A Case Report: The Efficacy of Human Amniotic Membrane Mesenchymal Stem Cells-Conditioned Medium (hAMMSC-CM) for Treating Plantar Pedis Trophic Ulcer in Leprosy Patients

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Abstract: Leprosy is an endemic disease in many countries with consequences of neuropathy that may leads to plantar trophic ulcer. The management of trophic ulcers is difficult due to its recurrent and recalcitrant characteristics. Placental derived cells are known to release factors with potential immunomodulatory and trophic activities. In this study we use the human amniotic membrane mesenchymal stem cells-conditioned medium (hAMMSC-CM) gel to promote the wound healing of trophic ulcer. A-50-years-old female presented with a trophic ulcer over the sole of her right foot since 3 years ago. The patient gave a history of decreased sensation over the right foot and was a known case of lepromatous leprosy released from treatment 2 years back after completing multibacillary multi-drug therapy for 1 years. Cutaneous examination revealed a single non-healing ulcer over the plantar aspect of the right foot with pale granulation tissue and seropurulent discharge at the base. Touch and pain sensation were lost over the foot and up to the lower leg one-third of the leg. The result of microbiology *Mycobacterium leprosy* examination was bacterial index (BI) 0 and morphological index (MI) was negative. A diagnosis of plantar pedis trophic ulcer in a treated case of leprosy was made. The ulcer managed by given topical hAMMSC-CM gel and covered with transparent film dressings. Application of gel was done every 3 days, and weekly evaluation included measurement of the size and the depth of the ulcer was done. The patient was treated with hAMMSC-CM for 8 weeks. The ulcer was significantly resolved, but there was no complete healing.

1 INTRODUCTION

Trophic ulcer that may occur in leprosy patient due to their nerve impairment is a condition of an ulcer that lack of nutrition. It is defined as a pressure ulcer caused by external trauma to a part of the body that is in poor condition because of disease, vascular insufficiency or loss of afferent nerve fibres by Mosby in 2016 (Puri, Venkateshwaran, and Khare, 2012). Plantar tophic ulcer is a physical disability that occurs in 10-20% of leprosy patient. Conventional therapies such as normal saline dressings and surgical debridement do not show a satisfactory improvement (Sari, Listiawa, and Indramaya, 2018).

2 CASE

A 50 years old female presented with an ulcer over the sole of her right foot since 3 years ago. The patient gave a history of decreased sensation over the right foot and was a known case of lepromatous leprosy released from treatment 2 years back after completing multibacillary multi-drug therapy for 1 years. She had been treated intermittently with topical antibiotics in addition to normal saline dressing for her ulcer, but complete resolution was never achieved. Cutaneous examination revealed a single non-healing ulcer measuring 1,8 x 1,8 x 0,4 cm over the plantar aspect of the right foot with pale granulation tissue and seropurulent discharge at the base.Touch and pain sensation were lost over the

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foot and up to the lower leg one-third of the right leg. Nerve thickening and regional lymphadenopathy were absent. The routine laboratory examinations were normal. There's no increasing of blood glucose level and no glucose in urine examination. The result of microbiology *Mycobacterium leprosy* examination was bacterial index (BI) 0 and morphological index (MI) was negative.

Figure 1: (a). A single non-healing trophic ulcer measuring 1,8 x 1,8 x 0,4 cm over the plantar aspect of the right foot with pale granulation tissue and seropurulent discharge at the base. (b), (c), and (d) the healing process of the ulcer treated with hAMMSC gel every 3 days for 8 weeks.

Based on the clinical findings, a diagnosis of plantar pedis ulcer in a treated case of leprosy was made. The initial treatment for the ulcer was sterile normal saline debris. After obtaining a clean ulcer, wound area and wound depth measurements were performed. Then the ulcer was given framycetin gauze dressing (FGD) and sterile gauze above the FGD but there was no clinical improvement after 8 weeks. On the next following week, the ulcers begin to be given topical hAMMSC-CM gel and covered with transparent film dressings. Application of gel was done every 3 days, and weekly evaluation included measurement of the size and the depth of the ulcer was done. The patient was treated with hAMMSC-CM for 8 weeks. The ulcer was significantly resolved, but there was no complete healing.

3 DISCUSSION

The global registered prevalence of leprosy by The World Health Organization (WHO) at the end of 2015 was 0.2 cases per 10.000 people. The number of new cases reported globally in 2015 was 2,9 new cases per 100.000 people. As the latest data published by WHO in the *Weekly Epidemiological Record* defines leprosy as a public health problem in countries where the prevalence of the disease exceeds one case per 10.000 inhabitants (Oliviera *et al.,* 2017). Indonesia was in the third rank of leprosy endemic country. About 5.284 new cases reported by East Java health profile in 2015.

Skin ulcers, such as those seen in patients with leprosy, are a serious public health problem. Infected chronic ulcers can lead to amputation of the affected limbs. The well-being and selfesteem of these individuals may be diminished by pain, loss of the ability to walk, and loss of independence. The appearance and unpleasant odour of the lesions may lead to social isolation. Most lesions in patients with leprosy are located on the lower limbs. Lower-extremity ulcers in patients with leprosy patients can be divided into two categories: leprous ulcers (due to the disease itself) and neuropathic ulcers (due to nervous system involvement). Plantar ulcers can cause a loss of protective sensitivity or lack of sensation in the plantar region from damage to the tibial nerve. Other factors increase the risk of developing plantar ulcers, including paralysis, volume loss of intrinsic foot muscles, loss of the fat pad under the metatarsal head, anhidrotic skin, biomechanical alterations, and/or deformities (e.g., foot drop, structural bone alterations) (Walsh, DeJong, Meyers, and Portaels, 2015; Rohatgi, Naveen, Salunke, Someshwar, Jerajani, and Joshi, 2016).

An expert in leprosy, Dr. Paul Wilson Brand (1914–2003), made major contributions to the understanding of the pathogenesis of the neurological complications of leprosy. It was considered that leprosy patients had nonhealing tissues and that nothing could be done about them. Neuropathic ulcers on the sole of feet usually develop at sites exposed to repetitive high pressures during activities of daily living like walking or working (Puri, Venkateshwaran, and Khare, 2012; Rohatgi, Naveen, Salunke, Someshwar, Jerajani, and Joshi, 2016).

The excessive pressure causes a hypertrophic reactive response of the local keratinocytes causing local hyperkeratosis. Hence at points of abnormal weight bearing and friction, callus formation may

occur. This callus finally cracks and breaks leading to ulceration. Hence, the risk of an ulcer is even higher when a callus is present. Importance of shaving callus at the margins of the ulcer and callus removal has shown reduction in dynamic plantar pressures in the forefoot by 30% during barefoot walking (Walsh, DeJong, Meyers, and Portaels, 2015; Rohatgi, Naveen, Salunke, Someshwar, Jerajani, and Joshi, 2016).

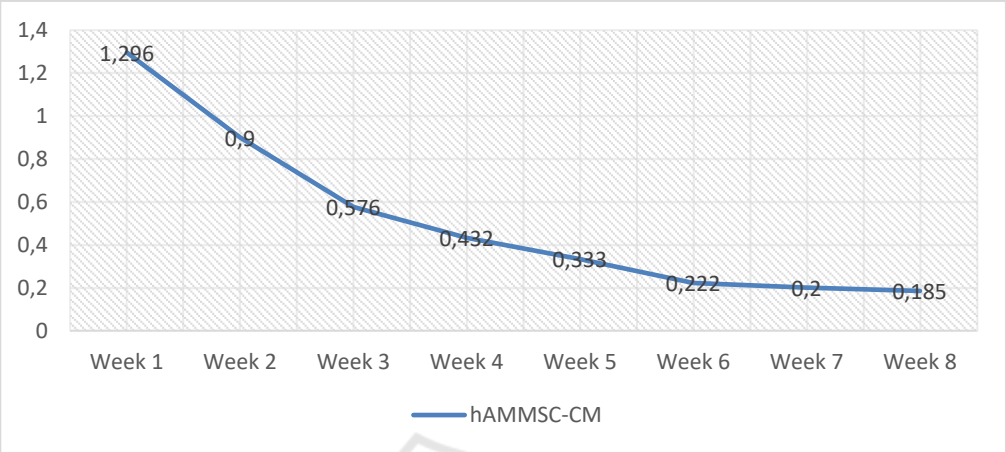

Figure 2: Healing progression. Ulcer treated with hAMMSC-CM not healed completely in 8 weeks.

The slow recovery of natural wound healing has resulted in the entry of exogenous wound healing treatments. Many treatments have proved to quicken the healing. This resulted in the discovery of more advanced treatments, such as tissue engineering, gene therapy, platelet-rich plasma, growth factors (GF) and stem cells (SC) therapy. Among these, SC has become the centre of attraction in wound healing by promoting microvascular remodelling. Reports have shown that SC plays a major role in strengthening wound healing by secreting a multitude of trophic and survival signals including GF, chemokines and cytokines. They serve as a tool among cells to communicate and these molecules can be traced in the conditioned medium (CM) or spent medium harvested from cultured cells (Jayaraman *et al*., 2013).

Lee et al. (2011) reported that CM of human embryonic stem cell (hESC)-derived endothelial precursor cells (EPC) containing high level of GF and cytokines such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), fractalkine, granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-6 were successfully used in the treatment of excisional wound healing in rats (Jayaraman *et al*., 2013).

In embryonic development, the mesodermal layer harbors multipotent progenitors that give rise to bone, cartilage, muscle and other mesenchymal tissues. Based on this embryonic perspective and previous reports from our group and others, a hypothetical and comprehensive scheme, proposed that in adult bone marrow (BM), a population of mesenchymal stem cells (MSCs) could likewise give rise to a spectrum of mesenchymal tissues by differentiating along separate and distinct lineage pathways (Caplan and Correa, 2011).

The approach has a high proliferative capacity, immunomodulatory activity, is low immunogenic, and non-tumorigenic. In its clinical application, hAMMSC-CM has a few advantages such as procurement procedures pose no morbidity, and there were unlimited amount of stem cells available due to its shorter expansion and doubling time than other adult stem cells. Mechanism of stem cells in tissue healing process is associated with the ability of stem cells to produce growth factors and cytokines that are necessary in healing. In vitro condition, these metabolites are also secreted in stem cell-conditioned medium (Rennie *et al.,* 2012; Skardal *et al.,* 2012).

Prakoeswa et al (2018) reported their analytical experimental approach study comparing the topical hAMMSC-CM and the framycetin gauze dressing (FGD) in ulcer healing. Ulcer healing in hAMMSC-CM group was significantly better with significant clinical and statistical differences ($p < 0,005$). Therefore, stem cell-conditioned medium can be used as a treatment modality because it contains growth factors and cytokines that are essential for cell regeneration (Rennie *et al.,* 2012; Prakoeswa *et al,* 2018).

4 CONCLUSIONS

It was found clinically that there were improvements in wound healing treated with hAMMSC-CM gel. Its may due to the mesenchymal stem cells that contain many growth factors. The further study to analyze the macrophage, vascular endothelial growth factor, fibroblast growth factor 2, transforming growth factor β1, keratinocyte growth factor, and epidermal growth factor is needed to prove the hypothesis.

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