"EFFICACY OF TOPICAL STATIN MEDICATION ON DIABETIC FOOT ULCER WOUND HEALING DYNAMICS- A RANDOMIZED CONTROLLED TRIAL FOR PERIOD OF ONE YEAR"

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LIST OF ABBREVIATIONS USED

DM	-	Diabetes Mellitus
DFU	-	Diabetic foot ulcer
PAD	-	Peripheral arterial disease
HbA1c	-	Glycosylated Haemoglobin
HMG-CoA reductase	-	3-Hydroxy-3-methylglutaryl-Coenzyme A reductase
LDL	-	Low density lipoprotein
TGF	-	Transforming growth factor
PDGF	-	Platelet derived growth factor
VEGF	-	Vascular endothelial growth factor
IGF-1	-	Insulin like growth factor -1
MMP	-	Matrix metalloproteinases
NO	-	Nitric Oxide
ABPI	-	Ankle Brachial Pressure Index
ROS	-	Reactive Oxygen species
TCC	-	Total Contact cast
IWDGF	-	The International Working Group on the Diabetic Foot
IL-6	-	Interleukin 6
IFN-gamma	-	Interferon gamma
TFN- alpha	-	Tumour necrosis factor alpha

IL-10	-	Interleukin 10
ICAM-1	-	Intercellular adhesion molecule-1
LFA-1	-	Lymphocyte function-associated antigen-1
MAC-1	-	Monocyte chemotactic protein-1
FPP	-	Farnesyl pyrophosphate
K6	-	Keratin 6
MRSA	-	Methicillin resistant Staphylococcus aureus
OATP	-	Organic anion transporting polypeptide
HBEGF	-	Heparin binding epidermal growth factor
HIV	-	Human immunodeficiency virus
HBsAg	-	Hepatitis B surface antigen
SD	-	Standard deviation
SES	-	Socioeconomic scale
BMI	-	Body mass index
Hb	-	Haemoglobin
FBS	-	Fasting Blood sugar
TLC	-	Total Leucocyte count
SE	-	Standard error
P.gingivalis	-	Porphyromonas gingivalis
PEDIS	-	Perfusion Extent Depth Infection and Sensation

ABSTRACT

Efficacy of topical statin medication on diabetic foot ulcer wound healing dynamics- A randomized controlled trial for period of one year

Back ground

DM is a widespread, non-communicable disease seen among world population.[1]Amongst the complication of DM, DFU is seen quite often. Diabetic foot disorders consists of the ulcers over the foot related with infection, PAD and neuropathy. It is a noteworthy reason for lower extremity amputation. Infection of DFU is one among the common complications seen in DM patients. One of the major problem in health sector is antibiotic resistance. Increasing antibiotic resistance has restricted the use of systemic antibiotics. In this situation topical antiseptics with broad spectrum of activity and minimal or no resistance plays an important role in management of infected ulcers.

One of the newer modalities for wound healing are topical statins that have been conventionally used for treatment against hyperlipidemia. Statins can facilitate healing of ulcers because of their wide spectrum of effects comprising of immunomodulatory, antioxidative, anti-inflammatory, antibacterial actions as well as enhancement of vascular function. Most of the studies done to evaluate the results of topical statin therapy on diabetic wounds, have been executed over animal and invitro human tissue. The lack of clinical studiesalongwith the need to explore the potential of topical statins in treating diabetic foot ulcers made us to compare healing of diabetic foot ulcers, between topical atorvastatin with saline and saline dressing alone.

Objectives

To test the effectiveness of topical statin medication along with conventional therapy (saline dressing) versus conventional therapy alone by comparing mean percentagewound area reduction in diabetic foot ulcers.

Methods

This hospital based randomised controlled study was done between Jan 2019 to Dec 2019.Total 60 cases of chronic diabetic foot ulcers between 18-70 years were selected and randomized using a computer generated randomization chart in to two groups, group A(test group) where topical atorvastatin emulgel with normal saline dressing was done and group B(control group) where dressing was done with saline done. Demographic data including duration of disease, hypertension, neuropathy and HbA1c levels were recorded. The wound healing was calculated as mean reduction in ulcer area and mean percentage reduction in ulcer area. The wound healing was then compared between two groups.

Results

Of the 60 patients, 52(86.67%) were males and 50(83.33%) aged more than 50 years. 51 of 60 or 85% belonged to low socio economic status. All of these parameters and ulcer parameters such as mode of onset, site of ulcer showed no significant difference in distribution among the groups. Presence of neuropathy, hypertension and HbA1c levels which are implicated as risk factors for DFU healing did not show statistically significant difference in distribution between groups. Ulcer healing was faster in group A (topical atorvastatin with saline dressing group) compared to group B (saline dressing group), mean percentage reduction in ulcer area was 31.08% and 16.11% in group A and group B respectively (p-value=0.0001). In

the control group, there was a decrease in bacterial colonization with number of patients with positive culture report on day 0 being 16(53.33%) and those on day 14 being 7(23.33%). This was statistically significant(p value=0.004). In the test group, the number of patients with positive culture report on day 0 were 19(63.33%) and those on day 14 were 5(16.67%). This was statistically significant reduction in bacterial population in the test group(p=0.0001) which was more significant than the effect showcased by the control group.

Conclusion

When topical atorvastatin along with normal saline was applied to diabetic foot ulcers, a significant decrease in ulcer area with respect to dressing with only normal saline was noted. There was also a significant decrease in bacterial colonization after application of topical atorvastatin with saline as compared to saline alone.

Keywords Diabetic foot ulcer, topical atorvastatin, wound healing

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INTRODUCTION

Diabetes mellitus is one of the major non-communicable diseases worldwide with a plethora of clinical manifestations. It is a major problem in healthcare and a worldwide public threat which has increased dramatically in last 20 years.[1]The prevalence of diabetes worldwide is 6.4% and is likely to rise to 7.7% by 2030.[2] The prevalence in the population aged between 20-70 years is supposed to increase from 285 million in 2010 to 438 million by year 2030.[3]

Diabetics are predisposed to many complications, the notable ones include diabeticneuropathy and peripheral vascular disease both of which lead to diabetic foot ulcers.[2] These ulcers have a complex pathogenesis comprising of vascular, mechanical, oxidative, inflammatory,endothelial, nutritive factors.[4]

Diabetic foot is most common cause of hospitalization in diabetics.[5] The lifetime risk of foot ulcers in diabetic patients appears to range from 15-25%.[6]Diabetic foot ulceration leads to physical and emotional distress along with productivity and financial loss, thus impacting on overall quality of life.[7] Impaired healing and wound infection complicate ulcers, placing the patients at higher risk for osteomyelitis, gangrene and eventuallylimb amputation.[8]Nearly three quarters of lower limb amputations are because of diabetic foot ulcers.[9] It is estimated that every 30 seconds, one leg is amputated due to diabetic foot ulcer worldwide.[10]

Diabetes being a multiorgan systemic disease, requires a holistic approach for management of diabetic foot ulcer in order to receive optimal outcomes. Besides a strict glycaemic control, management of these ulcers includes an initial assessment of wound healing strategies and a thorough knowledge of the causative factors responsible for improper wound healing. Effective wound healing mandates an adequate debridement, antibiotic therapy and optimum non-weight bearing. Despite

extensive studies and technological developments, there is presently no solitarytreatment modality with quantifiable clinical outcome present.Several modalities have been tried on diabetic foot ulcers locally for enhancing the wound healing ability such as topical phenytoin, insulin, honey, etc with newer approaches including negative pressure therapy on wounds, hyperbaric oxygen treatment. One such novel therapeutic modality for wound healing that has reflected promising outcome in the recent times are statins.

Statins like Atorvastatin, Simvastatin, Rosuvastatin, Pravastatin, etc are HMG-CoA reductase inhibitors that reduce cholesterol synthesis and also reduce LDL levels in blood, thus playing a major role in treating hyperlipidemias.Recently statins have reflected diverse pleiotropic effects other than lipid lowering activity. They have shown promising evidence as new therapeutic modalities for conditions like psoriasis, alopecia, wound healing such as in vascular cutaneous ulcers, pressure ulcers, periodontitis.[11] The wide spectrum statin effects comprise of immunomodulatory, antioxidative, anti-inflammatory, antibacterial actions and enhancement of vascular function.Various animal studies have expressed benefits of statins on wound healing.[11]

Statins can interfere with the synthesis of bacterial protein and inhibit manybiosynthetic and bacterial cellular processes. This attributes to statins its antibacterial

property, making them more alluring to use for wound healing. The conventional antibiotic side effects or fear of antibiotic resistance can also be avoided.[12]Oral statins, similar to other systemic medications, can cause a number of adverse effects such as myopathy and hepatotoxicity. So delivery of statins topically may be a feasible option other than oral intake to maximize their effects and decrease risk of sideeffects.[11]The

use of statins in wound healing is thus, rational and promising carrying a good prognostic impact. Studies have been done to observe the effects of wound healing in vascular cutaneous ulcers, pressure ulcers, psoriasis, etc.Many studies have also been done to evaluate the results of topical statin therapy on diabetic wounds over animal and invitro human tissue. However there is data lacking in human studies.Till now oral statins have been studied more than topical statin therapy over cutaneous ulcers, diabetic foot ulcers, for anastomotic healing.

There is a need to study the effect of topical statin on the healing of diabetic foot ulcers. This study is an attempt to nullify this gap in information by using topical atorvastatin.It would help us to understand the role of topical atorvastatin on wound healing in diabetic foot ulceration as compared to the conventional therapy of saline dressing alone.

AIMS AND OBJECTIVES

To test the effectiveness of topical statin medication along with conventional therapy (saline dressing) versus conventional therapy alone by comparing mean percentage wound area reduction in diabetic foot ulcers.

REVIEW OF LITERATURE

DIABETES MELLITUS:

Diabetes is a complex, chronic illness requiring continuous medical care with multifactorial risk reduction and treatment strategies beyond glycemic control.[13]The American Diabetes Association defines diabetes mellitus as "a group of metabolic diseases characterizedby hyperglycemia resulting from defects in insulin secretion, insulin action, or both."[14]

Epidemiology

Diabetes has emerged to be one of the significant causes of morbidity and mortality worldwide in the past few decades.In the year 2019, the global prevalence of diabetes amounted to 463 million people which is estimated to rise to 700 million by 2045. Every 1 in 5 of the people who are above 65 years old have diabetes. There were 4.2 million deaths reported due to diabetes in the year of 2019.It has been estimated that 374 million people are predisposed to develop type 2 diabetes as of 2020.[15]India is the second most affected country by diabetes mellitus after China.[16] As per the latest

International Diabetes Federation Atlas, there are more than 77 million people with diabetes in India as of 2020.[15] Nearly 1 million Indians die due to diabetes every year.[17] According to the Indian Heart Association, India is estimated to have 109 million people with diabetes by 2035.[18]

Pathophysiology

Genetic and environmental risk factors affect inflammation, autoimmunity and metabolic stress states at the cellular level. These lead to destruction of beta cell mass affecting insulin production and/or its activity. Inadequate insulin secretion along with diminished tissue response to insulin affects the complex pathways of hormone action at multiple levels. The deficient action of insulin on target tissues forms the basis of abnormalities in carbohydrate, fat, and protein metabolism in diabetes. The chronic hyperglycemia of diabetes consequently leads to long-term damage and dysfunction of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels.[19]

Classification

Diabetes can be classified into the following general categories[14]:

1. Type 1 diabetes (β-cell damage, because of autoimmune mechanisms, leads to absolute insulin deficiency)
2. Type 2 diabetes (β-cell insulin secretion hampered along with insulin resistance)
3. Gestational diabetes mellitus (development of diabetes in the second or third trimester of pregnancy which was not overt diabetes before gestation)
4. Specific types of diabetes:

For example,

- monogenic diabetes syndromes (example- neonatal diabetes and maturity-onset diabetes of the young)
- diseases of the exocrine pancreas (example- cystic fibrosis and pancreatitis)
- drug- or chemical-induced diabetes (example- with glucocorticoid use, in the treatment of HIV/AIDS, or after organ transplantation)

Diagnosis of Diabetes Mellitus[14] (Table 1)

Diabetes can be diagnosed based on any of the following four tests

IIbA1C	>= 6.5%
Fasting Blood Sugar	>= 126 mg/dL
2 hour plasma glucose during 'Oral Glucose Tolerance Test'	>= 200 mg/dL
Random plasma glucose	>= 200 mg/dL with classic symptoms of hyperglycemia and hyperglycemic crisis

Complications[20]

Uncontrolled diabetes has certain acute complications such as hyperglycemia withketoacidosis or the non ketotic hyperosmolar syndrome.The long term injurious effects of diabetes are categorized into microvascular and macrovascular complications.The microvascular complications comprise of diabetic retinopathy leading to progressive loss of vision, nephropathy which is a leading cause of renal failure and neuropathy. Diabetic neuropathy is described the presence of clinical features of peripheral nerve dysfunction in diabetics after eliminating other causes. Peripheral neuropathy in diabetics may manifest in forms such as sensory, multifocal and autonomic neuropathies.Distal sensorimotor symmetric polyneuropathy is considered as the most common type of diabetic neuropathy, leading to foot ulcers, amputations and charcot joints.

Autonomic neuropathy manifests as gastrointestinal, genitourinary and cardiovascular

clinical features. The increased risk of atherosclerosis, platelet adhesion and hypercoagulability in diabetic patients form the basis of macrovascular complications

in them. Diabetics are more at risk for cardiovascular, cerebrovascular and peripheral arterial disease. Hypertension and abnormalities of lipoprotein metabolism are longterm manifestations diabetes mellitus.Chronic hyperglycemia leads to impairment of growth and susceptibility to certain infections.

Lower limb complications in Diabetes Mellitus[20]

Diabetes leads to a multitude of complications in lower extremities as a result of its microvascular and macrovascular effects. The combination of distal sensorimotor peripheral and autonomic neuropathy, vasculopathy and infection makes the foot of a diabetic patient susceptible. Consequently, they develop decreased sensations of pain, temperature, proprioception with small muscle wasting leading to foot deformities and dry skin leading to callus formation. All these put the patients' foot at risk for the formation of ulcers. Repetitive trauma to foot due to decreased sensations have a superadded effect on the vulnerable foot for ulcer formation.

DIABETIC FOOT ULCER

Definition[21]

Diabetic foot' is defined as "Infection, ulceration or destruction of tissues of the foot of a person with currently or previously diagnosed diabetes mellitus, usually accompanied byneuropathy and/or peripheral arterial disease in the lower extremity."

Epidemiology

Diabetic foot is one of most common, expensive as well as severe complications of diabetes.

Estimates suggest 15% of diabetics carry a risk of forming diabetic foot ulcers in their lifespan, from where15-20% cases eventually result in need for amputation.[22]Every half minute one lower limb or part of lower limb gets lost worldwide because ofdiabetes.[23] In India, approximately 40,000 lower limbs are lost in amputation every year. Of these, more than 75% cases are due to neuropathy with superadded infection, which are potentially avoidable.22

Surgical Anatomy of foot

The foot is an integrated complex of tendons, ligaments, muscles and bones arranged in arches with an intricate neurovascular framework. It provides a pedestal to carry entire weight of body and forms crux of locomotion.The foot skeletal framework comprises of 7 tarsals, 5 metatarsals and 14 phalanges.The hindfoot comprises of talus and calcaneum. The midfoot includes cuboid, navicular and cuneiforms and the forefoot includes phalanges and metatarsals.[72,73]

Skin and Subcutaneous tissue

The skin over the plantar aspect or the sole of the foot is thicker and more sensitive than that of the dorsal aspect. The subcutaneous tissue deep to the plantar skin is more fibrous and compact than the loose tissue deep to the dorsal skin. Fibrous septa attach the skin to the plantar aponeurosis preventing excessive movement of skin during walking. This improves the plantar grip during locomotion. These 'modified skin ligaments' also concentrate the subcutaneous fat over the weight bearing areas of heel, sole's lateral margin as well as across plantar aspect of heads of metatarsals. This helps those areas to act as shock absorbing pads.

The skin over the sole has characteristic lack of sebaceous glands and hair follicles and marked presence of numerous sweat glands.[72,73]

Deep fascia

The deep fascia is a distal extension of the inferior extensor retinaculum over dorsum offoot. This is called 'plantar fascia' over sole of foot, where it has a more complex distribution being thicker in the central area and weaker in the medial and lateral areas.The plantar fascia holds paramount importance in the maintenance of the longitudinal arches ofthe foot. It also protects the plantar aspect of foot from injury and keeps the parts of foot intact.The stronger central portion is termed as the 'plantar

aponeuroses. It extends from the calcaneum distally and ends within its own modifications namely the 'fibrous digital sheaths' enclosing the 5 flexor tendons and 'superficial transverse metatarsal ligament' over metatarsal heads.[72,73]

Muscles of foot

Each foot comprises of 20 muscles. There are 2 on the dorsal aspect, 4 in the intermediate position and 14 muscles on sole of foot. The muscles of the sole of foot are divided into four layers within four compartments.

All the muscles of the sole together stabilize the foot during the support phase of stance by maintaining the integrity of the arches of the foot.[72,73]

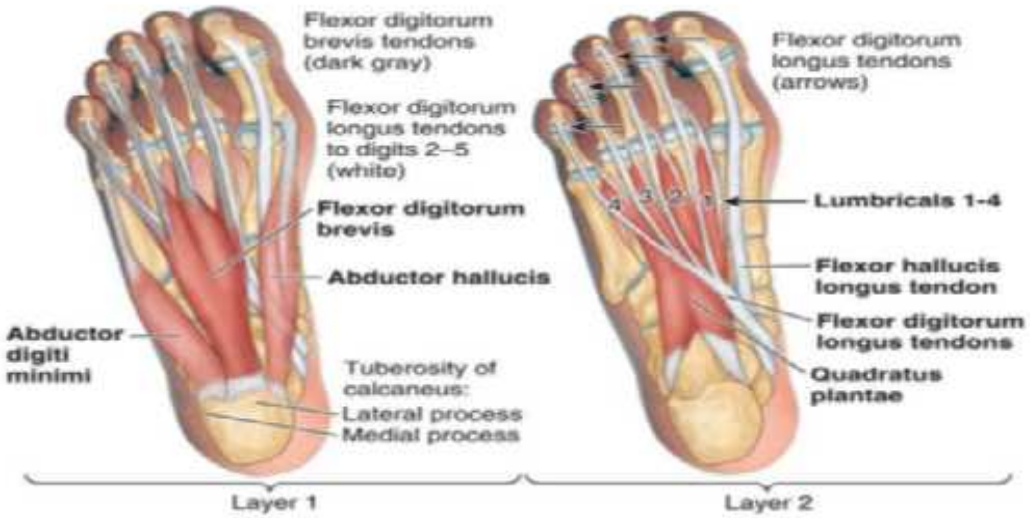


Figure 1a: Muscles of the sole of the foot- 1st and 2

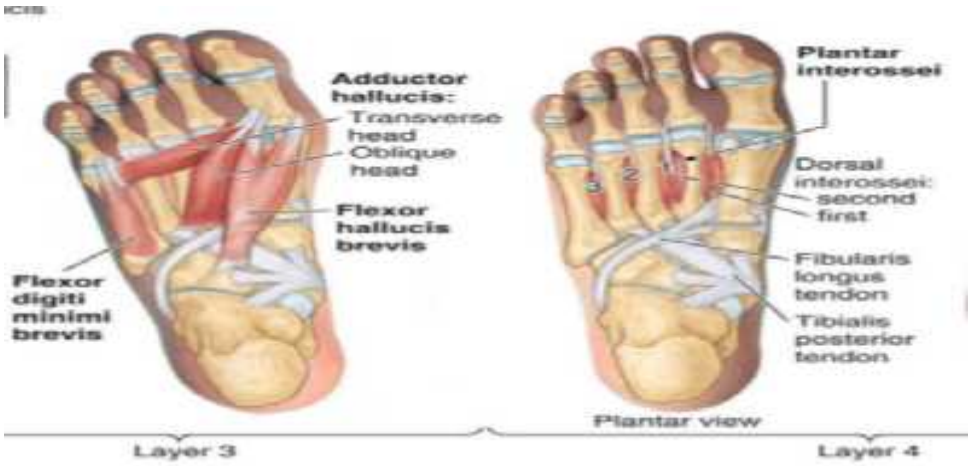


Figure 1b: Muscles of the sole of the foot- 3rd and 4th layer

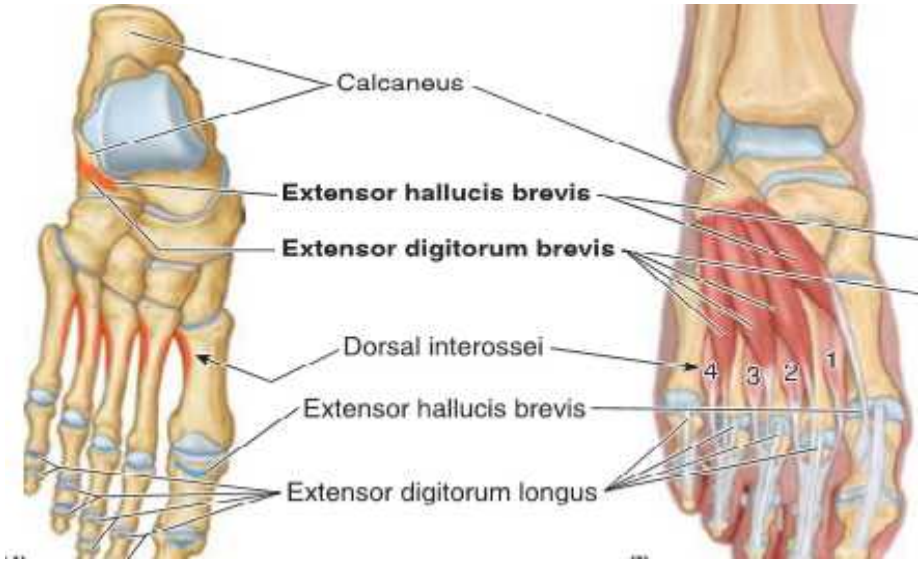


Figure 2 : Muscles of the dorsum of foot

Nerve supply of foot

The muscles of sole of foot are innervated by medial and lateral plantar nerves that are tibial nerve's end branches.

The Extensor digitorum brevis muscle on the dorsum of foot is innervated by lateral branchof deep fibular nerve

Cutaneous nerve supply of foot is by the following nerves:

1) Superficial fibular(peroneal) nerve – medial and lateral cutaneous branches
2) Deep fibular(peroneal) nerve
3) Sural nerve
4) Saphenous nerve
5) Medial and lateral plantar nerves

Arterial supply of foot

Dorsum of Foot-

Supply by Dorsalis pedis artery that is Anterior tibial artery's continuation

Dorsalis Pedis artery branches: -Lateral tarsal artery

-Arcuate artery

-First dorsal metatarsal artery

-Deep plantar artery (terminal branch)

PLANTAR ARCH

Sole of Foot

Supply by end branches of Posterior tibial artery:

-Medial plantar artery

-Lateral plantar artery

Plantar arch that is created by anastomoses between deep plantar artery and lateral plantar artery, gives plantar metatarsal arteries to toes.

Venous drainage of foot

The Dorsal venous arch drains majority of blood in foot through digital veins and communicating veins from sole into great saphenous vein medially and short saphenous vein laterally.On the sole of foot, medial and lateral plantar veins traverse along with their ***corresponding*** arteries and ultimately unite to form posterior tibial venae comitantes.[72,73]

Arches of the Foot[74,72,73]

Arches of foot comprise of bones of foot held together by ligaments, tendons and muscles forming an intricate elastic structure. These arches allow foot to support entire weight of body.

Different arches of foot are as follows:

-Longitudinal arch: 1) Medial longitudinal arch

2) Lateral longitudinal arch

- Transverse arch

Functions of the arches of the foot:

1) Uniform distribution of the weight of the body over the entire foot
2) To act as a propellant for locomotion such as during walking, running and jumping
3) To enable the foot to adapt to change in surface contour

Maintenance factors for the foot arches:

The foot allows to sustain the integrity of the arches by a number of factors

PASSIVE SUPPORTS:

1) Ligaments of the foot –

-The most significant in maintaining the arches

-Spring ligament or Plantar Calcaneonavicular ligament, Short and Long Plantar

ligaments

2) The plantar aponeurosis
3) The structure of the bones

DYNAMIC SUPPORTS:

1) The action of intrinsic and extrinsic muscles

2) The action of the long tendons extending into the foot

- Flexor digitorum longus, Flexor hallucis longus, Fibularis longus, Tibialis posterior

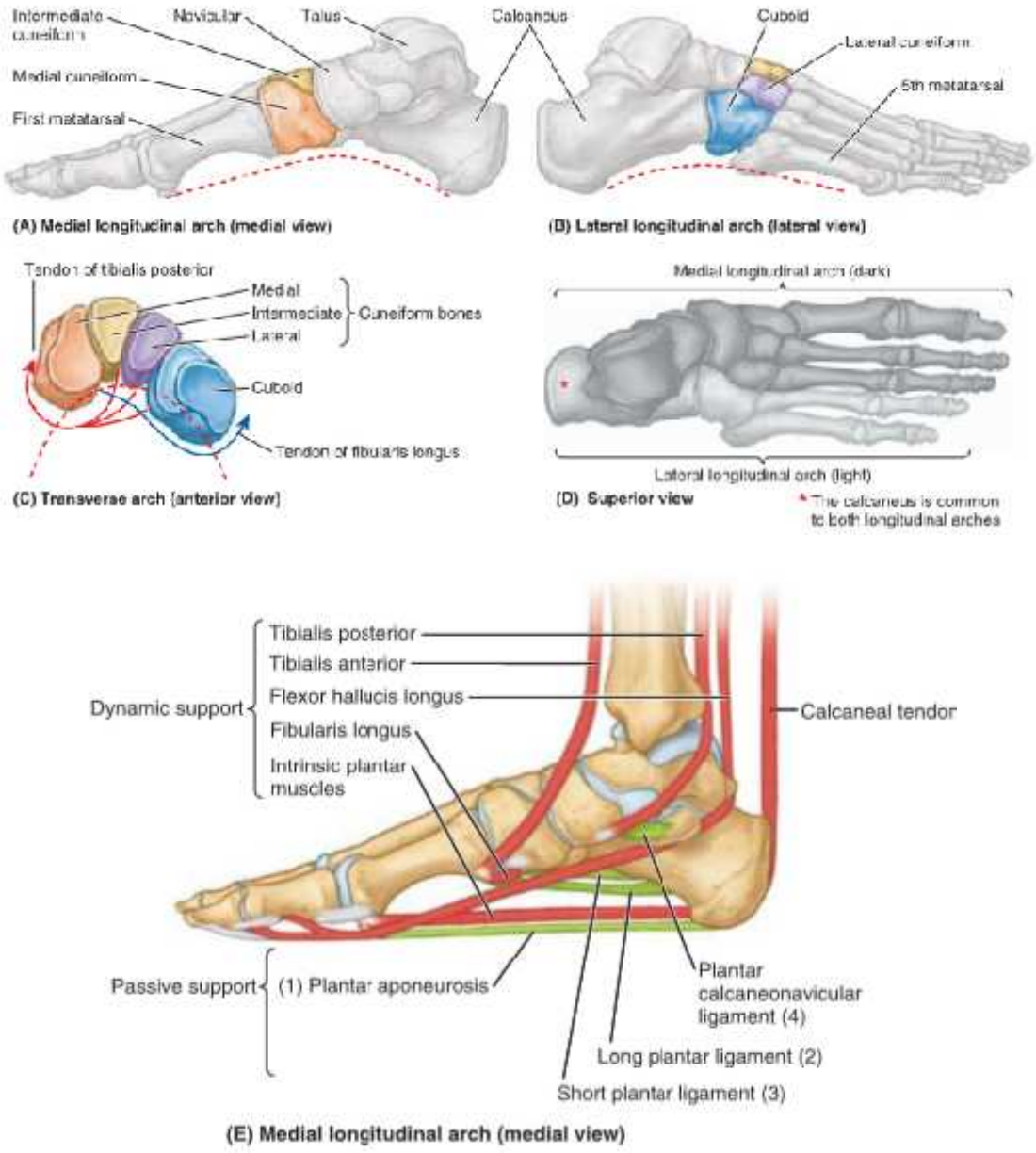


Figure 3 : Arches of foo

Wound healing

This is an intricate dynamic mechansim that replaces nonviable tissue. Chronic conditions suchas diabetes mellitus predispose the skin to impaired wound healing.[24]A thorough understanding of the physiology of wound healing holds paramount importance in dealing with local and systemic factors that hamper the normal healing process. This in turn allows to develop wound healing strategies that allow a faster road to recovery.

Physiological wound healing is described under four distinct phases which progress in a linear fashion.

1. Haemostasis

Disruption of tissue integrity leads to vascular injury both at the microvacular and macrovascular level. Damaged arteries constrict by smooth muscle contraction due to rise in cytoplasmic calcium levels. The decreased tissue blood flow due to vasoconstriction cause tissue hypoxia andacidosis. This leads to synthesis and release of vasoactive substances such as nitric oxide and adenosine causing reflex vasodilatation of arterioles and arteries. The process is further accentuated by mast cells which secrete histamine causing vasodilatation and increase in vascular permeability. Thus, a state of increased inflammatory cells in extracellular space is created.

The phase of haemostasis finally leads to clot formation which is supported by the intrinsic and extrinsic pathway along with platelet activation mechanism. The activated platelets also facilitate the wound healing process by releasing more than 300 crucial signaling molecules such as transforming growth factor or TGF, platelet

derived growth factor or PDGF and the vascular endothelial growth factor or VEGF. These in turn stimulate other platelets, endothelial cells and leucocytes.

The final step of this phase is the derivation of prostaglandins and leukotrienes from arachidonic acid as a result of the injured cell membrane in wounds. These provide the adequate stimulus to initiate the inflammatory phase.[24,25]

2. Inflammation

The 'first responders' of this phase are neutrophils, which accelerate a series of events such as the leukotriene stimulation and complement cascade. These in turn lead to 'chemotaxis' or the passage of neutrophils down the chemical gradient toward the wound. Neutrophils further promote the destruction of bacteria by phagocytosis, degranulation and release of toxic products like neutrophil elastase, cathepsin and proteases and finally by production of oxygen free radicals. In diabetics, decreased chemotaxis and phagocytosis has been implicated to impair this phase of wound healing. Macrophages dominate the wound healing cascade by 48-72 hours by releasing products likeepidermal growth factor and transforming growth factor. These promote angiogenesis, granulation tissue formation and modulate the inflammatory process. The last contribution in this stage is provided by the lymphocyte after 72 hours. They produce extracellular matrix and remodel collagen thus, regulating wound healing.[24,25]

3. Proliferation

This step in the wound healing process marks the repair stage of the wound.It comprises of the following stages:

Angiogenesis

The initiating event of neovacularization is the production of transforming growth factor orTGF and platelet derived growth factor or PDGF from platelets once the platelet plug is formed. As a consequence of hypoxia, vascular endothelial growth factor or VEGF is released which togetherwith other cytokines begin genesis of blood vessels and repair of damaged vessels.

Fibroblast migration

The formation of clot stimulates the production of growth factors causing fibroblast proliferation.The fibroblasts lay down extracellular matrix formed by hyaluronan, fibronectins and proteoglycans and ultimately collagen type 3 which provides the foundation of granulation tissue.The fibroblasts eventually transform phenotypically into myofibroblasts. The latter combine with the extracellular matrix proteins to initiate wound contraction. Collagen is the primary source providing strength to the regenerating tissue.

Epithelialization

This is marked by the proliferation of epithelium over the wound area covering the exposed surface and formation of attachments with the extracellular matrix. The movement of epithelial cells across the wound is supported by the embryological process of ‘epithelial-mesenchymal transition’. This stage is complete by 24 hours in wounds healing by primary intention.

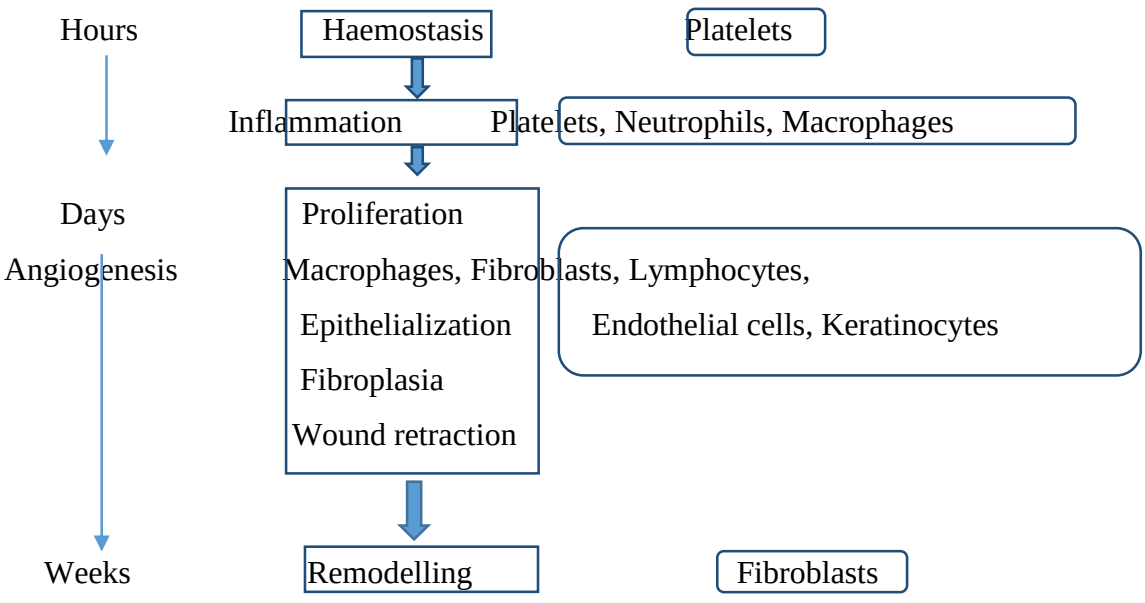
Wound retraction

Myofibroblasts are the pioneer mediators of this step which begins 7 days after the inciting stimulus to the tissue. The intercellular space is reduced by the actin-myosin interactions. Wound contraction occurs at a rate of 0.75mm/day.[24,25]

4. Remodelling

The last phase of wound healing aims at organization of the wound at the cellular level. This is achieved by maintaining a delicate balance between production and destruction of extracellular matrix proteins and formation of normal epithelium.Maturation of the scar tissue is further supported by replacement of the collagen type 1 with collagen type 3. Under normal circumstances, the wound area achieves a tissue strength of 50 % of its original by 3 months and 80 % of its initial capacity in the long run.[24,25]

FLOWCHART 1: Physiology of wound healing



PATHOPHYSIOLOGY OF DIABETIC FOOT ULCER

Crux of diabetic foot pathology is formed by hyperglycemic state of diabetes. The mechanism behind diabetic foot ulcer formation comprises of interplaybetween neuropathy, vasculopathy and infection.

NEUROPATHY

Chronic hyperglycemia leads to oxidative stress to neurons.

Metabolic derangements such as nonenzymatic glycation and polyol pathway hyperactivity causes production of free radicals. This has a direct toxic effect on neurons along with reduction in nitric oxide production. The decrease in nitric oxide subsequently leads to endothelial dysfunction and thus reduced blood flow to the nerves.

This state of neuropathy is presented in the sensory, motor and autonomic parts of the nervous system.The sensorimotor neuropathy is typically symmetrical and is initiated from the distal extremities with an insidious progression centrally

Sensory-The loss of protective sensation of pain in the diabetic foot makes it more susceptible to trauma and consequently ulcer formation.

Motor- The nerve supply to intrinsic foot muscles is hampered due to destruction of the motor fibres. This leads to an imbalance between the flexion and extension of the foot due to atrophy of the intrinsic muscles. Hyperextension of the metatarso-phalangeal joint with flexion of the proximal or distal interphalangeal joints leads to claw toe and hammer toe deformities, respectively. The resultant foot deformities exposes bony prominences which act as pressure points. This makes the foot a vulnerable target for skin erosion and ulcer formation.

Autonomic- There is loss of sympathetic tone which causes increased arteriovenous shunting and inefficient nutrient flow. The functional impairment of sweat and sebaceous glands makes the foot dry and at a risk for skin breach. This predisposes the foot to ulcer formation and also increase the risk for superadded infection.

Charcot Arthropathy

An amalgamation of motor, sensory and autonomic neuropathies in diabetics results in this condition. Decreased sensation along with muscle atrophy and joint laxity causes structural and functional deterioration of the foot. The accelerating condition is marked bypathological fractures, joint dislocation and disablement of the foot framework.Autonomic neuropathy leads to structural impaired vascular smooth muscle action and resultant increased blood flow to the bone. Consequently, inflammation and bone resorption occurs in the bones of the foot further aggravating the arthropathy changes in the diabetic foot. The ultimate result is the damage to the arches of the foot which leads to 'rocker bottom' appearance of the foot. [26,22,27,72]

VASCULOPATHY

The persistent hyperglycemic state has a longstanding effect on the vasculature, ultimately progressing to peripheral arterial disease.The major hallmarks of vasculopathy are endothelial cell dysfunction and smooth cell abnormalities.There is increased release of thromboxane A2 which causes vasoconstriction and accelerated platelet aggregation resulting in plasma hypercoagulability.All these factors cumulatively cause lower limb ischemia in diabetics putting the foot in jeopardy for ulceration.

The diabetic vasculopathy has a specific binary manifestation- A nonocclusive microcirculatory impairment in the blood vessels of retina, peripheral nerves and

kidney and a macrocirculatory disablement signified by atherosclerosis of blood vessels of the cardiac and peripheral vascular system.

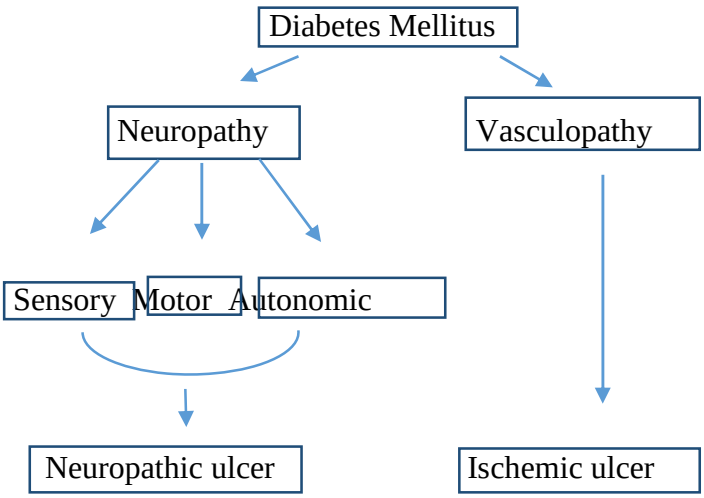
Microvascular- Thickening of the capillary basement membrane hampers the leukocyte migration and creates a functional microcirculatory ischemia of the tissues after injury. This is further accentuated by the loss of neurogenic vasodilatory response to injury, making the foot more vulnerable to infection.

Macrovascular- Peripheral arterial disease is the hallmark of this complication. The lower extremity arterial disease presents primarily due to an accelerated atherosclerosis of tibial arteries. This finally results in critical limb ishaemia and potential limb loss.[26,27,28,72]

INFECTION

The entry of microorganisms through the sites of ulceration leads to the proliferation of microbes which ultimately causes an inflammatory reaction and advent of infection in the diabetic foot.The onset of infection in the foot can have disastrous consequences due to the presence of inter-communicating compartments. This along with the absence of pain sensation, which causes the patient to continue ambulation, allows rapid spread of the infection into deep seated soft tissues and even bone leading to osteomyelitis.Structures like plantar aponeurosis, fascia and muscle tendons do not possess the ability to resist infection. The combination of hyperglycemia, neuropathy and vasculopathy further reduces the body's immunity facilitating progressive spread of infection. One of the significant immune system changes observed in diabetics is increased T lymphocyte apoptosis which further delays healing.[26,27]

FLOWCHART 2: Pathophysiology of diabetic foot ulcer



Intrinsic Factors affecting wound healing

Proliferative phase of wound healing in diabetics is hampered when growth factors like TGF and IGF-1 are absent and suboptimal functioning of enzymes is involved. Consequently, this prevents the proliferation of the cells and neovascularization in the tissue.

Matrix metalloproteinases (MMPs) are enzymes that maintain an optimal balance between production and degradation of extracellular matrix.Diabetic wounds manifest increased levels of MMPs which results in unwanted degradation of extracellular matrix rich in tissue proteinsfavoring wound healing.Additionally, defective migration of endothelial progenitor cells to the wound site occurs due to reduced NO.The cumulative effect of all these factors results in delayed and impaired wound healing. [28,29]

Biomechanics of the diabetic foot

The foot is exposed to an interplay of forces and their effects exerted on the foot structures. This forms the basis of foot biomechanics. In a diabetic foot appearing normal from the exterior, abnormal biomechanics is often the etiological factor behind abundant callus, foot deformities and foot ulcers. These ulcers show poor healing and tend to recur.The basis of deranged biomechanics in a diabetic foot is formed by presence of peripheral neuropathy and elevated plantar pressure especially over sites of bony prominences. Due to loss of sensations over areas with lack of subcutaneous tissue and fat, the underlying tissue is exposed to damage and leads to callus formation. Once haemorrhage sets in the callus, the site acts as a precursor to an ulcer. Additionally, trauma alongwith peripheral vascular disease act as significant factors causing tissue breakdown.[30,31]

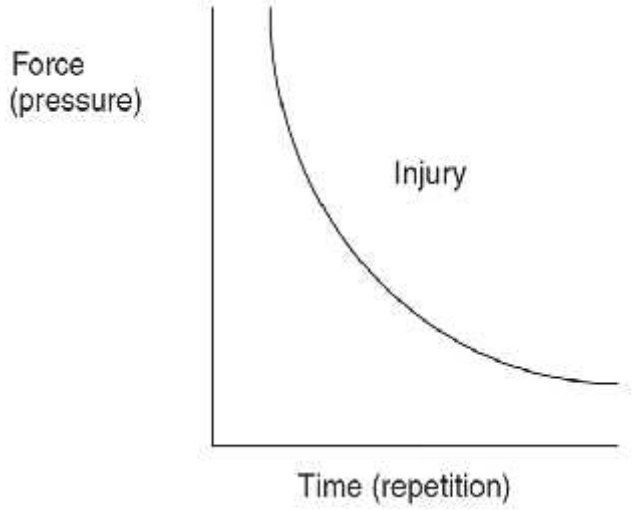
The usual sites of ulcer in a diabetic foot are the plantar aspect of forefoot, midfoot and toes followed by the dorsum of heel and toe.Besides callus formation, the other intrinsic factors which increase plantar pressure are tissue quality, motor neuropathy leading to atrophy of foot muscles, and presence of foot deformities. Deformities such as clawing of toes, hammer toes and charcot's arthropathy expose the heads of metatarsals, tips of toes and the midfoot, respectively to ulcer formation. Tissue breakdown is further accentuated by the absence of subcutaneous fat in these regions when the foot is deformed.[31]

Restricted joint movement at the metatarso-phalangeal, particularly that of the first toe, along with sub-talar joints, cause elevated plantar pressure. This is due to non-enzymatic glycosylation leading to stiffening of collagen at the joint capsules.

The most pronounced extrinsic factors rising the plantar pressure are ill-fitting footwear and barefoot walking.[30,31]

There are three mechanisms which can give rise to elevated pressure in a diabetic foot predisposing it to ulcer formation.

1. *Increased duration of pressures*- A low pressure applied for a lengthy time duration leading to ischemia and consequent tissue breakdown. Usually noticed due to incorrect footwear or placement of the heel over aver a flat surface for long.
2. *Increased magnitude of pressures*- A large magnitude of force is applied over a relatively small area of skin such as trauma due to a sharp nail or glass piece in a diabetic patient with peripheral neuropathy. A 'foot slap' can cause the same nature of trauma. In this case, weak dorsiflexor muscles cause a decreased slowing down of the forefoot after the foot touches the ground.
3. *Increased number of pressures*- Failureof the tissue to maintain integrity because of recurrent loading as seen in a neuropathic diabetic foot. The foot is subjected to repeated injury due to loss of sensations. This is also referred to as 'mechanical fatigue'.Kosiak, in his study on ischemic ulcers, noted an reverse relationship between force and time. With a rise in force, there is a fall in the time period or frequency of force(s) essential to cause tissue damage.[32]



GRAPH 1: Relationship between Force and Time

The patient should undergo foot biomechanical assessment which comprises of testing for range of motion of joint, manual muscle strength testing and static and dynamic gait analysis. The treatment may include a limb salvage procedure followed by usage of off-loading devices such as prosthetics, custom accommodative and/ or functional orthotics, bracing and custom molded shoes. Occasionally the limb salvage surgery has to be followed with elective balancing procedures. The latter includes tendon transfer and lengthening, bony reconstruction and fusion procedures.[33]

Classification of diabetic foot ulcer

A) The major classification is as follows:

1. The Neuropathic foot – Neuropathy is predominant in this condition
2. The Neuroischemic foot – Vasculopathy is predominant although neuropathy plays a major contributory role
3. The Ischemic foot- Vasculopathy is predominat

Types of Diabetic foot ulcer[34,35]: TABLE 2

Feature	Neuropathic	Ischemic	Neuroischemic
Sensation	sensory loss	pain	degree of sensory loss
Callus/necrosis	callus present and often thick	necrosis common	minimal callus; prone to necrosis
Wound bed	pink and granulating, surrounded by callus	pale and sloughy with poor granulation	poor granulation
Foot temperature and pulses	warm with bounding pulses	cool with absent pulses	cool with absent pulses
Other	dry skin and fissuring	delayed healing	high risk of infection
Typical location	weight-bearing areas of the foot, such as metatarsal heads, the heel and over the dorsum of clawed toes	tips of toes, nail edges and between the toes and lateral borders of the foot	margins of the foot and toes

B) The Wagner classification[36]: TABLE 3

Grade	Description of Ulcer
0	Intact skin in patients who are at risk
I	Superficial ulcers with exposed subcutaneous tissue
II	Exposed tendon and deep structures
III	Ulcers extend to the deep tissue and have either associated soft tissue abscess or osteomyelitis
IV	Ulcers include feet with partial gangrene
V	Feet ulcers with more extensive gangrenous tissue

C) The most widespread used and accepted classification is the University of Texas Wound Classification System[36] – TABLE 4

Stages	Description
Stage A	No infection or ischemia
Stage B	Infection present
Stage C	Ischemia present
Stage D	Infection and ischemia present
Grading	
Grade 0	Epithelialized wound
Grade 1	Superficial wound
Grade 2	Wound penetrates to tendon or capsule
Grade 3	Wound penetrates to bone or joint

D) The PEDIS Classification[36]- TABLE 5

GRADE	1	2	3	4
PERFUSION	Normal	Non critical	Critical limb ischaemia	-
EXTENT	Size	of	Ulcer	(cm^2)
DEPTH(TISSUE LOSS)	Full thickness	Deep involving muscle	Bone and/or joint	-
INFECTION	None	Mild	Moderate/severe	Systemic Inflammatory response syndrome
SENSATION	Intact	Loss of perceptive sensation	-	-

Assessment of diabetic foot

Physical Assessment

This begins with a thorough history taking of the patient enquiring about any past history of ulcers, amputation, trauma, history of intermittent claudication and rest pain, loss of sensations to lower extremity.

The general physical examination of the patient should look for any signs of anemia, sepsis with or without fever.Local examination should reflect the presence of any active infection in the form of ulcer, wet or dry gangrene of the lower limb. Other lesions such as skin cracks and fissures, presence of fungal infection, macerated skin especially in web spaces and calluses should be looked for. Presence of deformities such as the claw toes, hammer toes, hallux limitus, hallux rigidus and pes cavus determine the chronicity of the diabetic infection in the foot. There is possibility of high arched foot with visible muscle wasting on the plantar and dorsal aspect of the foot.On palpation, the temperature denotes the underlying pathology with a cold foot signifying peripheral ischaemia and a warm foot pointing towards active infection of the limb.According to 'The International Working Group on the Diabetic Foot' and the 'Infectious Disease Society of America', there is a validated clinical criteria for recognising and classifying diabetic foot infection depending on its severity.

Table 6: Classification of Diabetic foot infection[34]

Clinical criteria	Grade/severity
No clinical signs of infection	Grade 1/uninfected
Superficial tissue lesion with at least two of the following signs: — Local warmth — Erythema >0.5–2cm around the ulcer — Local tenderness/pain — Local swelling/induration — Purulent discharge Other causes of inflammation of the skin must be excluded	Grade 2/mild
Erythema >2cm and one of the findings above or: — Infection involving structures beneath the skin/ subcutaneous tissues (eg deep abscess, lymphangitis, osteomyelitis, septic arthritis or fascitis) — No systemic inflammatory response (see Grade 4)	Grade 3/moderate
Presence of systemic signs with at least two of the following: — Temperature >39°C or <36°C — Pulse >90bpm — Respiratory rate >20/min — $PaCO_2$ <32mmHg — White cell count 12,000mm^3 or <4,000mm^3 — 10% immature leukocytes	Grade 4/severe

Screening for Peripheral neuropathy

One of the simple and effective methods used for testing the peripheral neuropathy involves using a 10g (Semmes-Weinstein) monofilament. A 10 g monofilament, when placed over the sole and bent, exerts a buckling force of 10g.The inability to sense this pressure is termed as sensory absence.Despite its affordability and portability, this test can have a variable accuracy in testing for neuropathy.The other commonly used simple method is using a standard 128 Hz tuning fork to test for Nvibration. In order to increase the accuracy in diagnosis, this test can be combined with other modalities like neurothesiometer and biothesiometer, that are intricate handheld instruments to evaluate vibration[37,74].

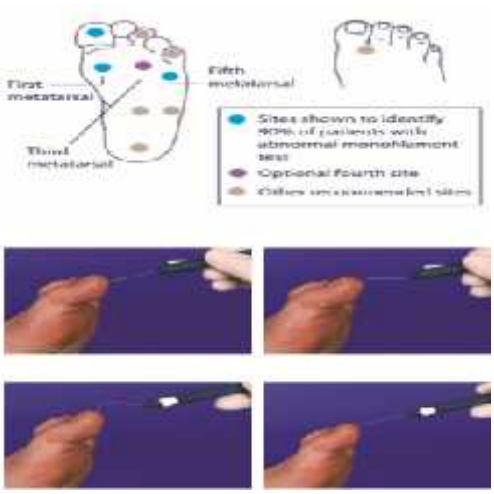


FIGURE 4: Tuning Fork test FIGURE 5: Monofilament test

Screening for Peripheral arterial disease

Peripheral arterial disease is present in approximately 40% of patients with diabetic foot ulcers.Along with palpation of the lower limb peripheral arteries, the ankle brachial pressure index(ABPI) can be a reliable measure to quantify the extent of peripheral arterial disease.It is the ratio of the maximum ankle systolic blood pressure to the arm systolic blood pressure quantified using a doppler. Normally the ABPI ranges between 0.9-1.3.

The limitations of this technique are user dependency, availability of equipment, lack oftraining. There is a possibility of a false high value owing to diabetics having calcified arteries with poor compressibility.

The severity of the vasculopathy is estimated as per the following table[37] – TABLE 7

ABPI	Interpretation
0.91-1.3	Normal
0.70-0.90	Mild Obstruction
0.40-0.69	Moderate Obstruction
<0.40	Severe Obstruction
>1.3	Poorly compressible vessel

Those with ABPI more than 1.3 due to noncompressiblevessels can be subjected to alternative tests such measurement of systolic pressures in toes, pulse volume assessment,duplexultrasound or transcutaneous oxygen quantification.Derangement in these tests prove the presence of peripheral arterial disease.[34,37,38]

Prevention of diabetic foot ulcer

Prior to managing diabetic foot ulcers, patients should take necessary steps in his daily life to prevent the formation the ulcers.

These include:

1. Examination of own's foot every day to look for areas of skin breach
2. Care of skin and nails of the foot
3. Maintenance of clean and dry foot
4. Usage of correct footwear
5. Cessation of smoking

Such measures will help to maximize the care of the foot thus preventing any form of skin epithelium breach.[34,37] Education of the patient and his caregiver alongwith good family support is crucial for these measures to be successful.

MANAGEMENT OF DIABETIC FOOT ULCER

This comprises of investigations and treatment

Investigations

1. Complete haemogram to evaluate for hemoglobin and total leucocyte count to rule out anemia and infection respectively.
2. Renal function tests to rule out diabetes induced nephropathy.
3. Xray of foot (Anteroposterior and lateral view) to rule out osteomyelitis, foot deformities, Charcot's arthropathy changes; also to grade the diabetic foot ulcer
4. Wound swab culture and sensitivity to look for bacterial presence and specific antibiotic treatment for it
5. Fasting blood sugar level and HbA_1C to know extent of glycemic control, the latter being a reliable predictor of wound healing
6. Urine ketone bodies to rule out diabetic ketoacidosis
7. Doppler study of lower limb to look for diabetic vasculopathy changes

Treatment

The principle aim of diabetic foot ulcer management is wound closure.The target is to treat the diabetic foot ulcer at an early stage to facilitate its faster healing.

Multidisciplinary approach

With the advent of newer resources and the presence of standard guidelines to aid in management, diabetic foot care has emerged to be a speciality that can be taken care of at a multidisciplinary care center. This has brought a significant reduction in the wound healing time, frequency of amputation and extent of amputation.= Multidisciplinary diabetic foot care encompasses a holistic management of a diabetic foot ulcer with the help of a surgeon, diabetologist, physiotherapist and nurse. The

introduction of 'diabetic clinic' has been pioneer in setting new benchmarks in this arena.[38,75]

The key components of diabetic foot ulcer wound management are:

1. Treating underlying disease process
2. Local ulcer care with control of localized infection
3. Pressure offloading

Treatment of a diabetic foot ulcer mandates coordinated partnership between the patients and treating team of doctors. Patient education about keeping adequate diabetic control with proper foot care and hygiene is paramount to attain overall positive results in the ulcer treatment.[34,75]

1. Treating underlying disease process

It is crucial to identify the underlying causative factor the diabetic foot ulcer. If detected during foot assessment, it should be treated along with the local wound care for a holistic approach to wound management

- Treatment of peripheral ischemia :

Those having critical limb ischaemia and superadded ulcers need treatment with the medical therapy besides being considered for revascularization procedures. This not only accelerates the wound healing process but also delays a possible future amputation for the patient

- Achieving optimal diabetic control:

It is necessary to aim for a tight glycemic control with proper tackling of risk factors like raised blood pressure, hyperlipidaemia and smoking. Optimal blood glucose aids wound healing, prevents adverseeffectson immune system and inhibits infection.Nutritional deficiencies should be diagnosed if any and addressed accordingly.[38,42]

- Identification of physical cause of trauma

It is important to do the patient's foot and footwear examination. The footwear reflects whether it is a proper fit and type and helps to rule out presence of pebbles, sharp objects etc which might traumatize the foot.

2. Local ulcer care with control of localized infection

A balanced debridement, frequent inspection, avoidance of infection and excessive moisture to prevent maceration are crucial. Ulcer care is a dynamic and evolving concept. It includes four components which tackle the various pathophysiological factors leading to a chronic diabetic foot ulcer.

The framework for optimal local ulcer treatment includes:

- Debridement of tissue
- Control of inflammation and infection
- Moisture care
- Advancement of epithelial edge

Debridement of tissue

The process of debridement is executed in allwounds, especially in those which are chronic in nature. It involves eliminating necrotic and nonviable tissue and consequently formation of granulation tissue.[39] The frequency and extent of debridement required depends on the rate of healing of the ulcer.

The necessity of repeated wound debridements in certain ulcers is in order to maintain its wound bed. At every dressing, the need for debridement is assessed. Not opting to debride a wound and choosing the wrong method of doing it can both delay ulcer healing and lead to hazardous consequences.When undertaken in the operation theatre, this procedure is followed by aggressive cleansing of the deep seated cavities in and around the wound.

The various modalities of ulcer debridement are as follows:

1.Surgical-

Although there is no proven best method of debriding an ulcer, surgical debridement is considered as a gold standard technique in practice. Regular, sharp debridement with scissors and scalpel and /or forceps have the benefits of removal of slough, hyperkeratotic and necrotic tissue, and callus till viable bleeding tissue is visualized. It helps to drain pus or any other discharge, reduces pressure over the ulcer and allows clear inspection of the underlying floor and base of the ulcer. Surgical debridement helps to optimize efficacy of topical preparations on the ulcer which in turn promotes faster healing.In patients with compromised peripheral vascular status needing revascularization, sharp debridement is not advocated. This is due to the risk of injury to the tissues with poor blood supply. A selective 'toothpick' approach rather than a more aggressive technique is considered more suitable in such patients.

2.Enzymatic

This method involves using enzymatic agents such as krill or papain derived collagen, collagenase from crab, dextrans and mixture of streptokinase and streptodornase. Removal of nonviable tissue is undertaken and healthy tissue is left behind. Ischemic ulcers are best suited for this debridement technique.The disadvantage of this modality is its high cost.

3.Biological

Sterile maggots can ingest microbes and nonviable tissue without affecting viable tissue. These have also been efficacious in treatment of ulcers infected with Methicillin resistant Staphylococcus aureus.The other alternative are larvae of greenbottle fly, which can remove the slimy layer and exclude microbes in wound. These are especially found to be efficacious in diabetic foot ulcers.

The other benefits of these biological agents include regulation of proteases, degradation of the extracellular matrix, promotion of fibroblast migration and potential improvement of skin perfusion. Another discovery are Lucilia sericata larvae are a source growth factorsBiological debridement is not that efficacious in treatment of neuropathic ulcers as the larvae cannot remove callus.

4. Hydrosurgical debridement

In this modality, saline or sterile water is targeted at a high speed towards the ulcer in a coordinated manner. This form of mechanical debridement allows accurate identification and elimination of necrotic tissue from ulcer.

5.Autolytic debridement

Moisture laden dressing makes the dead and necrotic soft and excludes them from the floor of the ulcer by naturally produced enzymes. This is due to the stimulation of the neutrophils and macrophages of the host immune system. Some of the useful dressings in this regard are hydrocolloids and hydrogels.

However the presence of moisture predisposes to maceration and should be avoided as much as possible . This method of debridement is contraindicated in patients with peripheral ischaemia with or without the presence of dry gangrene.[34,38,39]

Control of inflammation and infection

Infected diabetic foot ulcers are directly proportional to morbidity and mortality and need aggressive management. All infected ulcers require antibiotic therapy. Apart from systemic antibiotic therapy, these wounds also benefit from topical antimicrobial application. With the rise in antimicrobial resistance like Methicillin resistant Staphylococcus aureus and because of infection with Clostridium difficile, the use of topical antimicrobial formulations provides high local concentration over the increased wound bioburden without penetrating the intact skin or deeper tissues.

Topical antibiotic therapy also benefits patients with vasculopathy leading to poor peripheral vascular supply.

The patients are usually started on broad spectrum antibiotics, especially targeting Staphylococcus aureus and beta haemolytic Steptococcus. These are later switched to alternate drugs depending on the culture sensitivity results and if signs of inflammation do not improve.The apt antibiotic period to be followed depends on extent of infection along with patient's response.

Besides daily need of debridement in cases of infected ulcers with necrotic tissue, surgical intervention and antiobiotic treatment are required in the presence of abscess or necrotizing fasciitis. In cases with gangrene, extensive bone or joint involvement or crepitus, this can even lead to amputation.[34,40]

Role of Biofilms in chronic persistent infection

Biofilms refer to complicated amalgamation of multiple microbial populations which proliferate over chronic wounds. These usually are devoid of clinical features signifying infection and may not be routinely detected on cultures.

The antimicrobial agents cannot penetrate the wound surface due to an extra polymeric substance which converts biofilm matrix into slimy layer. As a protective mechanism, the host's immune system produces an inflammatory response, releasing increased amounts of proteases such as matrix metalloproteinases(MMPs) and elastase and reactive oxygen species (ROS). This leads to disintegration of adhesions connecting biofilm to tissue.[41]

The gold standard for treatment of such wounds include appropriate wound bed preparation with regular and repeated debridement, vigorous wound cleansing and usage of antimicrobial dressings to prevent reformation of the biofilms.

Moisture care

The appropriate dressing choice for the patient depends on the ulcer and patient. Factors usually considered are ulcer site and ulcer size, type of discharge from the ulcer, condition of surrounding skin and risk of infection to the ulcer.It is of absolute importance to identify and apply a balanced dressing for the diabetic foot ulcer which can maintain an optimum local environment with the correct moisture content, treating the ulcer discharge as well as local infection.

However, owing to complexities associated with diabetic foot ulcers which can hamper the outcome of the dressings such as vasculopathy, there is no ideal dressing choice for the same.The optimal dressing is chosen after thorough local examination of the ulcer. Factors considered include location and extent of ulcer, quantity and nature of ulcer discharge, condition of adjacent skin, ulcer bioburden and quality of life.The requirement of regular inspection and assessment of the ulcer usually asks for a daily change of dressing.[34,38,40]

Table 8: Types of ulcer dressings[34]

TYPES	ACTIONS	INDICATIONS	PRECAUTIONS AND CONTRAINDICATIONS
ALGINATES	-Promotes autolytic debridement -Absorbs fluid -Moisture control	Moderate to high exuding wounds	Dry and necrotic wounds
FOAMS	-Absorbs fluid -Moisture control	Moderate to high exuding wounds	-Dry and necrotic wounds -wounds with minimal exudate
HONEY	Rehydrate wound bed Autolytic debridement Antimicrobial action	-Moderate exuding wounds -Critically colonised wounds	-May cause drawing pain due to osmotic effect -Known sensitivity
HYDROCOLLOIDS	-Absorb fluid -Promote autolytic debridement	-Moderate exuding wounds -Combined use with silver for antimicrobial activity	-dry/necrotic wounds -high exuding wounds -May encourage overgranulation and maceration
HYDROGELS	-Rehydrate wound bed -Moisture control –Promote autolytic debridement -Cooling	-Dry/low to moderate exuding wounds -Combined use with silver for antimicrobial activity	-highly exuding wounds -where anaerobic infection is suspected -May cause maceration
IODINE	Antimicrobial action	-Critically colonised wounds or clinical signs of infection -Low to high exuding wounds	-Dry necrotic tissue -Known sensitivity to iodine -short-term use recommended (risk of systemic absorption)
Low-adherent wound contact layer (silicone)	-Protect new tissue growth -Atraumatic to periwound skin	-Low to high exuding wounds -Use as contact layer on superficial low exuding wounds	-May dry out if applied for long -Known sensitivity to silicone
SILVER	Antimicrobial action	-Critically colonised wounds	-Some may cause discolouration -Known sensitivity
POLYURETHRANE FILM	-Moisture control -Transparent (allow visualisation of wound)	-Primary dressing over superficial low exuding wounds	-moderate to high exuding wounds

Table 9: Guide for Wound Management[34]

TYPE OF WOUND TISSUE	THERAPEUTIC GOAL	ROLE OF DRESSING	WOUND BED PREPARATION	PRIMARY DRESSING	SECONDARY DRESSING
Necrotic, black, dry	To remove devitalised tissue	-Hydration of wound bed -Promote autolytic debridement	Surgical or mechanical debridement	Hydrogel Honey	Polyurethane film dressing
Yellow with slough and low exudate	-To remove slough -provide clean wound bed for granulation tissue	-Rehydrate wound bed -Promote autolytic debridement	-Surgical or mechanical debridement -antiseptic wound cleansing	Hydrogel Honey	Polyurethane film dressing Low adherent (silicone) dressing
Yellow with slough, moderate to high exudate	-To remove slough -provide clean wound bed for granulation tissue -Exudate management	-Absorb excess fluid and maceration -Promote autolytic debridement	-Surgical or mechanical debridement -antiseptic wound cleansing	Absorbent dressing (alginate/foam)	polyurethane film dressing
Granulating, with exudate	-Promote granulation -Provide healthy wound bed for epithelialisation	-Maintain moisture balance -Protect new tissue growth	Wound cleansing	-Hydrogel -Absorbent dressing (alginate/foam) -Low adherent (silicone) dressing	-Pad and/or bandage -Avoid occlusive bandages
Epithelialising red with low exudate	Promote epithelialisation and wound maturation (contraction)	Protect new tissue growth		-Hydrocolloid -Polyurethane film dressing Low adherent (silicone) dressing	-Pad and/or bandage -Avoid occlusive bandages
Infected Low to high exudate	-Reduce bacterial load -Exudate management	-Antimicrobial action -Moist wound healing	Antiseptic wound cleansing	Antimicrobal dressing	-Pad and/or bandage -Avoid occlusive bandages

Dressing application and ulcer monitoring

Regular examination and assessment of the ulcer and the dressing is crucial for desired results. Ideally for infected ulcers, daily inspection of wound with change of dressing is advised. Dressing type may require change with improvement in condition of ulcer.

Prior to the application of the dressing, ulcers should be cleansed with ideal choice of antiseptic solution depending on the healing stage of the ulcer. This helps to eliminate devitalized tissue, remove the biofilm and decrease the discharge from the ulcer thus, preparing the wound bed for healing..[34,40,41]

The measurement of the ulcer is undertaken by methods such as:

1. Grid tracing- Using a transparent sheet and graph paper

2. Digital planimetry- Using softwares such as 'Tissue Analytics' and 'WoundMatrix'

Advancement of epithelial edge

Debridement of ulcer edges eliminates the physical restrictions to epithelial growth on ulcer floor.

The line demarcating viable from necrotic tissue might become a source of infection and therefore the devitalized tissue needs to be debrided and eliminated.[34,38,40]

Adjuvant therapies

For ulcers which do not heal despite all conventional measures, adjunctive treatment modalities may be considered. These include:

1. **Negative pressure wound therapy**- Local application of subatmospheric pressure causes a fall in ulcer discharge, edema and increases the local angiogenesis. The latest modality is the negative pressure wound therapy with instillation therapy using antiseptic agents such as Polyhexamethylene Biguanide.

2. **Hyperbaric oxygen therapy**- Exposure to high concentration oxygen at a high pressure can cause proliferation of keratinocytes and fibroblasts and improve the capacity of white blood cells to destroy bacteria
3. **Ultrasonic stimulation**- Vibration of inflammatory wound tissue has been noticed to improve healing
4. **Laser therapy**- This has the potential to form new blood vessels locally, inhibit inflammation and promote extracellular matrix synthesis.
5. **Electrical Stimulation**- It can encourage fibroblast activity and form new blood vessels locally along with expressing antibacterial effects.

Owing to their higher cost as compared to the standard treatment therapies, these are opted for in situations which can guarantee faster ulcer improvement with minimal amputations and overall enhanced functional quality of life.[39,40]

3. Pressure Offloading

The major causes leading to onset and dismal healing of diabetic foot ulcers arethe vertical plantar pressure and the horizontal component of ground reaction force indicated by the plantar shear stress. The latter has a predominant contribution in progression of diabetic foot ulcers. Offloading of susceptible regions of foot for balanced pressure redistribution is necessary for those with peripheral neuropathy. This promotes wound healing and prevents recurrence of the ulcers in the long run.[38] Absence of offloading or inadequate offloading puts the foot at risk of tissue damage and ulceration.

The patient's footwear should be examined at every clinic visit with an intention to identify pressure areas in it. These are then corrected or other options, that can achieve the same results, are used.

Reliable indicator of good offloading is significant lack of undermined edges of ulcer on regular inspection.The ideal modality is 'Total contact cast'(TCC). It is a well shaped cast covering patient's foot and leg manufactured with plaster or fiberglass that distributes pressure equally over sole. It allows compliance of patient as it is not easy to remove. These are of relatively low cost and are usually indicated for effective offloading of ulcers located in the forefoot or midfoot. Typically, using a 'Total contact cast' for single ulcer on sole can hasten recovery by almost six weeks.

However it has demerits such as skin irritation, prevention of daily inspection, disruption of quality of life in terms of disturbed sleep, high cost, low availability, need for trained practitioner for application.'Total contact casts' are contraindicated in cases of superadded neuropathy and vasculopathy due to risk of further inducing new ulcers owing to skin irritation and lack of sensation. They are also not suitable for infected diabetic foot ulcers or ulcers with osteomyelitis as they do not allow daily wound inspection. The IWGDF recommends the use of offloading interventions to treat uncomplicated neuropathic ulcers. Non-removable walkers along with 'Total contact casts' are preferred in neuropathic ulcers.

Devices that can be removed such as Scotchcast boots, 'removable cast walkers', healing sandals, crutches, walkers are preferred for infected and ischaemic ulcers. Although removable modalities are more user- friendly and do not hamper the quality of life, they can be removed by patients at their own discretion and thus lead to lower efficacy.

The 'removable cast walker' has a lightweight and semirigid frame to support the limb. It has the benefit of a rocker type of sole that allows forefoot to get offloaded while walking or standing. The base of foot is broad enough to provide adequate space for dressings. Some 'removable cast walkers' have added advantages

of intermittent pneumatic compression for decreasing edema and some have extra layers of foam like soft material to offer a total contact.

The 'Instant totalcontact cast' is a revised version of the 'removable cast walker' with an additional layer of cohesive plaster bandage. This allows the 'instant total contact cast' to be as efficacious as the 'total contact cast' along with being easily applicable like a 'removable cast walker'.

In situations where above ankle devices are contraindicated, shoes that can offload forefoot as well as cast shoes are preferred. Some of the alternative options for off-loading forefoot ulcers are felted foam and custom insoles. These can attain pressure reduction at the ulcer site till upto 50%.[34,38,39]

Table 10: OFFLOADING DEVICES[34]

TYPE	KEY POINTS
REMOVABLE CAST WALKERS	- Similar pressure decrease to TCCs - More acceptable, easy to remove - Can be used on infected and ischemic wounds - Reduced healing rate compared with TCCs
SCOTCHCAST BOOTS	- Lighter, padded cast covering foot to ankle - Can be made nonremovable
HEALING SANDALS	-Designed to limit dorsiflexion of metatarsophalangeal joints - Light weight, stable, reusable Can increase risk of falling in patients with poor balance
CRUTCHES,WALKERS, WHEELCHAIRS	-Provides complete offloading of foot -Patients need good upper strength

FIGURE 6a -
REMOVABLE CAST WALKER

FIGURE 6b -
SCOTHCAST BOOT

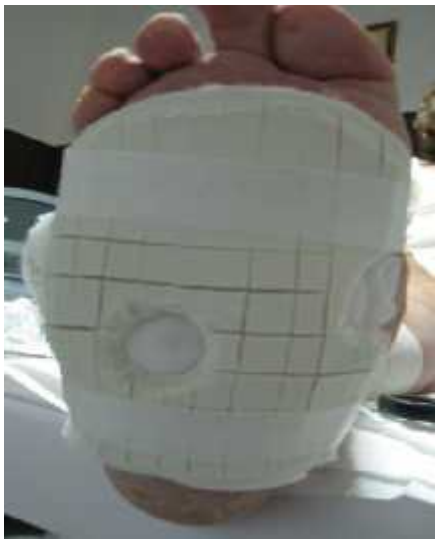


FIGURE 6c-
FELTED FOAM FOR OFFLOADING

Management of diabetic foot infection

Almost one third of diabetic patients develop diabetic foot ulceration in their lifetime of which 50% of the ulcers get infected. Almost half of these patients never manifest routine signs like erythema, warmth, swelling or pain. This is because of neuropathy and vasculopathy that are typically in an immune compromised host.[41]This makes it even more challenging to tackle the infection in the ulcers.The most common organisms involved are Staphylococcus aureus and Pseudomonas aueriginosa. Thus, it is of absolute importance to combat the infection present in the

diabetic foot ulcers for a holistic treatment of the ulcer both in terms of ulcer size and bioburden.[41,42]

Antimicrobial therapy

Antimicrobials are microbe killing products. They comprise of disinfectants, antiseptics and antibiotics.

Disinfectants-

These are chemicals used to inhibit the growth of or kill microorganisms on objects such as alcohol, glutaraldehyde and sodium hyperchlorite.

Antiseptics-

These are products that can prevent growth of or kill microbes in an ulcer or over normal skin, for example iodine, chlorhexidine, silver. When used over an ulcer, they directly hamper the bioburden.They have an extensive antimicrobial spectrum with longlasting activity.However they have disadvantage of being toxic to host cells like keratinocytes or leukocytes.Both disinfectants and antiseptics are both bacteriostatic and bactericidal.

Antibiotics-

These are natural or synthetic chemicals which have selective action either through topical or systemic route. They ideally have a specific cell target with narrow activity range. These are comparatively less toxic but can lose their benefits to bacterial resistance.[43,44]Initially, empirical antibiotics with a broad spectrum activity are used. These include cephalosporins such as ceftriaxone or cefotaxime alongwith metronidazole for anaerobic coverage. These are eventually changed to the definitive antibiotics as per the wound swab culture report obtained. Deep seated cultures from the ulcer are mandatory for accurate identification of the causative microorganism.

Topical Antimicrobial Therapy

The role of topical antimicrobial agents in dealing with diabetic foot infections is more pronounced because of antimicrobial resistance as noted in methicillin-resistant Staphylococcus aureus infection and side effects of systemic route treatment. These provide antimicrobial environment over ulcer. This property is significant in cases of reduced antibiotic tissue penetration such as in patients with vasculopathy.

Additionally, application of topical antimicrobials is more beneficialin patients who do not manifest the classical infection features buthave clinical risk of raised bacterial population. These ulcers usually present as non-healing wounds with increased discharge, odour and darkened granulation tissue.[41]

The disadvantages of topical antimicrobial agents include less penetration which restricts their usage to ulcers without cellulitis or aggravated soft tissue infection. There is risk of systemic absorption when applied over large surface ulcers, irritant dermatitis and allergies. Occasionally they may restrict usual healing of wound and even alter the normal cutaneous flora. It may be difficult to apply the topical antimicrobials in accurate dosage requiring frequent reapplications.[44]For an ulcer responding to the topical antimicrobial treatment, ideally it should be continued for two to three weeks, following which the ulcer is assessed for further course of management.[43]

ROLE OF SALINE DRESSING

Normal saline or Sodium chloride solution has been one of the first few certified agents that can be referred as standard for treatment of diabetic foot ulcers. In daily practice, it is used in the concentration of 0.9% weight/volume which is similar to the normal extracellular fluid composition.

Saline soaked guaze dressings are a type of nonadherent or low-adherence dressing that can provide a moist environment to the wound without being traumatic. It has the advantages of being inexpensive, simple to use with a hypoallergenic nature. Owing to its physiologic nature and isotonic content, normal saline acts as an effective wound cleansing agent by eliminating the necrotic debris and dead tissue. However it does not have any proven antimicrobial property.[45]

STATINS

Statinsare a class of chemical compounds which are routinely used in the treatment of hyperlipidaemia and in the prevention of cardiovascular events. Initially introduced in the 1980s, these are excellent in their activity and are tolerated well as hypolipidaemic drugs. Some of the commonly used statins in day to day practice are Atoravastatin, Rosuvastatin, Fluvasatin, Pravastatin, Simvastatin.[76]

In recent times, statins have been considered as a novel therapeutic treatment modality for wound healing due to its pleiotropic effects. The most pronounced are its anti-inflammatory, antioxidative, immunomodulatory, antibacterial, improvement of microvascular and reperfusion properties. These have been studied with promising results in various studies over the last two decades. Atorvastatin, a second generation synthetic statin, has showcased the maximum consistent results in this regard.[11,46]

In recent times, topical statin delivery system has been suggested as feasible option over oral statins. Topical application prevents the possible adverse effects of oral statins and ensures prolonged action at the local site. Some of the commonly used methods to deliver drugs topically are transdermal patches, emulgel, nanoemulgels.

Chemical structure

The statin compounds can be either be derived from fungi or artificially synthesized in nature. Pravastatin, Lovastatin, Simvastatin are from fungi whereas Atorvastatin, Rosuvastatin and Fluvastatin are synthetically derived.

The statin structure includes two segments:

1) Pharmacophore- This is comprised of Dihydroxyheptanoic acid that is HMG-CoA enzyme's analogue.

2)Pharmacophore moiety- This further has two divisions

a) Hydrophobic ring which binds to substrate analogue with covalent bonds and also to HMG-CoA reductase. Ring structure is partially reduced naphthalene(lovastatin, simvastatin, pravastatin), or pyrrole(atorvastin), or indole(fluvastatin) or pyrimidine(rosuvastatin).

b) Side groups on rings that characterize hydrophobicity or solubility, thus deciding pharmacokinetics of the drug.[47]

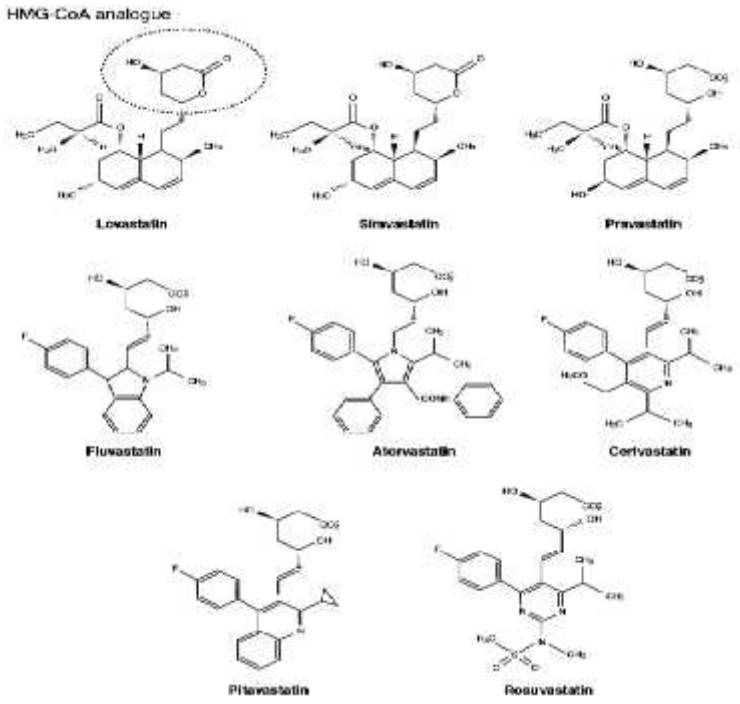


Figure 7– Structure of Statins[48]

Chemical and Physical properties

Rosuvastatin and atorvastatin possess extra interactions with hydrogen. The hydrophilic nature of rosuvastatin and pravastatin is owed to presence of methane sulphonamide and polar hydroxyl groups, respectively. Compounds such as atorvastatin, simvastatin, fluvastatin are more lipophilic.[47]

Topical gel formulations usually exhibit good homogeneity with pH of 7.6-7.8. They are unlikely to cause skin irritation on application and maintain structural and functional stability on being stored at 4 degrees temperature and 60% relative humidity. The hydrogels and hydroalcoholic gels allow a greater dissolution of drug due to its high aqueous component.[46]

Mechanism of action

The statins exert their hypolipidaemic property by competitively inhibiting 3-Hydroxy-3-methyl glutaryl coenzyme A or HMG CoA from converting into mevalonate. Usually this done by HMG CoA reductase. This is rate limiting pedestal in synthesis of cholesterol.

Consequently, there isreduction in cholesterol synthesis and a compensatory breakdown of intermediate and low density lipoprotein.[76] Increased statin doses of atorvastatin, simvastatin, rosuvastatin decrease level of triglyceride present due to high 'Very low density lipoprotein'.[77]

Additionally, statins can also decrease atherogenesis, inflammation and overall cardiovascular morbidity in hyperlipidaemic patients. Recent studies reflected benefits in using statins for alopecia, psoariasis, keloids, inflammatory diseases, bone fractures and chronic wounds.

EFFECTS OF STATINS ON WOUND HEALING

The diverse actions exhibited by statins are primarily attributed to linkage with numerous nuclear hormone receptors along with decreased isoprenylation of mevalonate's downstream targets.

1) **Anti-inflammatory Action** –

The anti-inflammatory property of statins is due to the following mechanisms:

a) Decreased release of C reactive protein, chemokines, proinflammatory cytokines such as IL-6, IFN-gamma, TNF-alpha with increase of anti-inflammatory cytokine such as IL 10.

b) Inhibiting trans-endothelial leucocyte migration through decreased generation of adhesion molecules such as ICAM-1 or Intercellular adhesion molecule-1, LFA-1 or lymphocyte function-associated antigen-1 and MAC-1 or Monocyte chemotactic protein-1.

c) Inhibition of Th-1 type T cell chemokine receptors

d)Reduction of polymorphonuclear leucocyte infiltration

2) **Epithelialization-**

Statins decrease the production of FPP(Farnesyl pyrophosphate), which is a crucial intermediate during cholesterol synthesis by mevalonate pathway.FPP has agonistic action for glucocorticoid receptor. The glucocorticoid receptors inhibit wound healing partially through repression of genes encoding for keratin in keratinoytes such as K6(keratin 6). Studies using in-vitro and ex-vivo human culture models, have shown that decreased FPP induces K6 (keratin 6) expression which causes increased keratinocyte migration, enhanced epithelialization and wound closure.

3) **Angiogenesis-**

Statins can decrease vasoconstriction by hampering tissue reaction to angiotensin-2 and decreasing production of endothelin-1. They also facilitate vasodilatation by increasing the production of nitric oxide synthase from endothelium. Rise in endothelial progenitor cell action by statins lead to formation of new blood vessels in ischemic tissue. Angiogenesis is also promoted by enhancing the synthesis of vascular endothelial growth factor or VEGF.Ability of statins to accelerate neoangiogenesis and oxygenation to ischemic tissue holds therapeutic significance in the treatment of diabetic ulcers with vasculopathy.

4) **Antibacterial action-**

Statins have been shown to possess antibacterial property in multiple invitro studies for both systemic bacteremia and local wound infections. This was even apparent against vancomycin resistant Enterococci and MRSA. In patients with chronic or diabetic wounds, excessive bacterial burden has significant role to play in impaired, delayed wound healing. Using statins for local wound management carries the benefits of enhancing epithelialization as well as combating the wound infection.[11,49]

Pharmacokinetics

Following oral administration, statins have a variable intestinal absorption(30-85%).Most of the statins are used as beta-hydroxy acid except in case of simvastatin as well as lovastatin. These are initially inactive lactones that are eventually activated in liver. All statins have an extensive first-pass hepatic uptake through the organic anion transporter(OATP) and metabolism by the cytochrome P450(CYP 3A4) mechanism. This leads to their systemic bioavailability ranging between 5-30% of the administered dosage. More than 95% of statins and their metabolites are protein

bound in plasma with peak plasma concentration concentrations being achieved in 1-4 hours. The exceptions to this are rosuvastatin and atorvastatin that possess half-lives of 20 hours and that of simvastatin being 12 hours. More than 70% of statins are metabolized by liver and excreted in faeces.[73]

When topically applied in the form of gel or emulgel formulations, statins have shown to be tolerable over the wound surface with no adverse effects. These have allowed adequate local drug release and enhanced permeation through the wound area for therapeutic action.[46]

Adverse effects- On reviewing the available literature, there are no observed side effects of topical statin therapy.[51,53,54,56,57]

Research studies on Statins related to wound healing

The potential of statins to facilitate ulcer healing has opened new avenue in treating diabetic foot ulcers.

Wide spectrum of statin's pleiotropic have been evaluated over the years in both invitro and invivo studies.

Johansen et al studied effect of 6 months high dosage atorvastatin versus low dose atorvastatin in the diabetic foot ulcer treatment. High dosage of drug was linked with remarkable fall in the inflammatory marker, C-reactive protein as compared to the low dosage.[50]

Statins, when used through the oral route, can manifest adverse effects like myopathy and hepatotoxicity in the long run. In recent times, topical statins have been in the limelight for wound healing. This is because it provides better drug delivery to site of action with prolonged and sustained action and less side effects. Besides, the extensive hepatic first pass metabolism can be avoided which thus prevents the decreased bioavailability at the site of action.[11]

Out of most of the topical statin preparations prepared, the most promising results have been seen with atorvastatin in wound healing. This is attributed to its properties of big half life, highly active metabolites, affinity for lipid and protein binding.[11,46]

Local atorvastatin therapy was evaluated by Toker et al in which topical atorvastatin was applied on streptozocin induced diabetic rats with diabetic wounds. The control group received a mixture of lanoline and vaseline. The results reflected that topical atorvastatin had a higher healing rate and could accelerate wound surface area reduction. It also led to better histological characteristics in the test group such as epidermal and dermal regeneration, enhanced granulation tissue synthesis and angiogenesis.[51]

Considering the potency of topical statins when used for diabetic wound healing, Farghaly et al did a study with the aim to develop different gel formulations of atorvastatin. These included hydrogel, hydroalcohol, microemulsion, alcoholic and anhydrous gel formulations in a concentration of 1% weight/weight and were applied in a dose of 100mg each over 2 cm^2 of induced diabetic ulcer. The manufactured formulations were tested for physical and rheological characteristics, extent of drug release and strength of wound healing in streptozocin-induced rats with diabetes. Outcome expressed that alcoholic gel base could allow maximum drug release. The invivo wound healing capacity, which was compared based on percent wound contraction, was statistically significant with hydroalcoholic, hydrogel and anhydrous gel forms. All the gel formulations were tolerated well locally with no side effects.[52]
Currently there is no commercially available preparation of topical atorvastatin. In this study, topical atorvastatin emulgel(5% weight/weight) was formulated and applied over diabetic foot ulcers in a dose of 10mg gel/cm^2 or 0.5mg atorvastatin/cm^2.

This was based on the principles followed in the study by Farghaly et al.[52]Out of all the gel formulations, the emulgel was noted to be an effective topical delivery vehicle as it has advantages of gel and emulsion both. Emulgel can be a drug reservoir as droplets of emulsion permit the enveloping of hydrophobic drugs with accelerated permeation through skin.[46,52]

Farsaei et al in his study, assessed the action of topical atorvastatin for treatment of pressure sores in seriously ill patients. The results showcased that topical atorvastatin in addition to the standard care for 2 weeks led to enhanced improvement in pressure ulcers.[53]A randomized clinical trial was done assessing effect of topical atorvastatin on posthaemorrhoidectomy pain and wound healing in which 1 gram of 2% atorvastatin emulgel or 20mg of drug was applied over the wounds every 12 hours for 14 days.[54]

The mechanisms by which topical statins could accelerate wound healing were evaluated by Sawaya et al in the Miller School of medicine, University of Miami. It was proven that statins could expedite in vivo epithelialization by stimulating expression of lnc-RNA and Gas5. Mevastatin, which was used topically in the test group, could reverse FPP induced c-Myc expression. c-Myc is an indicator of chronic wounds and diabetic foot ulcers. Additionally, statins could promote keratinocyte driven angiogenesis by stimulating production of VEGF and heparin binding epidermal growth factor or HBEGF. These highlighted the benefits of using topical in treatment of diabetic foot ulcers. Following two weeks, statistically significant wound healing was observed in the test group.[55]

In order to study the efficacy of topical delivery of statins against local inflammation, two studies used simvastatin gel to treat periodontitis. One of the studies included only diabetic patients with periodontitis. Results of both the studies

reconfirmed the anti-inflammatory action of topical statin. There was decrease in probing depth and raised clinical attachment level gain with remarkable fill in intrabony defect over regions treated with locally delivered simvastatin.[56,57]

Statins have a significant antibacterial effect that can be utilized in the treatment of infected wounds. Rego et al explored the potential of topical simvastatin as compared to saline in treating open infected skin wounds of rats. Culture results showed that the test group benefited markedly in terms of treating the local infection. This was a promising finding for treatment of diabetic foot ulcers which often have superadded infection impairing its healing[58].This study also attempts to study the antibacterial property of topical atorvastatin.

Majority of the studies conducted on topical atorvastatin are in vitro or animal models with very few clinical studies being available. The clinical studies researching topical statin therapy, have been performed on pressure ulcers, chronic vascular cutaneous ulcers or inflammatory conditions such as periodontitis or cutaneous psoriasis. Also, oral statins have been studied more than topical statins on diabetic foot ulcers. These studies have consistently reflected the pleiotropic effects of statin for wound healing. However there is no clinical study evaluating the benefits of atorvastatin used topically for diabetic foot wound healing. There is a need for confirmation of the advantages as well as adverse effects of statins by superior quality clinical trials prior to usage in clinical practice. This study is an attempt towards the same.

MATERIALS AND METHODS

Source of data were diabetic foot ulcer patients admitted under department of general surgery at KLES Dr.Prabhakar Kore Charitable Hospital and Medical Research Centre, Nehru Nagar, Belagavi, in the year 2019 between January to December.

a) **Study design**: A randomized control trial

b)**Duration of data collection**: 1 year

c)**Study Period**: January 2019 to December 2019

d)**Study Population**: Patients with diabetic foot ulcers, admitted in general surgical wards, measuring less than 6 x 6 cm^2 of Wagner grade 1

e) **Selectioncriteria**:

1) **Inclusion criteria**

-Type 2 Diabetic patients with diabetic foot ulcer

- Age between 18 to 70 years.

-Ulcers measuring less than 6x6 cm^2

-Wagner's classification grade 1

-Fasting blood sugar between 140-200 mg/dL

2) **Exclusion criteria**

-Pulseless limb

-Immunocompromised

-Osteomyelitis

-Skin malignancy,cellulitis

-Diabeticketoacidosis

-Diabetic gangrene

-Connective tissue disorder

f) **Sampling procedure**:

A computer generated randomization chart was used

g)**Sample size**:

Total sample size of 60 cases. 30 in group A and the other 30 in group B.

Sample size calculation

$$\underline{n = (z_{\beta} + z_{\alpha/2})^2 \times 2\ p\ (1-p)}$$

$$e^2$$

N =sample size,**p**=prevalence

$\mathbf{z_{\alpha/2}}$ = Critical value in a normal distribution. For a 95% confidence interval, the standard value is 1.96. αis called the 'level of significance' or the probability of making a type 1 error

$\mathbf{z_{\beta}}$ = Standard value for a (1-β) of more than 90%. β is probability of making a type 2 error with (1- β) referred to as the ' power of study'

e = Effect size. It denotes the quantitative measure of the magnitude of the experimental effect. It is a standard value which is chosen from a range of -1 to +1 with 0 indicating no effect.

Given,

Total number of diabetic foot ulcer patients admitted in hospital in period of January 2018 till December 2018 =240

Total number of patients admitted in the hospital from January 2018 till December 2018= 4218

Prevalence(p)= Total number of admitted patients with diabetic foot ulcer

Total number of admitted patients

$p = \frac{240}{4218} = 0.0569$, $(e)^2 = (0.2)^2$

$z_\beta = 1.24$ (>90%)

$z_{\alpha/2} = 1.96$ (95% confidence interval)

$n = \frac{(1.24 + 1.96)^2 \times 2(0.0569)(0.9431)}{(0.2)2}$

$n = \frac{(3.2)^2 \times (0.1073)}{(0.2)^2(0.2)^2} = \frac{1.099}{} = 27.47$

Considering the loss of follow ups, this number is rounded off to 30 in each control and intervention group.

h)**Instruments used for data collection**:

Ethical clearance was given by the Ethical research committee of JNMC, Belagavi.Data collection instrument was used for data collection.

All patients satisfying inclusion criteria were considered as subjects for the study. The patients were then included into study after taking written and informed consent. Selected patients were randomized into two groups namely, control group (n=30) and test group(n=30) on the basis of the computerized randomization chart.

Demographic data of the patients was noted in a predesigned proforma.Detailed history of the patient was taken.

Thorough clinical examination was done. Test for vascularity was done by palpating peripheral pulses.

In both the groups initially normal saline wash was given.

Topical management and dressing was done as follows-

GROUP A- TEST GROUP

In this group, dressing was done using topical atorvastatin alongwith saline wash.

Topical atorvastatin was applied in the form of : Atorvastatin gel (5% w/w)

Dose: 0.5mg of atorvastatin/cm^2 or 10mg of gel/cm^2. [52]

The formulation was an Emulgel comprising of Atorvastatin and excipients like methylparaben and propylpraben, Tween 80, Triethanolamine, Liquid Paraffin and Pemulgen.[54]

The formulation was optimized. It was prepared by the KLE College of Pharmacy.

A 10 mg measuring applicator was used for the application of the emulgel.

GROUP B- CONTROL GROUP

In this group, standard treatment that is conventional wet saline dressings were done.

Pressure offloading was executed for patients with ulcers on sole of foot.

Routine blood investigations and special investigations were carried out whenever indicated.

INVESTIGATIONS:

The following investigations were done for all patients in intervention and control group

1) Complete blood picture
2) Mini renal profile
3) Liver function test
4) Fasting blood sugar
5) Urine routine and microscopy
6) Urine ketone bodies
7) HbA1c
8) Wound swab for culture and sensitivity on day 0 and day 14

9) Xray Foot- Anteroposterior and lateral view

10) Colour Doppler of lower limb(as and when was required)

11) ECG

12) Chest Xray (posteroanterior view)

13) HIV and HbSAg

Empirical antibiotics ceftriaxone and metronidazole orcefotaxime and metronidazole were started and later specific antibiotic therapy was started after culture and sensitivity report was obtained.

Insulin or Oral hypoglycemic medicines were used in patients as per advice by the physician to maintain an adequate glycemic control.

i) Outcome

Patients in control and intervention group underwent daily diabetic foot ulcer dressings from day 0 till day 14.They were followed up for a total of 15 days. Wound healing in terms of percent reduction in ulcer size over 15 days after beginning of statin medication was stated as study's end point.

Simultaneouly the patients were made aware about restriction in diet and exercise to inhibit recurrence.Observation of healing of ulcer was done in terms of decrease in wound area at beginning(D_0) and at end of study(D_{14}). During every dressing, any discharge from the ulcers were also noted.

Calculation of wound area:

The dimensions of the ulcer i.e. length, width and area were measured by outlining the ulcer over a sterile transparent film placed over it. This was followed by placing the film over graph paper and counting the number of squares also referred to as 'grid tracing'. The length of the smallest square is 1mm.

The ulcer dimensions are measured on day 0(x) = initial wound area andday 14(y) = final wound area. The reduction in area and percentage reduction in area are calculated as follows:

Wound area on D0 = x

Wound area on D14 = y

Reduction in wound area = x-y

% Reduction in wound area = $\frac{x-y}{x}x100$

Assessment of antibacterial property of topical atorvastatin as compared to normal saline was done by sending a wound swab for culture and sensitivity test on day 0 and day 14 of the dressing.

The culture and sensitivity test reports, both of day 0 and day 14, for all patients were obtained and documented.

The patient was therefore followed up for a period of 15 days with dressings changed every day.

All the data collected from the patients was then coded and tabulated in Microsoft excel spreadsheet. The data was statistically analyzed.

STATISTICAL METHODS

The present study is done in KLE'S Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi and the findings are tabulated as below.

During the study year from January 2019 to December 2019, 60 patients with diabetic foot ulcers are randomized into study (Topical Atorvastatin and saline dressings) and control (saline dressings) groups. These groups were studied for the effect of topical atorvastatin and saline dressings versus saline dressing alone on decrease in size of ulcer.

Total of 60 patients met the selection criteria. Categorical data was denoted as rate and percentage. The expression of continuous data was in form of mean +/- standard deviation or SD. Comparison of categorical results were carried out using Chi square test and of continuous results by dependent t test and independent t test.

Probability value (p value) of less than or equal to 0.05 was considered as statistically significant.

RESULTS

The present study is conducted in KLE'S Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi, from January 2019 to December 2019,

In total, 60 patients with diabetic foot ulcers are randomized into test (Topical atorvastatin with saline dressings) and control (saline dressings) groups. These groups were studied for the effect of topical atorvastatin with saline dressings versus saline dressings alone on reduction in size of the ulcer.

Data collected was included into spreadsheets of Microsoft excel. The data was analysed and the results obtained were tabulated as represented below.

'P' value less than 0.05 considered significant statistically.

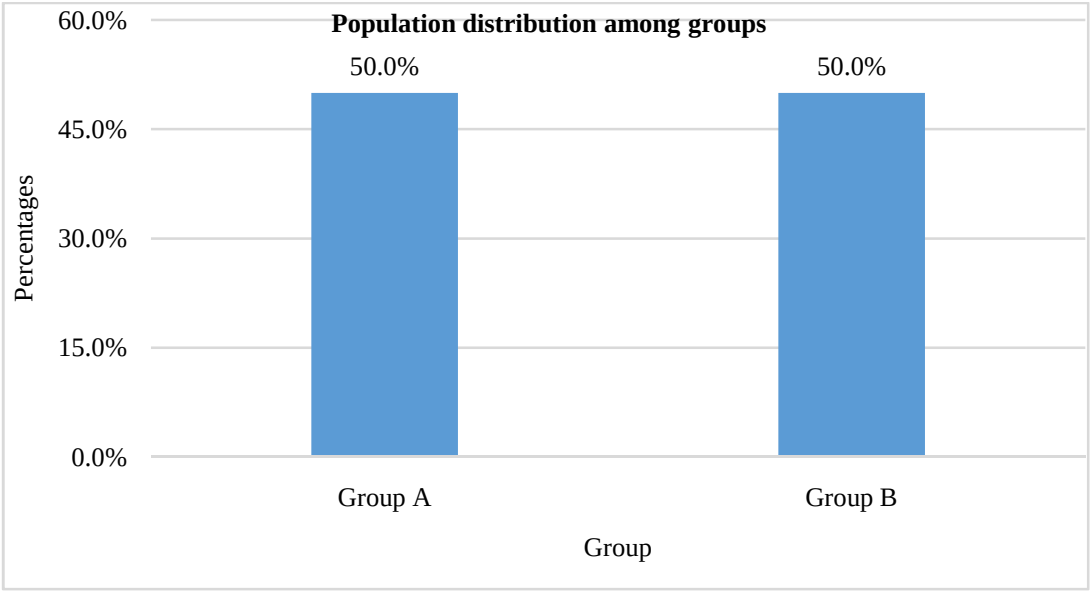
Results:A total of 60 subjects were present in the final analysis.

1. Population distribution in study

Table 11: Descriptive analysis of group in the study population (N=60)

Group	Frequency	Percentages
Group A	30	50.00%
Group B	30	50.00%
TOTAL	60	100.00%

Graph 2: Bar chart of group in the study population (N=60)



Among the study population 30(50%) participants were in group A and remaining 30(50%) participants were in group B. (Table 11 and Graph 2)

Group A - TEST GROUP - population where topical atorvastatin with saline dressing done.

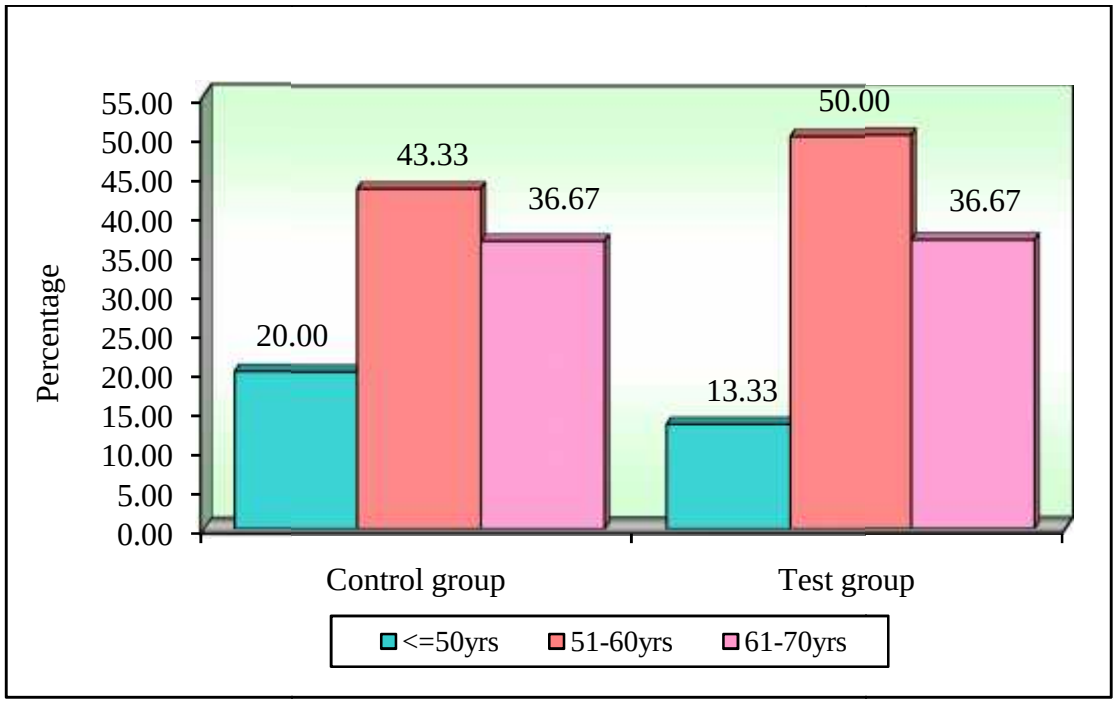
Group B - CONTROL GROUP - population where saline dressing done.

2. Age, Gender, Socio-economic status

Table 12: Comparison of control group and test group by demographic profile(age, gender and socio-economic scale)

Profile	Control group	%	Test group	%	Total	%	χ^2	p-value
Age groups								
<=50yrs	6	20.00	4	13.33	10	16.67	0.5429	0.7623
51-60yrs	13	43.33	15	50.00	28	46.67		
61-70yrs	11	36.67	11	36.67	22	36.67		
Gender								
Male	28	93.33	24	80.00	52	86.67	2.3077	0.1287
Female	2	6.67	6	20.00	8	13.33		
SES								
Low SES	25	83.33	26	86.67	51	85.00	1.0196	0.6006
Middle SES	4	13.33	4	13.33	8	13.33		
High SES	1	3.33	0	0.00	1	1.67		
Total	30	100.00	30	100.00	60	100.00		

Graph 3- Cluster Bar graph: Comparison of control group and test group by age groups

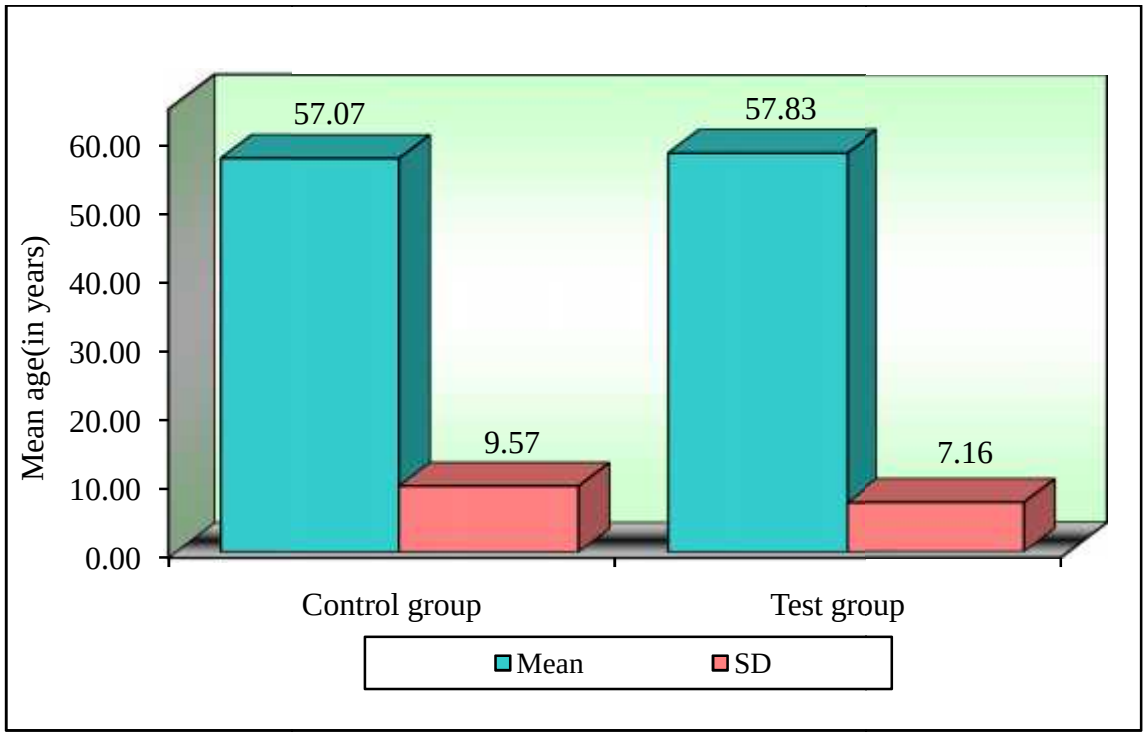


Among the control group, 6(20%) were aged below 50 years, 13(43.33%) were aged between 51-60 years and 11(36.67%) were aged between 61-70 years.Among the study group, 4(13.33%) were aged below 50 years, 15(50%) were aged between 51-60 years and 11(36.67%) were aged between 61-70 years.The total number of patients included in the study were found to be 10(16.67%) aged below 50 years, 28(46.67%) aged between 51-60 years and 22(36.67%) aged between 61-70 years. No statistically significant difference between the groups as per the age distribution was noted (p value = 0.7623) (Table 12 and Graph 3)

Table 13: Comparison of control group and test group with mean age by independent t test

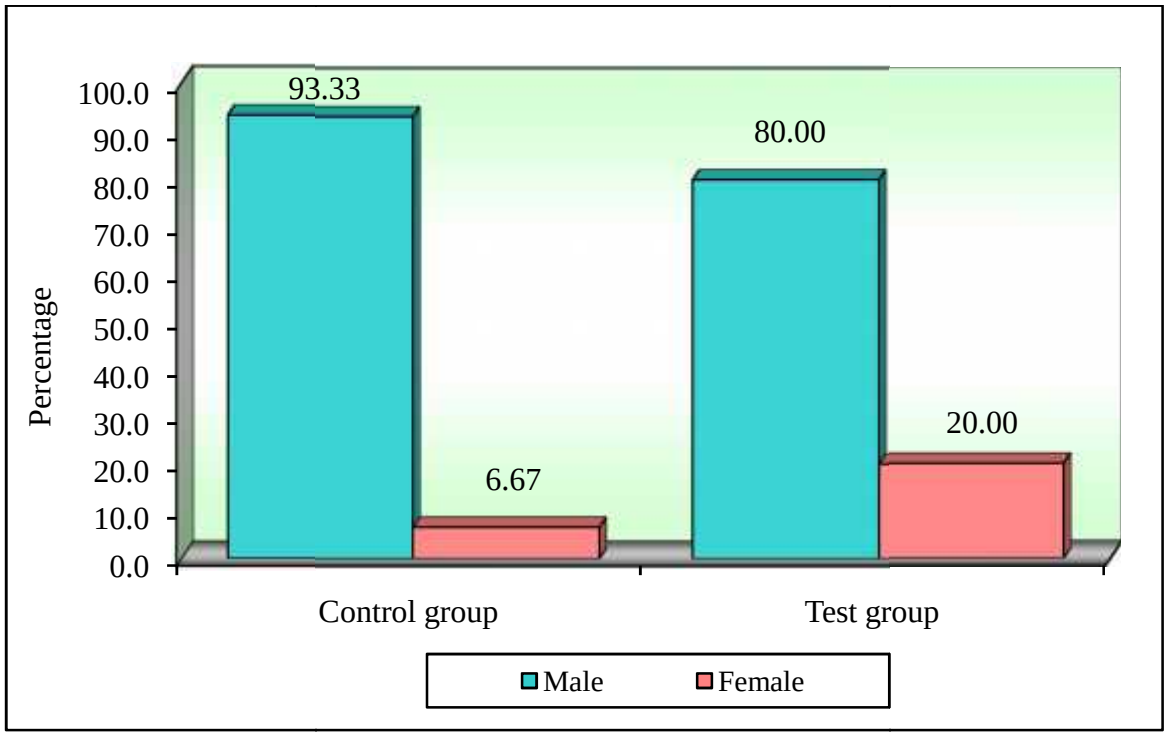
Groups	Mean	SD	SE	t-value	P-value
Control	57.07	9.57	1.75	-0.3514	0.7266
Test	57.83	7.16	1.31		

Graph 4: Cluster graph showing comparison of control group and test group with mean age(in years)



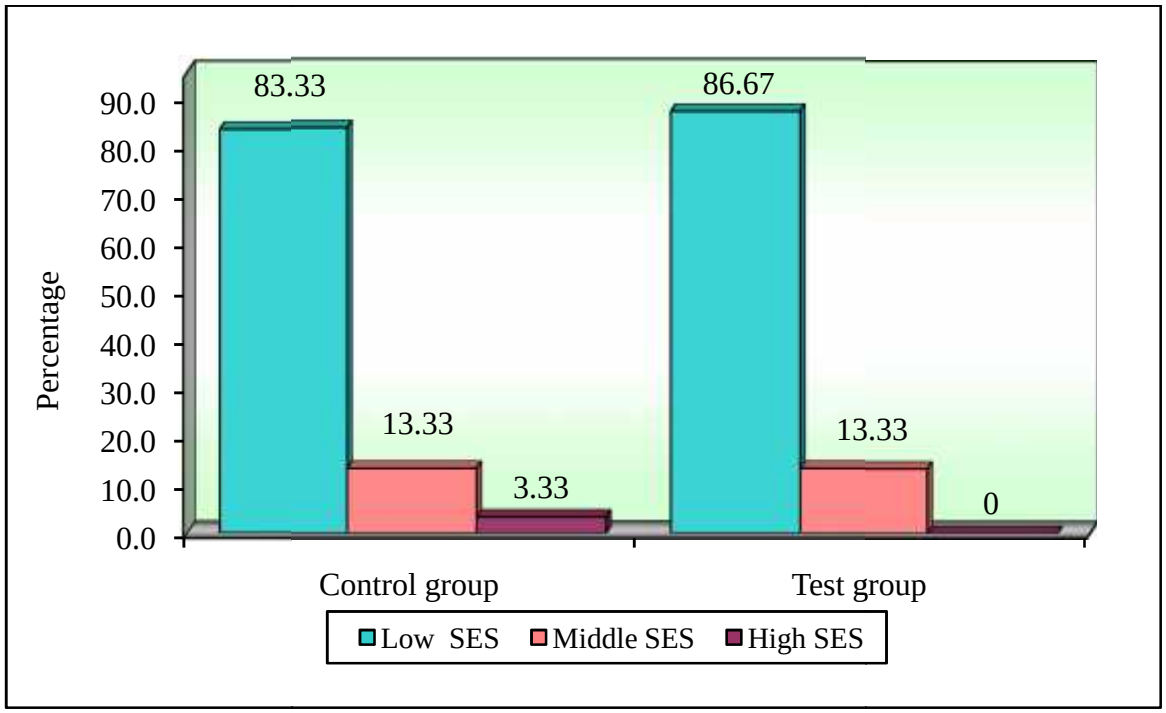
The mean age in both the control and test groups were 57.07 and 57.83 years, respectively. This was a closely comparable value between the two groups (p value = 0.7266)(Table 13 and graph 4)

Graph 5: Cluster graph showing comparison of control group and test group by gender



In the control group, 28 (93.33%) were males, 2 (6.67%) were females. In the test group, 24 (80%) were males, 6 (20%) were females. As per the gender distribution, no statistically significant difference was noted in both the two groups(p value 0.1287).(Table 12 and graph 5)

Graph 6: Cluster graph showing comparison of control group and test group by socio economic status



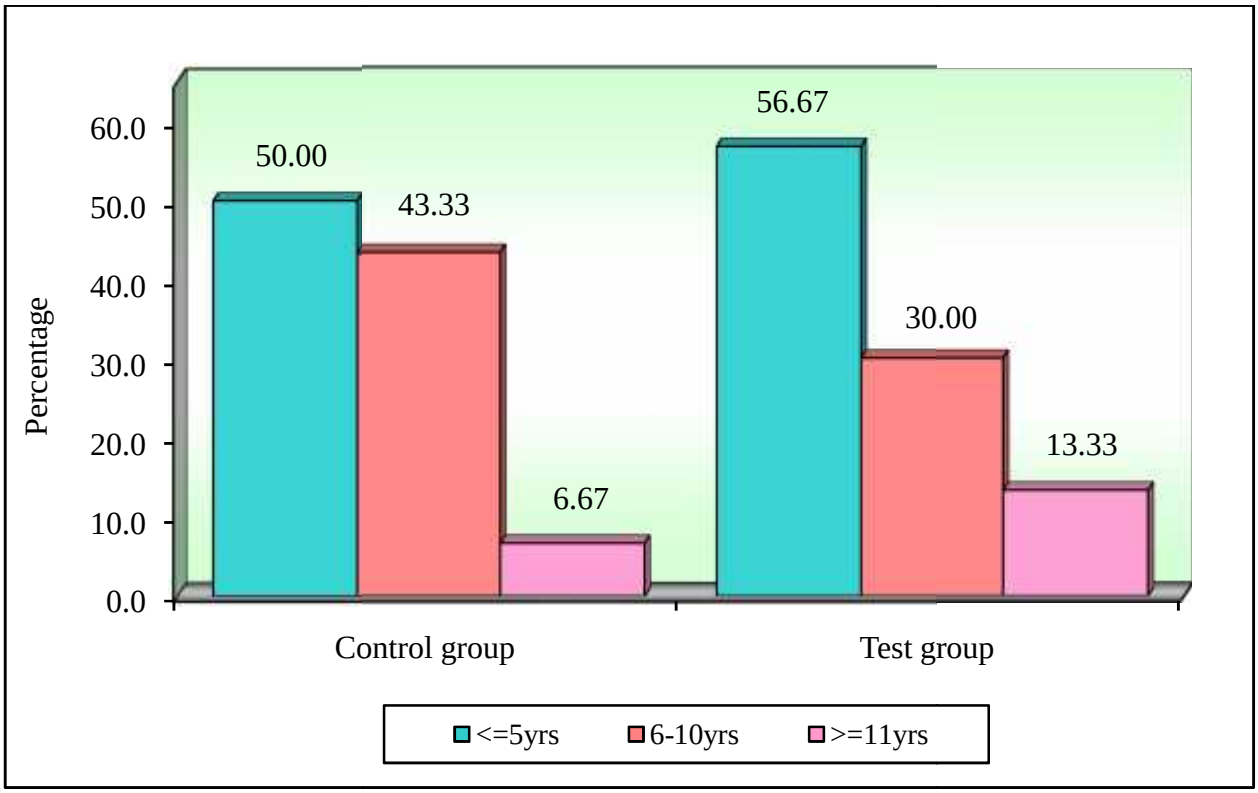
In the control group, 25 (83.33%) belonged to low socioeconomic status, 4 (13.33%) belonged to middle class, 1(3.33%) were belonged to high class. In the test group, 26 (86.67%) belonged to low socioeconomic status, 4(13.33%) belonged to middle class. There was no patients belonging to high socioeconomic status in the test group. No statically significant difference between the groups as per socio economic status distribution was noted. (P value 0.6006). (Table 12 and graph 6). The classification was done based on Modified B.G Prasad classification for economic status.[59]

3. Duration of diabetes, Onset of ulcer and Duration of ulcer

Table 14: Comparison of control group and test group by duration of diabetes, onset and duration of ulcer

Profile	Control group	%	Test group	%	Total	%	χ^2	p-value
Duration of diabetes								
<=5yrs	15	50.00	17	56.67	32	53.33	1.5189	0.4679
6-10yrs	13	43.33	9	30.00	22	36.67		
>=11yrs	2	6.67	4	13.33	6	10.00		
Onset of ulcer								
Traumatic	21	70.00	21	70.00	42	70.00	0.0000	1.0000
Spontaneous	9	30.00	9	30.00	18	30.00		
Duration of ulcer								
<4 weeks	22	73.33	21	70.00	43	71.67	0.0820	0.7740
>4 weeks	8	26.67	9	30.00	17	28.33		
Total	30	100.00	30	100.00	60	100.00		

Graph 7: Cluster graph showing Comparison of control group and test group by duration of diabetes



In the control group, 15 (50%) had diabetes for less than 5 years, 13 (43.33%) had diabetes duration ranging between 6 to 10 years, 2 (6.67%) had diabetes for more than 11 years.

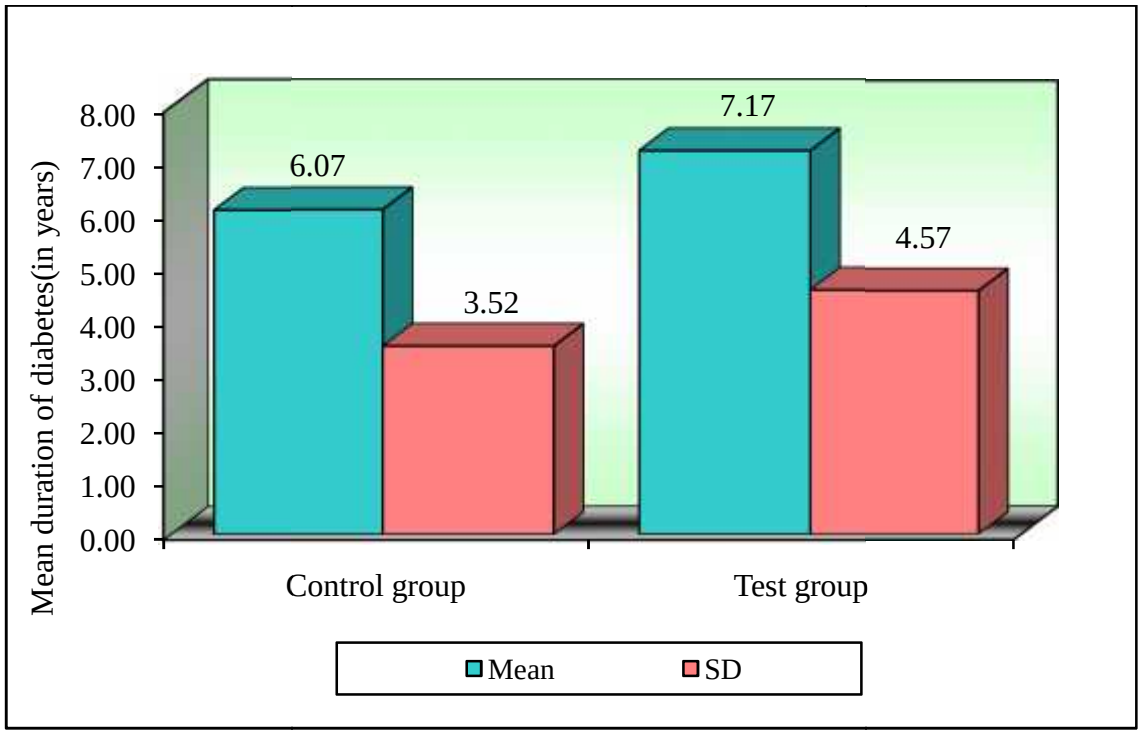
In the test group, 17 (56.67%) had diabetes for less than 5years, 9 (30%) had diabetes duration ranging between 6 to 10 years, 4 (13.33%) had diabetes for more than 11 years.

No statistically significant difference between the two groups(p value= 0.4679) as per the duration of diabetes was noted. (Table 14 and graph 7)

Table 15: Comparison of control group and test group with mean duration of diabetes(in years) by independent t test

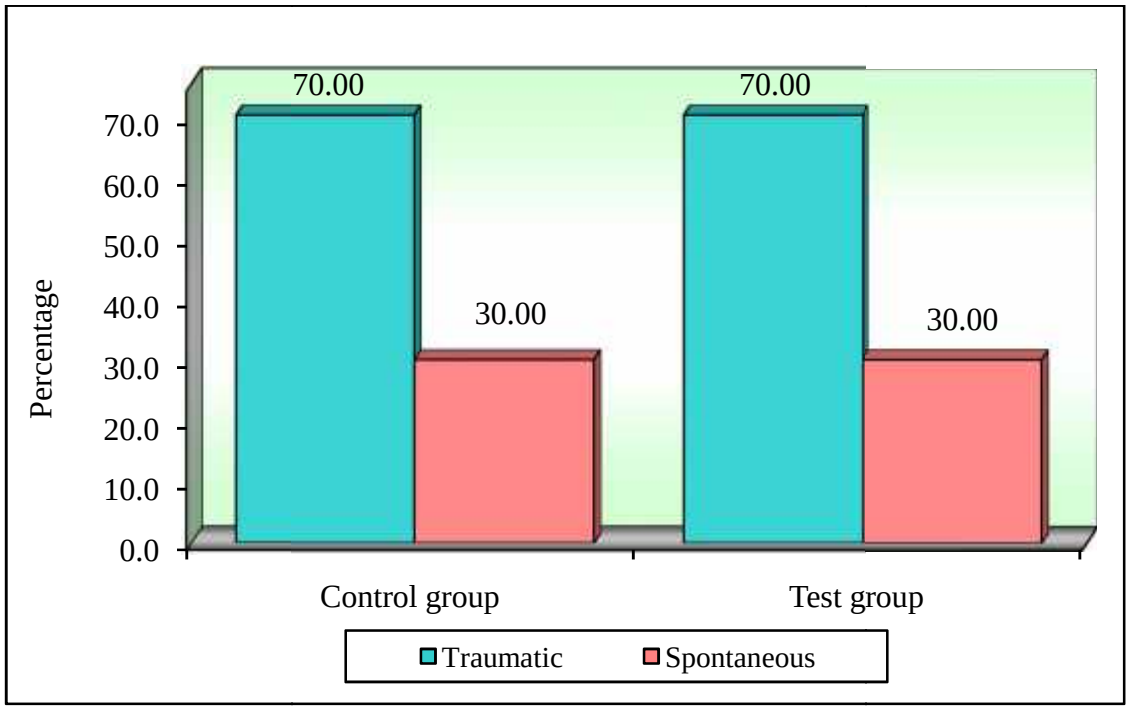
Groups	Mean	SD	SE	t-value	P-value
Control	6.07	3.52	0.64	-1.0459	0.3000
Test	7.17	4.57	0.83		

Graph 8: Cluster graph showing comparison of control group and test group with mean duration of diabetes (in years)



The mean duration of diabetes in both the control and test groups were6.07 and 7.17 years, respectively. No statistically significant difference between the two groups was noted (p value = 0.3000)(Table 15 and graph 8).

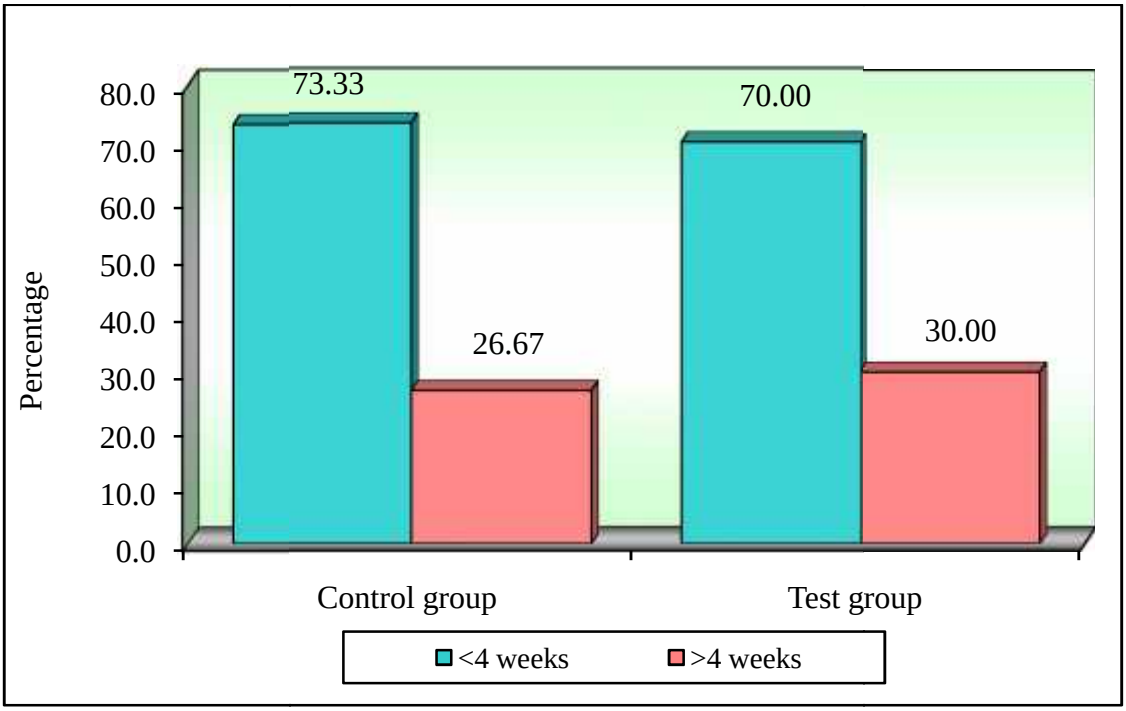
Graph 9: Cluster graph showing comparison of control group and test group by onset of ulcer



In both the control and test groups**,** 21 (70%) had traumatic onset and 9(30%) had spontaneous onset.

No statistically significant difference between two groups as per the onset of ulcer was noted (p value=1.0000)(Table 14 and graph 9).

Graph 10: Cluster graph showing comparison of control group and test group by duration of ulcer



In the control group, in 22(73.33%) patients the duration of the ulcer was less than 4 weeks and in 8(26.67%) patients the duration of the ulcer was more than 4 weeks.

In the test group, in 21(70%) patients the duration of the ulcer was less than 4 weeks and in 9(30%) patients the duration of the ulcer was more than 4 weeks.

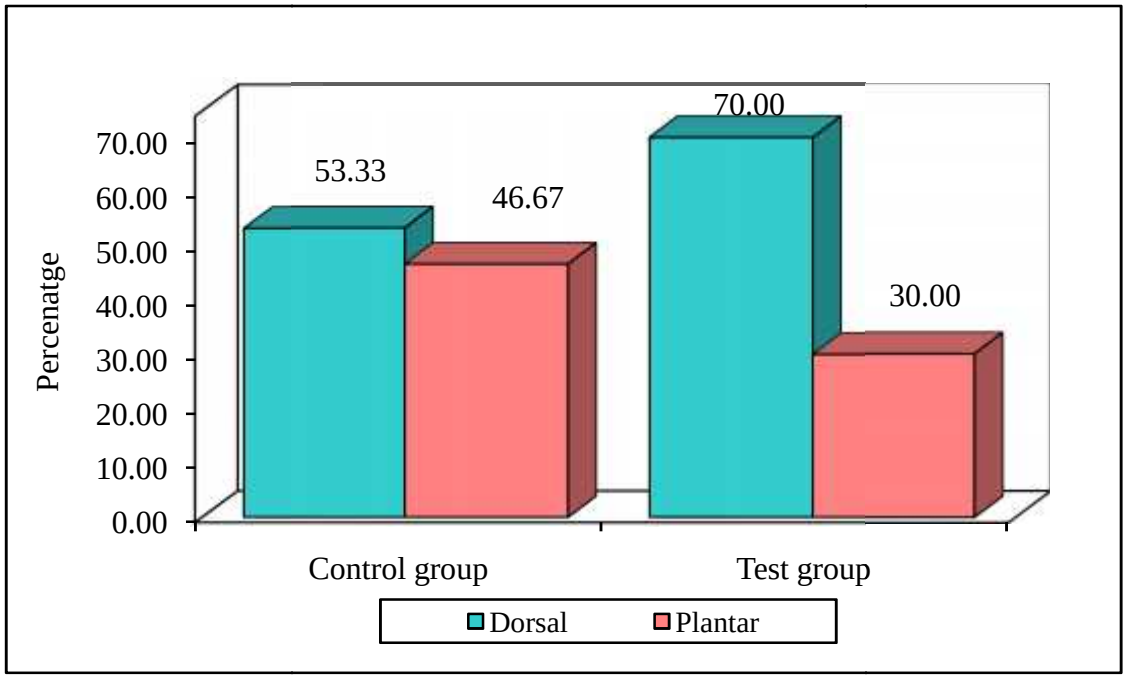
No statistically significant difference between the two groups as per the duration of the ulcer was noted(p value= 0.7740)(Table 14 and graph 10).

1. **Site of ulcer**

Table 16: Comparison of control group and test group by site of ulcer

Site	Control group	%	Test group	%	Total	%	χ^2	p-value
Dorsal	16	53.33	21	70.00	37	61.67	1.7630	0.1840
Plantar	14	46.67	9	30.00	23	38.34		
Total	30	100.00	30	100.00	60	100.00		

Graph 11: Cluster graph showing comparison of control group and test group by ulcer site



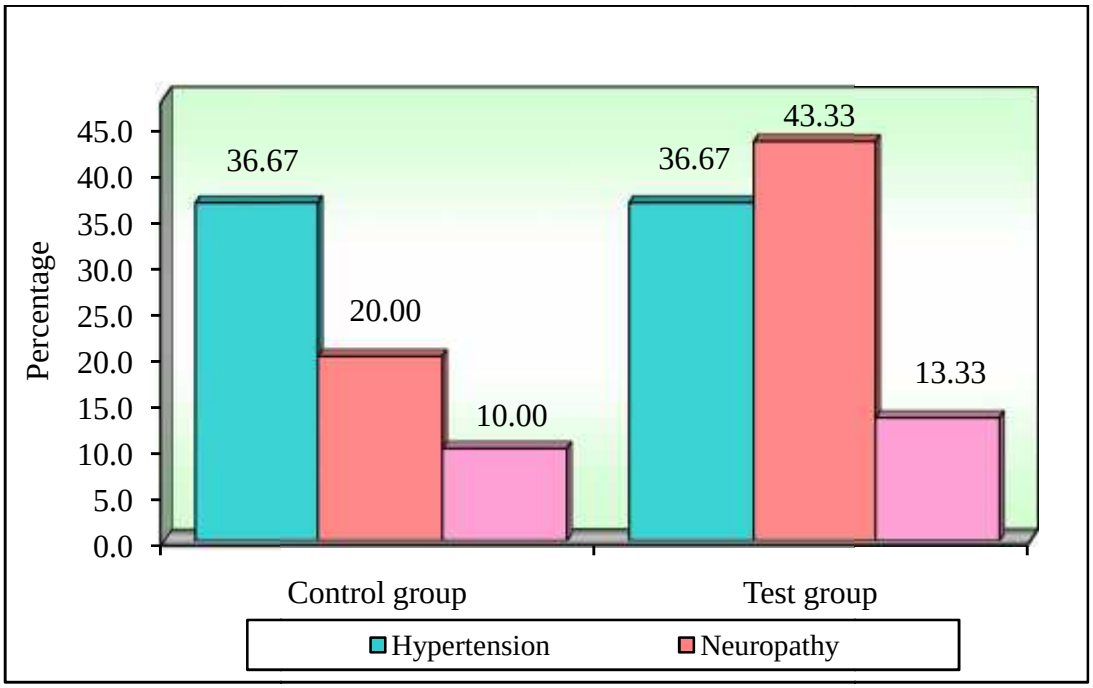
In the control group, 16 (53.33%) had ulcer on the dorsum and in 14 (46.67%) patients, the ulcer was present on the sole. In the test group, 21(70%) had ulcer on the dorsum and 9(30%) had ulcer on the sole. No statistically significant difference between the groups as per the site of ulcer was noted(p value= 0.1840)(Table 16 and graph 11).

5.Systemic Diseases

Table 17: Comparison of control group and test group by systemic disease

Profile	Control group	%	Test group	%	Total	%	χ^2	p-value
Hypertension								
Yes	11	36.67	11	36.67	22	36.67	0.0000	1.0000
No	19	63.33	19	63.33	38	63.33		
Neuropathy								
Yes	6	20.00	13	43.33	19	31.67	3.7740	0.0520
No	24	80.00	17	56.67	41	68.33		
Peripheral vascular disease								
Yes	3	10.00	4	13.33	7	11.67	0.6690	0.4130
No	27	90.00	26	86.67	53	88.33		
Total	30	100.00	30	100.00	60	100.00		

Graph 12: Cluster graph showing comparison of control group and test group by presence of systemic diseases



In both the control and test groups, 11 (36.67%) patients had hypertension and 19(63.33%) did not. No statistically significant difference between both the groups as per hypertension was noted(p value= 1.0000). (Table 17 and graph 12).

In the control group, 6(20%) had neuropathy while 24 (80%) patients did not. In the test group, 13 (43.33%) had neuropathy while 17(56.67%) did not. No statistically significant difference between both the groups as per the neuropathy distribution was noted(P value 0.0520)(Table 17 and graph 12).

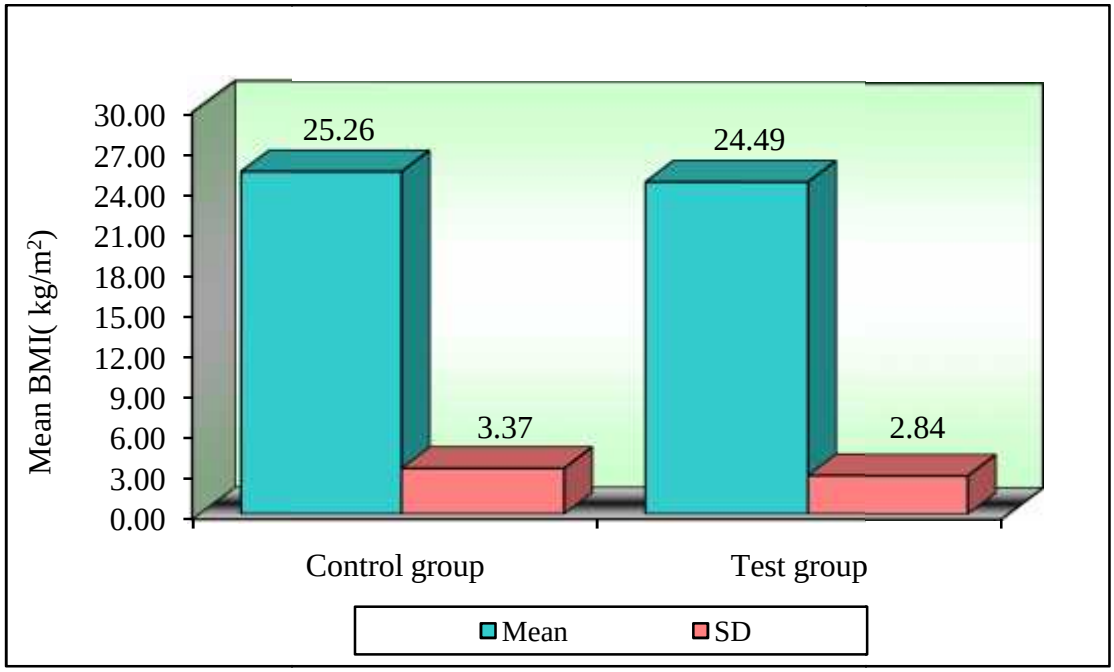
In the control group, 3(10%) had peripheral vascular disease while 27(90%) patients did not. In the test group, 4(13.33%) had peripheral vascular disease while 26(86.67%) did not. No statistically significant difference between both the groups based on the peripheral vascular disease distribution was noted(P value 0.4130)(Table 17 and graph 12).

6.Body Mass Index (BMI)

Table 18: Comparison of control group and test group with mean BMI(kg/m^2) by independent t test

Groups	Mean	SD	SE	t-value	P-value
Control	25.26	3.37	0.62	0.9523	0.3449
Test	24.49	2.84	0.52		

Graph 13: Cluster graph showing comparison of control group and test group with mean BMI(kg/m^2)



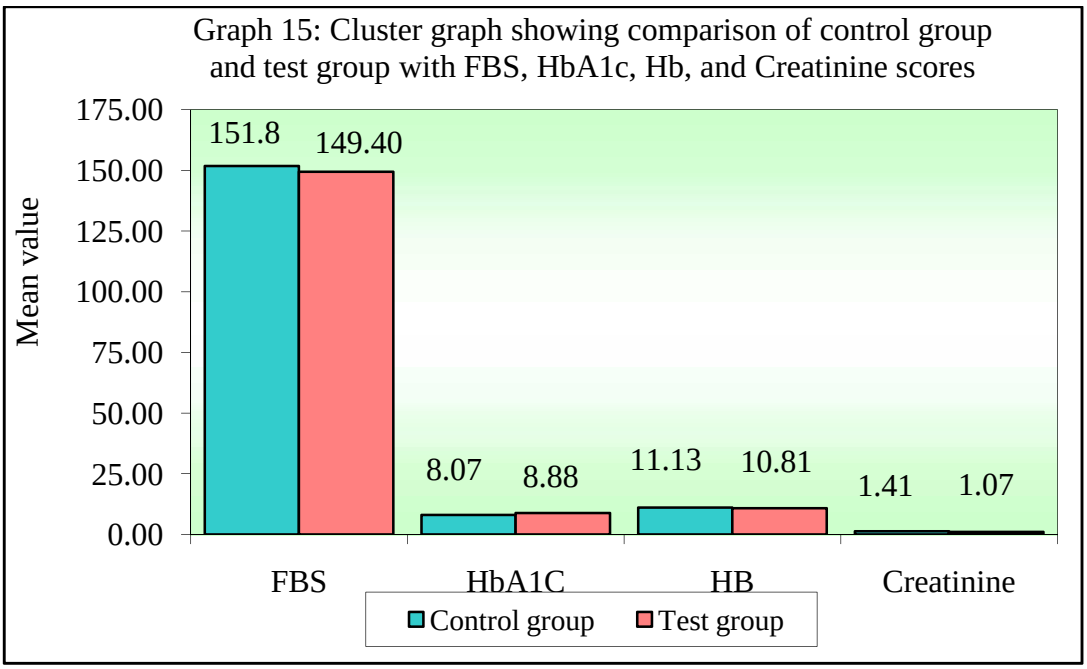
The mean BMI in the control group is 25.26kg/m^2 and in the test group is 24.49kg/m^2. As per the BMI distribution, the difference between both the groups was not statistically significant (P value 0.3449)(Table 18 and graph 13).

7. Fasting blood sugar, HbA1c, Haemoglobin, Creatinine

Table 19: Comparison of control group and test group with FBS, HbA1C, Hb, and Creatinine scores by independent t test

Parameter	Groups	Mean	SD	SE	t-value	P-value
FBS(mg/dL)	Control	151.83	13.82	2.52		
	Test	149.40	15.64	2.86	0.6387	0.5256
HbA1c(%)	Control	8.07	1.52	0.28		
	Test	8.88	1.73	0.32	-1.9250	0.0591
Hb(g/dL)	Control	11.13	1.38	0.25		
	Test	10.81	2.41	0.44	0.6182	0.5389
Creatinine(mg/dL)	Control	1.41	0.50	0.09		
	Test	1.07	0.42	0.08	2.8083	0.0068

Graph 14: Cluster graph showing comparison of control group and test group with FBS, HbA1c, Hb, and Creatinine scores



The mean 'Fasting blood sugar' in the control group is 151.83mg/dL and in the test group is 149.40mg/dL. No statistically significant difference between the two groups as per the fasting blood sugar was noted(p value = 0.5256)(Table 19 and graph 14).

The mean HbA1c in the control group is 8.07% and in the test group is 8.88%. No statistically significant difference between the two groups based on the HbA1c was noted(p value = 0.0591)(Table 19 and graph 14).

The mean haemoglobin in the control group is 11.13g/dL and in the test group is 10.81g/dL. No statistically significant difference between the two groups as per the haemoglobin was noted(p value = 0.5381)(Table 19 and graph 14).

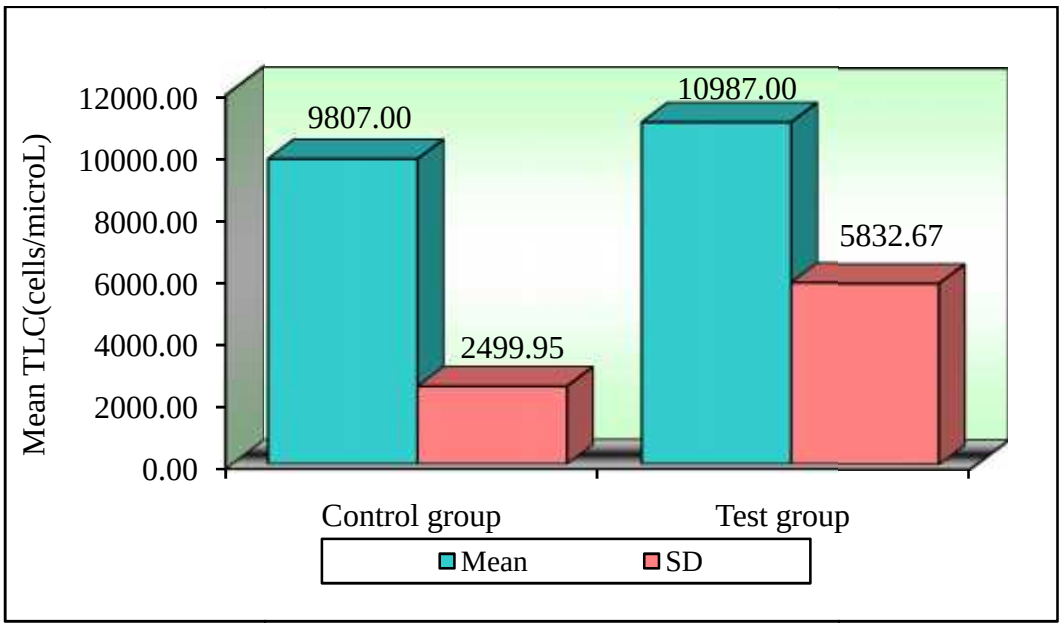
The mean creatinine in the control group is 1.41mg/dL and in the test group is 1.07mg/dL. This was a statistically significant difference between the two groups(p value = 0.0068)(Table 19 and graph 14).

8. Total Leucocyte Count (TLC)

Table 20: Comparison of control group and test group with mean TLC(Total Leucocyte Count)(cells/microL) by independent t test

Groups	Mean	SD	SE	t-value	P-value
Control	9807.00	2499.95	456.43	-1.0185	0.3127
Test	10987.00	5832.67	1064.90		

Graph 15: Cluster graph showing comparison of control group and test group with mean TLC(cells/microL)



The mean 'Total leucocyte count' in the control group is 9807.00cells/microL and in the test group is 10987.00cells/microL. No statistically significant difference between the two groups based on the 'total leucocyte count' value was noted(p value = 0.3127)(Table 20 and graph 15).

9. Wound Healing property (Ulcer area reduction)

The reduction in area and percentage reduction in area are calculated as follows:

Wound area on D0 = x

Wound area on D14 = y

Reduction in wound area = x-y

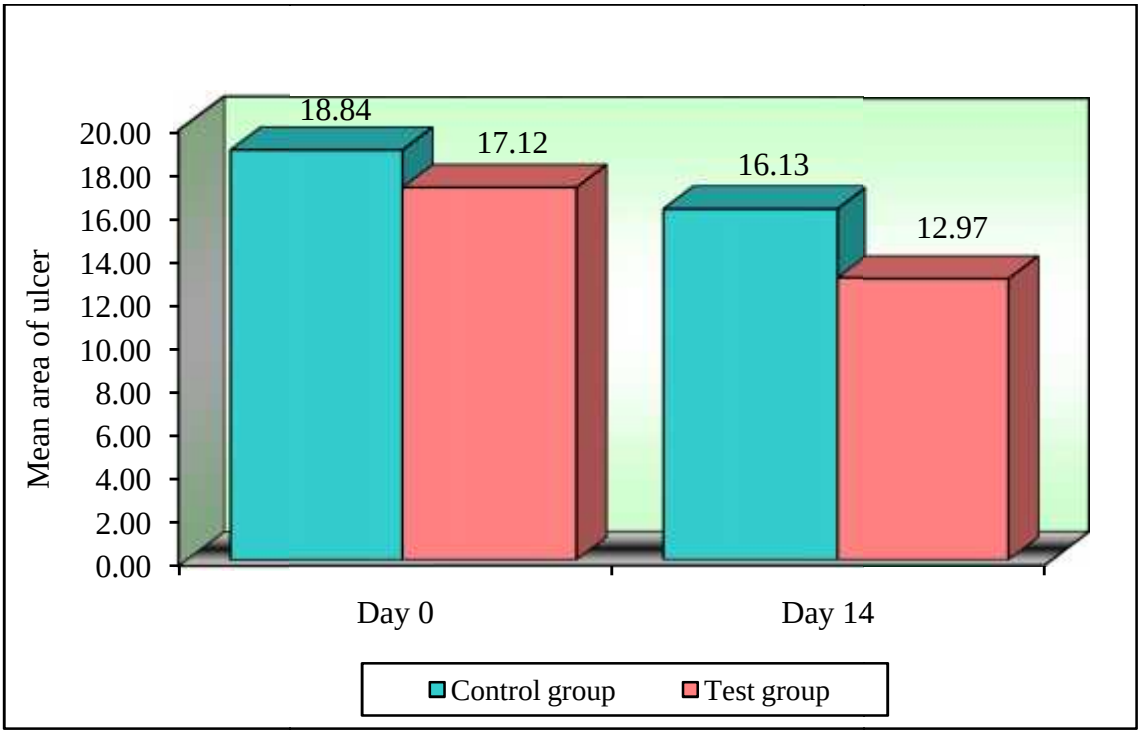
% Reduction in wound area (**Wound Contraction**)=$\frac{x-y}{x}$ X 100

Table 21: Comparison of control group and test group with Mean Area of Ulcer (cm^2) on day 0 and day 14

Time	Groups	Mean	SD	SE	t-value	P-value
Day 0	Control	18.84	11.01	2.01		
	Test	17.12	12.82	2.34	0.5590	0.5783
Day 14	Control	16.13	9.80	1.79		
	Test	12.97	10.49	1.92	1.2053	0.2330
Difference	Control	2.72	1.92	0.35		
	Test	4.15	3.37	0.61	-2.0283	0.0471*

*p<0.05

Graph 16: Cluster graph showing comparison of control group and test group with Mean Area of Ulcer on day 0 and day 14



By independent t test, the mean area of the ulcer on day 0 in the control group is 18.84cm^2 and in the test group is 17.12cm^2. Similarly, the mean area of the ulcer on

day 14 in the control group is 16.13$cm^2$and in the test group is 12.97cm^2. The difference between the two groups as per the mean area of the ulcer was not statistically significant both on day 0(p value = 0.5783) and day 14(p value = 0.2330)(Table 21 and graph 16).

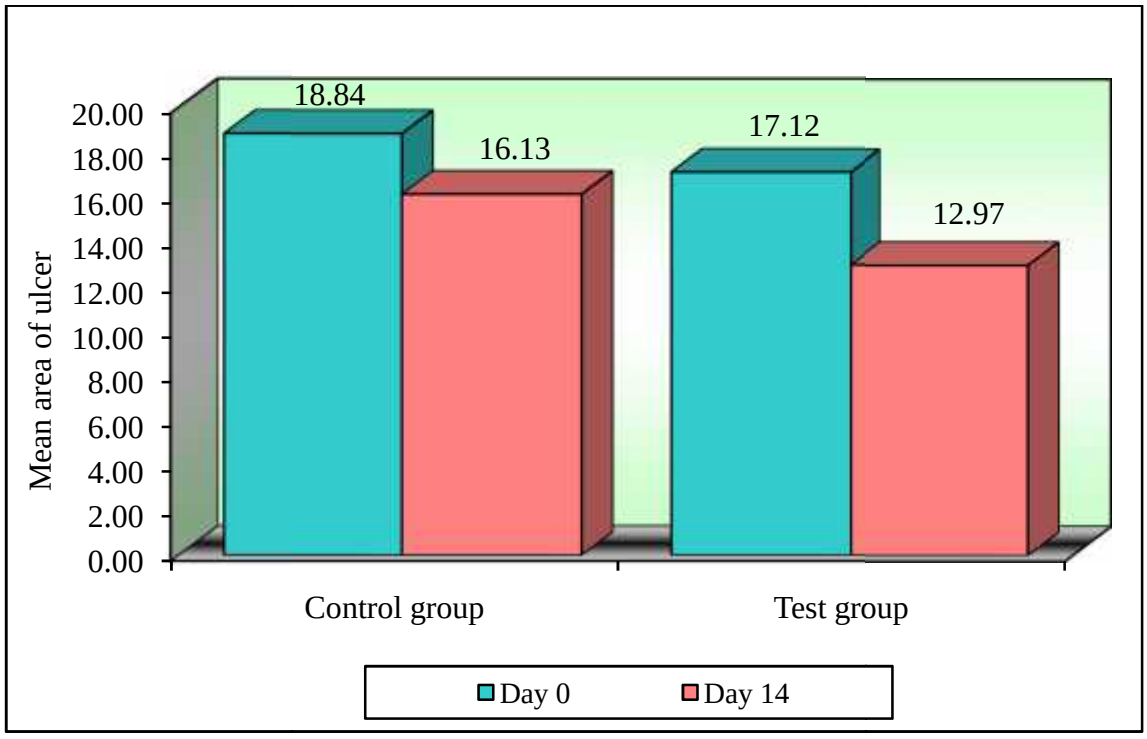
The difference in the mean areas of ulcer over 14 days in the control and test groups were 2.72cm^2 and 4.15cm^2, respectively. This result comparing the two groups was statistically significant (p value = 0.0471). Thus, this signified that the ulcer area reduction was statistically significant in the test group in comparison to the control group(Table 21 and graph 16).

Table 22: Comparison of Area of Ulcer on day 0 and day 14 in control group and test group

Groups	Time points	Mean	Std.Dv.	Mean Diff.	SD Diff.	t-value	P-value
Control	Day 0	18.84	11.01	2.72	1.92	7.7517	0.0001*
	Day 14	16.13	9.80				
Test	Day 0	17.12	12.82	4.15	3.37	6.7511	0.0001*
	Day 14	12.97	10.49				

*p<0.05

Graph 17: Cluster graph showing comparison of Area of Ulcer on day 0 and day 14 in control group and test group



By dependent t test,the mean area of the ulcer in the control group on day 0 and day 14 was 18.84cm^2 and 16.13cm^2, respectively with the mean difference in ulcer size being 2.72cm^2. The reduction in area of the ulcer over 14 days in the control group was statistically significant(p value = 0.0001). The mean area of the ulcer in the test

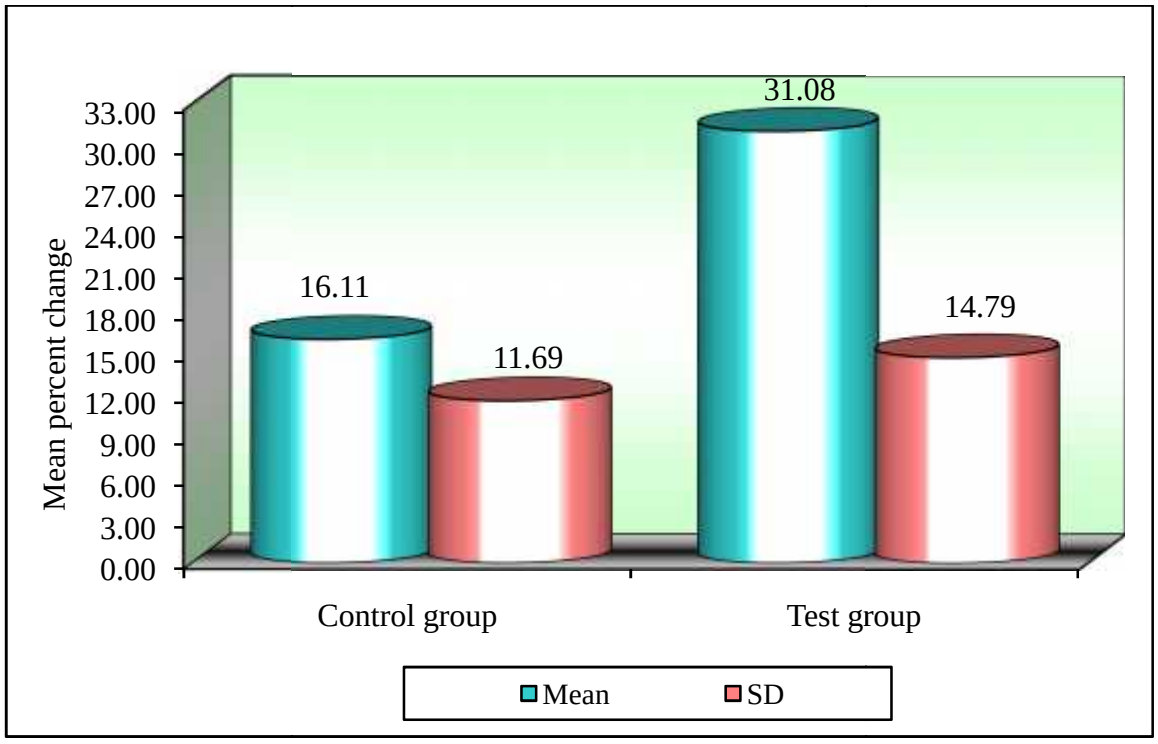
group on day 0 and day 14 was 17.12cm^2 and 12.97cm^2, respectively with the mean difference in ulcer size being 4.15cm^2. The reduction in area of the ulcer over 14 days in the test group was statistically significant (p value = 0.0001)(Table 22 and graph 17).

Table 23: Comparison of control group and test group with mean percentage reduction in area of Ulcer on day 0 and day 14 by independent t test

Groups	Mean	SD	SE	t-value	P-value
Control group	16.11	11.69	2.13	-4.3519	0.0001*
Test group	31.08	14.79	2.70		

*p<0.05

Graph 18: Cluster graph showing comparison of control group and test group with mean percent changes in area of Ulcer on day 0 and day 14



By independent t test, mean percentage reduction in area of ulcer over 14 days in both control and test groups are 16.11% and 31.08%, respectively. The mean percent reduction in ulcer area over 14 days was significantly more in the test group than in the control group(p value = 0.0001). This signified that the reduction in ulcer area over 14 days was more in the test group where topical atorvastatin was applied along with saline for dressing(Table 23 and Graph 18).

10. Antimicrobial Property

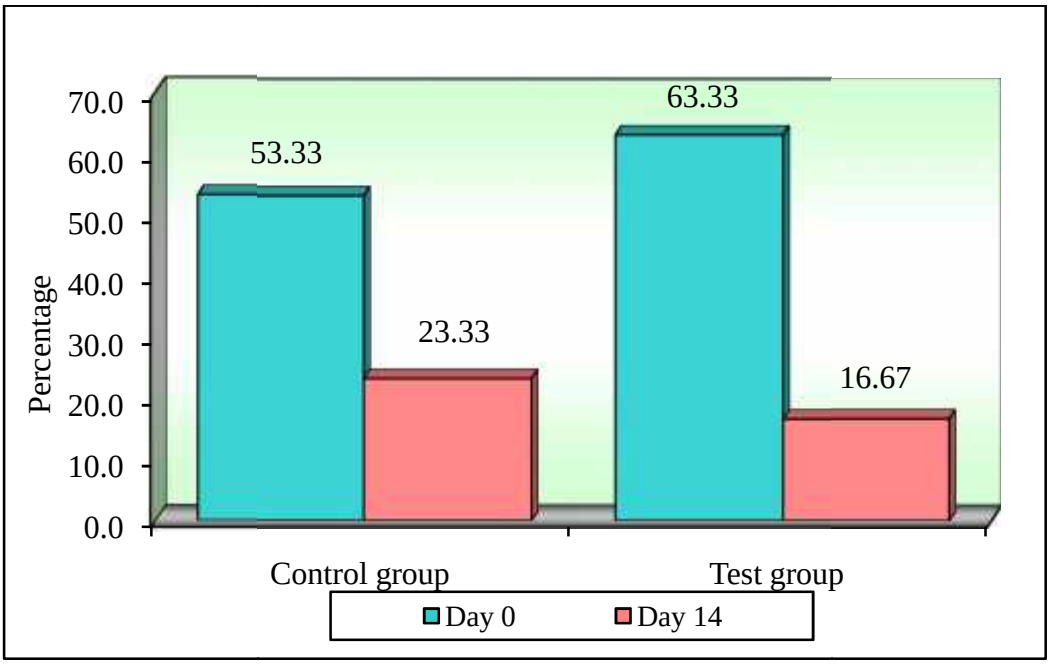
The presence of bacterial colonization in diabetic foot ulcers is detrimental in the process of its healing. This study evaluated the antimicrobial capacity of topical atorvastatin in comparison to normal saline.

Presence of wound infection

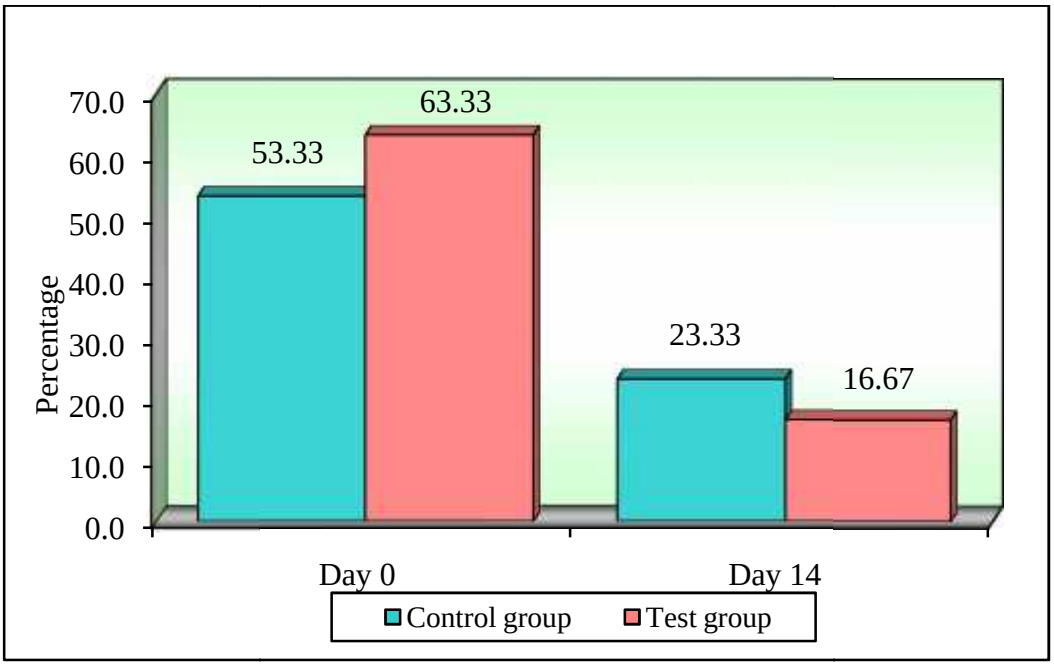
Table 24 : Comparison of control group and test group with Culture on day 0 and day 14

Culture at	Control group	%	Test group	%	Total	%	χ^2	p-value
Day 0								
Absent	14	46.67	11	36.67	25	41.67	0.6170	0.4321
Present	16	53.33	19	63.33	35	58.33		
Day 14								
Absent	23	76.67	25	83.33	48	80.00	0.4170	0.5186
Present	7	23.33	5	16.67	12	20.00		
Total	30	100.00	30	100.00	60	100.00		
Between Day 0 and 14	Mc Nemar χ^2, p=0.0040		Mc Nemar χ^2, p=0.0001					

Graph 19: Cluster graph showing comparison of control group and test group with Culture present on day 0 and day 14



Graph 20: Cluster bar graph showing comparison of Culture present on day 0 and day 14 for control and test groups



On the day 0, the control group showed 16 (53.33%) patients and the test group showed 19(63.33%) patients with positive culture report. This was not a statistically significant difference between both the groups as per the presence of positive culture report (p value = 0.4321). On the day 14, the control group showed 7 (23.33%) patients and the test group showed 5(16.67%) patients with positive culture report. However, this was not significant statistically (p value = 0.5186)(Table 24 and graphs 19,20).

In both the control and test groups, there was a reduction in the bacterial population.

In the control group, the number of patients with positive culture report on day 0 were 16(53.33%) and those on day 14 were 7(23.33%). This was statistically significant reduction in bacterial population in the control group(p=0.0040)(Table 24 and graphs 19,20).

In the test group, the number of patients with positive culture report on day 0 were 19(63.33%) and those on day 14 were 5(16.67%). This was statistically significant reduction in bacterial population in the test group(p=0.0001)(Table 24 and graphs 19,20). On considering the p value in the control group(0.004) and in the test group(0.0001), it was thus deduced that dressing with topical atorvastatin with saline expressed a greater decrease in bacterial bioburden than saline dressing alone as the p value is very very low in the test group.

Bacteria isolated

Table 25: Comparison of control group and test group by Organism grown from culture on Day 0

Organism	Control group	%	Test group	%	Total	%
Absent	14	46.67	11	36.67	25	41.67
Acinetobacter baumanni	2	6.67	0	0.00	2	3.33
Coagulase negative staphylococcus	0	0.00	1	3.33	1	1.67
Enterobacter cloacae	1	3.33	1	3.33	2	3.33
Enterococcus faecalis	1	3.33	1	3.33	2	3.33
Escherichia coli	1	3.33	1	3.33	2	3.33
Klebsiella oxytoca	1	3.33	0	0.00	1	1.67
Klebsiella pneumoniae	4	13.33	5	16.67	9	15.00
Kluyveraascorbata	0	0.00	1	3.33	1	1.67
MRSA	2	6.67	0	0.00	2	3.33
Proteus mirabilis	0	0.00	2	6.67	2	3.33
Proteus vulgaris	0	0.00	1	3.33	1	1.67
Pseudomonas aeruginosa	1	3.33	4	13.33	5	8.33
Staphylococcus aureus	2	6.67	2	6.67	4	6.67
Staphylococcus epidermidis	1	3.33	0	0.00	1	1.67
Total	30	100.00	30	100.00	60	100.00

In the control group, the most common bacterial organisms obtained from the day 0 culture included Klebsiella pneumonia (13.33%), Methicillin resistant Staphylococcus aureus(6.67%), Staphylococcus aureus(6.67%) and Acinetobacter baumanni(6.67%). In the test group, the most common bacterial organisms obtained from the day 0 culture included Klebsiella pneumonia(13.33%), Pseudomonas aeruginosa(13.33%),Proteus mirabilis(6.67%) and Staphylococcus aureus(6.67%)(Table 25).

DISCUSSION

Diabetes is one of the major non-communicable diseases with an ever rising prevalence both worldwide and in India. Diabetic foot ulcer is one of the major complications ofdiabetes with an estimated life incidence of 15-25% in the diabetic population.[60] Improper or inadequate treatment of diabetic foot ulcers carry major risk of amputation of the lower limb and can even lead to mortality.

Wound healing in diabetic foot ulcers involves a complex interplay of numerous factors, both intrinsic and extrinsic to the patient. Only proper tackling of all these factors such asneuropathy, vasculopathy, local infection, immunity can guarantee an optimum healing of the ulcer. Since the beginning, management of diabetic wounds involves an apt glycemiccontrol with anti-diabetic medication, antimicrobial treatment and local dressing.

Over the decades, various newer advances have been attained for the local wound care of diabetic foot ulcers. Due to the rising antibiotic resistance and possibility of adverse effects with other treatment options, there has always been a search for an allrounder modality that could be therapeutically effective, inexpensive, accessible with minimal or no side effects.

Statins, that are being used for the treatment of hyperlipidemias, have reflected huge potential in the process of wound healing due to its proven pleiotropic properties. Some of them are its anti-inflammatory nature, immunomodulatory, epithelilialization, reperfusion, and antibacterial capacity.[11]

Many studies have previously showcased the advantages of topical statin therapy for the treatment of conditions such as chronic vascular ulcers, pressure ulcers, periodontitis, psoriasis, etc.[11,53,56] However, there is a deficiency of adequate

clinical trials on the efficacy of topical statins on healing of diabetic foot ulcer. Available research in this regard is limited to invitro and animal models.

This study was an attempt to provide necessary knowledge regarding the benefits of topical atorvastatin over diabetic foot ulcers in terms of the time taken for reduction in ulcer size and bacterial population as compared to a conventional dressing modality of saline dressing.

This randomised control study was executed under the department of general surgery at KLES Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Nehru nagar. Belagavi, between January 2019 to December 2019. A total of 60 patients who satisfied the selection criteria and were willing to participate comprised the study population. These patients were categorized into two groups of 30 each, where the test group(group A) received treatment with topical atorvastatin emulgel along with saline dressing and control group(group B) received saline dressing alone (Group B).

The entire study population comprised of 10(16.67%) patients aged below 50 years, 28(46.67%) aged between 51-60 years and 22(36.67%) aged between 61-70 years. This clearly signified the prevalence of majority of the diabetic foot ulcers in patients who were more than 50 years of age. This was in agreement with the study done by Yazdanpanah et al that signified increasing age as a key risk factor for diabetic foot ulcer.[61]

The test group comprised of 24 (80%) patients who were males and 6 (20%) who were females whereas the control group included 28 (93.33%) male patients and only 2 (6.67%) female patients. This emphasized the male predisposition for diabetic foot ulcers in this study. ThanhDinh et al in his study proved that men have a higher

risk for foot ulceration due to them being at risk of more severe neuropathy, decreased joint stability and higher foot pressures. However, the presence of risk factors such as neuropathy nullified this difference between the two genders.[62] In this study, both the groups were comparable for the age distribution(p value= 0.7623) .

This study had divided the population in both the groups based on the patients' socio-economic status as per the modified BG Prasad classification into low(85%), middle(13.33%) and high(1.67%) socio-economic status groups.[59] On comparing both the groups based on socioeconomic status, no statistically significant difference was found. These findings were in congruence with the study done by Bikramjit et al which signified that the severity of the diabetic foot ulcer was directly proportional to the socio-demographic condition of the patient. A poor economic and educational status of the population could indirectly affect the progression, outcome and prognosis of the patient with diabetic foot ulcer.[63]This observation stresses on the importance of education, public awareness and overall socio-economic upliftment for the optimum management of the patient.

On the basis of the location of the ulcer, the control group had 16 (53.33%) patients with a dorsal ulcer and 14 (46.67%) with plantar ulcer. The test group had 21(70%) patients with ulcer on the dorsum and 9(30%) with that on the sole. Although both the groups were comparable in terms of the location of the ulcer, these results did not match with that observed by Younis et al. The latter assessed the frequency of foot ulcers in diabetics and found 61.22% of the ulcers on the plantar aspect, 30.80% on the dorsum and 8.08% of the ulcers on both the dorsum and sole of the patients.[64]

Alex et al, in his study to examine the crucial risk parameters for foot ulcers in diabetics, observed the significant contribution of hypertension and peripheral

neuropathy in the progression of ulcer. It was found that the superadded presence of callosities and foot deformities in diabetics with hypertension and neuropathy further increased the risk of developing foot ulcers. However, in their study, the majority of the population did not have hypertension(67.8%), peripheral neuropathy(81.1%) and peripheral vascular disease(90%).[65] Similarly, in our study the majority did not have hypertension(63.33%), peripheral neuropathy(68.33%) and peripheral vascular disease(88.33%). This finding stressed the importance of adequate glycemic and blood pressure control in patients by implementation of anti-diabetic and antihypertensive medication.

In a study done by AlGoblan et al, the body mass index has been found to be closely linked to the incidence of diabetic foot ulcers. 87.2% of the patients were either overweight or obese as compared to only 12.9% with normal weight. A high body mass index is reflective of obesity which in turn directly stimulates the development of diabetic foot ulcers.[66]Similarly, our study depicts the average BMI in the control group as 25.26 kg/m^2 and in the test group as 24.49 kg/m^2.This was not a statistically significant difference between the two groups(P value 0.3449).

Xiang et al studied whether effective control of the glycemic status in diabetics had any outcome on diabetic foot ulcer healing. The study stressed upon the need of a reasonable target for HbA1c that is a range between 7.0% to 8.0% during treatment. This ensured faster ulcer healing and also hampered the mortality rates in patients.[67]The desired range of HbA1c was consistent with our study findings. In the control group the mean HbA1c was 8.07% and that in the test group was 8.88%. This was not a significant statistical difference excluding bias from the result.

This study has considered parameters such as age group, sex, socioeconomic status, site of ulcer, hypertension, neuropathy, peripheral vascular disease and HbA1c

as risk parameters for foot ulceration in diabetics. On comparison of these factors, there was no statistically significant difference(p value >0.05) between the two groups of study population. This plays an important role in excluding bias from the study.

On analyzing the change in size of the ulcer over 14 days, it was observed that the mean area of the ulcer in the control group on day 0 and day 14 was 18.84 cm^2 and 15.86 cm^2, respectively with the mean difference in ulcer size being 2.98 cm^2. This was a statistically significant decrease in area of the ulcer over 14 days in the control group(p value = 0.0001). The mean area of the ulcer in the test group on day 0 and day 14 was 17.12 cm^2 and 13.46 cm^2, respectively with the mean difference in ulcer size being 3.66 cm^2. This was a statistically significant reduction in area of the ulcer over 14 days in the test group (p value = 0.0001).

The difference in the mean areas of ulcer over 14 days in the control and test groups were 2.72cm^2 and 4.15cm^2, respectively. This was a statistically significant result comparing the two groups(p value = 0.0471). Thus, this signified that the ulcer area reduction was statistically significant in the test group in comparison to the control group.The mean percentage reduction in area of ulcer over 14 days in the control and test groups are 16.11% and 31.08%, respectively. This difference is statistically significant difference over 14 days in the test group in comparison to the control group(p value = 0.0001). This signified that the reduction in ulcer area over 14 days was more in the test group where topical atorvastatin was applied along with saline for dressing.

This result was in synchrony with one of the oldest studies done to assess topical atorvastatin's efficacy on diabetic wound healing. Toker et al assessed the wound healing in streptozocin induced diabetic rats with local atorvastatin. The

control group received a mixture of lanoline and vaseline. Inspite of the disadvantage of this study being done on animal model, it proved that topical atorvastatin could cause a faster wound healing and also stimulate epithelialization and neovascularization.[56]

Farsaei et al in his study, concluded that application of 1% topical atorvastatin ointment for fourteen days on stage 1 and 2 pressure ulcers resulted in a statistically significant fall in both the stage and area of the ulcer. This is one of the few studies done with topical atorvastatin which have cemented the positive effect of this drug on wound healing in humans.[58]Diabetic foot ulcers pose as a major challenge to the treating surgeon by virtue of its complex pathophysiology and presence of multiple interconnected factors which impair wound healing. To achieve optimum results in wound healing, it is important to acquire a balance between patient's glycemic status and local wound environment. Topical atorvastatin has emerged to be a novel therapeutic modality which can surpass the disadavantages of other antimicrobial dressings, such as antibiotic resistance, as well as achieve guaranteed wound healing through a multitude of mechanisms.

There is a deficiency of human clinical studies studying the effects of topical statin on diabetic foot ulcers. Those which are available have been executed on other types of ulcers such as chronic vascular ulcers or pressure ulcers. Till now there is no agreement regarding the best statin drug for wound healing especially diabetic wound healing.[50] However, topical atorvastatin has been considered one of the best pioneer topical modalities amongst the available statins.[50,51]

Statins have been found to possess their antibacterial property through binding and disruption of cellular components such as wall lipoteichoic acids, lipopolysaccharides and proteins. They can also act synergistically as an adjunct to

antibiotics and carry the ability to promote the immune system acticity. Studies have confirmed the antibacterial efficacy of atorvastatin to combat a variety of bacterial infections. Atorvastatin is one of the most potent statins with antimicrobial activity.[68,69]

In both the control and test groups, there was a reduction in the bacterial population. In the control group, the number of patients with positive culture report on day 0 were 16(53.33%) and those on day 14 were 7(23.33%). This was statistically significant reduction in bacterial population in the control group(p=0.0040)(Table 19 and graphs 20,21).

In the test group, the number of patients with positive culture report on day 0 were 19(63.33%) and those on day 14 were 5(16.67%). This was statistically significant reduction in bacterial population in the test group(p=0.0001)(Table 19 and graphs 20,21). On considering the p value in the control group(0.004) and in the test group(0.0001), it was thus deduced that dressing with topical atorvastatin with saline expressed a greater decrease in bacterial bioburden than saline dressing alone as the p value is very very low in the test group. This statistical interpretation was as per explanation by James R Knaub.[69]

Similar to our findings, Rego et al concluded in his study that simvastatin could reduce the positive culture reports in comparison to saline when used over infected skin ulcers on rats. Following treatment, test group showed only one rat with positive culture whereas, all rats of control group had positive report for culture. This was expressive of the antibacterial strength of topical statins.[58]

Das et al attempted to determine the antibacterial capacity of atorvastatin. The minimum inhibitory concentration noted for the organism P.gingivalis was 0.8 microgram/ml dilution. Their study found that periodontal microbes were highly

sensitive to very low concentration of atorvastatin and thus showed scope in the treatment of periodontitis.[70]

Masadeh et al in his study to compare the antibacterial activity of different statins, found that as compared to other statins, atorvastatin has the statistically significant ability to act against organisms such as Pseudomonas aueriginosa, Proteus mirabilis and Staphylococcus aureus. These were also the common organisms found in this study's test group thus explaining the reduction in the bacterial colonization by topical atorvastatin. The other common organism found in our study group was Klebsiella pneumonia, against which studies have reflected atorvastatin's antibacterial action. However, the minimum inhibitory concentration is not statistically significant when compared with other statins such as rosuvastasin.[71]

This clinical study is one of the first attempts in exploring the benefits of topical atorvastatin on diabetic foot ulcer healing. The patients receiving the drug topically, acquired faster wound healing in comparison to the control group both in terms of ulcer size reduction and absence of bacterial colonization. The patients did not experience any adverse effects during the course of the application of the drug and tolerated it well.

LIMITATIONS OF THE STUDY

This study had a population of 60 patients, 30 in test and control groups each who were followed for a period of 15 days. It was conducted at a single center which is one of the other limitations of this study.

FUTURE SCOPE OF STUDY

There is no commercially available formulation of topical atorvastatin that can be used daily. This stresses the need of formulating an ideal formulation that can be applied for general clinical practice on a regular basis.

It is advised to follow up the patients till attainment of complete healing of ulcer. This would also recognize complications if any, like amputation that might manifest later. Thus, a holistis decision can be made about this new modality.

There is a need of large scale clinical trials and multicentre studies which can warrant the efficacy and characteristics of topical atorvastatin in diabetic foot ulcer healing.

CONCLUSION

We conclude that application of topical atorvastatin along with normal saline to diabetic foot ulcers, has reflected significant decrease in ulcer area with respectto dressing with only normal saline.

In adjunct to this, topical atorvastatin also showcased antimicrobial property by virtue of negative bacterial colonization after the designated duration of dressings in the test group. However, this was not statistically significant. During the course of this study, the application of this topical modality witnessed no adverse effects which thus, marks the safety of this formulation.

The efficiency of topical atorvastatin on diabetic foot ulcer healing is due to its diverse properties such as anti-inflammatory, antibacterial, antioxidative and immunomodulatory actions.

In future, there is a need to procure and avail for such topical formulations of atorvastatin for use over diabetic foot ulcers in clinical practice. Additionally, there is a requirement for multicenter, large scale studies that can establish its efficacy on application necessary for acceptance in day to day practice.

SUMMARY

Statins have showcased their impact in the process of wound healing through their multitude of positive effects. Topical atorvastatin carries the potential of being an effective treatment modality for the treatment of diabetic foot ulcers, both in terms of faster reduction in ulcer size and its antimicrobial property. The dearth of an efficient clinical trial proving the same has been the reason behind undertaking this study.

The intention of this study was to test the effectiveness of topical atorvastatin alongwith conventional saline dressing versus saline dressing alone in the healing of diabetic foot ulcers by comparing the mean ulcer area percentage reduction in the two groups.

This study was conducted on 60 selected patients admitted in KLE Dr.Prabhakar kore charitable hospital and MRC, Belagavi for diabetic foot ulcer management.

The participants of the study were randomised into two groups, the test group(Group A) and the control group (Group B). Group A patients underwent dressing with topical atorvastatin emulgel and saline whereas Group B patients had their dressing done with saline alone.

There was no statistically significant difference in the distribution of participants between the two groups in terms of age, gender, socioeconomic status, duration of diabetes and that of diabetic foot ulcer, site of ulcer, mode of onset of ulcer, hypertension, body mass index, peripheral vascular disease, fasting blood sugar and HbA1c.

The mean percent decrease in area of ulcer over 14 days in the test group was 26.21 %, whereas that in the control group was 17.36 %. There was a statistically

significant difference in the mean percent reduction in ulcer area in the test group(p value = 0.0024). This reflected that the reduction in ulcer area was more in the test group where topical atorvastatin was applied along with saline for dressing as compared to the conventional saline dressing.

Both the groups reported a decrease in the bacterial bioburden in the diabetic foot ulcers over the course of 14 days. However, the test group showed a greater reduction of bacterial colonization as compared to the control group, although not statistically significant, thus confirming the antimicrobial property of topical atorvastatin.

This study highlighted that dressing with topical atorvastatin had the capacity of a faster reduction in the diabetic foot ulcer size as well as the property of treating the local infection. Thus, this confirmed the effective wound healing strength of topical atorvastatin when applied over diabetic foot ulcers.

In a country like India, with its rising prevalence of diabetics and patients with diabetic foot ulcers, there is a necessity of an efficient topical drug for wound dressings which does not harbour the hazard of antimicrobial resistance and can be an easy and safe alternative for faster wound healing. Topical atorvastatin fits into this mould perfectly and is a promising option for the treatment of diabetic foot ulcers in the near future.

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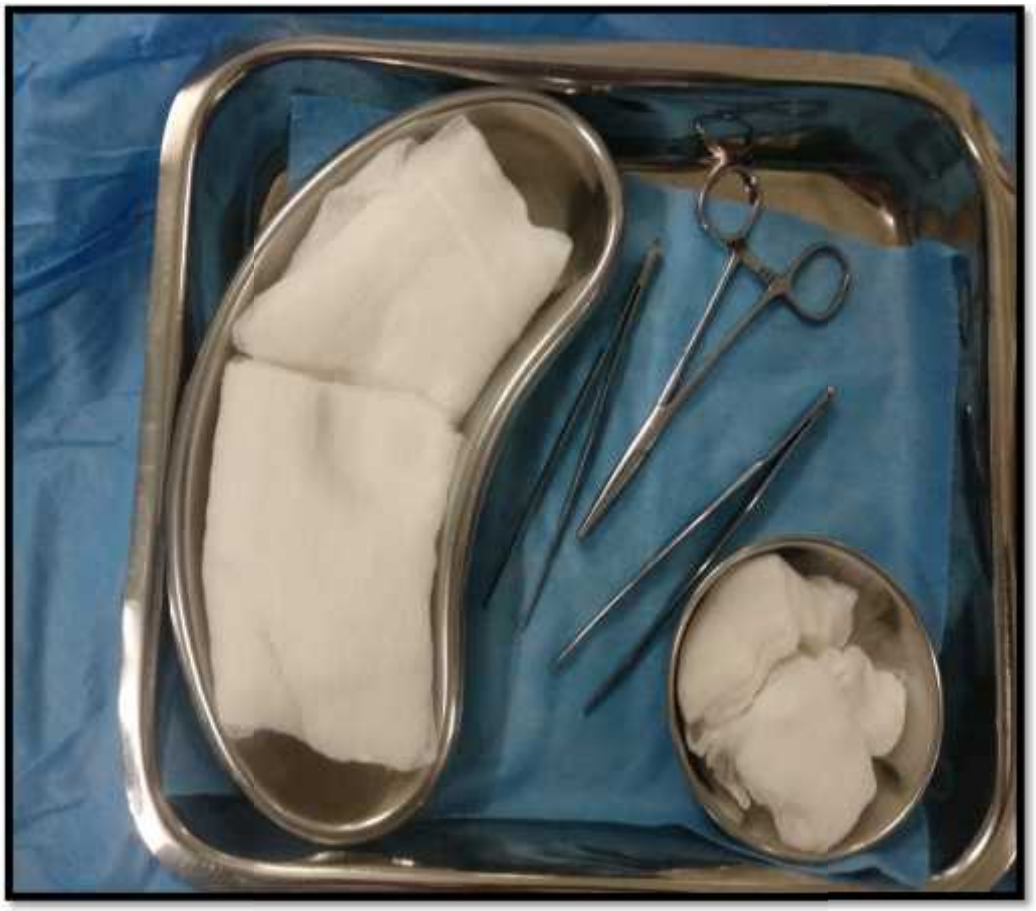
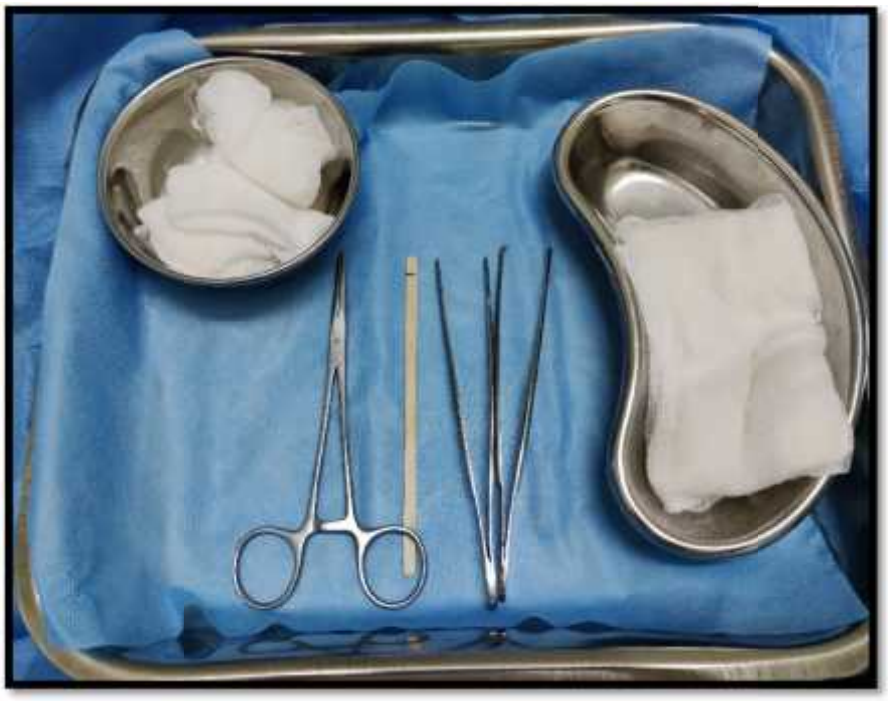
ANNEXURE I – PHOTOGRAPHS

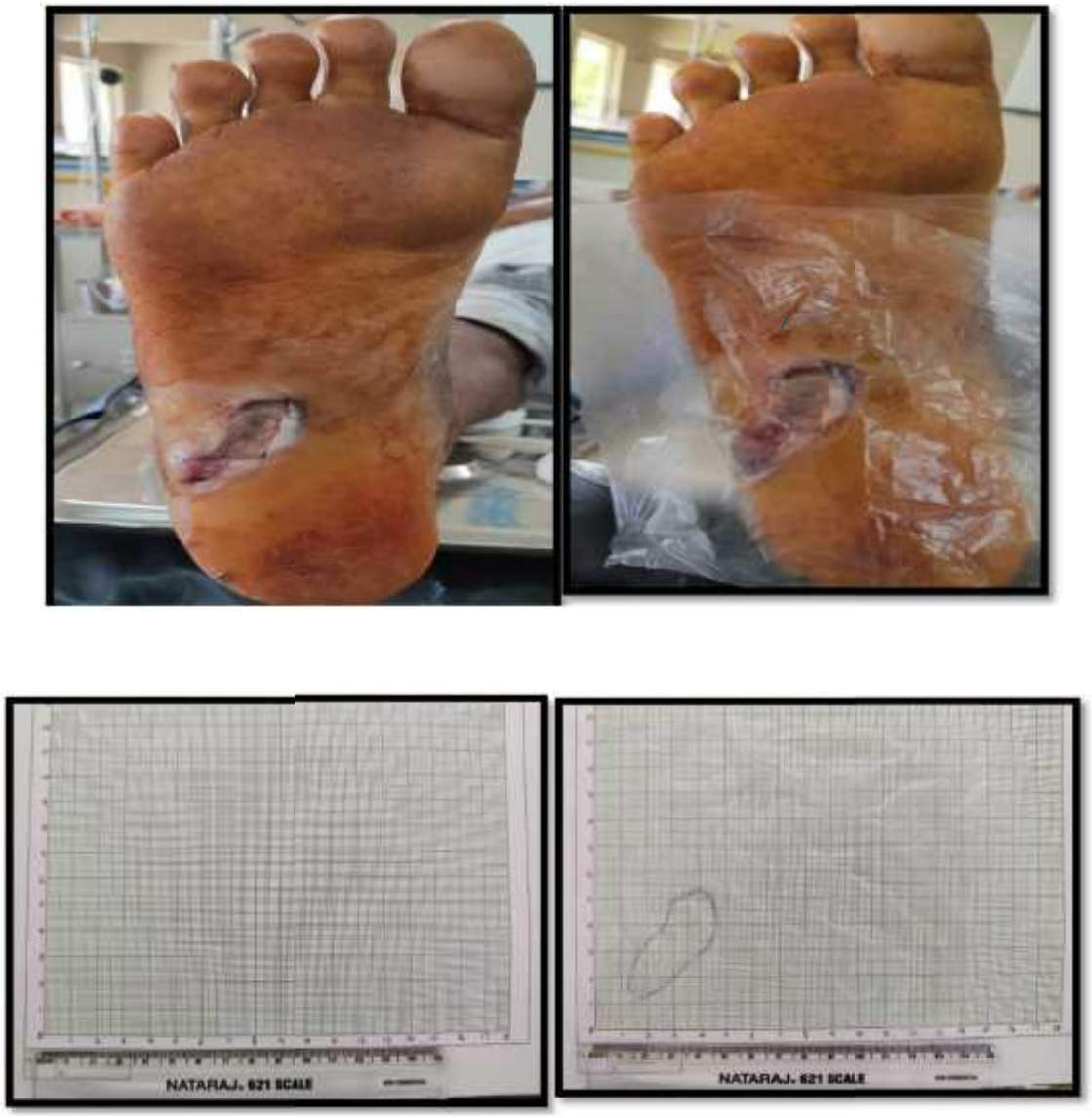


PHOTOGRAPH-1: DRESSING EQUIPMENT

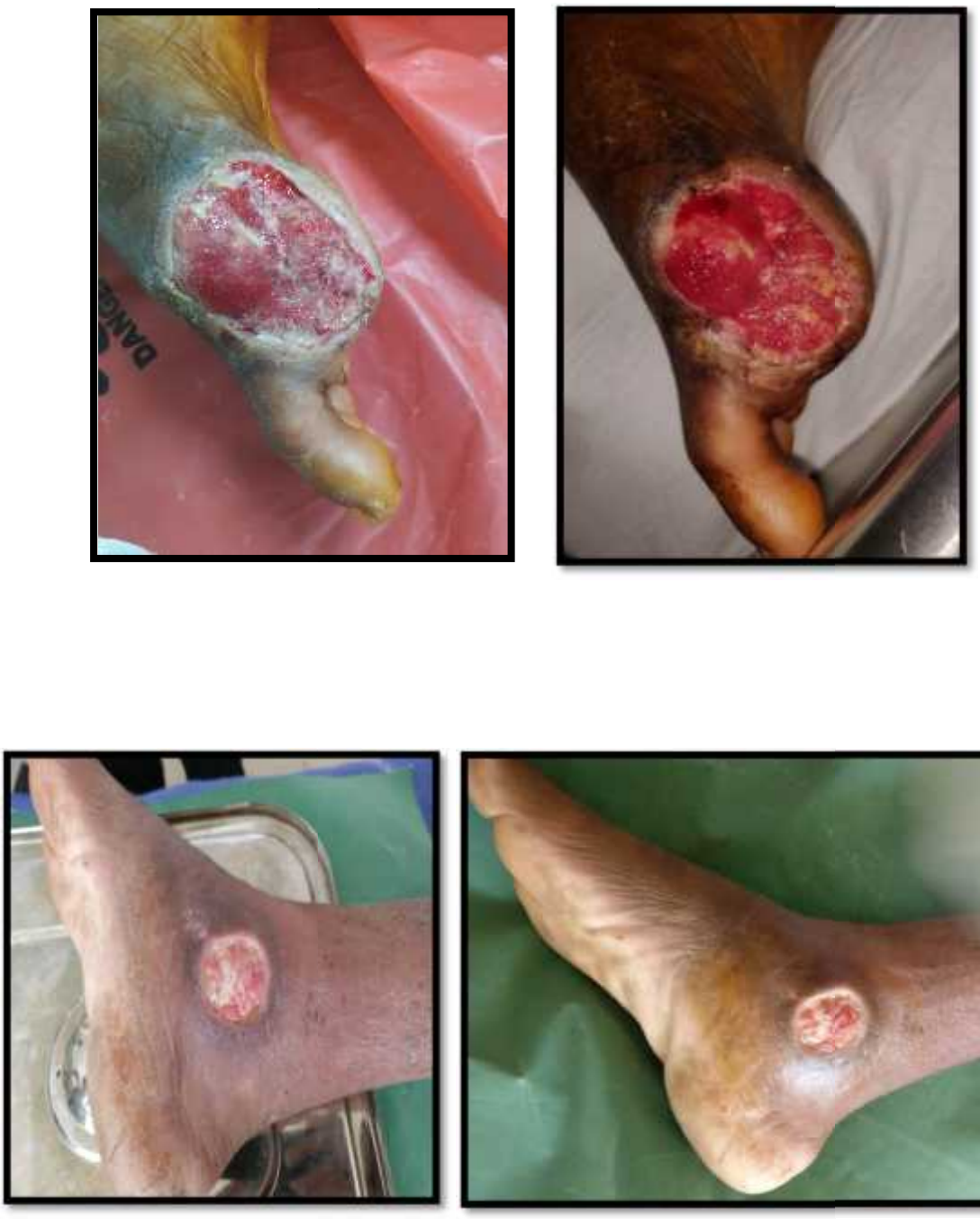


Photograph -2: 5% W/V Atorvastatin Emulgel with 10 Mg Measuring Applicatorin Dressing Tray

Photograph-3: 0.9% Normal Saline



Photograph-4: Grid Tracing For Measurement of Ulcer Area



Photograph -5. Ulcer on Day 0 and Day 14 in Saline Group

Photograph- 6: Ulcer on Day 0 and Day 14 in Atorvastatin Emulgel Group

ANNEXURE II – CONSENT FORM

CONSENT FOR PARTICIPATION IN RESEARCH STUDY

INFORMED CONSENT

Purpose of the study

I have been informed by**REG NO. BH0118002**, Post Graduate in M.S.General Surgery under the guidance of Dr._____**,** Professor Department of General Surgery, J.N. Medical College, KLE University, Belagavi is conducting a study to compare topical statin with saline versus saline dressing alone in healing of diabetic foot ulcers at KLE'S DR.PRABHAKAR CHARITABLE HOSPITAL AND MEDICAL RESEARCH CENTRE, BELAGAVI.

Diabetic foot ulcers are a serious complication of diabetes, leading to disability and early mortality. Diabetic foot ulcers are the most common cause of non traumatic amputation around the world and the most costly type of chronic wound. Infection in a diabetic foot is limb threatening and at times life threatening, and therefore must be treated aggressively. The selection of wound dressings is also an important component of diabetic wound care management. The purpose of this study is to find if topical atorvastatin emulgel with saline dressing is better than saline dressing alone in healing of diabetic foot ulcers.

Study procedure

Once you have signed the informed consent, necessary personal information and detailed medical history will be taken by the investigator. After this based upon randomisation you will be treated with topical atorvastatin emulgel with saline dressing or saline dressing alone. You will be subjected to examination of the foot ulcer along

with measurement of the ulcer dimensions, bacterial colonisation by culture and sensitivity and follow up will be done till 15 days of your hospital stay.

Potential risks

Allergic reaction and skin irritation to the drug used in the study are the possible risk factors

Benefits

The benefit of study is use of topical atorvastatin emulgel with saline dressings may help healing of diabetic foot ulcers faster as compared to saline dressing alone and thereby decreasing morbidity, hospital stay and need for amputation.

Financial incentive for participation

You will not receive any payment for taking part in this study.

Alternatives

Your participation in this study is entirely voluntary. You are free to refuse to participate or withdraw from the study at any time. You will still receive standard medical care from the hospital. The investigator holds the right to terminate the study at any time

Privacy

To protect my privacy, all the collected information will be given a number rather than using my name. Any information collected during the study will remain confidential. My medical files will be reviewed only at the hospital (or study doctor's office) to check the information and verify the result without breaking my confidentiality.

Authorization to publish results

The information about me will be analysed together with other study participants.Results of this study will be published and presented to scientific groups

for scientific purposes, but I will never be individually identified in the presentation of the study results.

Institutional policy

In case I have any questions related to the study, in future or in case of study related injury or illness, I can contact Dr. **REG NO. BH0118002**, Department of General Surgery, KLE University's J.N Medical College, Dr._____, Professor Dept. Of General surgery, KLE University's J.N Medical College, Belagavi.

Voluntary participation

My participation in the study is voluntary. In case I need any further information regarding my rights as study participant, I may contact Dr. Roopa M Bellad, Professor of Paediatrics, as Chairman of J. N. Medical College Institutional Ethics Committee on Human Subjects Research, Phone No.0831 2473777 ext-1527 at J. N. Medical College, Belagavi. My doctor will take care of me during this study. I am free to stop participation in this study at any time and for any reason.

CONSENT FORM

Study title: Efficacy of topical statin medication on diabetic foot ulcer wound healing dynamics– A Randomized controlled trial for period of one year at KLEs Dr Prabhakar Kore Charitable Hospital, Belagavi.

Please initial box

i. I confirm that I have read and understood the information sheet for the above study and have had the opportunity to ask questions. ☐

ii. I understood that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

iii. I understood that sponsor of the clinical trial, others working on the sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understood that my identity will not be revealed in any information released to third parties or published. ☐

iv. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purposes. ☐

v. I agree to take part in the above study. ☐

Subject's name:

Signature / left thumb impression of subject:

Date:

Name of person obtaining informed consent:

Signature of person obtaining informed consent:

(If a patient has limited ability to read and write, an impartial witness should be present during the entire informed consent discussion and patient's legally acceptable

representative should sign on the patient's behalf.) In these instances the patient his/her thumb impression taken in place of signature.

Patient's legally acceptable representative's statement: **NA** ☐

I, as the patient's legally acceptable representative was present during the consenting procedure and understand the preceding information describing this study. All of the questions regarding the study and the patient's participation in it have been answered to my satisfaction. I state that all aspects of the study were clearly presented during the consent procedure. The patient is willing to participate in this study and I sign below on his/her behalf testifying to this effect.

Name of the patient:

Name of representative:

Relationship to the patient:

Signature of representative:

Date:

Impartial witness declaration:

By signing the consent form I attest that the information was accurately explained to and apparently understood by the patient and the representative (if applicable) and that the informed consent was freely given by the patient.

Name of impartial witness:

Signature: Date –

ANNEXURE-III

PROFORMA

Group: ☐ I.D ☐☐☐ NO:

1.Name of the patient :_____

2.Age : ☐☐

3.Gender : 1. Male 2. ☐ Female

☐☐ ☐☐ ☐☐☐☐ 4.DOA :

☐☐ ☐☐ ☐☐☐☐ 5.DOD :

6.Date of ☐☐ ☐☐ ☐☐☐☐ interview :

☐☐☐☐☐☐ 7.IP no :

8.Address : 1.Belagavi 2.Outside Belagavi ☐

9.Phone no : ☐☐☐☐☐☐☐☐☐☐

10.Occupation : 1-Unemployed
2-Unskilled
3-Semi-skilled ☐
4-Skilled
5-Professional

11.Education : 1-Illiterate
2-Primary (1^{st}-7^{th} std) ☐
3-High school (8^{th}-10^{th} std)
4-Intermediate
5-Degree and above

12.Socio-economic status :1-Low ☐
2-Middle
3-High

Screening -

13.H/O diabetes : 1-Yes 2-No

14.If yes, type of diabetes :

Type 1	
Type 2	

15.H/O other illness : 1-Yes 2-No

16.If yes :1-Malignancy
2-Asthma/COPD
3-HIV/AIDS
4-Autoimmune disorders
5-Hemoglobinopathy

17.Urine for ketone bodies :
1- Positive 2-Negative

18.Applicant is willing to give consent :
1-Yes 2-No

19.**Final result**

1-Inelgible
2-Elgible but refused
3-Elgible and participating

Data collection instrument :

1.Duration of ulcer -1.<4 weeks 2.>4 weeks

2.Location of ulcer- 1.Left foot
2.Right foot

3.Mode of onset- 1.Traumatic
2.Spontaneous
3.Pressure

4.Other

4.Associated symptoms- 1.Fever

2.Pain

3.Discharge

5.Duration of diabetes-

6.On medication for diabetes-1.Yes

2.No

7.If Yes, type of medication-1.Oral hypoglycemic agents

2.Insulin

8.Complication:

	Yes	No
Neuropathy		
Vasculopathy		

9.H/O hypertension-1.Yes

2.No

10.Medical history:

	Yes	No
Peripheral neuropathy		
Nephropathy		
PVD		
CVD		

11. Amputation 1. Yes

2. No

If yes, DATE

REASON

Examination:

Height	Weight	BMI

1.

Pulse rate	Blood pressure	Temperature	Respiratory Rate

2.

3. Foot Deformity:

1- Toe deformity 2 – Charcot's foot

4. Wound Observations:

	Day 0	Day 7	Day 14
1. Site of ulcer			
2. Shape 1 – oval 2 – circular 3 – irregular			
3. Margin 1- Regular 2- Irregular			
4. Edge 1- Indistinct, diffuse 2- Attached to base 3- Not attached, hanging 4- Rolled in 5- Hyperkeratotic/ callous like 6- Fibrotic/ scarred			
5. Floor 1- Red granulation tissue 2- Pale granulation tissue 3- Slough/necrotic tissue			
6. Base 1- Fascia, tendons 2- Soft tissue 3- Bone			

7. Discharge 1- None 2- Serous 3- Purulent 4- Serosanguinous 5- Sero-purulent			
8. Surrounding skin 1- Edema 2- Eczema 3- Pigmented 4- Normal			

5.Wagner Grading:

1	
2	
3	
4	
5	

6. Peripheral pulsations of lower limb:

	Right lower limb	Left lower limb
1. Dorsalis pedis		
2. Anterior tibial		
3. Posterior tibial		
4. Popliteal		
5. Femoral		

7.Sensory system examination-i) Touch

ii)Pain

iii) Temperature

8.Regional lymph node examination-

ANALYSIS PLAN

1. Ulcer dimensions:

	D_0	D_{14}
Length (c.m)		
Width (c.m)		
Area ($c.m^2$)		

2. Wound culture and sensitivity on D_0 and D_{14}

a) Wound culture and sensitivity on D_0

1. No growth 2. Growth present ☐

If 2-

Organism present-

Sensitivity-

b) Wound culture and sensitivity on D_{14}

1. No growth 2. Growth present ☐

If 2-

Organism present-

Sensitivity-

3. 1)Wound area on D_0 =

2)Wound area on D_{14} =

3)Wound area reduction =

(Area on D_0-Area on D_{14})

4) Percentage wound area reduction =

$\frac{(\text{Area on } D_0 - \text{Area on } D_{14})}{\text{Area on } D_0} \times 100$

Investigations

1.Complete blood count

2.Fasting blood sugar-2 consecutive readings

3.Serum creatinine

4.Blood urea

5.X-ray foot-anterio posterior and lateral view

6.Urine analysis-routine and microscopy

7.Wound tissue culture

8.HbA_1C

9.UKB

10.Colour Doppler if it is indicated

ANNEXURE IV

ETHICAL CLEARANCE

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed to be University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
Website: http://www.jnmc.edu
E-Mail : dome@jnmc.edu
Phone: (+ 91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/09 **Date: 24/11/2018**

To,

REG NO. BH0118002
PG student in Surgery,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"EFFICACY OF TOPICAL ATORVASTATIN MEDICATION ON DIABETIC FOOT ULCER WOUND HEALING DYNAMICS – A RANDOMIZED CONTROLLED TRIAL FOR PERIOD OF ONE YEAR, AT KAHER;S DR. PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE, BELAGAVI-590010",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Arathi Darshan)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

ANNEXURE V

ATORVASTATIN EMULGEL FORMULATION METHODOLOGY AND CERIFICATION

Atorvastatin 5%w/w emulgel

Formulation ingredients table.

Ingredients	Quantity	Category
Atorvastatin	5gms	Active ingredient
Light liquid paraffin	20ml	Vehicle for drug
Tween 80	6gms	Emulsifier
Pemulgen-Tr -1 polymer	3gms	Gelling agents
Triehanolamine	0.05gms	pH adjusting agent
Sod methyl parbens	0.05gms	Preservative
Sod propyl parbens	0.005gms	Preservative
Distilled water qs	100gms	vehicle

Method of preparation: Atorvastatin drug was dissolved in warm (40^{o}c) liquid paraffin on magnetic stirrer for 2hrs. Drug was completely dissolved in light liquid paraffin. Tween 80 was added to the liquid paraffin drug mixture and stirred for 10 minutes. In 10m of water methyl and propyl parbens were dissolved and added to the drug mixture solution, stirred vigorously using propeller at 2500 rpm. Pemulgen-TR-1 was dissolved in 50 ml of warm water on magnetic stirrer for 1hr and kept overnight at room temperature for complete hydration. Gelling solution was mixed to the drug mixture using propeller for 30 minutes and pH was adjusted to 6.8 to 7.2 with triethanolamine. Final weight of the emulgel was adjusted with water and stored in air tight container at room temperature.

To whom so ever it may concern

This to certify that Atorvastatin 5%w/w emulgel was prepared in the department of Pharmaceutics of KLE College of Pharmacy Belagavi. In the preparation all ingredients were used as per specifications of Indian pharmacopeia. Preparation of emulgel protocol and procedure was developed and executed under the guidance of Mr. U. B. Bolmal, asst professor, department of pharmaceutics, KLE college of Pharmacy Belagavi.

DT: 19/02/2020

place: Belagavi.

(Mr. U. B. Bolmal)

Pharmaceutics
KLE College of Pharmacy
BELAGAVI - 10.

ANNEXURES VI – KEY TO MASTER CHART

S.NO - Serial number

IP NO - Inpatient number

SEX - 1. Male 2. Female

SOCIOECONOMIC STATUS – 1. Low 2. Middle 3.High

DURATION OF ULCER- 1. <4 weeks 2.>4 weeks

LOCATION OF ULCER– DRF Dorsum right foot, PRF Plantar right foot, DLF Dorsum left foot, PLF Plantar left foot

MODE OF ONSET – 1.Traumatic 2. Spontaneous 3. Pressure 4.Other

HYPERTENSION – 1.Yes 2.No

NEUROPATHY - 1.Yes 2.No

PVD (Peripheral Vascular Disease)- 1.Yes 2.No

CULTURE: P Bacterial growth present AB Bacterial growth absent

TEST GROUP

S.NO	IP NO	AGE	SEX	SOCIOECONOMIC STATUS	DURATION OF DIABETES	ONSET	DURATION OF ULCER	SITE	HYPERTENSION	NEUROPATHY	PVD	BMI	FBS	HbA1C	Hb	TLC	CREATININE	AREA D0	AREA D14	REDUCTION IN AREA	% REDUCTION IN AREA	CULTURE D0	CULTURE D14	ORGANISM
1	969623	51	1	1	5 years	2	2	PLF	2	2	2	21.48	140	7	8	7200	0.7	36	30	6	16.66	P	P	PSEUDOMONAS AERUGINOSA
2	965464	70	1	1	10 years	2	1	DRF	2	1	2	23.43	155	6.9	13.3	11610	1.6	36	33	3	8.33	P	P	PSEUDOMONAS AERUGINOSA
3	965303	57	1	1	5 years	2	1	DRF	2	2	2	25.72	140	8.2	9	5600	1	9	7.5	1.5	16.66	P	AB	KLEBSIELLA PNEUMONIAE
4	965237	56	1	1	20 years	1	2	PRF	1	1	2	21.48	145	7.6	8	13810	0.8	20	17.8	2.2	11	P	AB	STAPHYLOCOCCUS AUREUS
5	960615	55	1	1	9 years	1	1	DRF	2	1	2	26.66	150	9.5	11.7	7360	1.96	2	1.5	0.5	25	P	P	STAPHYLOCOCCUS AUREUS
6	960921	53	1	1	10 years	2	1	DRF	2	1	2	20.76	140	7.2	13	10500	0.79	4	3	1	25	P	AB	KLEBSIELLA PNEUMONIAE
7	959823	64	1	1	10 years	2	1	DLF	1	1	2	24.6	140	8.6	12.5	27300	0.88	24	22	2	8.33	P	AB	ENTEROBACTER CLOACAE
8	954942	46	1	1	2 years	2	1	DRF	1	2	2	24	140	7.1	8.4	13300	1.7	6	4	2	33.33	AB	AB	AB
9	951618	53	1	1	14 years	1	1	DRF	2	1	2	26.02	150	12	13.3	8400	1.2	4	2.25	1.75	43.75	P	AB	ENTEROCOCCUS FAECALIS
10	927360	70	1	1	10 years	1	2	DLF	1	1	1	23.43	140	10.1	10	7200	1.2	36	30	6	16.66	AB	AB	AB
11	939326	61	2	2	15 years	1	1	DLF	1	2	2	23.43	140	11	11	8120	1.1	8	6	2	25	AB	AB	AB
12	920358	64	1	1	5 years	1	1	DLF	1	2	1	19.08	140	8.6	9	9200	0.6	20	16	4	20	P	AB	KLEBSIELLA PNEUMONIAE
13	944271	50	1	1	4 years	1	1	DLF	2	2	2	19.08	140	10.2	10	4400	0.6	25	20	5	20	P	AB	KLEBSIELLA PNEUMONIAE
14	940042	65	1	1	5 years	1	2	DLF	1	1	2	23.43	140	9.4	11	7600	0.7	20	16	4	20	AB	AB	AB
15	945324	51	1	2	5 years	1	1	DLF	2	2	2	19.53	140	9.1	9.2	5400	0.5	20	16	4	25	P	AB	PROTEUS MIRABILIS
16	949394	52	1	1	5 years	2	2	DRF	2	2	2	23.43	140	10.1	10	9000	1.8	36	25	11	30.55	P	AB	ESCHERICHIA COLI
17	943516	47	1	1	5 years	1	1	DRF	2	2	2	23.14	150	9.7	9.4	10000	1.2	16	12	4	25	P	AB	COAGULASE NEGATIVE STAPHYLOCOCCUS
18	969012	66	1	1	7 years	1	1	DRF	1	1	2	28.12	150	7.3	9.3	15900	0.7	36	30	6	16.66	P	AB	PROTEUS VULGARIS
19	968509	65	2	1	5 months	1	2	DLF	2	2	2	27.34	177	8.3	8.4	11870	1.66	36	30	6	16.66	AB	AB	AB
20	967450	55	2	1	4 years	1	1	DRF	2	2	2	27.08	150	10.3	8.6	16510	0.8	6	4	2	33.33	P	P	PSEUDOMONAS AERUGINOSA
21	968742	57	1	1	5 years	1	1	PLF	2	2	2	27.34	160	7.6	13.1	4810	0.8	20	16	4	20	AB	AB	AB
22	965098	57	1	1	17 years	1	2	PRF	2	1	2	21.48	170	11.7	11.5	18970	0.9	36	30	6	16.66	P	P	PROTEUS MIRABILIS
23	963229	56	2	1	10 years	1	1	PLF	1	1	2	27.34	155	8.4	9.8	27110	1.4	24	20	4	16.66	AB	AB	AB
24	971431	67	1	1	6 years	2	2	PLF	1	2	2	25.39	160	11.3	11.1	1100	1	0.5	0.3	0.2	40	AB	AB	AB
25	971751	50	1	1	5 years	1	1	PRF	2	2	2	26.71	150	10.2	16.4	10420	0.8	6	4	2	33.33	AB	AB	AB
26	971956	54	1	2	2 years	2	1	DLF	1	2	2	26.1	200	11.2	15.6	10100	1.2	4	2	2	50	AB	AB	AB
27	972812	52	1	2	5 years	1	1	PRF	2	1	1	27.48	180	9.3	12.2	10700	0.83	9	7.5	1.5	16.66	AB	AB	AB
28	978452	66	1	1	4 years	1	1	DRF	2	1	1	27.48	140	6.4	15.3	13400	1	4	2	2	50	P	AB	KLEBSIELLA PNEUMONIAE
29	976750	70	2	1	7 years	1	2	PRF	2	2	2	25	120	6.2	8.9	9000	1.05	4	2	2	50	P	AB	PSEUDOMONAS AERUGINOSA

GROUP A TEST SHEET 1

GROUP B CONTROL SHEET 2

CONTROL GROUP

S.NO	I.P. NO	AGE	SEX	SOCIOECONOMIC STATUS	DURATION OF DIABETES	ONSET	DURATION OF ULCER	SITE	HYPERTENSION	NEUROPATHY	PVD	BMI	FBS	HbA1C	Hb	TLC	CREATININE	AREA D0	AREA D14	REDUCTION IN AREA	%REDUCTION IN AREA	CULTURE D0	CULTURE D14	ORGANISM
1	958902	67	1	1	10 years	2	1	DLF	1	1	2	23.89	200	9.9	12.3	10800	3.1	6	4	2	33.33	AB	AB	AB
2	958720	59	1	1	15 years	2	2	PRF	1	1	2	23.43	166	11.3	11.2	8220	1.73	16	14	2	12.5	P	P	KLEBSIELLA PNEUMONIAE
3	956630	51	1	1	1 year	1	1	DRF	2	2	2	27.34	150	11.8	11.7	8000	1.43	6	4	2	33.33	P	AB	STAPHYLOCOCCUS EPIDERMIDIS
4	955825	53	1	1	8 years	1	2	PRF	2	2	2	28.57	177	11.9	9.9	14200	0.89	16	14	2	12.5	P	P	ENTEROBACTER CLOACAE
5	955862	34	1	1	1 year	1	2	PRF	2	2	2	22.22	140	7.8	13.6	10670	1.5	6	5	1	16.6	P	AB	MRSA
6	953338	70	1	1	1 year	1	1	DRF	2	1	2	31.11	150	7.6	12.5	17090	1.5	30	27	3	10	P	AB	ACINETOBACTER BAUMANNI
7	936689	57	1	1	5 years	1	2	DLF	2	2	2	25.95	140	7.1	9.6	7200	1.25	36	30	6	16.66	P	P	ACINETOBACTER BAUMANNI
8	933981	62	1	2	2 years	1	1	DLF	1	2	2	25.97	144	6.8	10.2	7210	1.4	36	30	6	16.66	P	P	PSEUDOMONAS AEURIGINOSA
9	926926	70	1	2	4 years	1	1	PLF	1	2	2	27.34	140	6.6	11.3	9900	1.5	30	24	6	20	P	AB	KLEBSIELLA PNEUMONIAE
10	930526	50	1	3	10 years	1	1	DRF	2	1	1	31.11	144	6.9	10.2	10200	1.6	36	30	6	16.66	P	P	KLEBSIELLA OXYTOCA
11	930428	54	1	1	8 years	1	1	PRF	2	2	2	26.37	140	7.4	11.6	10000	1.6	36	30	6	16.66	P	AB	STAPHYLOCOCCUS AUREUS
12	926884	55	1	1	2 years	1	2	DRF	2	2	2	25.39	150	7.4	10.9	10100	1.8	12	10.5	1.5	12.5	AB	AB	AB
13	937656	62	1	2	10 years	1	1	PRF	1	2	2	22.49	155	7.4	9.6	9900	1.2	20	17.5	2.5	12.5	AB	AB	AB
14	926875	70	1	1	9 years	1	2	PLF	1	2	1	27.34	152	7.1	9.8	9200	1.4	30	24	6	20	P	P	ESCHERICHIA COLI
15	931614	40	1	1	5 years	2	1	DRF	2	2	2	24.43	150	6.4	10.1	9500	1.6	25	20	5	20	AB	AB	AB
16	929109	57	1	1	6 years	2	1	DRF	2	2	2	23.87	170	9.2	9.4	10100	1	6	4	2	33.33	AB	AB	AB
17	945384	53	1	2	3 years	1	2	DRF	1	2	2	21.48	140	6.2	9.8	10400	1.1	20	16	4	20	AB	AB	AB
18	949979	54	1	1	7 years	1	1	DLF	1	2	2	19.53	160	8.1	11.2	6120	1.4	12	9	3	25	AB	AB	AB
19	945872	60	1	1	12 years	1	1	DRF	1	1	2	20.76	140	7	10.7	10400	1.8	20	16	4	20	P	AB	ENTEROCOCCUS FAECALIS
20	944879	70	1	1	9 years	1	1	PRF	2	2	2	20.22	140	8.4	12.2	11000	1.3	16	12	4	25	AB	AB	AB
21	944073	42	1	1	6 years	2	2	PRF	1	2	2	27.68	144	8.6	11.2	9600	1.7	20	16	4	20	AB	AB	AB
22	970010	45	2	1	3 years	2	1	DLF	2	2	2	22.49	160	6.8	10.6	7630	0.6	6	5	1	16.66	P	AB	STAPHYLOCOCCUS AUREUS
23	968750	44	1	1	7 years	1	1	DRF	2	2	2	28.24	162	7.4	11.1	13930	1.5	6	6	0	0	AB	AB	AB
24	968767	65	1	1	4 years	2	1	PLF	2	2	2	22.05	140	9.7	14	9300	0.7	6.25	5	1.25	20	P	AB	KLEBSIELLA PNEUMONIAE
25	969336	58	1	1	3 years	1	1	PRF	2	2	2	29.15	160	9	13	10000	0.5	6	4	2	33.33	AB	AB	AB
26	969804	55	1	1	5 years	2	1	PLF	2	2	2	21.49	145	7.4	11.5	4350	1.6	24	20	4	16.66	AB	AB	AB
27	970920	65	2	1	4 years	2	1	DRF	2	2	2	27.34	161	8.2	9.1	9660	0.81	6	5.6	0.4	6.66	P	AB	MRSA
28	968058	59	1	1	10 years	1	1	DLF	1	1	1	29.09	140	7.1	9.3	6390	1.13	36	30	6	16.66	P	P	KLEBSIELLA PNEUMONIAE
29	973177	61	1	1	7 years	1	1	PLF	2	2	2	21.48	155	7.6	12.5	12440	2.2	20	19.2	0.8	4	AB	AB	AB
30	973638	70	1	1	5 years	1	1	PLF	2	2	2	30	140	7.9	13.7	10700	1.33	20	18	2	10	AB	AB	AB