ORIGINAL ARTICLE

Subtenon implantation of wharton's jelly-derived mesenchymal stromal cells in retinitis pigmentosa

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ABSTRACT

Introduction: Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous group of hereditary disorders in which there is progressive loss of photoreceptors and pigment epithelial function culminating in complete vision loss. Unfortunately, given the disease's devastating effects, it is untreatable and there is often little that can be done to improve visual outcomes in these patients. The lack of curative intervention creates a challenge in the management of RP and its progression. As such, the main goal is to slow down the apoptosis and loss of photoreceptors in order to delay visual deterioration.

Materials and Methods: We present two case illustrations of RP treated with WJ-MSC implanted in the deep subtenon space. Each patient underwent 4 sessions ranging from 1 to 3 months apart.

Results: At 3, 6, 9 and 12 months follow-up, the following were observed:(i) Both patients had no change in visual acuity and no further deterioration in vision or visual field.(ii) Optical coherence tomography showed a layer of hyperreflective material noted at the IO/OS junction area suggestive of a layer of new photoreceptors. The changes were noted in the macula and extramacular region for both patients.(iii) Both patients reported better discernment of colors and better vision at certain times during the day. (iv) No systemic or ocular adverse events were observed in the 12 month follow-up following the subtenon implantation of WJ-MSC.

Conclusion: Subtenon implantation of WJ-MSC appears to be a feasible and safe option to consider in delaying the progression of retinal degeneration and improving the quality of life affected by visual deterioration in patients with RP.

KEYWORDS:
Mesenchymal Stem Cells, Retinitis Pigmentosa, Subtenon implantation

INTRODUCTION

Retinitis pigmentosa (RP) is a group of hereditary degenerative disorder associated with retinal dystrophy of photoreceptors and an important cause of severe vision impairment. The degeneration of photoreceptors in RP is usually associated with gene mutations which maybe inherited as autosomal recessive (50–60%), autosomal dominant (30–40 %), or X-linked recessive. It has a very variable clinical course, and in the initial stages, the disease involves the destruction of the rod photoreceptors causing loss of night vision and progressive peripheral visual field loss, leading to tunnel vision. Further progression to later stages results in degeneration of cones leading to loss of central and colour vision followed by blindness at age 40–50 years.[1,2]

Part of the difficulty in treating RP is its complex and diverse genetic aetiology. While there are several different supportive treatments available, including supplementation with vitamin A and omega-3 fatty acids, or usage of neurotrophic factors and antioxidants, these therapies have often been shown to be ineffective, failing to address the root cause of the disease.[3]

Gene therapy has begun to show promising results for improving visual outcomes, as evidenced by new clinical trials like the one used to secure Luxturna's approval However, there are still several important challenges for gene therapies including its severely limited therapeutic target and extremely high cost.[4]

Consequently, scientific interest is particularly directed at restoratory therapy based on stem cells. The latter aims to recover cell density as well as to preserve the remaining retinal cells by improving intra/extracellular conditions.[6,7]

Mesenchymal stem cells are multipotent stromal cells with self-renewal and multi-differentiation abilities into various mesenchymal tissues, most notably bone, cartilage and adipose. Studies have also described the ability of MSC to differentiate into retinal progenitor cells, photoreceptors and retina neural-like cells whilst exerting neuroprotective and pro-regenerative effects via multiple paracrine factors.[5]

Özmert and Arslan recently reported the results of an open label, phase III clinical trial (NCT04224207) with WJ-MSCs. In this study, WJ-MSCs were implanted in the sub-tenon space in 32 patients (64 eyes) diagnosed with RP. In the 6-month follow-up period, a significant improvement in mean best corrected visual acuity (BCVA), outer retinal thickness values, mf-ERG results, and a decrease in the visual field mean deviation value were observed.[8]

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The authors did not observe any severe ophthalmic or systemic complication thus assuring its safety. An improvement in light perception and vision was observed 1 week after the treatment and no serious side effects were seen during that period.[8]

MATERIALS AND METHODS

This clinical study included four eyes from two patients attending the ophthalmology clinic of Shah Alam Hospital between November 2020 and November 2021. The study followed the tenets of the Declaration of Helsinki. The patients were informed about the details, aims, and the course of the study. Written informed consent was obtained from each subject before any of the study procedures or examinations were performed. The diagnosis of RP was established depending on the clinical history, ophthalmological findings, visual field (VF) test, optical coherence tomography(OCT), and electroretinography (ERG) test results of the patients.

The inclusion criteria were as follows: 1) a clinical diagnosis of RP confirmed by clinical history, fundus appearance, VF, OCT, and ERG; 2) subjects older than 18 years of age; 3) subjects who are able to do a reliable VF evaluation; 4) subjects who have at least 1-yearfollow-up results.

Patients with previous ocular surgery other than cataract extraction, ocular media opacities that would make the image quality insufficient for ocular imaging or affect the test results, coexisting ocular disease (e.g. retinal pathology other than RP, glaucoma, uveitis, strabismus, nystagmus), any other systemic disease (e.g., diabetes, neurological diseases, hypertension) that would have an impact on the results were excluded from the study.

A single experienced vitreoretinal surgeon (AO) performed all the surgical procedures and ophthalmic evaluations. Baseline ophthalmic evaluation of the patients included BCVA, applanation tonometry, slit lamp biomicroscopy, color fundus photography, OCT, VF, and ERG. Visual acuity was evaluated by using a Snellen chart at a distance of 3 m. OCT was performed using the Optovue (Optovue Inc, USA) with a standardized scanning protocol. VF examination was performed by (the Threshold 30-2 Humphrey VFby HFAII750 device (Carl Zeiss Meditec AG, Germany). ERG (ERG-Vision monitor, Monpack 3, Metrovision, France) Readings were recorded from each eye according to the International Society for Clinical Electrophysiology of Vision(ISCEV) guideline . All tests were performed with the same instrument and by the same technician.

Preparation of umbilical cord Wharton's jelly-derived mesenchymal cell

The WJ-MSC used in both patients were isolated from Wharton's Jelly of the umbilical cord that was collected from the single donor with the mother's consent and treated as follows:

The umbilical cord initially immersed in cord preservative solution was washed with 0.9% sodium chloride injection and soaked with filtered 70/75% medical grade ethanol for disinfection. After rinsing with 0.9% sodium chloride injection and measured by clamping and stretching both ends with hemostats, it was cut into 2–3cm small pieces. The Wharton's jelly was weighed, cut, washed, and centrifuged before seeding for culturing process in complete culture media. The cultured tissues are incubated in the CO_2 incubator with the parameters of 37°C, 5.0% CO_2, and 95% relative humidity. All cell preparation and cultivation procedures were conducted by Beike 23 Century International Stem Cell Laboratory, an MOH cGMP/cGTP accredited facility.

Culture expanded cells were cryopreserved at P4 using standard cryopreservation protocols until their use . Cells are characterized at the time of cryopreservation with flow cytometric analysis according to FDA and ISCT guidance to determine the expression of positive surface markers for CD90, CD73, CD105, CD29, ad negative for CD45, CD34, CD79a, CD14, and HLA-DR. Quality control analyses were carried for the following: mycoplasma analysis according to Ph.Eur2.6.7; endotoxin analysis were performed according to Ph.Eur 2.6.14. Microbial limit testing according to US-6.1, Sterility testing according to USP 7.1.

The average cell viability was over 90% and each patient received 10–11 million cells in 1.5 ml of electrolyte solution per eye.

Injection of WJ-MSC

A total of 1.5 ml of WJ-MSC suspension was injected into the subtenon space of each eye. The procedure was conducted under local anaesthesia with proparacaine hydrochloride drops (Alcaine, Alcon , USA) under sterile conditions. Subtenon injection using a 25 G subtenon curved cannula (BD, Visitec, UK) into the supero-temporal region was used for effective delivery of the 1.5ml of WJ-MSC. Post-operatively,Guttae maxitrol (neomycin + dexamethasone) eye drops were given qid for 1 week, oral Ibuprofen 200 mg three times a day for one week, and amoxycillin clavulonate 500 mg four times a day for three days.

RESULTS

Case 1

A 65-year-old Malay man known case of RP for more than 25 years with no prior history of mesenchymal stem cell (MSC) treatment . The current condition of both eye vision affects his daily living activities (DLAs) and his business.

Pretreatment assessment were done including, visual acuity, anterior and posterior segment slit lamp examination, color fundus and specific investigational test such as Automated visual field test, electrophysiology (Full field ERG and multifocal ERG) and OCT.

His vision was 1/60 and CF 1' for the right and left eye, respectively. He has both IOL implanted and had normal intraocular pressure. Both fundi showed typical RP changes minimally sparing the macula area. The visual field revealed a very constricted field in both eyes. Full-field ERG was not able to perform, and multifocal ERG showed an extinguished response in all fiverings in both eyes. His OCT revealed loss of the photoreceptor layer in all segments of both eyes.

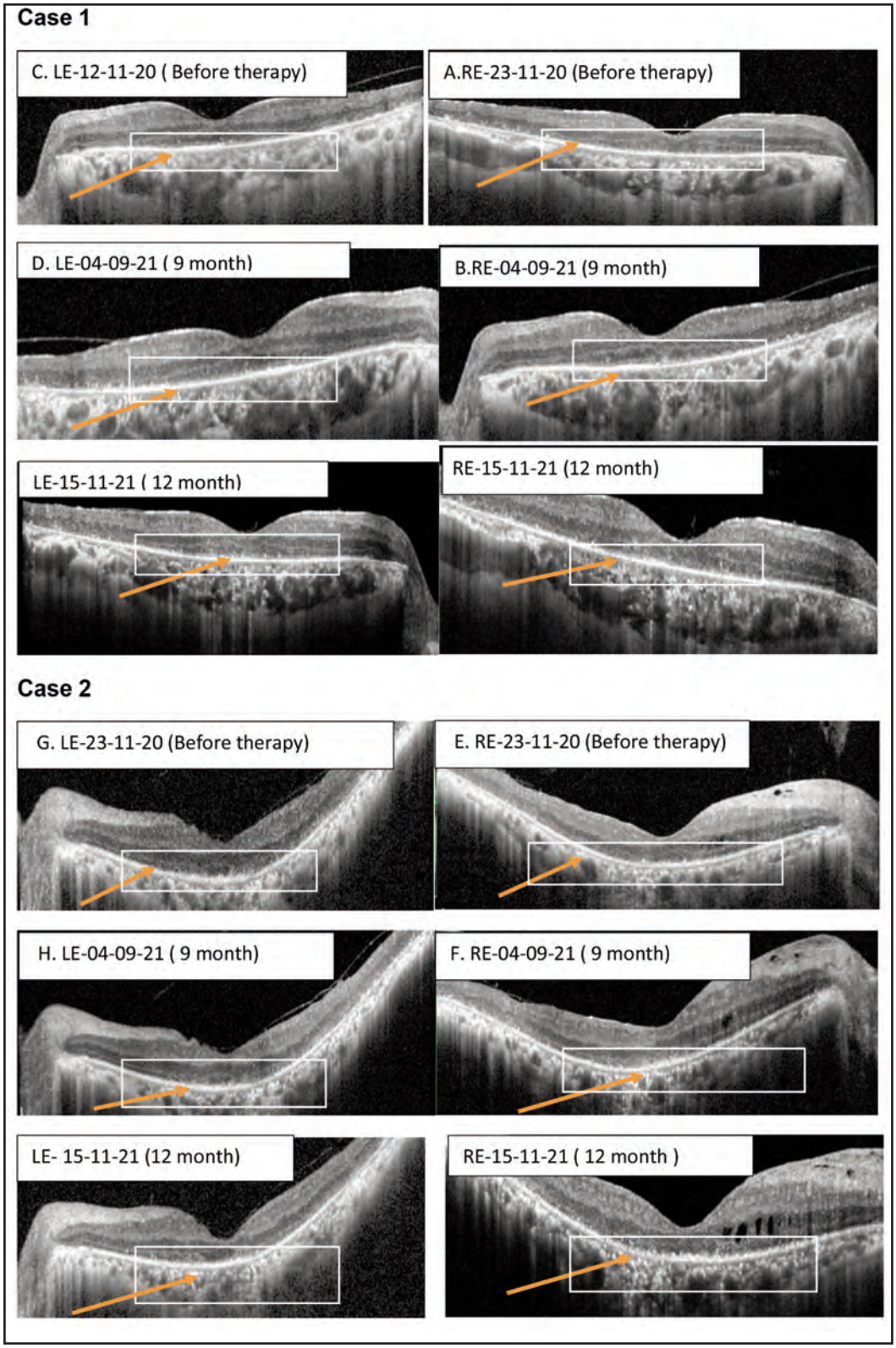

Fig. 1: OCT of Right Eye and Left Eye for both cases comparing the pre-treatment and 3rd and 4th post subtenon MSC implantations: (A,C &E,G): the OCT prior to MSC subtenon treatment- showing very minimal area of hyperreflectivity seen anterior to RPE layer.
(B,D& F,H): OCT after the 4th dose of MSC and post follow up at 9 and 12 month respectively; showing increased area of hyperreflectivity (orange arrow) at the interdigitation zone anterior to the RPE layer signifying possible regeneration of the outer segment of PRC.
The interdigitation zone is considered to be the contact cylinders formed by the apices of the RPE cells that encase part of the outer segment of the cones.

He underwent four sessions of WJ-MSC subtenon injection, which was carried out under aseptic technique. The injection interval was 4 weeks for the first three injections and 3 months for the fourth injection.

Post injection 9 months follow-up demonstrated no worsening in visual acuity charting and other slit lamp assessments. Subjectively patient reported ability to appreciate colors in more definite brightness and was also able to appreciate incoming vehicle while sitting at the passenger seat and able to read his handphone message much faster. The OCT findings noted an increase in hyperreflective material at the interdigitation zone layer of the photoreceptors cells (PRC) seen at the macula and extramacular region (Figure 1). After the third and fourth injection, a more definite hyperreflective layer can be seen on OCT. However, the central macula thickness did not show much increase in thickness.

Case 2
A 57-year-old Malay woman who was diagnosed with RP 20 years ago. She is currently coping with her job despite the progression of her tunnel vision which is gradually affecting her DLA. Her pretreatment assessment prior to WJ-MSC subtenon treatment revealed vision of 6/40 Ph 6/20 in the right eye (RE) and 6/30 Ph 6/20 in the left eye(LE). Both eyes had intraocular lens implanted and had normal intraocular pressure. Both fundi showed general RP changes with bony spicules, waxy pallor disc, and attenuated vessel in all four quadrants. Automated visual field showed bilateral grossly constricted field. Her ERG results were as expected,and revealed extinguished response in scotopic, photopic, and undetectable 30Hz flicker implicit time.

Her multifocal ERG response was reduced in all five rings in both eyes. OCT revealed the loss of PRC. She received a total of four sessions of subtenon MSC treatment with almost similar timing as case 1.

Post-treatment 9th and 12th month follow-up indicated her vision being maintained at 6/30 Ph 6/20 for both eyes. The OCT revealed a significant layer of photoreceptors with hyperreflective material at the interdigitation zone layer of the PRCs at macula and extramacular region(Figure 1).

Subjectively she claimed her vision is much clearer in the mornings, lasting a few hours until the afternoon. She was noted to be more confident and able to move faster at workplace as was observed by her staff and colleagues.

Post procedure, both patients received guttae maxitrol (neomycin + dexamethasone) four times a day for one week duration, T.Ibuprofen 200 mg three times a day for one week and T.amoxycillin 500 mg four times a day for three days to cover for the post-op inflammation and prevent infection.

DISCUSSION

Mesenchymal stem cells have been successfully isolated, cultured and expanded from several tissue sources including bone marrow, adipose tissue, amniotic membrane, dental pulp, umbilical cord blood and Wharton's jelly. They are considered as promising candidates for therapy to regenerate and repair degenerated retina cells in several retinal degenerative diseases.

In addition to this, it has been demonstrated in animal models that WJ-MSC can stimulate progenitor cells in the retina and elicit self repair mechanisms.[6] Such improvements could be largely due to the paracrine effects[7] of the implanted cells leading to a functional integration of grafted cells to substitute lost retinal photoreceptors or maintain their neuroprotective and neurotrophic effects to retain recipient functional photoreceptors.

For both of our cases, an increased area of hyperreflectivity demonstrated by OCT findings at the interdigitation zone layer of the PRCs was seen at the macula as well as extramacular region. This is likely attributed to the regeneration of the outer segment of the PRC which also coincided with subjective improvements in function as reported by both patients.

No further deterioration in visual acuity was observed and there were no serious adverse events or ophthalmic/systemic side effects reported during the 12 month follow-up.

To the best of our knowledge, there is no other clinical study in Malaysia using WJ-MSC application for RP. A similar study by Ozmert and Arslan from Ankara University , Turkey [9] reported efficacy and safety in their 6 month followup. We have followed up our cases for more than 12 months and thus far there is sustained improvement with no disease progression and with no adverse events demonstrating safety and efficacy of subtenon transplantation of WJ-MSC. However, long term followup is essential to evaluate the durability of the improved visual function and to determine when a booster treatment with WJ-MSC will be required.

CONCLUSION

We postulate that delivery of WJ-MSC by subtenon implantation in RP cases can induce repair and have significant immunomodulatory effects by inhibiting proinflammatory cytokines in the retinal microenvironment suppressing chronic inflammation and subsequent prevention of apoptosis.[5]

Although still far from routine clinical practice, regenerative stem cell-based therapies may become the standard means of treating severe retinal degeneration giving hope to those suffering from RP.

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