INTRODUCTION AND AIM OF THE WORK

Diabetes mellitus refers to a range of conditions characterized by an elevation of the blood glucose level, and the disorder is divided into two principal varieties; Type 1 is due to an autoimmune attack on the pancreatic B-cells that results in their destruction and Type 2 diabetics exhibit impairments of both insulin secretion and insulin action. Type 2 diabetes is reaching epidemic proportions in Western societies and is predicted to affect 300 million people worldwide by 2025 ***(Harris, 1998).***

Sustained hyperglycaemic values are the underlying pathological factor in the development of secondary diabetic complications. Excessive blood sugar values result in the development or progression of microvascular (diabetic retinopathy, diabetic nephropathy and diabetic neuropathy) and macrovascular (coronary heart disease, peripheral arterial occlusive disease, cerebral arterial occlusive disease) complications ***(Harris, 1998).***

Diabetic foot disease is a major health problem, which concerns 15% of the 200 million patients with diabetes worldwide. Major amputation, above or below the knee, is a feared complication of diabetes. More than 60% of non-traumatic amputations in the western world are performed in the diabetic population. Many patients, who undergo an

amputation, have a history of ulceration ***(Dalla and Faglia, 2006).***

Wound infections must be diagnosed clinically on the basis of local (and occasionally systemic) signs and symptoms of inflammation. Laboratory (including microbiological) investigations are of limited use for diagnosing infection, except in cases of osteomyelitis. Imaging studies may help diagnose or better define deep, soft-tissue purulent collections and are usually needed to detect pathological findings in bone. Plain radiography may be adequate in many cases, but MRI (in preference to isotope scanning) is more sensitive and specific, especially for detection of soft-tissue lesions. ***(Lipsky et al., 2004)***

Diabetic foot wound care in all stages needs multidisciplinary management to control mechanical, wound, microbiological, vascular, metabolic and educational aspects. Achieving good metabolic control of blood glucose, lipids and blood pressure is important in each stage, as is educational control to teach proper foot care appropriate for each stage. Aggressive management of diabetic foot ulceration will reduce the number of feet proceeding to infection and necrosis and thus reduce the number of major amputations in diabetic foot patients ***(Edmonds, 2008)***

An area of research that holds promise for the treatment of difficult-to heal diabetic ulcers is stem cell application ***(Falanga et al., 2006).***

Stem cells are the self renewing progenitors of several body tissues and are classified according to their origin and their ability to differentiate. Current research focuses on the potential uses of stem cells in medicine and how they can provide effective treatment for a range of diseases ***(Choumerianow et al., 2008).***

Adult stem cells are generally limited in differentiating into different cell types. However evidence have been issued that they have extraordinary flexibility to be trans-differentiated into other cell types ***(Jiang et al., 2002).***

The potential advantage of using stem cells from an adult is that the patient's own cells are reintroduced to the patient which means that the cells would not be rejected by the immune system ***(NIH, 2005).***

Human bone marrow is an ideal source of stem cells because it is readily available and easily secured with a relatively simple procedure. Also, bone marrow derived stem cells don't have antigens which would eliminate the potential for rejection ***(Yang., 2003).***

Progenitor cells (EPCs) are the key cellular effectors of postnatal vasculogenesis and play a central role in wound healing. In diabetes, there is a significant impairment in the number and function of circulating and wound-tissue EPC ***(Gallagher etal, 2006).***

Transplantation of autologus bone marrow stem cells could constitute a novel, clinically feasible and safe therapy for patients with diabetic foot ***(Bartsch et al., 2005).*** This can be done by transplanting a population of progenitor cells locally into the wound periphery which may be a useful and potentially safe adjunct to wound simplification and closure (***Roger et al., 2008).***

Aim of the work

Our aim is to evaluate the efficacy of the use of autologus undifferentiated bone marrow mononuclear layer transplantation as a line of therapy for diabetic foot ulcer cases.

Chapter 1
Diabetic Foot Ulcer

EPIDEMIOLOGY

Diabetes is one of the foremost causes of death in many countries and a leading cause of blindness, renal failure, and nontraumatic amputation. Global prevalence of diabetes in 2003 was estimated to be 194 million (***Singh et al., 2005***).

An estimated 14.6 million persons are currently diagnosed with the disease, while an additional 6.2 million people who have diabetes remain undiagnosed; this represents a six fold increase in the number of persons with diabetes over the past four decades (***Harris, 1998).***

Diagnosed diabetes is most prevalent in middle-aged and elderly populations, with the highest rates occurring in persons aged 65 years and older (***Frykberg et al., 1998).***

The prevalence of diabetes mellitus is growing at epidemic proportions in the United States and worldwide (***Boulton et al., 2005***).

By 2030, this figure is predicted to rise to 366 million due to longer life expectancy and changing dietary habits (***Wild et al., 2004).***

Foot disorders are a major source of morbidity and a leading cause of hospitalization for persons with diabetes. Ulceration,

infection, gangrene, and amputation are significant complications of the disease***. (Singh et al.2005).***

RISK STRATIFICATION

Following a thorough diabetic foot examination, the patient may be classified according to a cumulative risk category. This enables the physician to design a treatment plan and determine whether the patient is at risk for ulceration or amputation. Several risk stratification schemes have been proposed, assigning different weights to important risk factors for ulceration including peripheral neuropathy, arterial insufficiency, deformity, high plantar pressures, and prior history of ulceration or amputation (***Ramsey et al., 1999***).

Although no one system has been universally adopted to predict complications, Table 1 presents a simplified risk stratification that has been endorsed by an international consensus group and others (***Peters and Lavery, 2001***).

Table (1): Risk Categorization system.

Category	Risk Profile	Evaluation Frequency
0	Normal	Annual
1	Peripheral neuropathy (LOPS)	Semi-annual
2	Neuropathy, deformity and/or PAD	Quarterly
3	Previous ulcer or amputation	Monthy to quarterly

1. Risk for Ulceration:

One of the most common complications of diabetes in the lower extremity is the diabetic foot ulcer. An estimated 15% of patients with diabetes will develop a lower extremity ulcer during the course of their disease (***Reiber, 2001***).

Hospital discharge data indicate that the average hospital length of stay for diabetic patients with ulcer diagnoses was 59% longer than for diabetic patients without ulcers. (***Reiber et al., 1995***). While 7% to 20% of patients with foot ulcers will subsequently require an amputation, foot ulceration is the precursor to approximately 85% of lower extremity amputations in persons with diabetes (***Margolis et al., 2005***).

Risk factors identified include peripheral neuropathy, vascular disease, limited joint mobility, foot deformities, abnormal foot pressures, minor trauma, a history of ulceration or amputation, and impaired visual acuity. These and other putative causative factors are shown in Figure (1) (***Boulton et al., 2004).***

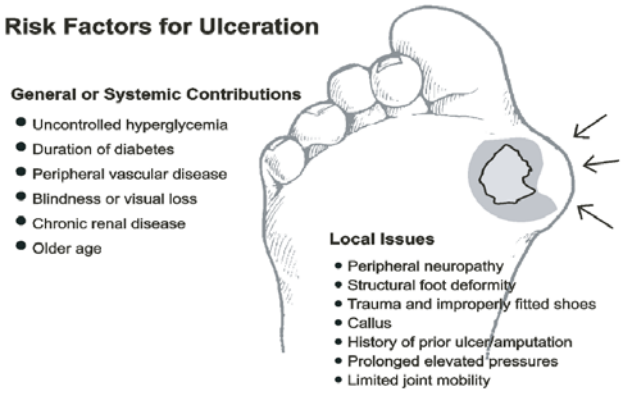

Figure (1): Risk factors for Ulceration ***(Boulton et al., 2004).***

Peripheral sensory neuropathy in the face of unperceived trauma is the primary factor leading to diabetic foot ulcerations (***Abbott et al., 2002***).

Approximately 45% to 60% of all diabetic ulcerations are purely neuropathic, while up to 45% have neuropathic and ischemic components; the differences between them as shown in table 2 (***Reiber et al., 1999***).

Table (2): Clinical features of neuropathic and ischaemic foot

Neuropathic	Ischaemic (neuroischaemic)
• Warm with intact pulses	• Pulseless, not warm
• Diminished sensation; callus	• Usually diminished sensation
• Ulceration (usually on tips of toes and plantar surfaces under metatarsal heads)	• Ulceration (often on margins of foot, tips of toes, heels)
• Sepsis	• Sepsis
• Local necrosis	• Necrosis or gangrene
• Oedema	• Critical ischaemia (urgent attention) foot pink, painful, pulseless, and often cold
• Charcot's joints	

The effects of motor neuropathy occur relatively early and lead to foot muscle atrophy with consequent development of hammertoes, fat pad displacement, and associated increases in plantar forefoot pressures (***Greenman et al., 2005***).

A large prospective population-based study found that elevated plantar foot pressures are significantly associated with neuropathic ulceration and amputation (***Lavery et al., 2003***).

Peripheral arterial disease rarely leads to foot ulcerations directly. However, once ulceration develops, arterial

insufficiency will result in prolonged healing, imparting an elevated risk of amputation ***(Gibbons, 2003).***

Mechanisms of Injury:

The multifactorial etiology of diabetic foot ulcers is evidenced by the numerous pathophysiologic pathways that can potentially lead to this disorder (***Rathur and Boulton, 2005***).

Among these are **two common mechanisms** by which foot deformity and neuropathy may induce skin breakdown in persons with diabetes (***Knox et al., 2000***).

The first mechanism of injury refers to prolonged low pressure over a bony prominence (i.e., bunion or hammertoe deformity). This generally causes wounds over the medial, lateral, and dorsal aspects of the forefoot and is associated with tight or ill-fitting shoes.

Shoe trauma, in concert with loss of protective sensation and concomitant foot deformity, are the leading event precipitating foot ulceration in persons with diabetes (***Lavery et al., 1998***).

When an abnormal focus of pressure is coupled with lack of protective sensation, the result can be development of a callus, blister, and ulcer (***Lavery et al., 2003***).

The other common mechanism of ulceration involves prolonged repetitive moderate stress (***Brand, 1988***).

This normally occurs on the sole of the foot and is related to prominent metatarsal heads, atrophied or anteriorly displaced fat pads, structural deformity of the lower extremity, and prolonged walking. Figure (2) summarizes the various pathways and contributing factors leading to diabetic foot complications.

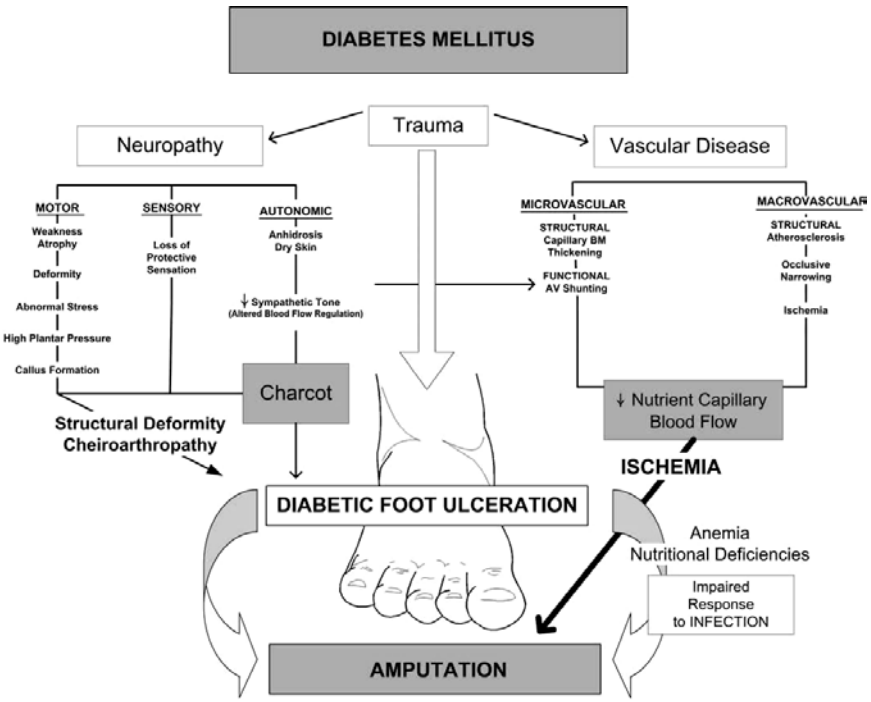

Figure (2): Diabetes mellitus is responsible for a variety of foot pathologies contributing to the complications of ulceration and amputation. Multiple pathologies may be implicated, from vascular disease to neuropathy to mechanical trauma ***(Brand, 1988).***

2. Risk for Infection

Infections are common in diabetic patients and are often more severe than infections found in nondiabetic patients. Persons with diabetes have an increased risk for developing an infection of any kind and a several-fold risk for developing osteomyelitis (***Shah and Hux, 2003***).

With an incidence of 36.5 per 1,000 persons per year, foot infections are among the most common lower extremity complications in the diabetic population (excluding neuropathy), second only to foot ulcers in frequency (***Lavery et al., 2003***).

Autonomic neuropathy often results in dry skin with cracking and fissuring, creating a portal of entry for bacteria (***Shaw and Boulton, 1997***).

Hyperglycemia, impaired immunologic responses, neuropathy, and peripheral arterial disease are the major predisposing factors leading to limb-threatening diabetic foot infections (***Lipsky et al., 2004).***

Uncontrolled diabetes results in impaired ability of host leukocytes to fight bacterial pathogens, and ischemia also affects the ability to fight infections because delivery of antibiotics to the site of infection is impaired. Consequently, infection can develop, spread rapidly, and produce significant and irreversible tissue damage (***Caputo, 1994***).

Additionally, attempts to resolve any infection will be impaired due to lack of oxygenation and difficulty in delivering antibiotics to the infection site. Therefore, early recognition and aggressive treatment of lower extremity ischemia are vital to lower limb salvage (***Sumpio et al., 2003***).

Even in the presence of adequate arterial perfusion, underlying peripheral sensory neuropathy will often allow the progression of infection through continued walking or delay in recognition (***Eneroth et al., 1999***).

3. Risk for Amputation

Generally, the lower extremity amputation rate is 15 to 40 times higher in the diabetic versus non diabetic populations, and

the rate is at least 50% higher in men versus women (***Moss et al., 1992***).

Survival rates after amputation are generally lower for diabetic versus non diabetic patients (***Larsson et al., 1998***).

Researchers have reported a 50% incidence of serious contralateral foot lesion (i.e., ulcer) following a lower extremity amputation, and a 50% incidence of contralateral amputation within 2 to 5 years of a lower extremity amputation (***Larsson et al., 1998***).

The same risk factors that predispose to ulceration can also generally be considered contributing causes of amputation, albeit with several modifications (Fig 3).

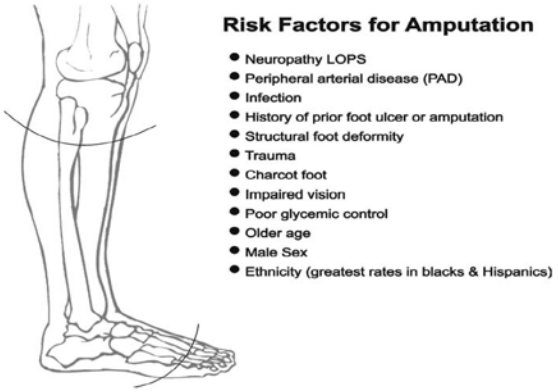

Figure (3): Risk factors of Amputation ***(Gibbons, 2003).***

Impairment of arterial perfusion may be an isolated cause for amputation and a predisposing factor for gangrene. Early diagnosis, control of risk factors, and medical management as well as timely revascularization may aid in avoiding limb loss (***Gibbons, 2003).***

While infection is not often implicated in the pathway leading to ulceration, it is a significant risk factor in the causal pathway to amputation (***Reiber et al., 1999***).

Lack of wound healing, systemic sepsis, or unresolved infection can lead to extensive tissue necrosis and gangrene, requiring amputation to prevent more proximal limb loss. This includes soft tissue infection with severe tissue destruction, deep space abscess, or osteomyelitis. Adequate debridement may require amputation at some level as a means of removing all infected material (***Frykberg, 2003***).

Amputation has also been associated with other diabetes-related comorbidities such as nephropathy, retinopathy, and cardiovascular disease (***Resnick et al., 2004***).

Aggressive glucose control, management of associated comorbidities, and appropriate lower extremity care coordinated in a team environment may indeed lower overall risk for amputation (***Driver et al., 2004***).

The best predictor of amputation is a history of previous amputation. A past history of a lower extremity ulceration or amputation increases the risk for further ulceration, infection, and subsequent amputation ***(Boyko et al., 2002*)*.***

Re-amputation can be attributed to disease progression, non healing wounds, and additional risk factors for limb loss that develop as a result of the first amputation. Tragically, the 5-year survival rate after a diabetes-related lower extremity amputation has been reported to be as low as 28% to 31% ***(Aulivola et al., 2004).***

CLINICAL ASSESSMENT OF THE DIABETIC FOOT

The evaluation of the diabetic foot involves careful assimilation of the patient's history and physical findings with the results of necessary diagnostic procedures (Pathway 1). Screening tools may be valuable in evaluating the patient and determining risk level ***(Robert et al., 2006).***

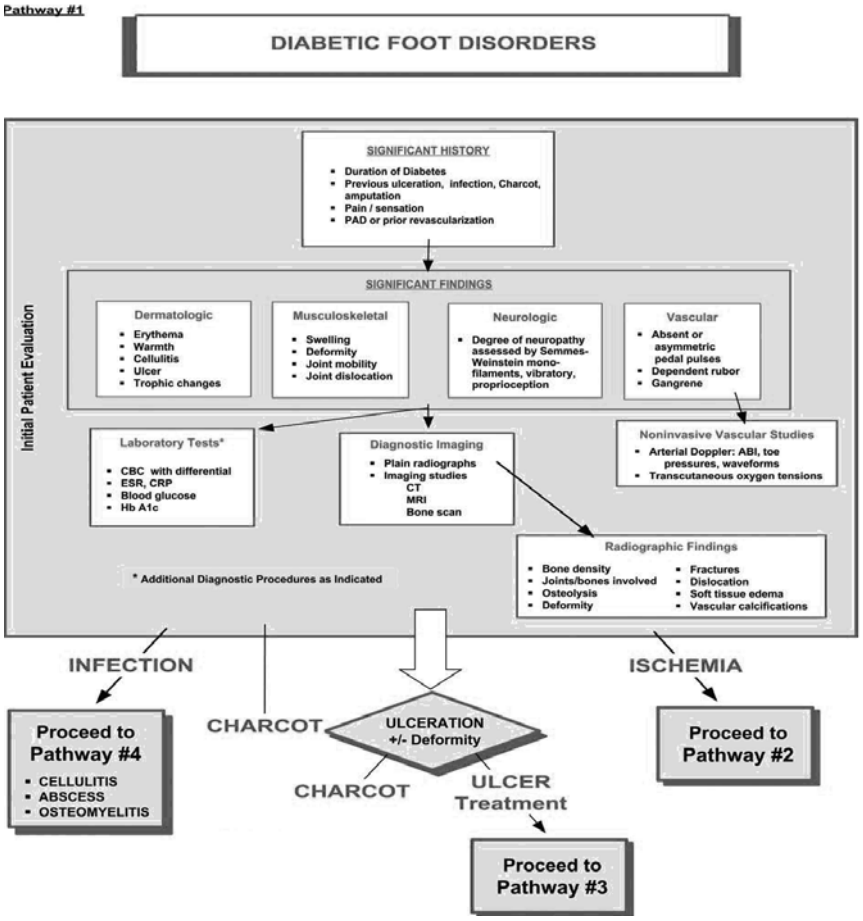

Fig (4) : Pathway (1): Clinical assessment of the diabetic foot ***(Robert et al., 2006).***