UTILITY OF AUTOLOGUS BONE MARROW STEM CELLS FOR TREATMENT OF CHRONIC LEG ULCER

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Background

Chronic leg ulcer is a major health problem which may associate different diseases as vascular, neoplastic, infectious diseases and trauma as well (1). The patients suffer from many complications as infection, septicemia, psychology troubles and physical inability leading to many economic troubles. the last line of treatment in cases with Critical lower limb ischemia is amputation with its high rate of morbidity and mortality(2).

Aim of the work

The study design was randomized controlled clinical trial in an attempt to assess the potential treatment of chronic leg ulcer through the use of the autologous bone marrow stem cells

Patients and Methods:

After approval from Research Ethics committee at Faculty of medicine Tanta University, Egypt. Sixty patients with chronic leg ulcer were included from vascular surgery department Tanta university hospitals,Egypt. Thirty patients of this study were selected from vascular surgery department Tanta university hospitals, biopsy from the edges of the ulcer will be examined pathologically before and after the stem cell transplantation. Another Thirty patients of this study received ordinary line of treatment served as control

Patient preparation:

The following clinical and laboratory investigations were done for each patient:

Complete history taking and clinical examination.

Laboratory investigations: venous blood sample was taken and the following were done {Complete blood count (CBC), B.T., C.T., PT, PTT ,Liver function test ,kidney function test ,Fasting blood glucose (FBG),2 hours post prandial(2PP) ,HBV Ags ,HCV Abs ,HIV Abs.

Abdominal ultrasonogrophy: to ensure that there is no hepatomegally or spleenomegally. Ulcer edge biopsy: for histopathological examination to exclude malignancy.

The patients were received recombinant human Granulocyte Colony Stimulating Factor (rhG-CSF)(GeneLeukim Injection- China). Each 1ml vial contains 300µg Filgrastim given by subcutaneous injection in a dose of 5 µg/kg per day for 3-5 days to mobilize stem/progenitor cells. Meanwhile, a perfusion of 10,000 units/day heparin for 3-5 days by intravenous drip was used to avoid the possible risks of embolism because G-CSF induced the increase of circulating blood cells.

Preparation of human bone marrow mononuclear cells (BM MNCs)

The aspirated B.M. was diluted at a ratio of 4:1 with clinical buffer.

Before use, the filter was wetted with clinical buffer (Clini MACS PBS/EDTA buffer 1000ml, CE approved for clinical use catalogue number #700–25, from Miltenyi Biotec Company) then the diluted B.M. passed through it by using sterile Pasteur tubes.

35ml of the diluted cell suspension was carefully layered over 15 ml of ficoll – paque (GE Electric, Pharmacia).in a 50 ml conical tube.

Centrifugation was done at 2000 rpm for 20 minutes at 20°c in a swinging out bucket rotor without brake.

The upper layer was aspirated leaving the mononuclear cell layer undisturbed at the interphase.

The interphase cells (stem cells, lymphocytes, monocytes, and thrombocytes) were carefully transferred to a new 50 ml conical tube.

Cells were washed by adding up to 40 ml of clinical buffer, mixed gently and centrifuged at 1200 rpm for 15 minutes at 20°c, then the supernatant was carefully and completely removed (this step was done twice).

Cell pellet was re-suspended in appropriate amount of clinical buffer (final volume of 300 µl of clinical buffer for up to 108 total cells).

In the operating room :

The patients were taken to the operating room and placed under general anaesthesia.

The ulcers were surgically debrided under sterile conditions to ensure a clean base with no scars, fibrotic or necrotic tissues. This allowed direct contact of bone marrow cells to a viable wound tissue base.

The cells were injected into the ulcer edge and the ulcer area by using a 3 ml syringes with 19 gauge*1.5 needle.

Thereafter, the wound surface was protected with ointment gauze (BIO-TULLE) and sterile dry gauze dressings. This dressing was left onto the wound for 24 hours then removed, the wound was washed with saline 0.9% only and a new dressing was used.

RESULTS

Demographic data

Demographic data	Age	Male	Female
Group I Stem cell group (N.=30)	Range =12-64 Mean= 48	17	13
Group II Control group (N.=30)	Range =15-61 Mean= 43	16	14

Ulcer Site

Ulcer site	GI N(=30)		GII (=30)	
	N	%	N	%
Lower 1/3 leg	12	40%	12	40%
Middle 1/3 leg	9	30%	6	20%
Upper 1/3 leg	3	10%	6	20%
Foot	6	20%	6	20%

Ulcer size

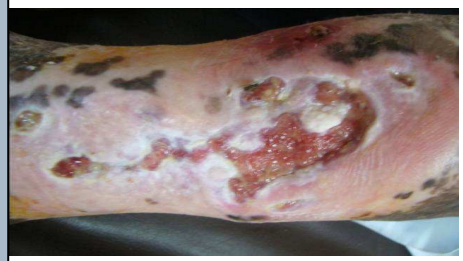
Ulcer size	GI N(=30)			GII (=30)		
	Mean	SD	P-value	Mean	SD	P-value
Before therapy	8.60	1.06	-	8.33	1.36	-
After 3 months	5.20	0.72	0.023**	7.50	1.37	0.162
After 6 months	3.20	0.65	0.019**	7.38	2.16	0.345
After 12 months	1.6	0.62	0.001**	6.83	2.30	0.430

Degree of ulcer healing

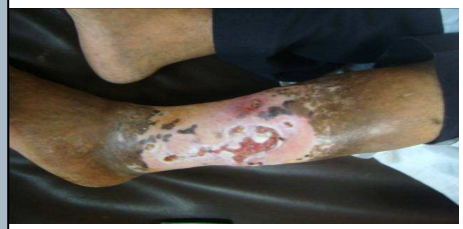
Degree of healing	GI N(=30)		GII (=30)	
	N	%	N	%
Good healing (75-100%)	14	46.8%	3	10%
Moderate degree Healing (50-75%)	6	20%	1	3.3%
Mild degree Healing (25-50%)	5	16.6%	2	6.7%
Poor Healing (0-25%)	5	16.6%	24	%80

Life-style impact

Life-style impact	GI N(=30)		GII (=30)	
	N	%	N	%
Pain relief	21	70%	9	30%
Walking ability	20	66.6%	3	10%
Decreased need for frequent dressing	24	80%	6	20%

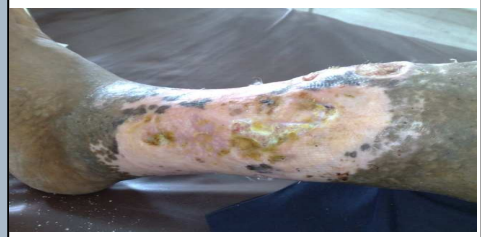


before stem cell therapy



After 2ms of stem cell therapy

After 4ms of stem cell therapy



After 6ms of stem cell therapy

Discussion

In this study, the thirty patients described shows heterogeneity in the types of ulcers treated by the actual administration of MNCs as they did not respond to traditional modalities of treatment for years. They also did not heal with traditional wound care, which included several applications of skin grafts. 14 out of 30 patients (46.8%) showed good healing ,6 out of 30 patients (20%) showed moderate healing and 5 out of 30 (16.6%) showed mild healing while 5 out of 30 (16.6%) showed no healing. On the other hand as compared with the control group 24 out of 30 patients (80%) showed no healing ,3 out of 30 patients (6.7%) showed mild healing and 1 out of 30 (3.3%) showed moderate healing while only 3 out of 30 (10%) showed good healing. In agreement with our study, **Bediavas et al.,2003** achieved similar results in three patients who had complete closure of their yearlong ulcers with use of BMA and cultured cells. All healed within 3 months; however, One patient required a bioengineered skin.In 2007, Badiavas et al., conducted another study injected BMA into the ulcers of 4 subjects but only one healed completely.

The largest study to date using bone marrow-derived mesenchymal stem cells with or without autologous skin graft was published by ***Yoshikawa et al., 2008***. This study included 20 patients with various non-healing wounds. The authors reported complete healing in 18 patients and showed regeneration of native tissue by histologic examination. The study supported the previous literature that bone marrow-derived stem cells are associated with dermal rebuilding, remodelling, increasing wound vascularity, and reduced fibroses.

Conclusion

Autologous transplantation of bone marrow derived mononuclear cells is a simple, safe and effective modality of treatment for chronic leg ulcer.

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