# INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects of insulin secretion and/or increased cellular resistance to insulin. Chronic hyperglycemia and other metabolic disturbances of DM lead to long-term tissue and organ damage as well as dysfunction involving various body systems ***(Jerry, 2009).***

The incidence of diabetes mellitus is increasing globally ***(Reiber, 2001)*** and elder diabetics had twice the risk of developing a foot ulcer, three times the risk of developing a foot abscess and four times the risk of developing osteomyelitis ***(Reed, 2004)*** Similarly diabetics are more prone to either local or higher amputations ***(Hall and DeFrances, 2003)***.

In 2001, approximately$10.9 billion was spent on diabetic neuropathy and associated complications as foot ulcers, up to 27% of total medical costs of diabetes ***(Gordois et al., 2003).***

Pathophysiologic factors involved in the development of diabetic foot ulcers are neuropathy, arterial insufficiency, musculoskeletal abnormalities, and poor wound healing. Microbial pathogens and poor nutrition also play a key role and compromise the healing process ***(Sumpio, 2000).***

The initiating injury may be from acute mechanical or thermal trauma or from repetitively or continuously applied mechanical stress ***(Peter et al., 2005).***

The initial management of diabetic patients consists of proper foot care to prevent ulcers. Feet should be kept always clean and dry. Patients with neuropathy should not walk barefoot and properly fitted shoes are essential. Glycemic control also is paramount in the prevention of diabetic neuropathy and the development of foot ulcers ***(Mark and Arthur et al., 2004)***.

Management of diabetic ulcers includes cleansing of the wound, debridement of any necrotic or gangrenous foreign bodies or exposed bone ***(Garyson et al., 1995)***, to reach a healthy base that will support granulation tissue and allow healing by secondary intention. ***(Chen et al., 2005)***

Initial regimen of antimicrobial treatment should usually be selected empirically, and then be modified on the basis of both the patient's clinical response and the results of culture and sensitivity testing ***(Teppler et al., 2000).***

Reconstructive surgery may also be indicated in patients with an unstable Charcot foot ***(Garapati and Weinfeld, 2004)***; however, many diabetic patients will need revascularisation to achieve timely and durable healing.

New treatments for diabetic foot ulcers continue to be introduced ***(Eldor et al., 2004),*** yet few are subjected to controlled or comparative studies of their efficacy ***(Sibbald and Mahoney, 2003),*** including the use of negative pressure dressings ***(Armstrong and Lavery, 2005),*** hyperbaric oxygen treatment ***(Roeckl et al., 2005),*** bioengineered skin equivalents ***(Saap et al., 2004),*** growth-factor therapy ***(Smiell et al., 1999)*** and bone-marrow-derived stem cells ***(Badiavas et al., 2003).***

The concept of stem cells originated at the end of the 19th century as a theoretical postulate to account for the ability of certain tissues (blood, skin, etc.) to self-renew for the lifetime of an organism even though they are comprised of short-lived cells ***(Paolo et al., 2008)***.

These are pluripotent cells in embryos ***(Jisun and Vincent, 2007)*** and can be isolated from normal blastocysts ***(Martin, 1981***).

Different types of stem cells could have different uses as in treatment of: Alzheimer's disease, Parkinsonism, degenerative conditions of bone and cartilage, and also for treatment of diabetic foot ulcers ***(Audrey et al., 1999)***.

In treatment of diabetic foot ulcers the transplanted stem cells have the ability to migrate to the damaged tissue sites and stimulate repairs by differentiating into skin-specific cells. In addition, experiments of influence of local application of

mesenchymal stem cells on cutaneous wound regeneration showing convertion into phenotypes of epidermal keratinocytes, sebaceous glands, follicular epithelial cells, and vascular endothelial cells by trans differentiation ***(Fu et al., 2007)***.

More general concerns regarding the use of stem cells, including the real risk of tumor formation following transplantation, also the wide array of social, political, legal, ethical, and economic issues must be considered ***(Bert et al., 2001).***

Yet, we remain convinced that the field of stem cell biology holds tremendous promise for furthering our understanding of the human body and our ability to treat its maladies ***(Yu et al., 2007).***

# Aim of the Work

To study advantages and disadvantages of treatment of diabetic foot ulcers using stem cells.

*Chapter (1)*

# PATHOGENESIS OF DIABETIC FOOT ULCERS

## Introduction

The most important complications of diabetes mellitus are neuropathy and diabetic foot. Manifestations of resulting complications range from simple to highly complex, including limb amputations and life-threatening infections. Foot infections in people with diabetes are common; this creates complex social problems owing to the financial burden resulting from the high cost of treatment and healing ***(Aguilar, 2009).***

In addition to severe morbidity, foot infections cause prolonged hospitalization and psychological and social problems for the patient and his family. Even though foot pathology in diabetic patients entails high medical costs, it also causes loss of productivity in patients ***(Ramsey et al., 1999)***. Portugal the International Diabetes Federation (IDF) said that the prevalence of DM around the world is 366 million ***(Mbanya, 2011)***.

On the other hand, the most common cause of non-traumatic amputations is diabetes mellitus, and 80% of these could be averted through adequate prevention and early intervention ***(Mbanya, 2011).***

Therefore, complications arising from diabetic foot represent an important medical challenge in growing proportions. Foot ulcerations represent 85% of all amputations. Hence, the association between ulcers and lower extremity amputations is patently obvious. Taking into account that the major risk factor leading to amputation is ulceration, around 15% of all foot ulcers will ultimately require amputation at some point ***(Mbanya, 2011).***

Other risk factors for amputation include a long history of diabetes, peripheral neuropathy and structural changes of the foot, peripheral vascular disease, poor glycemic control, a prior history of foot ulcers, previous foot surgery and/or amputation, retinopathy and nephropathy ***(Clayton et al., 2000, Aguilar & Rayo, 2000).***

Risk factors for the development of foot ulcers in diabetic patients should be evaluated from 3 different dimensions:

1. pathophysiology.
2. Anatomical and structural alterations
3. Environmental influences

**1. <u>Pathophysiology:</u>**

These changes occur at a biomolecular level and are caused by hyperglycemia, which leads to the development of neuropathy, as described in Fig 1. Over the past two decades considerable evidence has been accumulated to support the

potentially pathogenetic role of a number of mechanisms that lead diabetic persons to develop wounds. The major mechanisms are: ***(Aguilar, 2009)***

- Nerve hypoxia/ischemia
- Auto oxidative stress
- Polyol pathway overactivity
- Increased advanced glycation end-products
- Deficiency of gamma linolenic acid
- Protein kinase C, especially B-isoform increase
- Cytokines dysfunction
- Disorders of collagen molecules (elastin, proteoglycans)
- Endothelial dysfunction
- Mitochondrial dysfunction
- Growth factors deficiency
- Alteration of the immune mechanism
- Increased secretion of proteases

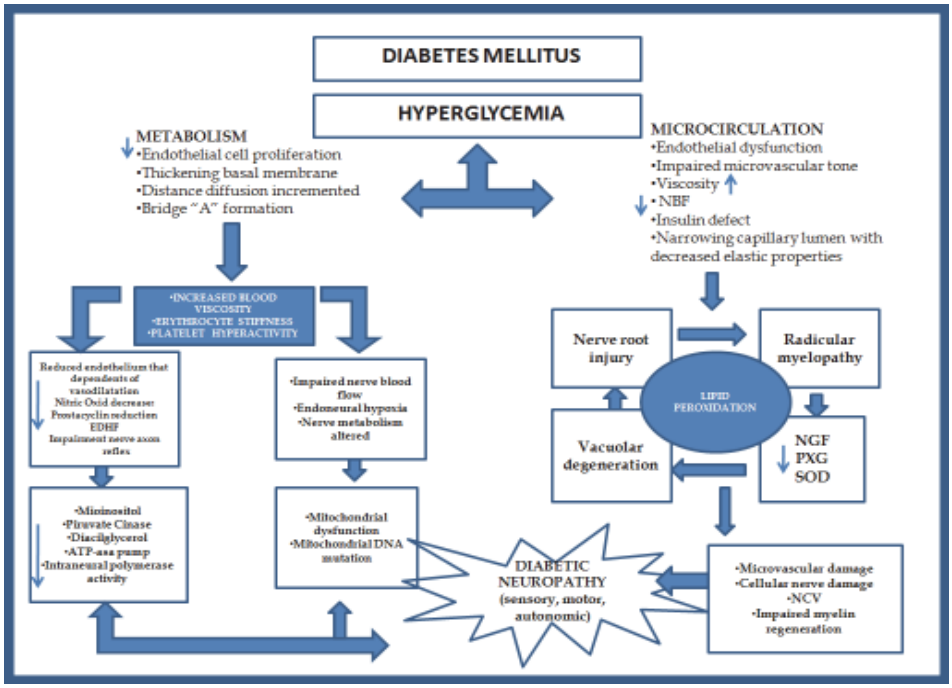


**Figure (1):** Role of different mechanisms in neuropathy.

The most important are oxidative stress and endothelium dysfunction; these mechanisms produce disorders in metabolism and microcirculation ***(Aguilar, 2009).***

EDFH= Endhotelium derived hyperpolarizing factor.

NBF = nerve blood flow.

NGF = nerve growth factors.

NVC = Nerve velocity conduction.

PXG = Glutathion Peroxidase.

SOD = Superoxide Dismutase.

Under normal circumstances, wound repair is a highly orchestrated event that involves the interaction of the elements described above. Each stage of the healing process entails this orchestrated effort ***(Aguilar, 2005)***.

The damaged tissue quickly releases tissue factor and other stimuli, such as expulsed collagen, to activate a variety of physical mechanical, biological or chemical events ***(Aguilar, 2005)***.

These changes cause damage to the nerve fiber and even peripheral vascular disease, which in turn takes its toll at the molecular level.

Endothelial dysfunction is the most serious impairment affecting microcirculation, owing to changes in the proliferation of endothelial cells, thickening of the basement membrane, decreased synthesis of nitric oxide, increased blood viscosity, alterations in microvascular tone and decreased blood flow ***(Aguilar, 2005).***

On the other hand the immune system is compromised by lowered leukocyte activity, inappropriate inflammatory response and the disruption of cellular immunity (inhibition of fibroblast proliferation, impairment of the basal layer of keratinocytes and reducing epidermal cell migration) ***(Aguilar 2005, Boulton , 2003).***

Another important factor that affects neuropathic foot microcirculation is the disability of the nerve axon reflex. The stimulation of the C-nociceptive fibers produces retrograde stimulation of adjacent fiber ***(Caselli, 2003)***.

These fibers instantly secrete a vasomodulator, such as substance P (SP), a calcitonine gene-related peptide (GCRP), neuropeptide Y (NPY), and histamine ***(Lyons, 2008).***

These peptides produce vasodilation (this response is known as Lewis Triple Flair Response) ***(Lyons, 2008).***

The Lewis response mechanism is:

Red spot due to capillary dilatation and flare due to redness in the surrounding area due to arteriolar dilatation mediated by axon reflex and wheal due to exudation of fluid from capillaries and venules ***(Lyons, 2008).***

This drawn up in Figure (2) for normal and diabetic patients.

The main substances are histamine and peptides. In the absence of this response the skin blood flow is affected when the injury occurs and this is one of the major factors related to impaired wound healing ***(Parkhouse, 1988).***

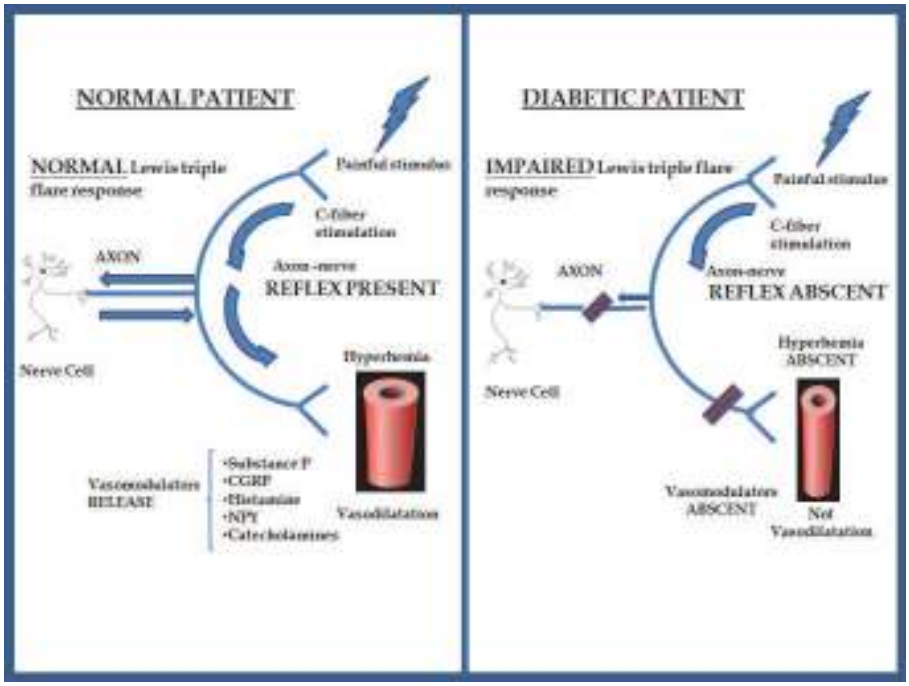


**Figure (2):** Injury and inflammation in normal subjects and diabetic patients.

The nerve axon reflex. Stimulation of C-nociceptive fibers produces retrograde stimulation of adjacent fibers to release vasomodulators. The results are hyperemia during injury or inflammation. The Lewis Triple Flare Response is absent in diabetic patients affecting wound healing ***(Lyons, 2008)*.**

## 2. Anatomical and structural alterations:

The anatomical and structural alterations that are the result of diabetic neuropathy are divided into three types: sensory, motor and autonomic.

## 2.1. Peripheral sensory neuropathy:

Close to 30-50% of all diabetic patients present peripheral sensory neuropathy. Sensory neuropathy is the most common predictor of foot ulceration in a patient with diabetes ***(Nather, 2011).***

The development of foot ulceration reported in sensory neuropathy occurs in 78% of cases. Peripheral sensory neuropathy initiates a series of events that together with peripheral motor and autonomic damage eventually result in foot ulceration ***(Nather, 2011).***

In a normal situation, treatment centers would tell the patient to continue walking or walk some with changes in gait. The recipients of this information are the sensory nerves. In diabetic foot, sensation is affected and the stimulus to refrain from ambulation does not exist. These are the causes that lead the patient to continue walking even when no sensation of pain is present, which in turn prolongs affectation and delays healing of the traumatized area. The damage to sensation that provides protection is the key element in the development of ulcers ***(Reiber et al., 1999).***

## 2.2. Peripheral motor neuropathy:

Motor neuropathy typically presents structural alterations of the dynamic anatomy of the foot and joints causing weakness and wasting of intrinsic muscles. This causes a loss

of balance in the gait because of damage to the muscles, as mentioned above, in addition to another characteristic: clawing of toes and plantar flexion of the metatarsal head ***(Carine et al., 2004).***

The atrophy of the interosseous and small intrinsic muscles of the foot acts to stabilize and hold the phalanges of the toes straight, as the long flexor and extensor tendons act through the insertions into the distal phalanges of the toes up into dorsiflexion, similar to a foot pressing the accelerator of the car.

Alterations in the morphology of the structure of the foot, toes, forefoot and limited joint mobility impaired the ability of the foot to absorb and redistribute the forces relayed to impact the ground while walking. Effects on the foot include the reduction of motion and changes to the angle of the subtalar and first metatarsophalangeal joints (MTPJ) ***(Carine et al., 2004).***

Vital musculoskeletal structures, such as equinus deformity through the shortening of the Achilles tendon and the collapse of the plantar fascia, facilitating abduct to adductor equine changes to the forefoot. In diabetic patients, the flexor tendons and extensor tips tend to be straight and rigid. If the intrinsic muscles are unable to do this, the toes shrink back to form what is called hammer toes and favor the thrust of one toe over another or a toe on the metatarsal head with the weight

forced to the anterior surface with high force ***(Carine et al., 2004).***

On the other side, the contraction that the hammer toes causes on the plantar fat pad and the metatarsal heads reduces soft tissue plantar MTPJ, making them more susceptible to fracture of the skin, including the bone next to the formation of traumatic ulcers, owing to inappropriate weight loads. The mechanism is related to the high pressure exerted on the foot that occurs during the gait, in turn caused by motor neuropathy, which itself in turn causes structural changes in the anatomy and sliding of the fat pads of the foot, in addition to occasional blows received ***(Carine et al., 2004).***

Figure (3) presents three different images at different stages of changes in architecture of the foot, allowing us to better understand the condition. In addition to the shortening and thickening of the joints, the decreased capacity of distribution of plantar pressure in DM patients contributes to the development of high foot pressure and ensuing ulcerations ***(Lyons et al., 2006)***.

Excessive pressure and structural deformities in individuals with neuropathy is a prerequisite for the development of wounds. Consequently, structural changes and of pressure favors the formation of calluses on various prominent parts of the foot, including the plantar region, the heel, the big toe, etc. The structure of the foot is a major