DENTAL PULP STEM CELLS FOR TREATING UNHEALED DIABETIC FOOT ULCER: A PIONEERING ATTEMPT :

Dr. Sankaranarayanan[1] M.D.S., **Dr. P. Ramachandran**[2]M.S., Mch., **Dr. V. R. Ravi**[3]M.B.B.S., M.S., **Dr. Divya A**[4]**,** MDS,

1. Prof& Head Department of Oral Pathology, Srimoogambigai Institute of Dental Sciences, Kulasekaram.
2, 3. Consultant Orthopedic Surgeon, Martuthi Hospital & Director, Mother Cell Regenerative Center
4. Reader, Department of Oral Medicine and Radiology, J.K.K Nattaraja Dental College, Komarapalayam, Namakkal District, Tamilnadu, India.

INTRODUCTION:

Chronic lower extremity ulcers are a physical and financial burden to the health and economic establishment world wide. Foot complications account for about 25% of all diabetic admissions with 50,000 lower-limb amputations performed annually. Most lower extremity wounds are caused by venous disease, arterial insufficiency, diabetic neuropathy or a combination of these factors. Other etiologies may include vasculitis, TAO/burns, and trauma.

The last decade has witnessed a dramatic increase in the mechanistic understanding of angiogenesis and arteriogenesis, the two processes by which the body responds to obstruction of large conduit arteries. This knowledge has been translated into novel therapeutic approaches to the treatment of peripheral arterial disease. In chronic wounds, the senescent cells, due to inhibition of fibroblast proliferation are unable to divide and hence, become unresponsive to growth factors. Stanley and osler showed that a human venous leg ulcer with more than 15% of senescent cells would be more difficult to heal.

Given the poor prognosis associated with critical limb ischemia (CLI) numerous interventions have been attempted, primarily based on stimulation of angiogenesis in order to allow formation of collateral blood vessels. According to the Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II) treatment for CLI should be focused on revascularization using surgical or per-cutaneous means.

Unfortunately less than half of the patients are eligible for these procedures, and efficacy is limited. Additionally, many patients require re-surgical interventional procedures due to high levels of restenosis, because of the complex anatomy of the vascular occlusion and/or the presence of other risk factors. Nonsurgical options for CLI are limited to medical therapy, which offers limited or no benefit. Noninvasive stem cell therapy has been proposed as an alternative for such patients. Recent articles on this disease have proposed stem cell therapy along with endovascular procedures also.

STEM CELL THERAPY IN PERIPHERAL VASCULAR DISEASE:

Cell therapy with stem cells and endothelial progenitor cells (EPCs) to stimulate angiogenesis as a potential treatment for ischemic disease is an exciting area of research in regenerative medicine. Recent evidence suggests that stem cells derived from bone marrow have the potential to treat many disorders given their plasticity and ability to differentiate into various types of tissues, nerve/ vessels and skin cells. Administration of concentrated mononuclear cells (stem cells) locally and the limb muscles has shown promising results for treating lower extremity ulcers.

Trials to date have reported clinical improvement and reduced need for amputation in CLI patients receiving autologous bone marrow or mobilized peripheral blood stem cells for stimulation of angiogenesis.

Even though autologus stem cell treatments are currently entering Phase III trials, practical and scientific pitfalls will limit widespread implementation.

Hurdles to overcome include: a) reduced angiogenic potential of autologous cells in aged patients with cardiovascular risk factors. b) Invasiveness/adverse effects of bone marrow extraction and G-CSF mobilization, respectively. c) Need for on-site cellular manipulation.

Mesenchymal stem cells (MSC) derived from various sources, like adipose tissue, cord lining, placental origin, bone marrow, endometrial origin and dental pulp stem cells have low immunogenic properties. That means these MSC have potential properties for treating many incurable diseases without any need for HLA matching. Subsequent to this basic character proved by animal and human study, many pharmaceutical companies have started conducting clinical trials using MSC stem cells.

In India stemputics along with Cadila Pharma Company have already completed phase1/2 studies. The DCGI has also approved for this clinical study. Many institutes are conducting various levels of study using cord lining MSC's for the treatment of type 1 diabetics.

Recently, the FDA of Canada has approved the allogenic MSC therapy for the treatment of GVHD following BMT and allogenic hematopoietic stem cell treatment. Hence allogenic MSC therapy also has gained wide acceptance.

DENTAL PULP STEM CELLS:

Dental pulp stem cells is being transplanted to mouse hind limb ischemia resulting in successful engraftment and an increase in the blood flow including high density of capillary formation.

The transplanted cells were in proximity of the newly formed vasculature and expressed several proangiogenic factors, such as VEGF-A, G-CSF, GM-CSF, and MMP3.

Dental pulp is a new stem cell source for cell-based therapy to stimulate angiogenesis/vasculogenesis during tissue regeneration.

The researchers reported that stem cells derived from dental pulp display increased immunosuppressive activity when compared to bone marrow mesenchymal cells and likely to have immunosuppressive activity with potential clinical applications in allogenic in vivo stem cell transplantation. Various animal studies using both autologus and allogenic dental pulp stem cells for bone regeneration has shown no immune reaction with complete bone regeneration.

Based on above basic and animal study we have decided to use dental pulp stem cells for treating diabetic wound.

CASE REPORT:

A patient aged 72 years referred to plastic surgeon for non healing anterior foot wound of the left leg post amputation. The patient is a known diabetic for the past 18 years and suffering from peripheral neuritis since 5 years. The patient also underwent coronary angio-bye pass graft (CABG) 3 years back. The stem cell treatment for this case is cleared by institutional ethical committee approval.

HISTORY OF THE PRESENT WOUND:

The patient had an accident while traveling in a bike and injured his left toe. The wound has not healed by conventional treatment. After 2 months the little finer and middle toe of the left leg was first amputated, even after that the wound has not closed. Later, vascular surgeon has amputated fore foot with remaining 3 toes. After the amputation also the wound has not healed for 3 months. There was bone necrosis in the amputated region. Since the wound healing is delayed in all his previous surgical procedure's the plastic surgeon has opted for stem cell therapy.

In this article we report a case of chronic lower extremity non healed diabetic ulcer treated with Dental pulp stem cell, which has started healing within 2 months, and has taken up 70% of the skin graft. From the available English literature this is the first time a dental pulp stem cell is being used to treat CLI globally. This has proved the safety of the procedure. However many such treatments should be attempted to prove the safety and efficacy of this treatment.

Our team has already treated more than 20 cases of CLI using bone marrow aspirate and with other sources of stem cells. This primary attempt of Dental Pulp Stem Cell treatment for unhealed diabetic ulcer has an encouraging results and more number of such cases should be tried for a better understanding and for its efficacy for routine clinical practice.

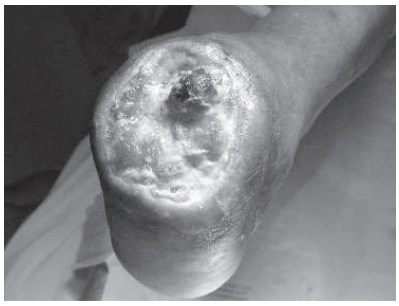

Figure 1. Forefoot amputated leg with unhealed ulcer.

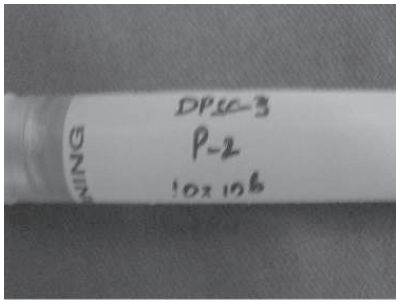

Figure 2. Dental pulp stem cells in vial

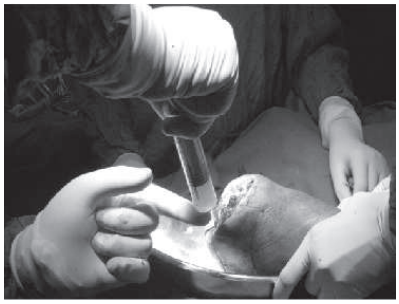

Figure 3. Dental pulp stem cell injection

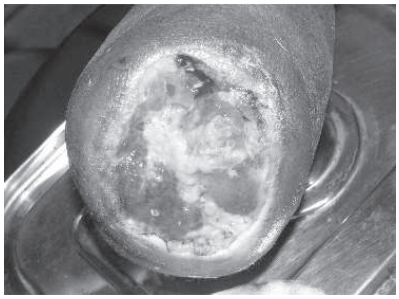

Figure 4. 18 days Post operative view.

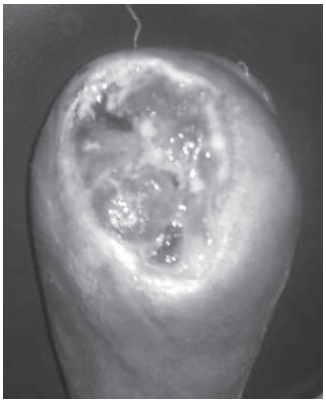

Figure 5. One month post-operative view

Figure 6. View the 70% of skin graft has taken up.

STEM CELL TRANSPLANTATION:

Since the patient has already under gone multiple surgeries including the CABG, he doesn't want to take his bone marrow stem cells.

As dental pulp stem cell has more vasculogenic potential we choose dental pulp stem cell for angiogenesis and for hastening the healing potential of this case. 35 million dental pulp stem cells were processed from the commercial stem cell lab. The laboratory is cGmp qualified one, and they are banking both cord and dental pulp stem cells. The stem cells were selected by cell surface marking identification. The markers were cd90 and cd106 and negative for cd34 and cd 45. This proves the postiveness of MSC. The cells are also karyotyped in a separate lab. The cells are obtained after checking cell viability and also negative endotoxin test.

The stem cells were mixed with 30 ml of platelet rich plasma and injected in to the left leg along the course of the posterior tibial, anterior tibial, dorsalis pedis, peronieal arteries. It is not given intra arterially. The patient is observed for a day in the hospital and discharged after one day. The patient was given broad spectrum antibiotic cover. The post transplant period is uneventful.

The first visit was after 18 days and second one after 30 days. After the granulation was healthy skin graft was given. After 2 weeks of post-op dressing the skin graft has taken up by 70%. The remaining healthy epithelialization expected within one month.

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Corresponding author :

Dr. Sankaranarayanan, M.D.S., D. Oral Laser (Vienna).,
Prof& Head Department of Oral Pathology
Srimoogambigai Institute of Dental Sciences
Kulasekaram K. K. District.
Director, Mother Cell Regenerative Center
Phone: +919443120843. Fax: +914651280740
Email: vedas72@hotmail.com.