INTRODUCTION

Diabetes is rapidly increasing in prevalence worldwide and surgery in patients with diabetic foot is becoming more common. Foot complications are a major cause of admissions in diabetic patients and comprise a disproportionately high number of hospital days because of multiple surgical procedures and prolonged length of stay in hospital ***(Guariguata et al., 2014).***

Diabetic foot is an umbrella term for foot problems in patients with diabetes mellitus. Foot disorders such as ulceration, infection and gangrene are the most common, complex, and costly sequelae of diabetes mellitus ***(Singh et al., 2005)***.

DFU is a common complication of DM that has shown an increasing trend over previous decades. The lifetime risk of a patient with diabetes developing an ulcer may be as high as 30% and up to 85% of all lower limb amputations in diabetes are preceded by foot ulcers. Diabetes is the most common cause of non-traumatic lower limb amputation ***(Armstrong et al., 2017).***

It is estimated that approximately 20% of hospital admissions among patients with DM are the result of DFU. Indeed, DFU can lead to infection, gangrene, amputation, and even death if necessary, care is not provided ***(Snyder et al., 2009).***

The optimal therapy for diabetic foot ulcers remains ill-defined. Saline-moistened gauze has been the standard method; however, it has been difficult to continuously maintain a moist antiseptic wound environment with these dressings. This has led to the development of various hydrocolloid wound gels, which provided more consistent moisture retention. Refinements in topical ointments have resulted in the addition of various pharmacological agents including growth factors and enzymatic debridement compounds. Hyperbaric oxygen therapy and culture skin substitutes are other wound therapies that have been advocated. All these therapies are associated with significant expense and are being utilized in some situations without sufficient scientific evidence demonstrating their efficacy. Therefore, the search for an efficacious, convenient, and cost-effective therapy continues.

Silver nanoparticles wound dressing is a novel method of treating chronic diabetic foot ulcers. It involves the controlled application of silver nanoparticles (Ag4O4) in the form of an alkaline water-based gel (hydrogel) to the wound site. has been shown to be an effective way to accelerate healing of various wounds ***(Verma, 2018).***

Till today, extremely limited data is available on the role of Silver nanoparticles wound dressing in healing of diabetic foot ulcers. Therefore, we endeavor to put forward a study to evaluate the role of Silver nanoparticles wound dressings in healing of diabetic foot ulcers.

AIM OF THE WORK

The aim of this study is to compare wound outcome, limb salvage, healing time of diabetes related foot ulcers and cost effectiveness in terms of duration of hospital stay between Silver nanotechnology dressings and Standard moist wound therapy (SMWT) in management of diabetic foot ulcers.

Chapter 1

NORMAL WOUND HEALING PROCESS AND IMPAIRED HEALING PROCESSES IN DIABETES

Wound healing is a dynamic and complex biological process that can be divided into four partly overlapping phases which are hemostasis, inflammation, proliferative and remodeling phases. These phases involve many cell types, extracellular components, growth factors and cytokines ***(Boulton et al., 2013).***

Diabetic foot ulcers (DFUs) are one of the most common and serious complications of diabetes mellitus as wound healing is impaired in the diabetic foot.

Diabetes mellitus causes impaired wound healing by affecting one or more biological mechanisms of these processes. Most often it is triggered by hyperglycemia, chronic inflammation, micro and macro-circulatory dysfunction, hypoxia, autonomic and sensory neuropathy, and impaired neuropeptide signaling. In addition, several anatomical foot deformities predisposing to the development of DFUs have also been associated with impaired wound healing. Moreover, diabetes affects the immune response and lowers resistance to infection. Understanding these mechanisms helps in specifically targeted treatment of diabetic foot ulcers ***(Dinh et al., 2005).***

Normal wound healing phases

Haemostasis

Hemostasis is the first step of tissue repair and platelets play a major role in this step. Exposure of circulating platelets to collagen of the injured tissue leads to their activation, aggregation, and adhesion to the damaged endothelium. Upon activation of the coagulation process, fibrinogen is converted to fibrin and eventually the thrombus and the provisional extracellular matrix is formed. Activated platelets release proteins that induce migration and adhesion of neutrophils and monocytes, as well as several growth factors that promote wound healing ***(Pradhan et al., 2009, Daniel et al., 2014).***

Inflammatory Phase

The inflammation phase of the wound healing is initiated immediately following injury, as inflammatory cells enter the wound site. The first cells that infiltrate the injured tissue are neutrophils. Adhesive molecules on the vascular endothelial surface surrounding the damaged tissue are activated and thus neutrophils adhere to the endothelium. Neutrophils then move through ruptured capillaries or through spaces between endothelial cells ***(Dinh et al., 2012).***

Neutrophils play a major role in infection control and tissue debridement. They are also involved with the wound healing process, as they produce several growth factors that promote cell

proliferation and proteases that degrade extracellular matrix. Circulating monocytes then rapidly differentiate into mature macrophages as they enter the tissue space. Activated macrophages phagocytose bacteria, foreign bodies, apoptotic neutrophils, and damaged tissue components from the wound. They also express a variety of pro-inflammatory mediators and cytokines. Resident mast cells also respond quickly to tissue injury and play an important role in the wound healing process. Mast cells undergo degranulation, which releases several cytokines that induce neutrophils recruitment, as well as proteinases that degrade extracellular matrix. T-lymphocytes enter the wound site in the late inflammatory phase and seem to have modulatory activity on tissue remodeling ***(Koh et al., 2011, Robert and William, 2014).***

The inflammatory phase of wound healing will persist if there is a need for it, ensuring that all excessive bacteria and debris from the wound is cleared. Protracted inflammation can lead, however, to extensive tissue damage, delayed proliferation, and result in the formation of a chronic wound ***(Daniel et al., 2014).***

Proliferative Phase

As the inflammation resolves and macrophages switch to an alternative activated anti-inflammatory form, the wound undergoes to the proliferative phase. Anti-inflammatory macrophages express a variety of anti-inflammatory mediators,

proteases, and protease inhibitors, as well as growth factors, such as vascular endothelial growth factor (VEGF) and tissue growth factor beta (TGF-β) that promote cell proliferation and protein synthesis. The provisional matrix begins becomes replaced by granulation tissue. Fibroblasts are activated by the growth factors released by macrophages and migrate into the wound along the provisional matrix. They then start to proliferate and produce collagen and other extracellular matrix molecules ***(Koh et al., 2011).***

Fibroblast proliferation is sustained by the angiogenesis of new capillaries. The development of new blood vessels from a pre-existing capillary network is required to provide oxygen and nutrients to the rapidly proliferating cells within the healing wound. Vasculogenesis, on the other hand, is the de novo formation of new blood vessels by recruitment of precursor endothelial progenitor cells (EPCs) from the bone marrow. EPCs are a population of adult stem cells with the ability to differentiate into epithelial cells and to promote endothelial regeneration and neovascularization in response to tissue ischemia ***(Brem et al., 2007).***

During the early proliferative phase new capillaries organize into a microvascular network throughout the granulation tissue. During the latter phase of healing process, the density of blood vessels diminishes. Granulation tissue, which derives from the characteristic granular appearance that results from the presence of ample new capillaries, is the new

connective tissue. This forms during the healing process and consists of fibroblasts, endothelial cells, inflammatory cells, extracellular matrix components and new blood vessels ***(Drela et al., 2012).***

Concurrent with the formation of granulation tissue, keratinocytes migrate from the wound edges or around skin appendages over the new matrix and start to re-epithelialize ***(Dinh et al., 2012).***

Epithelialization and Remodeling

The remodeling phase of the wound healing starts at about 2–3 weeks after initial injury and granulation tissue gradually transforms into mature scar tissue. The density of blood vessels decreases, and collagen is remodeled and organized. During the remodeling phase there is continual new collagen synthesis and collagen degradation. Wound contraction may also occur, during which myofibroblasts decrease the size of the wound by drawing the edges of the wound toward one another ***(Pradhan et al., 2009).***

Important factors in wound healing

1. Nutrition

Malnutrition adversely affects healing by prolonging inflammation, inhibiting fibroblast function, and reducing angiogenesis and collagen deposition. There are many essential

nutrients which are important for wound healing, including vitamin A (involved in epidermal growth), carbohydrates (for collagen synthesis) and omega-3 fatty acids (modulate arachidonic acid pathway) ***(Campos et al., 2008).***

2. Hypoxia

All wounds are hypoxic to some extent as their local vascular supply is disrupted. While a degree of hypoxia is required to facilitate re-epithelialization, sufficient oxygen is an essential requirement for wounds to heal ***(Kurz et al., 1996)***.

3. Infection

Wound infection causes delayed primary closure

4. Immunosuppression

Patients with HIV, cancer and malnutrition all have a degree of immunosuppression which can lead to delayed wound healing. In addition, any drugs which impair the inflammatory response can impede the healing cascade. Oral steroids, such as prednisolone, have been shown to decrease cytokine concentrations during wound repair, leading to reduced collagen deposition.

5. Chronic disease

Any chronic disease which affects the cardiorespiratory system may adversely affect the supply of oxygen and other

nutrients required for wound healing. Patients with diabetes have significantly impaired wound healing as they are relatively immunocompromised and higher blood glucose levels affect leucocyte function. Diabetes also causes long-term microvascular damage which affects both tissue oxygen levels and the supply of nutrients ***(Tsioufis et al., 2012).***

Chapter 2

DIABETIC FOOT ULCERS

DFU is a quite common presentation in patients with diabetic feet and once an ulcer has developed, there is an increased risk of ulcer progression that may ultimately lead to amputation ***(Leone et al., 2012).***

Pathophysiology and risk factor

Minor trauma leading to cutaneous ulceration is the precipitating event for diabetic foot problems. The presence of neuropathy, vascular insufficiency and an altered response to infection make the patient with diabetes more prone to ulceration and contribute to the chronicity of diabetic foot ulcers. Moreover, oxidative stress, altered inflammation responses and impaired wound healing have also emerged as critical factors ***(Jeffcoate et al., 2018).***

The classic chronic ulcer in diabetes mellitus is a relatively small, punctate wound that lies on the plantar surface beneath a deformed metatarsal head. This site is most often affected because of neuropathy and anatomic changes in the arch. Because of insensitivity, pressure is a major contributor to these wounds. In addition, the tips of the toes may develop pressure-related ulcers due to clawing, and the toes may develop gangrene from microvascular disease or micro emboli.

Ill-fitting shoes may lead to wounds in other areas of the foot. Once a wound develops, it often remains open for prolonged periods. The ulcer develops a rim of raised epithelium with some pale granulation tissue in the center. There is often callus formation around the ulcer. It is not uncommon for the wound to develop surrounding cellulitis, because there tends to be reduced ability to control local colonization of bacteria.

Unfortunately, there is also a tendency for the wound infection to ‘‘invade’’ inside the foot and travel along plantar fascial planes. For instance, a relatively small metatarsal wound may track well inside the foot. A plantar ulcer may track all the way through the foot to the dorsal side. In a similar fashion, a patient may present with a gangrenous toe that requires amputation. After the amputation, the wound between the remaining toes fails to heal.

Later, the patient may develop cellulitis and probing the wound reveals a wound that dissects well into the foot. The next procedure is a trans metatarsal amputation that also does not readily heal. Finally, the patient may require a below-knee or even an above-knee amputation. Overall, the rate of lower limb amputation in patients with DM is 15 times higher than patients without diabetes.

Factors causing altered wound healing in DFU

1. Hyperglycemia

Hyperglycemia can lead to non-enzymatic glycation of collagen and other proteins and to the formation of advanced glycation end products (AGE). These end products reduce the solubility of the extracellular matrix and perpetuate the inflammatory alterations observed in diabetes. Hyperglycemia may increase available nutrients for bacteria and may also impair local defenses. Leukocytes have different forms of impaired function in a hyperglycemic environment ***(Pradhan et al., 2009).***

2. Peripheral neuropathy

Peripheral neuropathy results in insensitivity and loss of the protective sensation thus facilitating trauma. It also alters proprioception and thus the distribution of pressure under the foot on standing and walking. Moreover, loss of neural supply to the intrinsic muscles of the foot leads to an imbalance between the flexors and extensors, clawing of the toes and increased prominence of metatarsals heads leading to the development of high pressure zones on the plantar surface while walking or standing ***(Boulton et al., 2018).***

This combination of insensitivity and high pressures applied to the foot places the patient at a great risk of neuropathic ulceration. Patients may also have peripheral autonomic dysfunction which results in increased resting blood

flow. This autonomic neuropathy also leads to loss of sweat gland function leading to dry skin and predisposing the skin to cracking and infection. Repetitive high pressure leads to the formation of callus tissue beneath weight bearing areas. The presence of callus in an insensitive foot is highly predictive of subsequent foot ulceration ***(Boulton et al., 2018).***

3. Peripheral arterial disease

Diabetes induced peripheral arterial disease affects both small and large vessels. Reduced blood supply exacerbates the changes brought about by neuropathy and is a major factor in the etiology of ulceration. Impaired hyperemic response to inflammation, increased capillary permeability leading to edema formation and the loss of other regulatory responses alter the diabetes patient's response to injury. In addition, diabetic arteries are rigid due to the presence of excessive calcium in their intima and media (Monckeberg's sclerosis). This environment places the foot and ankle at the risk of ulceration ***(Boulton et al., 2018).***

As a result, the foot appears atrophic, evidenced by the lack of hair growth on the dorsum, cool temperature and thin shining atrophic skin. Patients usually complain of pain in the limb as the ischemia progresses due to the oxygen and nutrient deprivation to the tissues ***(Boulton et al., 2018).***

4. Infections

The damage resulting from neuropathy and ischemia predisposes to foot infection. Infection may be bacterial or fungal. These infections are usually polymicrobial and include aerobic gram positive cocci such as Staphylococcus aureus, gram negative bacilli such as Escherichia coli, Klebseilla species and Proteus species and anaerobes such as Bacteroides sp. and Peptostreptococcus sp. Biofilms, or passive infections, consist of bacterial colonies that form on the surface of chronic wounds. More than 60% of all infections are caused by biofilms ***(Ahmad, 2016).***

Early recognition, proper assessment, and prompt intervention and good follow up are vital for proper management of DFU.

5. Edema

The basement membrane thickening may also make leukocyte migration more difficult. This extracellular matrix deposition may ‘‘trap’’ inflammatory cells and may contribute to the increased tendency for infection.

6. Impaired Angiogenesis and Vasculogenesis

Angiogenesis and vasculogenesis have been also described to be impaired in the non-healing diabetic wound. EPCs are reduced in patients with diabetes at risk of foot