 day after the surgery and topical antibiotic and steroid drops were administered four times a day for 1 month after surgery. Ophthalmic evaluations, including BCVA, anterior and posterior segment examinations, color fundus photographs, OCT VF, and RNFL analyses were performed at first, third, sixth months, and first year postoperatively. Fundus fluorescein angiography (FFA) was done before and 1 year after the treatment.

Statistical analysis

Statistical analyses were conducted using SPSS version 20 statistical package program. Descriptive data are presented as the arithmetic mean ± SD and median (Min − Max) for non-normally distributed numerical variables, and as the frequencies and percentage for categorical variables. Quantitative normally distributed data was tested with one-way analysis of ANOVA test (posthoc Scheffe). $p < 0.05$ was considered as statistically significant.

Results

Twenty nine eyes of 23 optic atrophy patients completed 1 year follow-up period. The mean age of the subjects was 41.8 ± 18.2 (range 19–82 years). Thirteen of them were male and 10 were female. While the operations were performed in both eyes of six patients, 11 patients received the therapy to the right and six patients to the left eye. Average age of clinical onset of the disease was 31.0 ± 18.7 years and the mean disease duration was 11.1 ± 7.9 years. Systemic and neurological examinations including MRI of the patients were done before treatment which revealed no contraindication for MSC implantation.

Six patients with intracranial hypertension, five patients with glaucoma, three patients with traumatic optic neuropathy, three patients with comprehensive optic atrophy due to previous intracranial mass, two patients with methyl alcohol intoxication, two patients with diabetic optic atrophy, one patient with previous central retinal vein occlusion, and one patient with anterior ischemic optic neuropathy were included to the study. The demographics of the patients were seen in Table 1.

After suprachoroidal UC-MSCs implantation there were statistically significant improvements in BCVA and VF results during 12 months follow-up ($p < 0.05$). The mean BCVA at baseline was 0.10 ± 0.21 (range 0.001–0.90) Snellen lines which improved to 0.15 ± 0.29 (range 0.001–1.00) Snellen lines at the end of the first year. The difference was statistically significant ($p < 0.05$). When we evaluate the results of VF tests, the mean deviation (MD) value at baseline was −26.11 ± 8.36 (range −14.18 to −34.41). At the end of the first year it improved to −25.01 ± 8.73 (range −12.56 to −34.41) which was statistically significant ($p < 0.05$). When we evaluate the mean RNFL thickness measurements at baseline and at 12 month follow-up the results were 81.8 ± 24.9 µm and 76.6 ± 22.6 µm, respectively. There was not a significant difference between the mean values ($p > 0.05$). The results of the test were shown in Tables 1 and 2. Figures 1 to 6 showed VF improvement and RNFL changes of patients.

All patients who underwent stem cell treatment revealed no signs of fluid collection, edema, persistent leakage on FFA. We found no morphological changes in OCT scans of the both eyes of the patients. The mean RNFL thickness measurements of the all treated eyes did not show any significant changes after treatment. There were no serious ocular adverse events during 1 year follow-up period.

Discussion

Multiple diseases like glaucoma, demyelinating optic neuritis, ischemic optic neuropathy, and hereditary optic neuropathy can damage retinal ganglion cell axons and optic nerve fibers and as a result irreversible death of these cells causes optic atrophy. Neurotrophic factors like basic fibroblast growth factor (bFGF), neural growth factor (NGF), ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF) play an important role for neuroprotection. Because of their ability to inhibit the apoptotic cascade, neurotrophic factors may represent a promising therapeutic strategy in degenerative diseases.[14,15]

The main mechanism of action of MSCs in degenerative diseases is to regulate the microenvironment with paracrine effect by secreting growth factor. They can be used as a source of trophic factors, promote survival of cells and activate intrinsic repair mechanisms.[16] An experimental study showed that MSCs express trophic factors such as VEGF, BDNF, and NGF.[17] Due to these properties of MSCs, they found a place in several preclinical studies of retinal and optic nerve diseases, which confirmed the neuroprotective and reparative effects.[18,19]

Table 1. Detailed information about the demographics, preoperative, and postoperative test values of the patients.

Patient no.	Eye	Sex	Diagnosis	Age	Onset age	Duration	VA-pre	RNFL-pre	VF-MD-pre	VA-post	RNFL POST	VF-MD-post
1	R	M	Glaucoma	19	5	15	0.001	84	34.41	0.001	85	34.41
2	R	M	Glaucoma	29	5	24	0.01	62	30.83	0.01	63	17.58
3	R	M	ICM	38	25	13	0.01	57	25.54	0.1	60	19.64
4	R	F	Trauma	28	8	20	0.01	54	32.93	0.01	57	32.96
5	L	M	ICH	42	38	4	0.1	64	28.67	0.2	57	28.84
6	L	M	ICM	50	44	6	0.01	63	32.42	0.01	59	28.74
7	R	M	MAI	63	60	3	0.01	110	24.4	0.05	115	23.1
8	L	M	DOA	52	45		0.01	95	26.5	0.03	97	22.9
9	R	F	MAI	34	32	2	0.01	67	27	0.01	75	24.9
10	R	F	Trauma	31	25	7	0.01	75	28.3	0.01	80	27.6
11	L	F	ICH	36	30	7	0.01	80	26.5	0.01	85	26.4
12	L	M	CRVO	53	37	16	0.001	100	29.74	0.05	78	29.93
13	R	M	Trauma	45	41	4	0.001	105	30.01	0.001	70	28.2
14	R	F	ICH	20	7	13	0.4	58	28.39	0.9	64	27.96
15	R	M	Glaucoma	82	70	12	0.002	106	30.58	0.002	100	30.6
16	L	M	ICM	45	42	3	0.9	100	29.58	1	100	31.27
17	R	M	Glaucoma	57	40	17	0.01	100	33.5	0.02	100	33.1
18	R	F	ICH	19	7	12	0.15	74	34.05	0.15	78	34.19
	L						0.01	86	34.33	0.05	66	34.17
19	R	F	ICH	21	13	8	0.05	98	19.42	0.05	99	19.05
	L						0.05	98	19.42	0.05	99	19.05
20	R	F	AION	64	60	4	0.1	68	28.61	0.1	62	27.26
	L						0.7	79	17.87	1	70	16.83
21	R	F	DOA	66	35	31	0.15	84	27.57	0.15	95	29.28
	L						0.01	122	30.13	0.01	75	31.67
22	R	M	ICH	41	37	3	0.01	107	14.63	0.01	101	12.56
	L						0.01	106	14.18	0.01	101	13.18
23	R	F	Glaucoma	70	45	25	0.01	75	31.41	0.01	57	31.35
	L						0.05	63	31.34	0.05	71	31.15

R: right eye; L: left eye; M: male; F: female; ICM: intracranial mass compressive optic neuropathy; ICH: intracranial hypertension (idiopathic); MAI: methyl alcohol intoxication; CRVO: central retinal vein occlusion; DOA: diabetic optic atrophy; AION: anterior ischemic optic neuropathy; VA: visual acuity (Snellen line); RNFL: retina nerve fiber layer (micron); VF-MD: visual field mean deviation.

Table 2. Results of the patients, before (preop) and 1 year after the treatment (postop).

Variable	*n*	Preop		Postop		*p*
		Mean ± SD	Median (min –max)	Mean ± SD	Median (min –max)	
Visual acuity (Snellen line)	29	0.10 ± 0.21	0.01 (0.001–0.90)	0.15 ± 0.29	0.03 (0.001–1.00)	0.002
RNFL thickness (micron)	29	81.8 ± 24.9	80.5 (54.0–122.0)	76.6 ± 22.6	78.0 (57.0–115.0)	0.346
VF (mean deviation)	29	–26.11 ± 8.36	–28.61 (14.18–34.41)	–25.01 ± 8.73	–27.96 (12.56–34.41)	0.035

RNFL: retinal nerve fiber layer; VF: visual field.

MSCs are considered as immunoprivileged since they do not express Major Histocompatibility Complex (MHC) on cell surfaces. This advantage allows autologous or allogenic use without risk of rejection. After the transplantation, patients do not need to get any immunosuppresive treatment for preventing rejection.[20,21] Furthermore, MSCs can diminish T cell proliferation, secret various immunomodulatory mediators and these properties are thought to be beneficial in treating neuroinflammatory disorders.[22]

Previous experimental studies have reported successful results with different types of stem cells. Bone marrow, adipose tissue, dental pulp, and umbilical cord have been used in animal models of glaucoma and optic nerve injury.[23–26] These studies showed that stem cells increase the regeneration of axons, re-establish neural connections and enhance the survival of RGCs with expression of growth factors.[27–31]

These cells have high proliferation capacity and their effects are long lasting. In a rat model of glaucoma, Yu

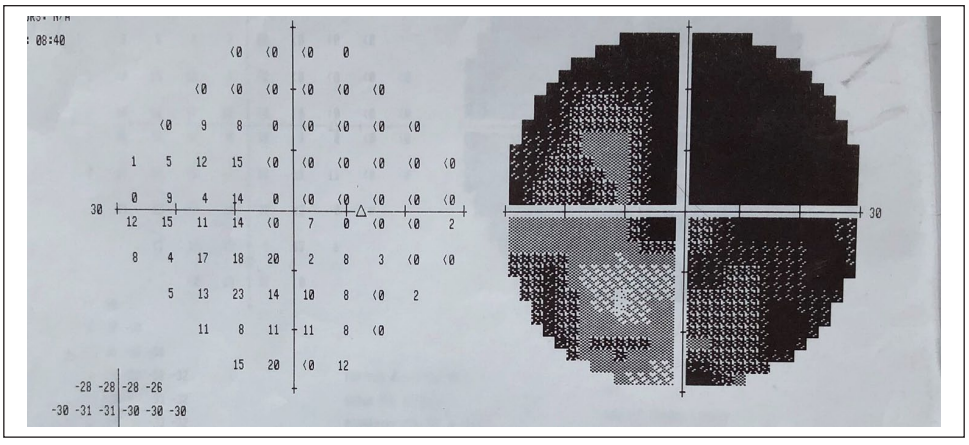

Figure 1. Visual field of a patient: before treatment.

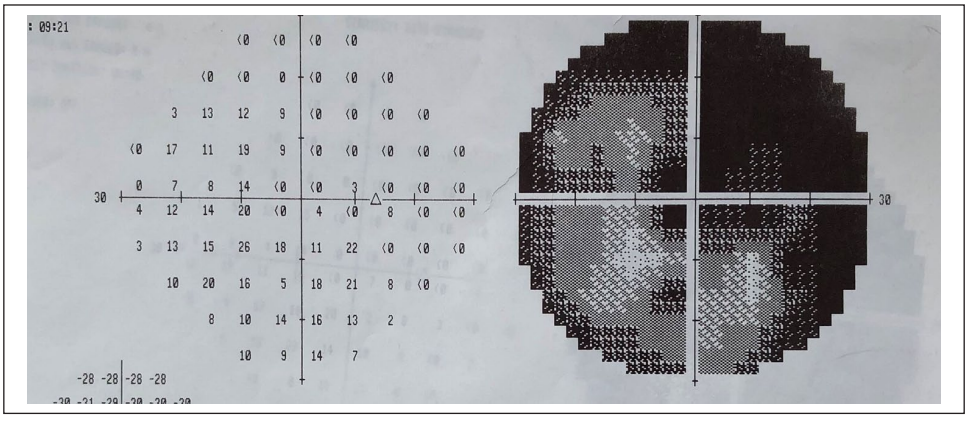

Figure 2. Visual field of a patient: 6 months after the treatment.

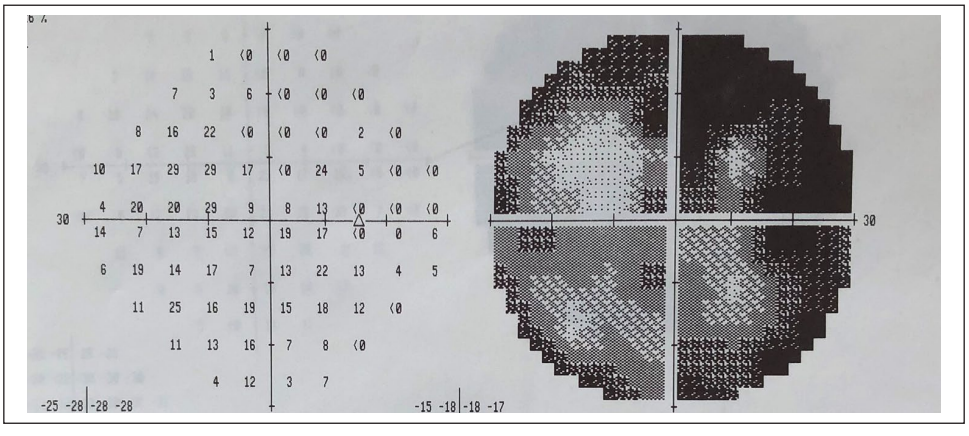

Figure 3. Visual field of a patient: 1 year after the treatment.

et al.[24] injected BM-MSCs into vitreous body and the cells were observed in the vitreous cavity of the eye during the first month after transplantation. A similar experimental optic nerve injury study with BM-MSCs showed that the cells were found inside the eye for up to 18 weeks.[32] In a rat study the researchers confirmed that these cells are not cleared from the tissue.[33] Although all types of stem cells are shown to be effective, it is not clear whether there is a difference between the effectiveness of these cell groups.[34,35]

In this clinical study we used umbilical cord tissue as a stem cell source which was not used in previous clinical

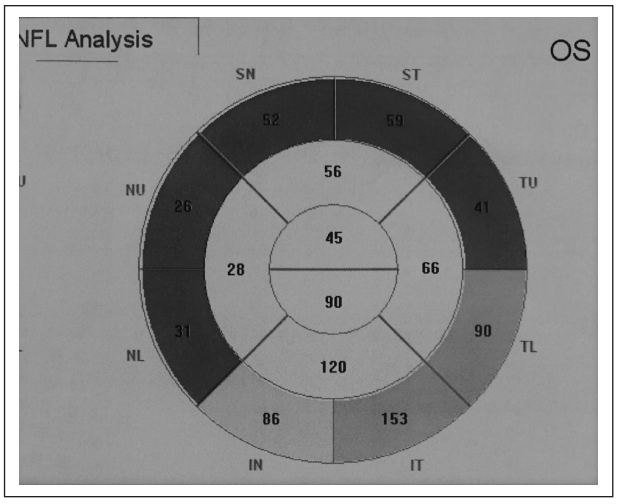

Figure 4. Retina nerve fiber thickness of a patient: before the treatment.

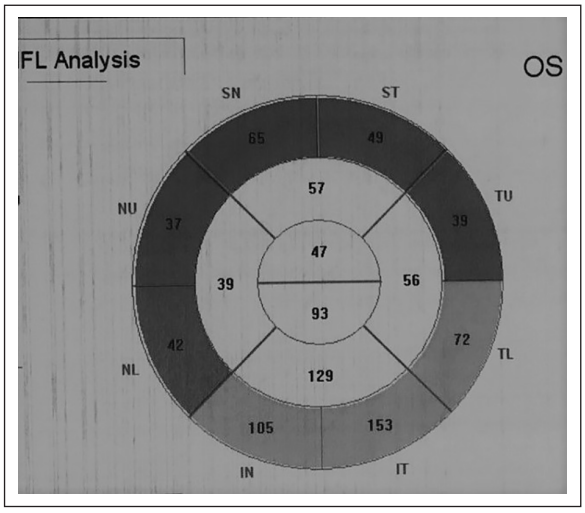

Figure 5. Retina nerve fiber thickness of a patient: 6 months after the treatment.

studies. These cells have advantages like higher proliferation potential, a larger portion of mesenchymal progenitor cells, more stable doubling time, faster self-renewal ability, lower immunogenicity and more primitive properties than other adult tissue derived MSCs. The collection of these cells is noninvasive and patients can be protected against any intervention or infection.[36,37]

Our results confirmed that UC-MSCs were safe in the treatment of optic atrophy during 12 month follow-up period. We found no morphological pathology like choroidal neovascular membrane, retinal edema, neovascularization, and tumorogenic mass in OCT and FFA scans of the both eyes of the patients. Suprachoroidal surgical technique was also proven to be safe in previous studies of our group.[12,13]

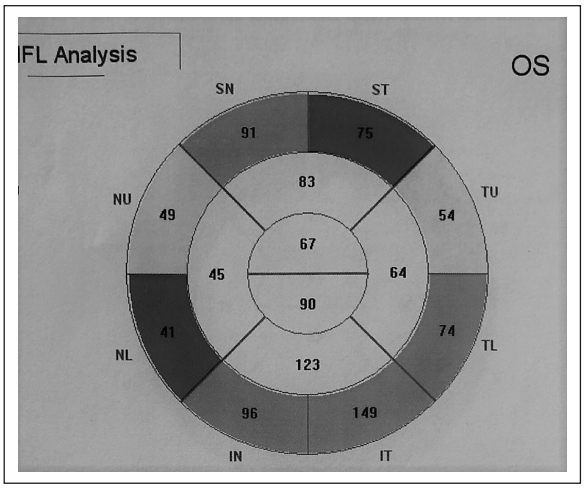

Figure 6. Retina nerve fiber thickness of a patient: 1 year after the treatment.

It is well accepted that the optical structural lesions are usually correlated with the functional damage. Previous studies mentioned improvements of RNFL in some of the cases after stem cell applications.[38,39] In this study, although we found improvements in RNFL thickness measurements of most of the cases the mean value did not show any significance. We believe that our result may be due to the heterogeneity in the severity and the etiology of the disease in included patients. To clarify the effect of the stem cells to the relationship between structure and function of the optic nerve, more data from future studies are needed.

In a previous clinical trial known as Stem Cell Ophthalmology Treatment Study (SCOTS), the results of various types of delivery techniques have been reported. In this study the researchers performed retrobulbar, subtenon, intravitreal and into the optic nerve injections for MSC transplantation. They reported no differences between these techniques.[38–40] It is important to address risks and safety issues as well as standardization and optimization of the delivery techniques. Subretinal implantation method is reported to have some ocular complications including choroidal neovascular membrane (CNV), epiretinal membrane (ERM), and retinal detachment.[41–44] Suprachoroidal, subtenon, and retrobulber approaches have the advantage of delivering the cells without surgical complications as this techniques include no removal of vitreous and no intervention to retinal tissues.[13,38–40]

SCOTS group reported results of two cases, one with autoimmune optic neuritis and the other with idiopathic bilateral optic neuritis. They observed no serious complications during the follow-up period.[38,40] Another recent study of SCOTS including 10 patients with bilateral visual loss due to non-arteritic ischemic optic neuropathy (NAION) reported that 80% of patients experienced visual acuity improvements and 20% remained stable. They also

reported that the duration of visual loss did not affect the response to the treatment.[39]

A previous case report of our group enrolled four eyes of four patients with diabetic optic atrophy, methyl alcohol intoxication, and intracranial hypertension. Suprachoroidal implantation of adipose tissue derived MSCs was performed and no systemic or ocular complications were observed in this study. All of the patients experienced improvements in visual acuity, VF, and electrophysiological recordings. There was also a thickening of the choroid in OCT of the four patients.[12]

This clinical study included 29 eyes of 23 patients. To the best of our knowledge this is the largest study including stem cell implantation in optic neuropathies to date. Considering the late stage spectrum of the patients in our study, regarding to the BCVA and VF, our results could not represent the potential response that would be received from the patients in early-moderate stages of the disease. The authors are also aware that a longer follow-up period is needed to understand the behavior of UC-MSCs implanted in suprachoroidal space and confirm the results of the study. Even with these facts and despite the heterogeneous etiology of the recruited subjects, the results of our study seems to be promising with no serious complications and give hope for future studies including patients with the early phase of their diseases.

Authors' note

The manuscript has not been published elsewhere previously and it is not under consideration in any other journal.

Author contributions

All authors have contributed significantly and are in agreement with the content of the manuscript.

Declaration of conflicting interests

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