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Efficacy of fibroblast transplantation in the healing of cutaneous leishmaniasis scar: A case report

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Abstract

Autologous fibroblast transplantation has been proven to be a promising method in wound healing with no side effects. This is the first study aimed to determine the efficacy and safety of autologous fibroblast cell injection to the atrophic scar caused by cutaneous leishmaniasis as an endemic disease in many middle-eastern countries. It causes chronic skin lesions and permanently disfiguring scars. Autologous fibroblasts were obtained from the patient's ear skin and were injected intradermally twice at 2-month intervals. Outcomes were measured using ultrasonography, VisioFace, and Cutometer. No adverse reaction was observed. The results showed improvements in epidermal thickness and density, melanin level, and skin lightening. Moreover, the skin elasticity in the scar area increased after the second transplantation. No improvement was observed in dermal thickness and density. A longer follow-up with more patients is recommended to investigate the effectiveness of fibroblast transplantation better.

Keywords

Skin regeneration, atrophic scar, autologous fibroblast, cutaneous leishmaniasis

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Introduction

Leishmaniasis is a parasitic infection caused by over 20 species of the genus *Leishmania*.[1] Cutaneous leishmaniasis accounts for about 1.5 million cases annually. It is an endemic in 98 countries, most prevalent in South America and middle-eastern countries, especially Iran.[2–4] This infection originates via female sandfly bites, causing chronic skin lesions and permanently disfiguring scars on exposed parts of the body, eventually leading to lifelong stigmatization and psychological consequences.[1,5] A majority of these painless lesions heal spontaneously with remaining atrophic scars.[6] It results from dermal atrophy and collagen degradation due to an acute inflammatory response. Current clinical approaches are limited due to many side effects and drug resistance,[7] and the need for long-term use.[8–10]

A new long-term therapeutic modality is to use the minimally invasive autologous fibroblast transplantation. Fibroblasts, the predominant cells of connective tissue, are responsible for synthesizing and organizing collagens. They help maintain skin integrity by secretion of soluble cytokines and growth factors.[11–15] This pilot study aimed to assess the safety and efficacy of autologous fibroblast transplantation to remedy the scar lesion of a patient infected with cutaneous leishmaniasis.

Case report

The patient was a 34-year-old man with a skin lesion caused by cutaneous leishmaniasis on his left cheek in his childhood. The exclusion criteria can be seen in Supplementary 1.

To prepare the skin sample for fibroblast cell isolation, 8-mm^2 skin biopsy was taken from the patient's ear using a punch. The biopsy site was checked regularly for any

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Table 1. Thickness and density of the epidermis and dermis in the scar lesion of the patient suffered from cutaneous leishmaniasis before and after FT.

Sonography		TE (μm)	TD (μm)	DE (μm)	DD (μm)
Before FT	Scar	93	969	176.94	41.92
	Ctrl	109	789	159.29	37.42
2 months after first FT	Scar	109	1266	137.44	31.92
	Ctrl	109	1562	144.48	44.83
2 months after second FT	Scar	110	1195	143.81	43.42
	Ctrl	110	992	139.26	33.37

FT: fibroblast transplantation; Ctrl: control.
Fibroblast transplantation could improve the epidermal thickness and density to a level similar to the control (right cheek). No improvement in dermal thickness and density was observed after cell transplantation.

complications. The sample was placed in phosphate-buffered saline (PBS) containing 2% penicillin/streptomycin and sent to the clean room and incubated in Dispase solution (Gibco, Germany) and then in 0.1% collagenase I (Sigma, Germany). After centrifuging of the suspension, the spindle-shaped cells were proliferated up to passage 3 and were karyotyped before transplantation (Supplementary 2).

The treatment site was cleaned with ethanol 70%. Then, a number of 107 cells/mL with cell viability above 90% in normal saline was injected at the volume of 100 μL/cm^2 into the middle layer of the lesion dermis, applying a 30-gauge needle.

The patient's right cheek quality was assumed as the control at each time point. Cell transplantation was done two times at 2-month intervals.

Ultrasound of the skin (Taberna Pro Medicum GmbH, Luneburg, Germany) was used to evaluate the change in dermal and epidermal thickness and density (Table 1, Supplementary 3). The VisioFace Quick system (Courage & Khazaka Electronic GmbH, Cologne, Germany) was used to determine melanin and erythema indexes, severity of pigmentation (Delta E value), and skin lightness (Delta L value). Cutometer (Cutometer Dual MPA 580; CK Electronics, Germany) was used to evaluate the scar elasticity.

The epidermal thickness improved after the first transplantation and became similar to the thickness of the epidermis on the right cheek. The density decreased gradually at the lesion site and reached a level similar to the epidermal density of the control. In dermal layer, no improvement was observed after cell transplantation.

As Figure 1 demonstrates, after transplantation, the erythema index experienced a slight decline and reached below the erythema level of the control area (Figure 1(a)). Melanin index of the scar area reached levels similar to the control area (Figure 1(b)). The Delta L and Delta E changes indicate skin lightening increase and pigmentation level reduction (Table 2).

Based on F1, Q0 (maximum recovery), and Q3 (viscoelastic recovery) values, skin elasticity slightly improved at 2 months post-second fibroblast transplantation. The R2 (gross elasticity), R5 (net elasticity), and R7 (skin firmness) parameters doubled, indicating a skin elasticity improvement in the scar area (Figure 2). The changes to the scar area are shown in Supplementary 4.

Discussion

This study aimed to determine the efficacy and safety of autologous fibroblast cell injection to atrophic scar in a patient suffering from cutaneous leishmaniasis using ultrasonography, VisioFace, and Cutometer. Our results showed improvements in epidermal features. No improvement was observed in dermal thickness and density.

Cutaneous leishmaniasis treatments include the use of Glucantime as the first line of treatment, with minimal use due to many side effects and drug resistance.[7] Trichloroacetic acid (TCA) peeling and topical 25% podophyllin solution with 48%–100% effectiveness are also used.[16-18] Topical paromomycin and pentavalent antimonials are restrictedly used, due to their effectiveness against limited species of *Leishmania*, the need for long-term use, and the local side effects.[8–10,19] Fractional CO2 laser, photodynamic therapy, heat therapy, and cryotherapy are also used.[20–23]

Since 1975, cell therapy has been used in treating burn wounds.[24] Autologous fibroblast transplantation was first used in 1995.[11,12] It was later used across multiple skin lesions, such as burned skin in diabetic patients, skin wrinkles, and atrophic acne scars.[25,26]

Fibroblasts synthesize various growth factors and cytokines that play a vital role in wound healing, stimulation, dermal milieu augmentation, epidermal differentiation process, dermal regeneration, and matrix deposition.[27–32]

Isolating the fibroblast cells is a simple procedure that requires a small biopsy at a non-visible site, typically the retro-auricular area. It has virtually zero risk of hypersensitivity reaction. Recent studies have measured improvements in facial contour defects with the durability of at least 12–48 months. They have shown that using fibroblast cells in the dermis layer help form granulation tissue and create an epithelial layer.[33]

To our knowledge, only the study by Nilforoushzadeh et al. has assessed the use of fibroblast transplantation in

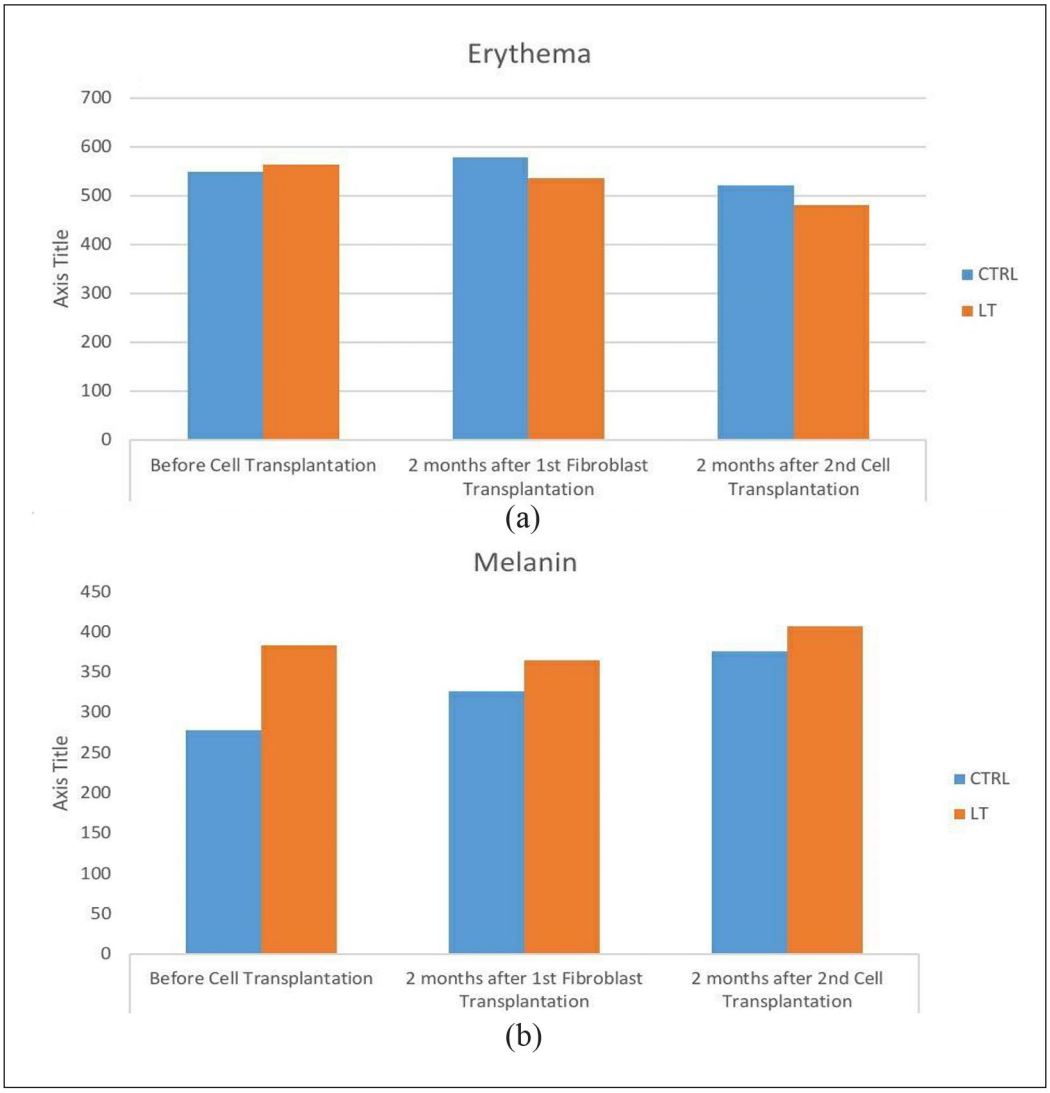

Figure 1. Melanin and erythema indexes before and after transplantation. (a) The erythema index of the scar area was similar to the control area before FT. It experienced a decline and reached below the erythema level of the control area after the second FT. (b) Melanin index of the scar area was higher than the control area before FT. It declined to levels similar to melanin index in the control area. The patient's right cheek quality was assumed as the control at each time point. FT: fibroblast transplantation.

Table 2. Assessment of VisioFace based on Delta E and Delta L values.

VisioFace		Delta E	Delta L
Before FT	Scar	10	−9
	Ctrl	Not perceptible by the human eye	
2 months after first FT	Scar	2	−2
	Ctrl	Perceptible through close observation	
2 months after second FT	Scar	2	−1
	Ctrl	Perceptible through close observation	

Ctrl: control.
The values were measured in the patients suffered from cutaneous leishmaniasis before and after fibroblast transplantation (FT). The patient's right cheek was assumed as the control in each time point.

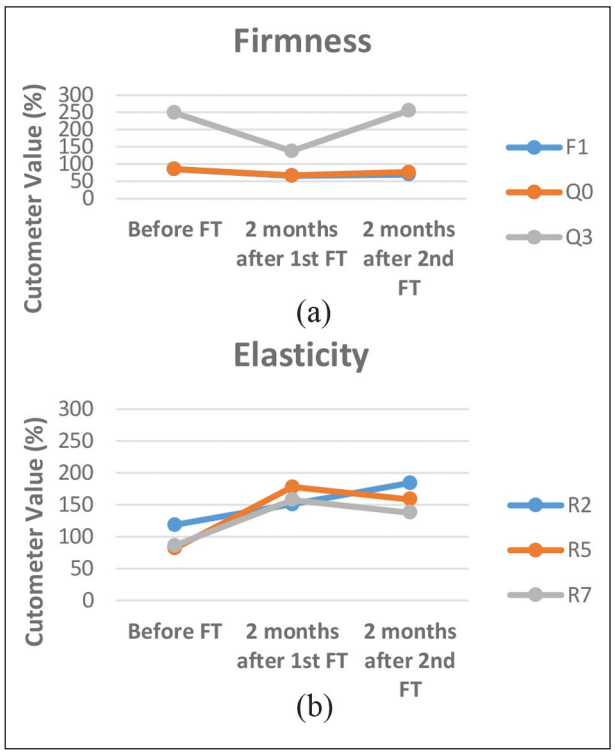

Figure 2. The (a) skin firmness and (b) elasticity based on Cutometer data.

treating scars caused by cutaneous leishmaniasis. In this study, autologous fibroblast and keratinocyte cell transplantation were performed on a patient suffering from cutaneous leishmaniasis two times with a 2-month interval, along with dermal abrasion. The quality of wound healing was not evaluated by any unique method; instead, the satisfaction of the patient and the doctor was evaluated as a criterion. As three different variables, including fibroblast, keratinocyte, and dermal abrasion, were used to treat the lesion, the obtained results cannot be attributed to only the efficacy of autologous fibroblast transplantation.[34]

The importance of our study is that the evaluation of the efficacy and safety of fibroblast transplantation in treating scars caused by cutaneous leishmaniasis has been investigated for the first time. As a pilot study with one patient, the results could not be statistically interpreted; however, it gave us excellent insight to conduct more comprehensive studies with a higher number of participants.

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Author contributions

F.F. designed the research study and oversaw the execution and writing process.
G.L. selected the patient, and performed the cell culturing and microbial tests.
L.M. performed cell culturing and cell preparation.
A.H.M.E.K. selected the patient and wrote the paper.
N.A. performed karyotyping and analyzed the data.
K.G.M. performed the cell injection, analyzed the data, and wrote the article.
All the authors involved in this project have read and approved the final manuscript.

Declaration of conflicting interests

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Ethical approval

The current study was registered at the IRCT (Iranian Registry of Clinical Trials) and the study protocol was approved by the Ethics Committee of Iran University of Medical Sciences. (IRCT registration number: IRCT20150715023218N1; ethics committee reference number: IR.IUMS.REC.1399.196).

Informed consent

A written informed consent was obtained from the patient for participation in the study and the rights of the subject were protected.

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Supplemental material

Supplemental material for this article is available online.

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