A COMPARATIVE STUDY OF PLATELET RICH PLASMA VERSUS NORMAL SALINE DRESSING IN DIABETIC FOOT

A DISSERTATION SUBMITTED TO

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY

In partial fulfillment of the regulations for the award of the

M.S.DEGREE EXAMINATION

BRANCH I GENERAL SURGERY

DEPARTMENT OF GENERAL SURGERY

STANLEY MEDICAL COLLEGE AND HOSPITAL

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY
CHENNAI

` **APRIL 2015**

CERTIFICATE

This is to certify that the dissertation titled **" *A COMPARATIVE STUDY OF PLATELET RICH PLASMA VERSUS NORMAL SALINE DRESSING IN DIABETIC FOOT* "** is the bonafide work done by ***Dr. V.SAKTHIVEL*****,** Post Graduate student (2012 – 2015) in the Department of General Surgery, Government Stanley Medical College and Hospital, Chennai under my direct guidance and supervision, in partial fulfillment of the regulations of The Tamil Nadu Dr. M.G.R Medical University, Chennai for the award of M.S., Degree (General Surgery) Branch - I, Examination to be held in April 2015.

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Date: September 2014 **DR.V.SAKTHIVEL**

ACKNOWLEDGEMENT

My sincere thanks to **Dr.AL.MEENAKSHISUNDARAM, MD., D.A.,**The Dean, Govt. Stanley Medical College for permitting me to conduct the study and use the resources of the College.I consider it a privilege to have done this study under the supervision of my beloved Professor and Head of the Department **Prof.Dr.S.VISWNATHAN,** who has been a source of constant inspiration and encouragement to accomplish this work.

I am highly indebted to my guide and Mentor **Prof. Dr.K.KUBERAN,** Professor of Surgery for his constant help, inspiration and valuable advice in preparing this dissertation.I express my deepest sense of thankfulness to my Assistant Professors **Dr.R.VIJALAKSHMI, Dr.G.CHANDRASEKAR,** for their valuable inputs and constant encouragement without

which this dissertation could not have been completed.I express my sincere gratitude to my guides **Prof. Dr. P.Darwin, Prof.Dr.J.Vijayan, Prof. Dr.K. Kamaraj**, former Heads of Department of General Surgery . I thank them for the constant support, able guidance, inspiring words and valuable help they rendered to me during my course.

I am extremely thankful to my patients who consented and participated to make this study possible.

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INTRODUCTION

"Diabetes Mellitus is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both".

The effect of Diabetes Mellitus includes long term damage, dysfunction and failure of various organs especially eyes, kidney, heart and blood vessels. Chronic complications are responsible for high morbidity and mortality and cause disproportionately high number of hospital days.

In 1921, Banting, Best and Macleod demonstrated pancreatic extracts lower blood sugars. In 1936, Antanio discovered oral hypoglycemic agents. W. R. Jordan described association of diabetes with foot lesions.[1]

The incidence of diabetes and its complications are on a rise, the risk of lower extremity amputations is 15 fold higher in diabetics as compared to non-diabetics.[2]

Essential to mention here that chronic diabetic foot ulcer is the leading cause of amputations in these patients, The incidence of diabetes and its complications are on a rise, the risk of lower extremity amputations is 15 fold higher in diabetics as compared to non-diabetics.[2] Essential to mention

here that chronic diabetic foot ulcer is the leading cause of amputations in these patients, also that 15% of all diabetics develop diabetic ulcer and the most commonest site being the foot also that 15% of all diabetics develop diabetic ulcer and the most commonest site being the foot. Although the fundamental pathophysiologic factors leading to diabetic ulcer remain incompletely understood, the triad of neuropathy, ischemia and infections commonly is considered the most important.

These diabetic ulcers are known to be resistant to conventional treatment and may herald severe complications if not treated wisely.[3,4,5]

The wound environment contains a variety of growth factors .Platelet rich plasma release. Platelet- derived growth factor is of particular relevance due to its chemotactic, mitogenic, angiogenic, and stimulatory effects leading to matrix formation and wound bed granulation. PDGF may be of significant benefit of diabetics as recalcitrant diabetic wounds have been found to be deficient in or absent of PDGF.[6] Platelet-derived growth factor (PDGF) is one of the numerous growth factors, or proteins that regulate cell growth and division. In particular, it plays a significant role in blood vessel formation (angiogenesis).

PRP is an effective concentration of multiple growth factors by virtue of platelets alone, which contain plasma proteins ,namely fibrin , fibronectin and vitronectct .This cocktail of GFs is pivotal in diabetic foot for modulation of tissue repair and regeneration.plasma proteins as a scaffold for connective tissue and epithelial migration.

PrepackagedGFs degranulation occurs in platelets occur upon "activation" on coming in contact with coagulation triggers.GFs secreted in turn bind to their respective transmembrane receptors expressed over adult mesenchymal stem cells, fibroblasts epidermal cells,endothelial cells.

The efficacy of certain growth factors in healing various injuries and the concentrations of these growth factors found within PRP are the theoretical basis for the use of PRP in tissue repair.

Platelet rich plasma contain following growth factors:

- platelet-derived growth factor
- transforming growth factor beta
- fibroblast growth factor
- insulin-like growth factor 1
- insulin-like growth factor 2
- vascular endothelial growth factor
- epidermal growth factor
- Interleukin 8
- keratinocyte growth factor

AIM AND OBJECTIVE OF THE STUDY

To compare the efficacy of Platelet rich plasma (PRP) dressing Vs conventional wound dressing in wound reduction in patients with chronic diabetic foot ulcers, admitted in Stanley medical college , Chennai.

REVIEW OF LITERATURE

Foot ulceration, sepsis and amputation are known and feared by almost every person who has diabetes diagnosed. Yet these are potentially the most preventable of all diabetics[14]. Life time risk for foot ulcers with diabetes is 15%[4]. Important factor to determine outcome of diabetic foot is severity and not ulcer site[15].

The incidence of diabetes and complications are on rise. In well-studied town of Framingham the prevalence of diabetes has risen from 0.9% in 1958 to 3% in 1993. India has dubious distinction of highest number of diabetics in the world. In the year 1995 there were 19.4 million diabetics which is expected to rise to 57.2 million by 2025.[5]

Diabetic foot being one of the most common complications, where 15% of all diabetics develop diabetic ulcers, the most common site being the foot. Every 2% rise in glycosylated hemoglobin increases the risk of lower extremity ulcers by 1.6 times and lower extremity amputation by 1.5 times[16].

Diabetes has highest risk factor associated with limb threatening ischaemia. Trivial trauma secondary to neuropathy and distorted pedal architecture causes ulcerations. 15% of all diabetics develop foot ulcer. 20% of admissions in diabetics are for foot problems.[4]

HISTORICAL BACKGROUND OF WOUND HEALING

- The treatment and healing of wounds are some of the oldest subjects discussed in the medical literature and probably earliest problems of human race.[17]
- Early surgeons like Ambrose, Pare, John Hunter, & Sir James Paget have given some scientific knowledge to their handling of wounds, particularly those resulted from war.[18]
- Halsted was intensely interested in wound healing process.
- In the early 1900's Carrel & his associates made investigations with the scientific approach to wound healing. Later Carrel (1916), Harvey & Howe's (1930), studied incised wounds & contributed to the knowledge of wound healing.[18]
- There is a saying; "If there were no regeneration, there would be no life; if everything regenerated, then, there would be no death".

HEALING, REGENERATION & REPAIR

Healing

"Body replacement of destroyed tissues by the living tissue" or "Integrated series of cellular & biochemical events which restores the functional integrity & regains the strength of injured tissue".

Regeneration

"It is a process of replacement of lost tissue by an identical type of fresh tissue". There is proliferation of surrounding undamaged specialized cells.[18] Seen in- [1] Epidermis [2] Endothelium [3] Liver cells [4] Mucous membrane.

Repair

It is the replacement of lost tissues by granulation tissue, which matures to form the scar tissue". This is inevitable, when the surrounding specialized cells do not possess the capacity to proliferate e.g. muscle & nervous tissue.

Repair begins during the early phases of inflammation but reaches completion usually after the injurious influence has been neutralised.[31]

During repair, the injured tissue is replaced by.[19]

- Regeneration of native parenchyma cells
- By filling of the defect with fibroblastic tissue (scarring).
- By a combination of these two processes.

HEALING:

Definition:

"Body replacement of destroyed tissues by the living tissue" or "Integrated series of cellular & biochemical events which restores the functional integrity & regains the strength of injured tissue"

Phases of Healing:

Wound healing & repair are complex processes that involves dynamic series of events.

[1] Coagulation

[2] Inflammation

[3] Fibroplasia, Angiogenesis, Proliferation & Granulation tissue formation.

[4] Epithelization

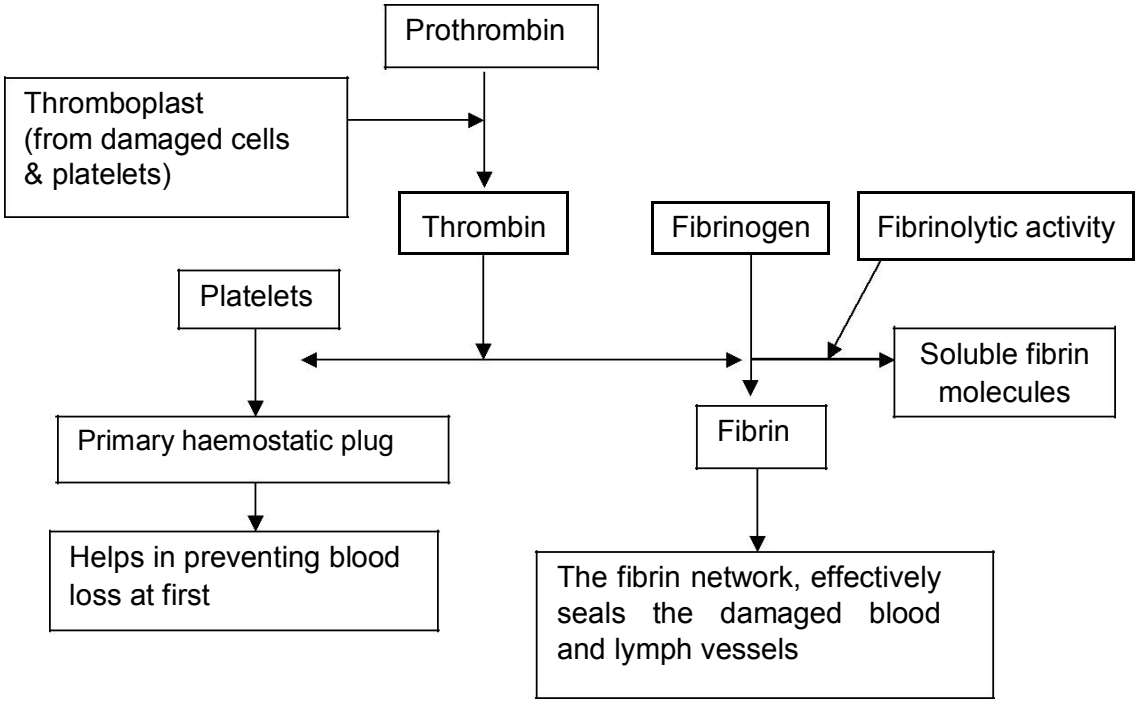
[5] Collagen Synthesis

[6] Wound contraction / Tissue Remodeling / Scar Maturation

COAGULATION :

- Helps in preventing blood loss, covering wound surface, & holding the wound edges together & thus contributing to the healing process.
- Knighton et al (1982) & Ross (1980) have shown equivocally that fibrin & platelets play an important role in initiating the wound healing.

Fig. 2.1 : Mechanism of Coagulation



GRANULATION PHASE OF WOUND HEALING :

- **Phases of wound healing coming under this phase are :** Fibroplasia, Angiogenesis, Proliferation

What is Granulation tissue[19]

'This is a highly vascular tissue, containing largely of

1. Fibroblasts [Proliferating fibroblasts + Products of Fibroblasts]
2. Endothelial cells lining capillaries of newly sprouting blood vessels
3. Macrophages
4. Pleuripotent Pericytes

Above all are embedded in a matrix consisting

1. Fibronectin
2. Proteoglycans rich in Hyaluronicacid & collagen [This collagen is at first mainly of Type-III, changing later to Type I]

Why named as 'Granulation Tissue'?

The term granulation tissue derived from it's pink, soft, granular appearance on the surface of wounds.[19]

FUNCTIONS OF GRANULATION TISSUE :-

- Fill the gap of the wound
- Supports the growing & migrating epithelial cells –The connective tissue matrix of granulation tissue forms nutritive substrate, over which regenerating epidermis can migrate & is gradually replaced by scar tissue

Factors which play important role in Granulation tissue formation:

- Chemotactic factors
- Growth Factors
- Structural molecules
- Proteases [Digests connective tissue matrix (Clark, 1985)]

ANGIOGENESIS OR NEO-VASCULARISATION :

Vital part of **proliferative phase** of wound healing & repair.

It is seen in[18]

- Embryonic development phase
- During repair process (throughout life span of an organism)
- Under certain pathological conditions

Without Angiogenesis, invasion of the wound bed by macrophages & fibroblasts would cease due to lack of oxygen & nutrients.[18]

In the initial stages, these vessels lack the basement membrane & have loose cellular junction (Gullino, 1981) & are fragile in nature. Due to this, on slightest touch, the vessels bleed profusely which is a characteristic feature of newly formed capillaries. The leakage facilitates the movement of cells & macromolecules into wound site.[18]

There are four steps in angiogenesis [18,19]

Step-I : Proteolytic degradation of basement membrane of parent vessel to allow formation of capillary sprout & subsequent cell migration[31] Angiogenic factors acts on capillary endothelial cells, which releases collagenase. This enzyme degrades the collagen of basement membrane.[18]

Step-II: Migration of endothelial cells towards the angiogenic stimulus

Fragmentation of the collagen of basement membrane, permits the migration of endothelial cells into the peri-vascular spaces.[18]

Step-III: Proliferation of endothelial cells, just behind the leading front of migratory cells

Endothelial cells migrate into the peri-vascular spaces where they form buds, which are added by the proliferation of cells with in & near parent vessel (Kalebie et al, 1983).[18]

Step-IV: Maturation of endothelial cells & organization into capillary loops

- **Functional Capillary Loops:** During dermal repair, these buds grow rapidly towards the free surface, where they branch at their tips & unite to form **functional capillary loops.**
- **Superficial Capillary Plexus** : On these loops, new buds develop, so that, a **superficial capillary plexus** rapidly forms in the granulation tissue.
- **Canalization :** Proliferation & branching of cords of endothelial cells later become canalized to form growing capillary buds of healing wound.

- **Fusion :** Capillaries originating from opposite sides of the wound fuse & establish a complete circulation with in the wound.

REMODELLING OF THE VASCULATURE:

There is constant remodeling of the vasculature, which involves obliteration of many of the capillaries (Marchesi, 1985).

As each capillary loop becomes functional, it brings nutrients & oxygen to nearby cells, enabling the fibroblasts to secrete materials for the matrix, through which macrophages & other cells can migrate further.

As the scar maturation proceeds, capillaries gradually regress & the red vascular rich wound tissue transforms into a white, relatively avascular cell poor scar (Zitelli, 1987)

The above proliferative & migratory processes are repeated sequentially, until wound bed is filled with granulation tissue

MACROPHAGIA [18]

- It is a point at which protecting & clearing functions of inflammatory response are linked to starting of reparatory process

What is Macrophagia?

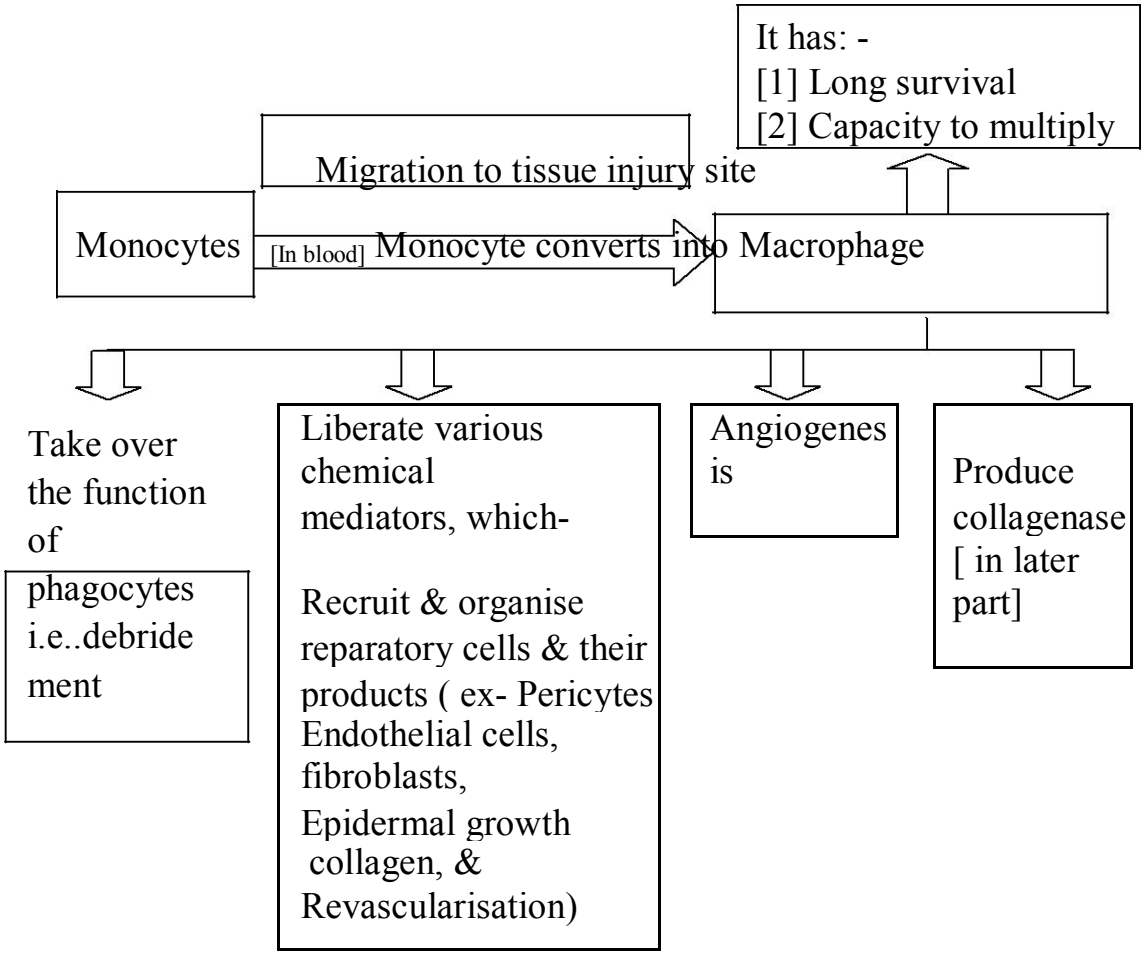
Macrophagia is

[1] Migration of Monocytes [from blood] to tissue injury site

[2] Conversion of monocyte to Macrophage after migration to tissue injury site. These are key cells in dermal repair

- Wound macrophages, which appear subsequent to the cells, play pivotal role in healing by liberating various factors

FIG. 2.2: Functions of Microphages



Macrophages & angiogenesis[18]

It appears that macrophages promote angiogenesis by liberating

ENDOTHELIAL

GROWTH FACTOR (EGF)

Macrophages & Collagenase Enzymes :

Table 3.1: Role of Collagenase

Phase of wound healing	**Sources of collagenase**	**Role of collagenase**
In early part of wound healing	Neutrophils	Collagen of wound debris is broken down by collagenase & converted to breakdown products of collagen, which is then cleared by phagocytes, so, they **assist in tissue debridement**
In later part of wound healing	Macrophages	This enzyme controls the amount of new collagen deposition.

Macrophages & Collagen:

Macrophages secrete lactate which stimulates collagen synthesis by fibroblasts.

Table 3.2 : Migration of Fibroblasts – Mechanism[18]

Phase of inflammation	Chemical which acts as chemotactic agent for fibroblasts in their migration :
Initial	By Fibrin – Fibronectin – Collagen Scaffold of wound base (Brown et al 1988)
Later	By : [1]Soluble chemical factor from macrophages (Wahl, 1981) [2]Collagen peptide (Postlethwait et al , 1978)

Fig. 2.3: Functions of Fibroblast in Wound Healing

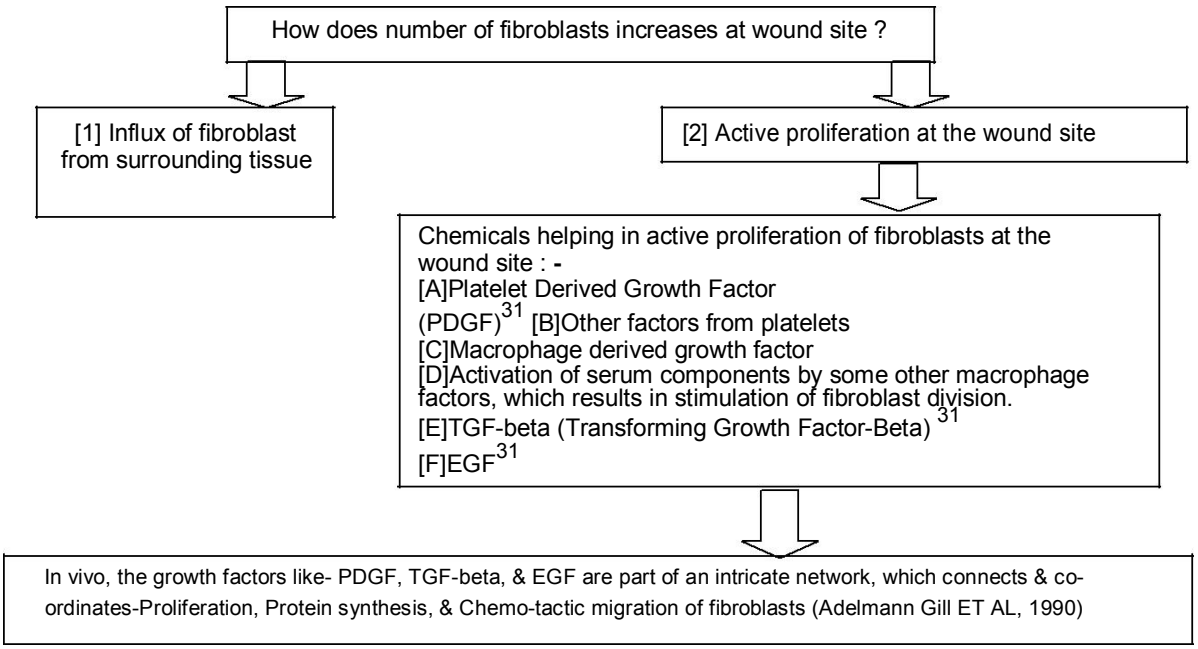
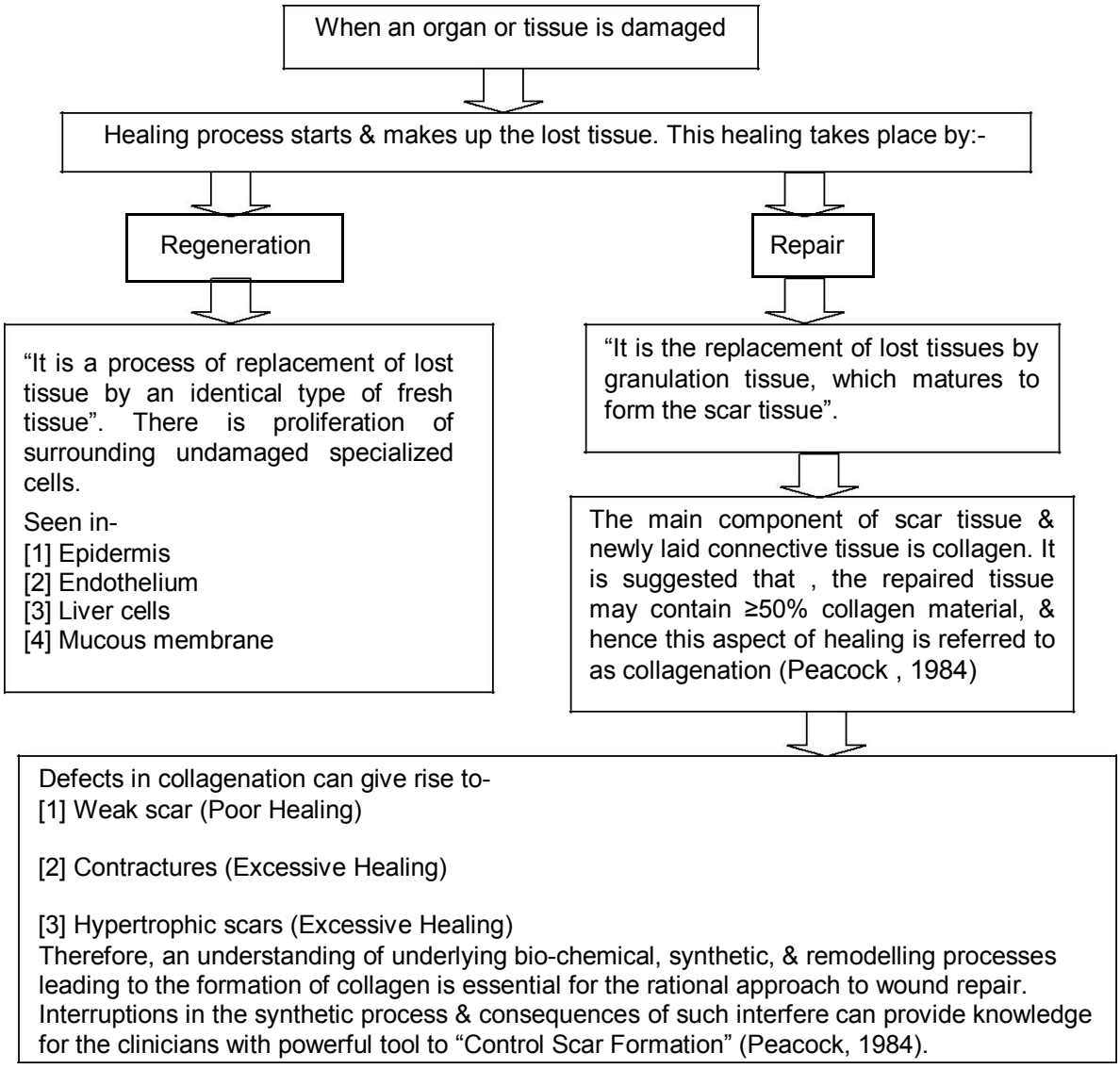


Fig 2.4 : Collagenation Mechanism



Collagen synthesis by fibroblasts begins early in wound healing, by day 03 or 05 & continues for several weeks, depending on wound site[19]

COLLAGEN FIBERS :

Functions of collagen [19]:

1. Support to the tissues.

2. Provides structural framework to other types of tissues.[19]

3. Acts as a medium where blood vessels & nerves are passing.

4. Bring & keeps the wound edges together & provides tensile strength for holding together ⇗This holding strength prevents the breakdown of tissue (organ) at the healed site.[19]

5. Fill the gap caused by the tissue loss.

- Collagen is the most abundant [25% of total body protein – Peacock, 1984) proteins of the connective tissue.[19]

  Collagen is essentially a product of fibroblasts.

- True fibrils form in the extracellular space & these collagen fibrils give strength to connective tissues.[19]

- A critical extracellular modification is Lysyl Hydroxy-lysyl Oxidation. It causes cross linkage between alpha chains of adjacent molecules & is the basis of the structural stability of collagen. Cross linking is the major contributor to the tensile strength of collagen.[20]

- **Collagen Deposition :** Collagen that gets deposited into the extra-cellular matrix of the healing wound has 4 successive phases of synthesis:
    1. Bio-synthesis of Tropo-collagen
    2. Fibril Formation
    3. Collagen Maturation
    4. Collagen Degradation.

Types of collagen.[19,21]: On the basis of bio-chemical composition of the chains that make up the triple helix of the collagen molecule, some 14 types of collagen can be discerned, of which the most well characterized are shown in following table.

Table 3.3: Types of Collagen

Type of collagen	CHAINS				Characteristics	Distribution
I	α 1 (I),	α 2(I)			Bundles of banded fibers with high tensile strength	Skin (80%), Bone (90%), Tendons, Most other organs
II	α 1 (II)				Thin fibrils, Structural proteins	Cartilage (50%), Vitreous Humour
III	α 1 (III)				Thin fibrils, Pliable	Blood vessels, Uterus, Skin (10%)
IV	A 1	A 2	α 3	α 4,α 5, α 6 (IV)	Amorphous	All basement membranes
V	α 1 [V, α 2(V)]		α3(V)		Amorphous, Fine fibrils	2-5% of interstitial tissues, blood vessels, Interstitial tissues
VI	α 1 (VI)	α 2 (VI)	α3 (V)			
VII	α 1 (VII)				Anchoring Filament	Dermal-Epidermal Junction
VIII	α 1 (VIII)	A 2 (VIII)			Probably Amorphous	Endothelium-Descement's Membrane
IX	α 1 (IX)	α 2 (IX)	α3 (IX)		Probably Role in maturation of cartilage	Cartilage
X	α 1 (X)					
XI	α 1 (XI)	A 2 (XI), α 2 (XI)				

DEGRADATION OF COLLAGEN AND OTHER ECM PROTEINS

- Net collagen accumulation, however, depends not only on synthesis but also on collagen degradation.
- Degradation of collagen and other ECM proteins is achieved by following enzymes.[19]

Metalloproteinases.[22]

- Helps in degradation of collagen and other ECM proteins
- These are dependent on zinc ions for their activity.

These enzymes are produced by.[19]

- Fibroblasts
- Macrophages
- Neutrophils
- Synovial cells
- Some epithelial cells

Their secretion is induced by

- Growth factors (PDGF, FGF),
- Cytokines (IL-l, TNF-a),
- Phagocytic stimuli

- Nevertheless, it is thought that the collagenases play a role in degrading collagen in inflammation and wound healing.[19]
- Degradation aids in the debridement of injured sites and also in the remodelling of connective tissue necessary to repair the defect.[19]
- Indeed, collagenases and their inhibitors have been shown to be spatially and temporally regulated in healing burn wounds. [19]

GROUND SUBSTANCE IN HEALING WOUND[18]

- Connective tissue consists of cellular and non cellular component (matrix). Matrix is again composed of fibres and ground substance.
- **Definition:** This is non-fibrous part of the matrix in which cells and fibres are embedded.
- **Consistency:** Except in mineralized connective tissue, the ground substance is a viscous gel.

Table 3.4: Constituents of Ground Substance

Water	**High proportion**
Mucopolysaccharides	It has been suggested that the fibroblasts, on the outer surface, have a layer of mucopolysaccharides (Peacock, 1984c) whose charge and orientation determine the aggregation and orientation of tropocollagens.
Fibronectin	Fibronectin is a glycoprotein with high molecular weight (Reese et at, 1983) There are two types of Fibronectin (a) Cell surface Fibronectin and (b) Plasma Fibronectin Functions: Fibronectin of connective tissue matrix acts as a glue between different matrix components and fibroblasts
Chondronectin	It is a specific adhesive between chondroblasts and type II collagen
Mucoproteins	
Glycoproteins	
Lamenin	
Entactin	

WOUND CONTRACTION[18]

- **Definition:** "Wound contraction may be defined as a process by which the size of the full thickness open wound is diminished by centripetal movement of the whole thickness of surrounding skin".

- The feature that most clearly differentiates primary from secondary healing is the phenomenon of wound contraction, which occurs in large surface wounds[19]

- Wound contraction is one function of granulation tissue which is critical for repair.

- The events of wound healing from injury to fibroplasias, occurs in almost all wounds. Certain events like wound contraction occurs characteristically in excision dermal wound and epithelization occurs in wounds of surface lining epithelium.

- In humans, the wound contraction is less because in most parts of the body the skin is somewhat firmly attached to subcutaneous tissue but it can occur in areas like back of neck and buttocks (Peacock, 1984).

- **Timing of Wound contraction:**

  Wound contraction starts from about 3rd or 4th day of wounding and continues up to 15th or 16th day and stops thereafter, irrespective of whether the wound is totally closed or not.

- **Rate of wound contraction:**

  The rate of wound contraction is about 0.6-0.75 mm/day (Peacock 1984).

  Wound contraction is not materially affected by size or shape of the wound but perhaps by the length of the wound perimeter (McGrath and Simon, 1983).

- **Mechanism of wound contraction [18]:**

  The mechanism of wound contraction is disputable and debatable. Many theories like Pull theory, Push theory / Picture Frame theory etc have been proposed but none of them appears to be satisfactory.

  Dollion (1987) pointed out that modified fibroblasts rich in actin filaments are responsible for wound contraction[19]

  Myofibroblasts are situated just under the advancing edges of the wound.

  In early phases of wound contraction, contractile epidermal cells in wound edges are suggested as a source of force (Baur et al, 1984).

  Wound contraction can be both beneficial or detrimental. Wound contraction can lead to distortion, disfigurement and impairment of function.

EPITHELIZATION[18]

- **Definition**: Epithelization is a process of wound healing involving body surfaces.

- Unlike healing by fibroplasias where lost parenchymal cells are replaced by non-specific connective tissue, in epithelialization lost epithelial cells are replaced by epithelial cells only. It is an example of healing by regeneration.

- **Stages of epithelization**: The whole process of epithelization thus includes the following stages (Peacock,1984).

  Mobilization and loosening of basal cells from their dermal attachment.

  Migration or movement of cells to a position of cell deficit.

  Proliferation or replacement of cells to a position of cell deficit and

  Differentiation or restoration of cellular function.

- **Epithelization which depends on several factors like:**

  Size of wound

  Location of wound

  Shape of wound

  Impairement of blood supply

  Pathological modification of wound.

- **Healing by epithelization occurs in:**

  Dermal wounds,

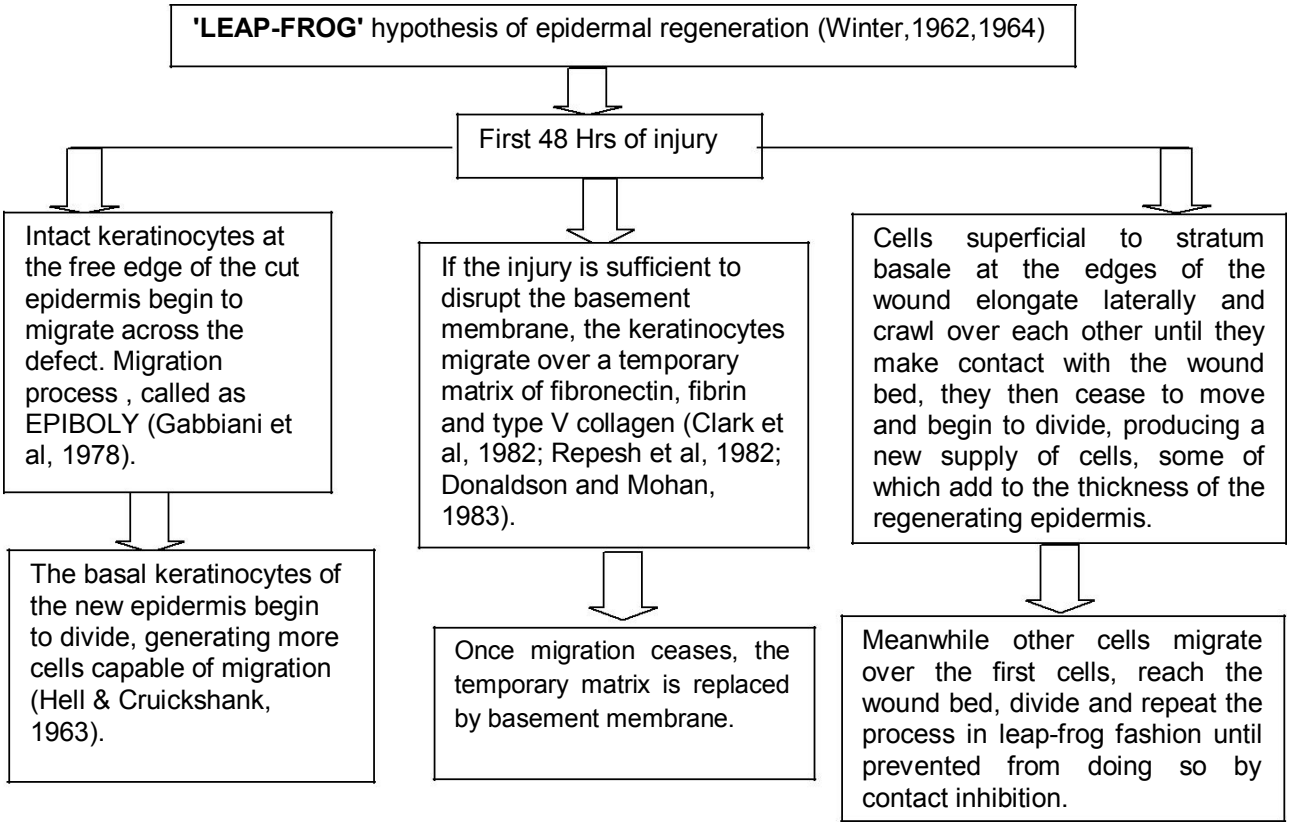
  Wounds of tracheobronchial surface,

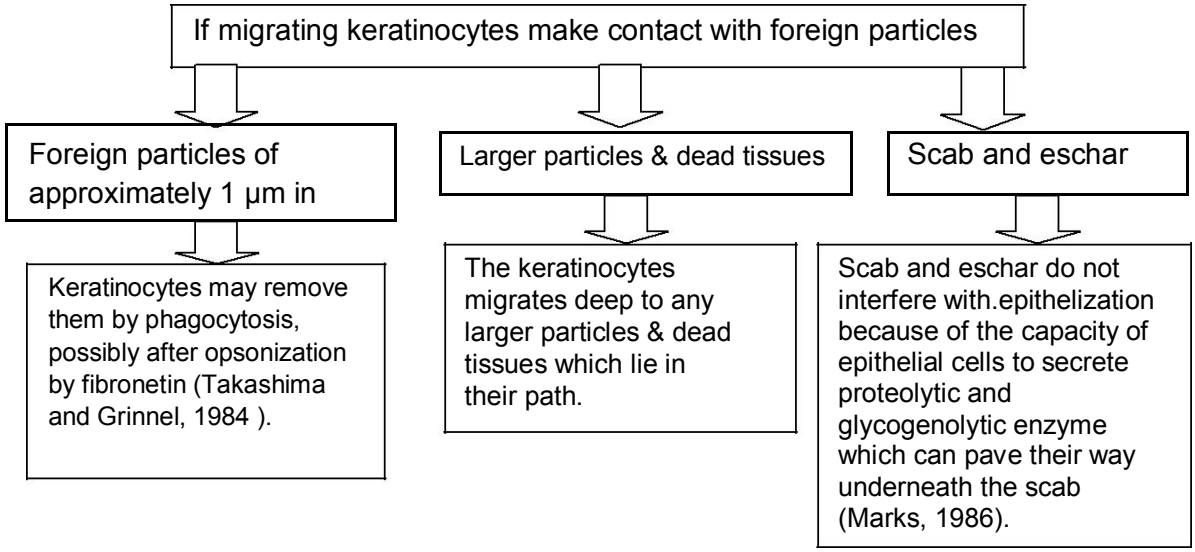
  Surface wounds in gut, urinary bladder, uterus etc.

- **Timing of Epithelization:**

  First 24 Hrs of injury :-Changes in the epidermis leading to re-epithelization begin within 24 hours of the formation of a cutaneous wound.

Fig. 2.5: Mechanism of Epithelization





WOUND HEALING[19]

- **MECHANISMS OF WOUND HEALING :**

  Wound healing, as we have seen, is a complex (but orderly) phenomenon involving a number of processes, including induction of an acute inflammatory process by the wounding, regeneration of parenchymal cells, migration and proliferation of both parenchymal and connective tissue cells, synthesis of ECM proteins, remodeling of connective tissue and parenchymal components, and collagenization and acquisition of wound strength.

- **TYPES OF WOUND HEALING :**

  Primary union or healing by first intention - The healing of a clean, uninfected surgical incision approximated by surgical sutures. The incision causes death of a limited number of epithelial cells and connective tissue cells; as well as disruption of epithelial basement membrane continuity.

  Secondary healing or healing by second intention

  - When there is more extensive loss of cells and tissue, as occurs in infarction, inflammatory ulceration, abscess formation, and surface wounds that create large defects, the reparative process is more complicated.

- The common denominator in all these situations is a large tissue defect that must be filled. Regeneration of parenchymal cells cannot completely reconstitute the original architecture.

- Abundant granulation tissue grows in from the margin to complete the repair. This form of healing is referred to as secondary union or healing by second intention.

Secondary healing differs from Primary healing in several respects:

Inevitably, large tissue defects initially have more fibrin and more necrotic debris and exudates that must be removed. Consequently, the inflammatory reaction is more intense.

Much larger amounts of granulation tissue are formed. When a large defect occurs in deeper tissues, such as in a viscus, granulation tissue with its numerous scavenger white cells bears the full responsibility for its closure, because drainage to the surface cannot occur.

Perhaps the feature that most clearly differentiates primary from secondary healing is the phenomenon of wound contraction, which occurs in large surface wounds. Large defects in the skin of a rabbit are reduced in approximately 6 weeks to 5 to 10% of their original size, largely by contraction. Contraction has been ascribed, at least in part, to the presence of myofibroblasts-altered fibroblasts that have the ultrastructural characteristics of smooth muscle cells. The deposition of connective tissue matrix, particularly collagen, its remodeling into a scar, and the acquisition of wound strength are the ultimate effects of orderly wound repair.

GROWTH FACTORS

Growth factors exert diverse effects on cell growth, metabolism, differentiation. Growth factors stimulate or inhibit progression through the cell cycle that Control cell viability or death, or that act principally to regulate cell differenciation.[26]

Their modes of action include

1. Autocrine

   Actions are mediated by a GF on its cell of origin after its secretion in to the extracellular environment.

2. Paracrine

   GF that is secreted by one cell has an effect on adjacent cells.

3. Juxtacrine

   GF is bound to the cell membrane or extra cellular matrix.

4. Intracrine

Actions occur inside the cell of origin.

The effects of GF are mediated by activation of specific receptors. These receptors are transmembrane proteins.[23,27]

The major growth factor families are

Table 3.5 GROWTH FACTORS [23,28]

Factor	Cell or Tissue of Origin	Selected Target Cells or Tissue	Selected Stimulatory (S) or Inhibitory (I) Actions	Clinical Trials
EGF	macrophages, monocytes	epithelium, endothelial cells	S: proliferation of keratinocytes, fibroblasts, and endothelial cells S: keratinocyte migration.	venous ulcers
FGF	monocytes, macrophages, endothelial cells	endothelium, fibroblasts, keratinocytes	S: proliferation of endothelial cells, keratinocytes, and fibroblasts. S: chemotaxis, ECM	diabetic ulcers, venous ulcers, pressure ulcers
GMCSF	macrophages, fibroblasts, endothelial cells	hematopoietic, inflammatory cells, neutrophils, fibroblasts	S: chemotaxis of endothelial cells, inflammatory cells S: keratinocyte proliferation, activation of neutrophils	venous and arterial ulcers
HGH	pituitary gland	hepatocytes, bone, fibroblasts	S: IGF-1 production	venous ulcers
IL-1	lymphocytes, macrophages, keratinocytes	monocytes, neutrophils, fibroblasts, keratinocytes	S: monocytes, neutrophils S: macrophage chemotaxis	pressure ulcers
PDGF	platelets, macrophages, neutrophils, smooth muscle cells	fibroblasts, smooth muscle cells	S: proliferation of smooth muscle cells and fibroblasts S: chemotaxis S: ECM, contraction	diabetic ulcers, pressure ulcers
TGF-β	platelets, bone, most cell types	fibroblasts, endothelial cells, keratinocytes, lymphocytes, monocytes	S: ECM, fibroblast activity S: chemotaxis I: proliferation of keratinocytes, endothelial cells	venous ulcers, pressure ulcers

PLATELET DERIVED GROWTH FACTOR (PDGF)

The wound environment contains a variety of growth factors. Platelet-derived growth factor is of particular relevance due to its chemotactic, mitogenic,angiogenic, and stimulatory effects leading to matrix formation and wound bed granulation . PDGF may be of significant benefit of diabetics as recalcitrant diabetic wounds have been found to be deficient in or absent of PDGF.[23]

Platelet-derived growth factor (PDGF) is one of the numerous growth factors, or proteins that regulate cell growth and division. In particular, it plays a significant role in blood vessel formation (angiogenesis).PDGF was discovered as a protein released from the alpha granules of platelets. It was purified from platelets as a highly basic 30- kilo delton dimeric protein. Purified PDGF was found to consist of two related chains, PDGF- A, PDGF-B, products of separate genes. PDGF binds to two cell surface receptors, PDGFR-α and PDGFR-β which also are related in structure and sequence but are distinct gene products. Both growth and their receptors are expressed factors in a wide variety cell and tissue types. PDGF- has been prepared and purified

STRUCTURE OF PLATELET-DERIVED GROWTH FACTOR

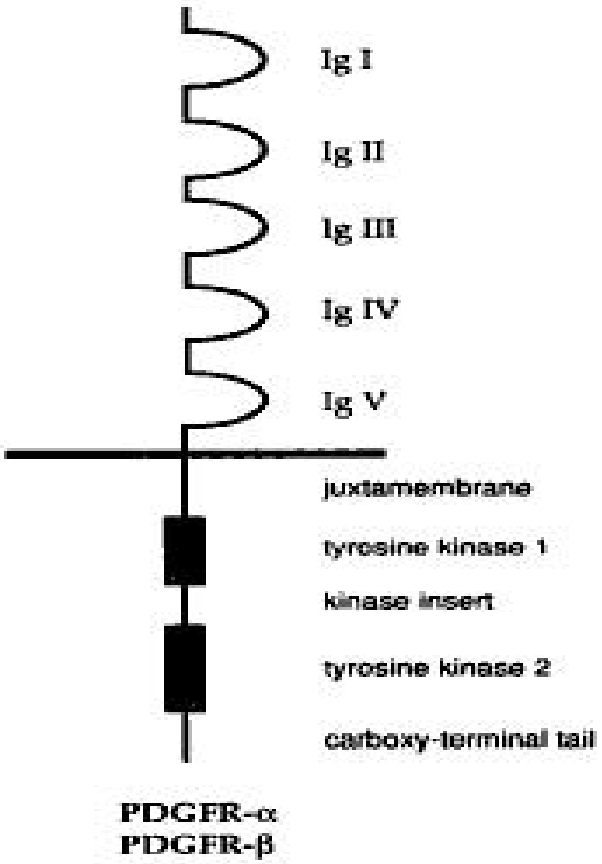
Mature PDGF-A and -B chains are 109 amino acids in length and are 60% identical. Both PDGF chains are synthesized as precursor proteins that undergo pro-cessing to yield mature glycoproteins. All three combinations of growth factor dimers have been isolated from tissues: AA, AB, BB in addition to platelet a granules, PDGF has been isolated from several cell types including macrophages and from aortic smooth muscle cells. Recently, two divergent members of the PDGF family were identified and termed PDGF-C and D.[23]

PLATELET-DERIVED GROWTH FACTOR RECEPTORS AND SIGNALING

The two PDGFRs are ligand-activated tyrosine protein kinases. The receptors are composed of an extracellular region that contains five Ig-like domains, a transmembrane segment, and an intracellular region with a tyrosine kinase domain that is split by a kinase insert of approximately 100 amino acids. The binding of PDGF to the extracellular region of the receptor induces receptor dimerization.

Both homo- and heterodimets can form, depending on the ligand and the relative receptor abundance. PDGFR-β homodimers bind only PDGF BB and DD; PDGFR-α homodimers bind PDGF AA, AB, BB, and CC; whereas PDGFR-αβ heterodimers bind PDGF BB, AB, CC, and DD.[23]

Fig 2.6 Structure of platelet derived growth factor receptors[23]



BIOI.OGIC EFFECTS

PDGF action is essential for normal development. One of the major actions of PDGF in the adult is in wound healing. Tissue injury leads to the rapid release of abundant PDGF A or B by degranulating platelets. Other short-term sources of growth factor include activated macrophages and endothelial cells. It is chemotactic for smooth muscle cells, fibroblasts, neutrophils, and monocytes and stimulates macrophage activation. It is a potent mitogen for fibroblasts and smooth muscle cells and stimulates their proliferation in collaboration with other growth factors. PDGF induces expression of fibronectin, of collagenase, and of some types of collagen, and these proteins participate in the tissue remodelling that occurs during wound healing.[23]

PLATELET RICH PLASMA(PRP):[57]

Platelet-rich plasma (PRP) is an autologous product, with large number of platelets in a small volume of plasma. It is derived by centrifugation of the whole blood . PRP is effective in improving the natural way of wound healing, soft tissue and bone reconstruction .

PRP incorporates high concentrations of fibrin, PDGF, into the graft mixture. Through recent studies, it has been learnt that PRP has wide uses in clinical wound healing. When added to small bony defects , PRP increases the bone density. In case of larger defects, it is used in combination with grafting material.

PRP[56] can also be exogenously applied to soft tissues, as it promotes tissue sealing and wound healing. When PRP is used pre operatively, it decreases the hospital stay and the post operative need of blood and blood products.

PRP in recent times, has also found its application in the field of cellular therapeutics and tissue engineering. Platelet-rich plasma (PRP) is a fibrin tissue adhesive. It is different from fibrin glue by the high platelet content. The platelets has a capacity to accentuate wound healing and osteogenesis.

PRP accelerates the hemostatic cascade to a stimuli, and also antagonizes the steroidal effect of delay in wound healing. PRP produces an antimicrobial effect, due to its high content of leukocytes.

PRP can be used as an effective hemastatic agent. PRP enhances epithelial, epidermal and endothelial regeneration. It promotes angiogenesis, collagen synthesis, soft tissue healing and reduces dermal scarring.

PRP which has a wide range of clinical healing applications in various fields such as,

Miscellaneous clinical application of PRP:[58]

Neurosurgery
Pituitary tumor removal
Skull base tumor resection
Intradural procedures involving tumor or release of tethered cords
Dural tumors
Acoustic neuroma excisions (dura tears during laminectomy)
Oral and Maxillofacial Surgery
Mandibular reconstruction
Alveolar cleft repair
Oral-nasal fistulas
Otorhinolaryngology-Head and Neck Surgery
Radical neck dissections
Pectoralis major myocutaneous flaps
Facial fractures
Reconstructions
Cosmetic Surgery
Full and split-thickness skin grafts donor sites and recipient sites
Skin flaps
Bone grafts
Metal implants
Tissue expansion
Aesthetic Surgery (Face Lifts, liposuction, etc)

Augmentation & reduction mammoplasty
Reconstructions
Urology
Radical retro-pubic prostatectomy, & retroperitoneal lymph node dissections
Periodontal Surgery
Dental implants
Guided Bone Regeneration
Orthopedic/Spinal Surgery
Total Hip Replacement
Total Knee Replacement
Scoliosis Repair
Spinal Fusion
All Open and Internal Reduction Fixation Operations
Hand and Foot Surgery
Bone Graft Surgery
Cardiothoracic Surgery
Sternotomy
Graft Conduit Sites
Esophagogastrectomy
General Surgery
Recurrent Hernia Repair
Anal Fistula
Bariatric Surgery

PRP is very effective for diabetic patients having chronic non-healing wounds. PRP also acts as carrier for growth factors, and so increases vascularization of tissue.

PRP decreases incidence of postoperative and intraoperative bleeding at both recipient and donor sites. It promotes the stability of grafted tissue due to its adhesive property.

PRP and FIBRIN GLUE :[60]

PRP involves taking 10ml patient's blood before procedure, centrifugation, and activating the platelets, application of the gel to the site. The platelets is in a reverse ratio to red blood cells opposite to naturally obtained clot. The healing is improved 2 to 3 times.

PRP has to be differentiated from fibrin glues.

PRP	FIBRIN GLUE
High concentration of platelets	Low platelet concentration
Less fibrinogen	More fibrinogen

MECHANISMS OF ACTION [61-64]

Hemostatic Response to Injury:

After an injury, the released subendothelial factors attract platelets and activate coagulation. Platelets produce factors such as thromboxane, adenosine, and serotonin, which stimulates coagulation and fibrin is produced.

Hemostatic plug is formed due to increased thrombin production and platelet activation. This reduces bleeding. This also aids the wound healing by thrombin-mediated cell activation and platelet- mediated angiogenesis.

Primary hemostatic plug is formed by activation of platelets, through the production of Vwf and fibrinogen that binds platelets to the vessel wall and to one another.

Secondary hemostatic plug results fromed by the action of thrombin , which is essential for the formation of fibrin. Then the platelets gets entrapped between them. The balance of all components determines the integrity of hemostatic plug .

PRP closely resembles the final step of the coagulation process, by the formation of a fibrin clot.

Growth Factors:[65]

PRP produces its effects via the release growth factors from alpha granules to accelerate wound healing . This process begins within minutes, and ninety percent of the GF are secreted within one hour. This process continues for about 7 days. The rate of wound healing is directly proportional to the amount of platelets found in the site.

PDGF-αα,	Chemotactic for fibroblasts and macrophages
αβ, ββ	Mitogenic for fibroblasts, smooth muscle cells and endothelial cells
TGF*- β1,	Mediates angiogenesis
β2	Chemotactic for fibroblasts, keratinocytes and macrophages
	Mitogenic for fibroblasts and smooth muscle cells
	Inhibits endothelial cells, keratinocytes and lymphocytes
	Regulates matrix proteins, including collagen, proteoglycans, fibronectin and matrix-degrading proteins
VEGF†	Chemotactic and mitogenic for endothelial cells
	Mediates angiogenesis
EGF‡	Mediates angiogenesis
	Mitogenic for fibroblasts, endothelial cells and keratinocytes
HGF§	Mediates regeneration
FGF‖	Mediates tissue organization and regeneration
FGF-9	Aids generation of new follicles

*TGF: Transforming growth factor, †VEGF: Vascular endothelial growth factor,
‡EGF: Epidermal growth factor, §HGF: Hepatocyte growth factor,
‖FGF: Fibroblast growth factor

Contraindications

Though treatment with autologous PRP is risk free, following are few conditions where it must be used carefully,

1. Coagulopathies
2. Thrombocytopenia
3. Anemia
4. Hemodynamic instability
5. Sepsis
6. Unstable angina

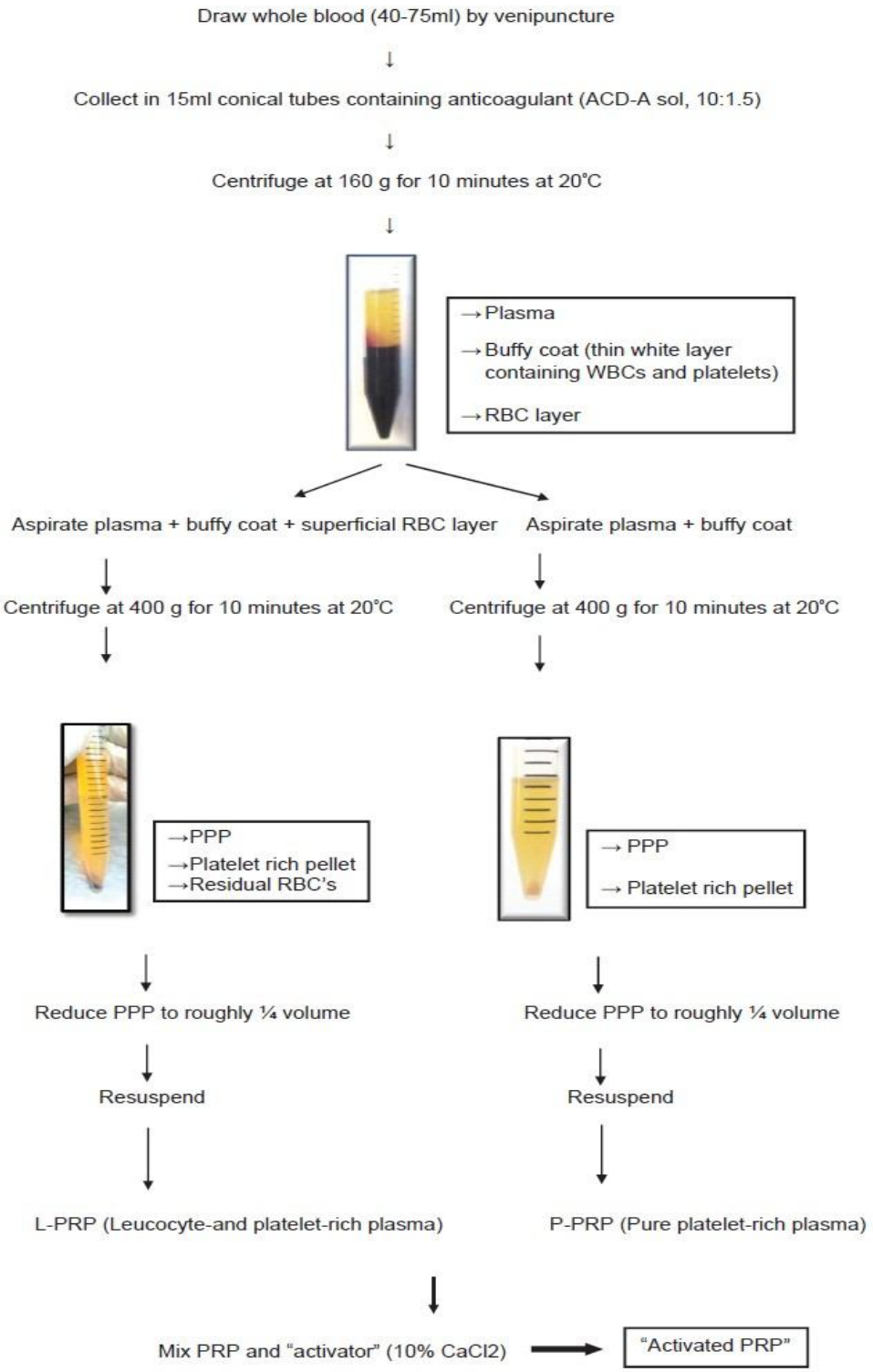
So it becomes mandatory to evaluate the hematological inidices in the pre-treatment period for every patients.

PREPARATION OF ACTIVATED PRP:

Activated PRP is prepared by two methods,

1. Manual double spin method
2. Automated method

MANUAL DOUBLE SPIN METHOD:[66]



AUTOMATED DEVICES

Various automated devices are available in the market for the production of activated PRP. Although time saving, these devices are expensive and also with unproven efficacy.

CLASSIFICATION OF PLATELET CONCENTRATES

Based on the quantity of fibrin and leukocyte, PRP can be broadly classified under following categories,

1. PRP-p(platelet –rich plasma-pure)
2. PRP-L(platelet –rich plasma and leucocyte)
3. PRF-p (platelet –rich fibrin pure)
4. PRF-L(platelet rich fibrin and leucocyte)

DIABETES MELLITUS

Definition :

"Diabetes [31-37] mellitus is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both"**.**

Cause of hyperglycemia may include:

- Insulin production decreased
- Reduced glucose consumption
- Raised glucose synthesis

"Level of glycaemia at which diabetes specific complications occur rather than on deviations from population based mean"

Classification[31-37]

TYPE I

Type	**Pathology**
I A	Autoimmune beta cell destruction ⇗ Insulin Deficiency
I B	Develop insulin deficiency by unknown mechanism causing destructive process of beta cells Lack immunologic markers

Type II

- Decreased insulin production
- Insulin resistance
- Raised glucose synthesis

  Various metabolic ,genetic modification leads to high blood sugar level in type 2 diabetes.

Initial phase of abnormal blood glucose is seen in DM as

- Impaired fasting glucose (IFG)
- Impaired glucose tolerance (IGT)

Diagnosis [31-37]

The National Diabetic Data Group & World Health Organisation have issued a diagnostic criteria for DM-2 based on the following facts:

- RBS ≥200 mgs / dL Or ≥11.1 m mol / L with symptoms of DM (Polyuria, Polydipsia, Weight loss)
- FBS ≥126 mgs / dL or ≥ 7.0 m mol / L
- 2 Hr Plasma Glucose (During Oral GTT) ≥ 200 mgs / dL or ≥11.1 m mol/L (Not recommended as a part of routine screening).

- Strong co-relation b/w ↑ FPG & ↑ HbA1c concentration but currently not recommended for the diagnosis of DM.

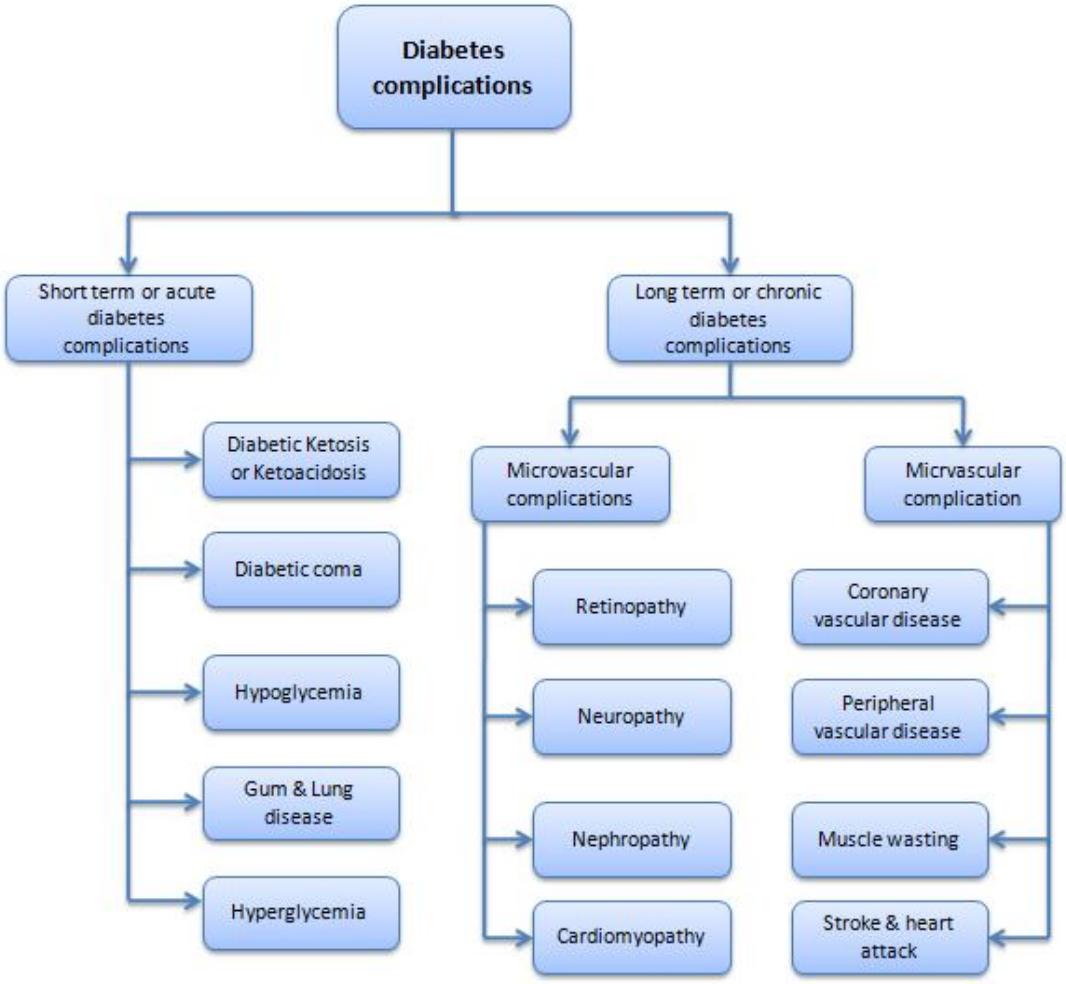
Table 3.6: Diagnosis of Diabetes Mellitus

Terms	Definition
Random Blood Glucose (RBS)	Blood Glucose levels not related to meals
Fasting Blood Glucose (FBS)	Blood Glucose levels when there is no caloric intake from past 8 Hrs
2 Hr Plasma Glucose (During Oral GTT)	The test should be performed using a glucose load containing the equivalent of 75 gms anhydrous glucose dissolved in water

Chronic Complications of DM [31-37]

Diabetes mellitus affects almost all the organs and caries high morbidity and mortality on a chronic basis.

Fig. 2.7: Chronic Complications of DM



- Increased duration of high blood glucose is related to chronic complication mostly recognized in fourth decade

- Patient with type 2 diabetes usually diagnosed with some complication due to asymptomatic high blood sugar levels for long time.

- Chronic hype rglycemia leads to increased microvascular changes.

    - All pathies related to diabetes are prevented by controlling the high blood sugar values.

    - Two to four times higher mortality was recognized in patient with chronic hyperglycemia is noted.

    - High HbAc1 ,fasting and postprandial blood sugar level are associated with above mentioned complication.

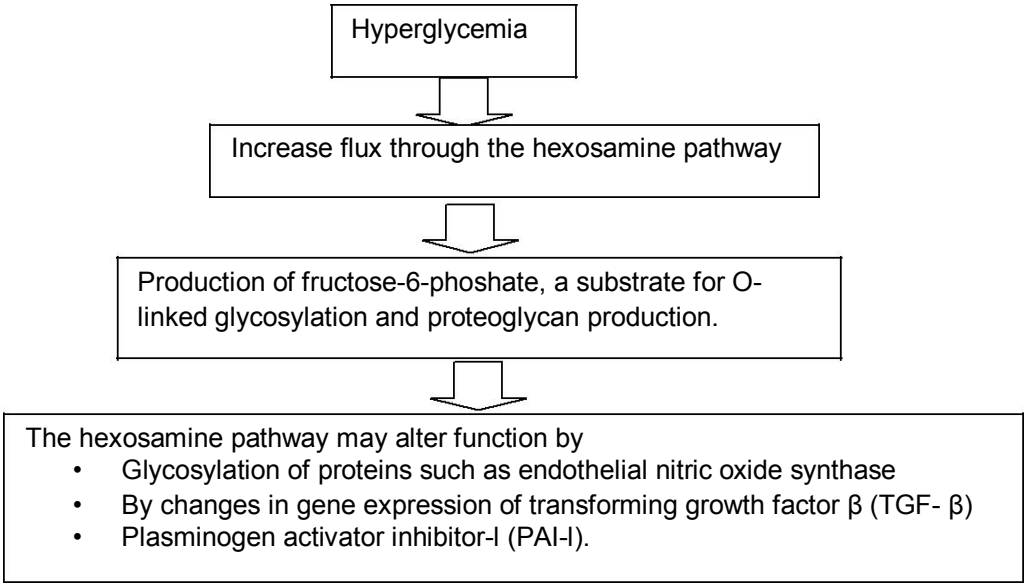
    - Increased lipid, and high blood pressure significantly contribute to macrovascular complications.

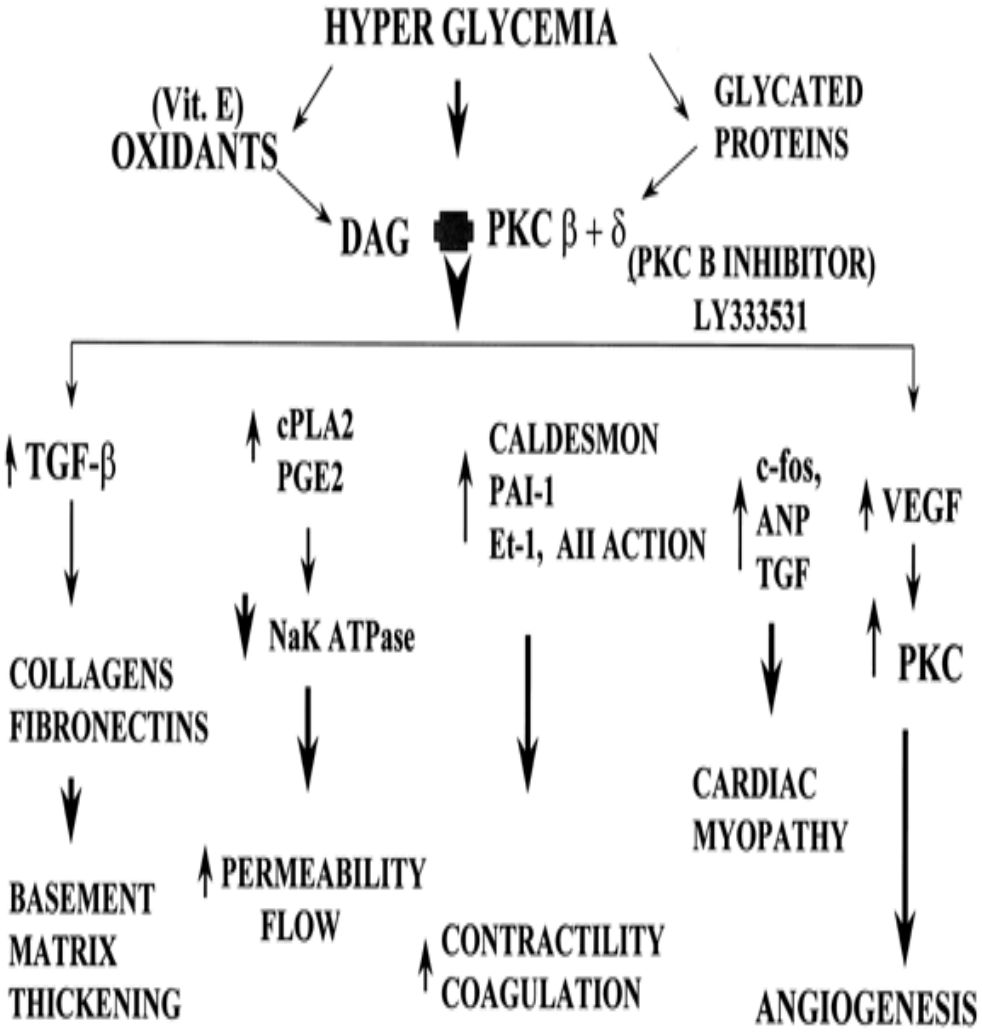
Mechanisms of complications [31-37]

Many Theory are proposed to relate high blood sugar value to chronic complications of DM (Fig. 2.8).

A hypothesis proposes that leading to :

Fig. 2.8: Mechanisms of Complication of Diabetes Mellitus




HYPER GLYCEMIA
(Vit. E)
OXIDANTS
GLYCATED
PROTEINS
DAG
PKC β + δ
(PKC B INHIBITOR)
LY333531
TGF-β
cPLA2
PGE2
CALDESMON
PAI-1
Et-1, AII ACTION
c-fos,
ANP
TGF
VEGF
NaK ATPase
PKC
COLLAGENS
FIBRONECTINS
CARDIAC
MYOPATHY
PERMEABILITY
FLOW
BASEMENT
MATRIX
THICKENING
CONTRACTILITY
COAGULATION
ANGIOGENESIS

Neuropathy And Diabetes Mellitus [31-37]

- The incidence of neuropathy is 32 percent in middle age ,to that of 60 years of age.[1,2] where it is about 50 percent.

- Neuropathy is directly related to blood sugar , and duration of diseas.

- May manifest as
    1. Polyneuropathy
    2. Mono-neuropathy
    3. Autonomic Neuropathy

1. Myelinated and unmyelinated fibers are affected.

2. Diabetic neuropathy is are diagnosis if of other possible etiologies are excluded.

Poly-neuropathy / Mono-neuropathy :

- The symmetric polyneuropathy is common form of neuropathy in diabetes.

- It presents as:
    1. Distal sensory loss - most frequent presentation
    2. Hyperesthesia
    3. Paresthesia
    4. Dysesthesia
- Symptoms includes a sensation of following, which begins in the feet & spreads proximally.
    1. Numbness,
    2. Tingling
    3. Sharpness
    4. Burning

- Physical examination reveals
    1. Sensory loss
    2. Loss of ankle reflexes
    3. Abnormal position sense.

- Worsening of lower limb Pain and rest pain is typically seen in diabetes patients.

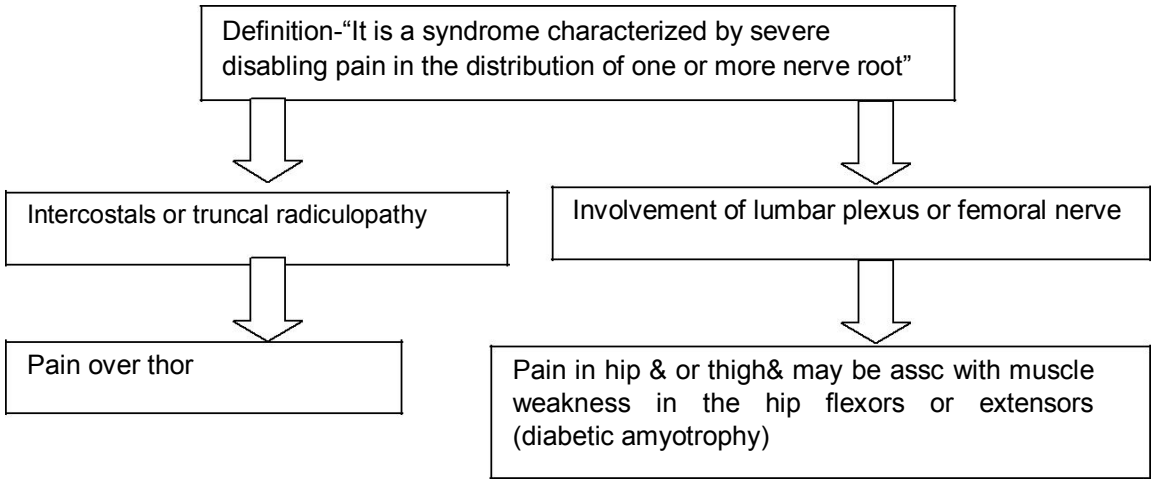
- Chronic and acute type of painful , neuropathy have been described.

- As duration of disease progress, neuropathy also progress in legs.

- With improvement in the sugar value, progression of neuropathy decreases.

Diabetic Neuropathy :

It may be accompanied by - Motor weakness



Treatment of diabetic neuropathy :

- Symptomatic treatment.
- Since pain of acute diabetic neuropathy may resolve over the first year,analgesics may be discontinued as progressive neuronal damage from DM occurs.
- Chronic, painful diabetic retinopathy is difficult to treat but may respond to

1. Tricyclic antidepressants - Amitryptiline, desipramine, nortriptyline
2. Gabapentin
3. NSAIDs (Avoid in renal dysfunctions)
4. Others (Mexilitine, Phenytoin, Carbamazepine, Capsaicin cream) Referral to pain management center may be necessary.

Lower Extremity Complications [31-37]

- Foot ulcers and infections are a major source ofmoibidity in individuals with DM.
- The reasons for the increased incidence of these disorders *in* DM involve the interaction of several pathogenic factor?

- Neuropathy
- Abnormal foot biomechanics
- Peripheral arterial disease
- Poor wound healing.

Neuropathy :

Neuropathy is present in over 80 percent of patients with foot ulcers.

Peripheral sensory neuropathy :

Interferes with normal protective mechanisms and allows the patient to sustain major or repeated minor trauma to the foot, often without knowledge of the injury

Motor and sensory neuropathy :

Lead to abnormal foot muscle mechanics and to structural changes in the foot (hammer toe, claw toe deformity, prominent metatarsai heads, Charcot joint).

Autonomic neuropathy :

Results in anhidrosis and altered superficial blood flow in the foot, which promote drying of the skin, and fissure formation.

Peripheral arterial disease and poor wound healing :

Impede resolution of minor breaks in the skin, allowing them to enlarge and to become infected.

Disordered proprioception :

Causes abnormal weight bearing while walking and subsequent formation of callus or ulceration.

Approximately 15% of individuals with DM develop a foot ulcer, and a significant subset will ultimately undergo amputation (14 to 24%) risk with that ulcer or subsequent ulceration.

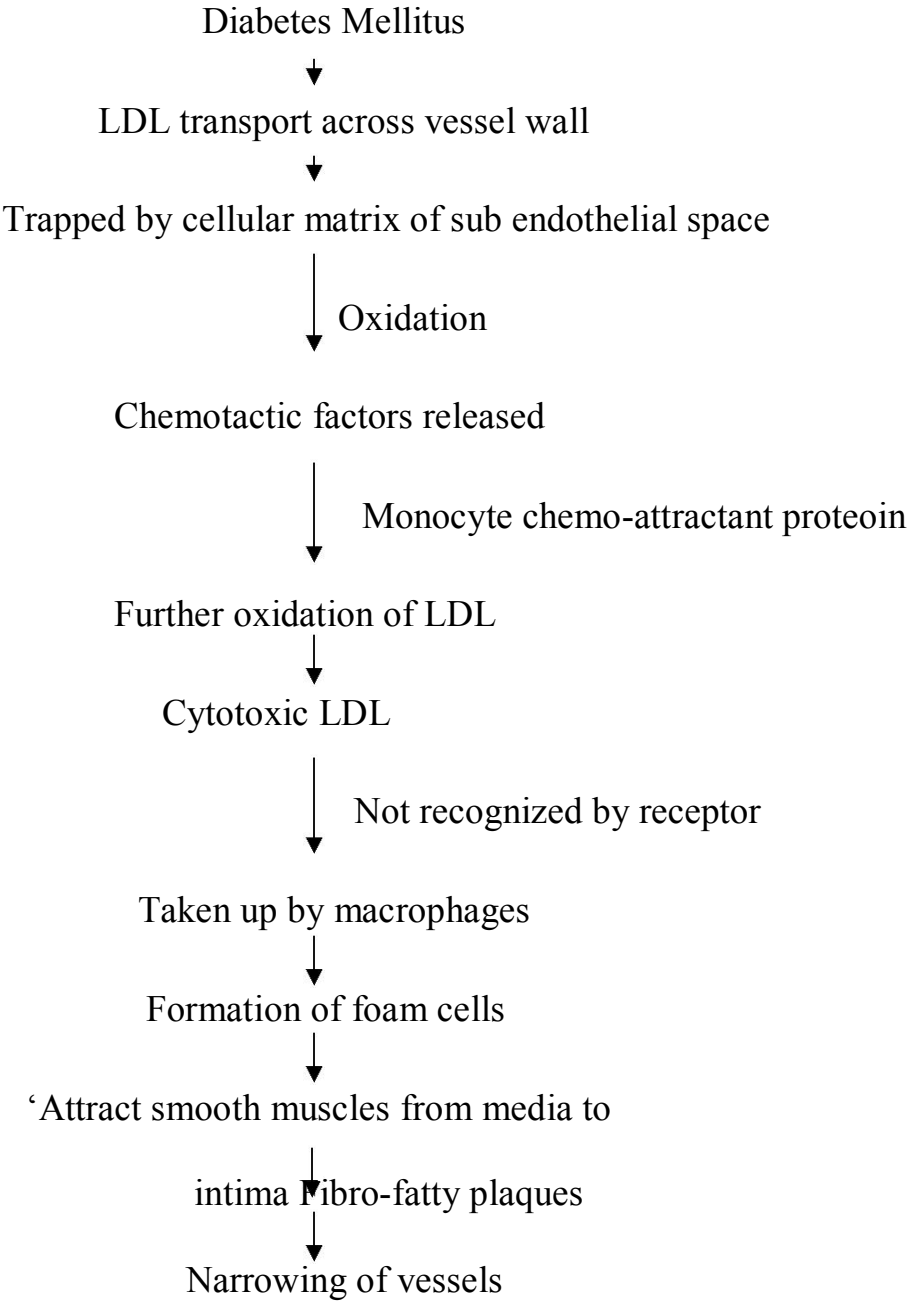
VASCULAR CHANGES IN DIABETES

1. Atherosclerosis: Chronic inflammatory process that can be converted into acute clinical event by plaque rupture[38,39.]

Development of atherosclerosis is accelerated in DM leading to increased morbidity and mortality. All the large vessels are involved in this process and clinical manifestations are apparent as a result of atherosclerotic narrowing and thrombosis of coronary, cerebral and leg vessels

l. Lipoproteins pathogenesis:[40,41]

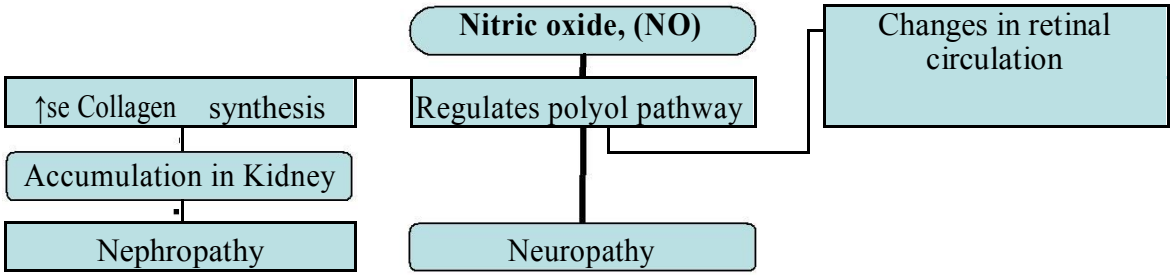
Fig 2.9: Pathophysiology diabetic vasculopathy



Π. Endothelium:

a. Nitric oxide, (NO): (EDRF-Endothelium derived relaxing factor)

Nitric oxide, (NO)[42]



b. Prostacyclin (PGI_2)[43]

PGI_2

↓

Potent vasodilator also inhibits

Platelet adhesion & aggression

↓

Prostacyclin stimulating factor

(Present in human arterial smooth muscle)

↓

↓se in Diabetics & arterial wall of patients with old MI

c. Thromboxane A_2($TX\text{-}A_2$):>>>>Vasoconstrictor- Conteracts effect of N.O

↑sed levels found in DM, HTN & hyperlipidemia.

d. Endothelin::>>>>Vasoconstrictor

↑sed levels found in DM around 3.5 times

Fig 2.10 Pathogenesis of diabetic ulcers.[44-46]

Diabetes Mellitus

↓

Neuropathy & limited joint mobility

↓

Atrophy of intrinsic muscles & clawing

↓

Pressure transfer from heel & toes to metatarsal heads

↓

No redistribution of pressure

↓

Ulceration

Predisposing factors for ulceration: [47]

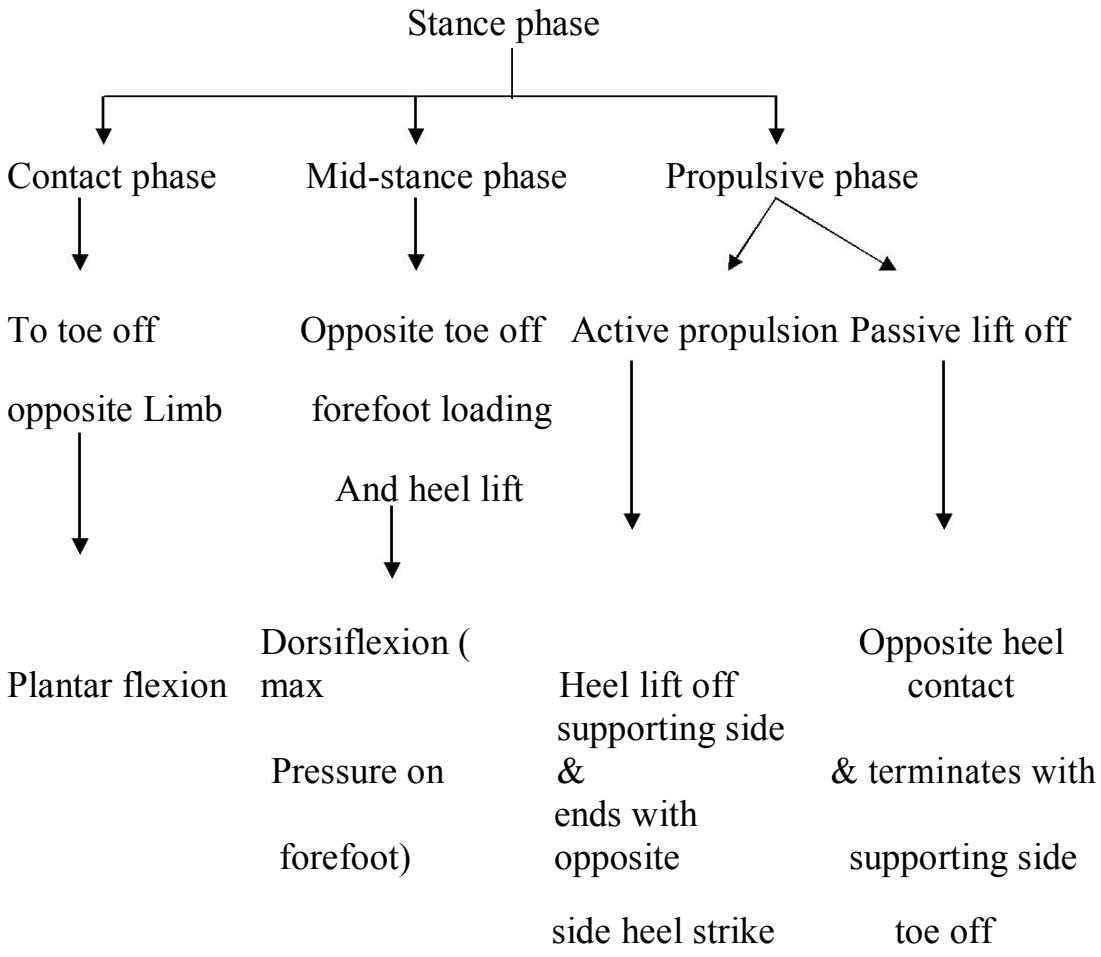
1) Limited joint mobility.
2) Peripheral neuropathy.
3) High plantar pressure.
4) Vascular diseases.

Biomechanics of diabetic foot [47]

Gait cycle:

1. **Stance phase**
2. **Swing phase**

Fig 2.11 Biomechanics of diabetic foot



Changes in foot caused by diabetes

1. **Peripheral neuropathy** [48]

   A. Dryness of skin

   B. Callus formation

2. High pressure at bony prominences

↓se plantar tissue thickness

↓

Weak intrinsic muscles of foot

↓

Imbalances of flexors & extensors causing clawing of foot

↓

Pulling away fat padding from metatarsal heads

3 Limited joint mobility [49]

Unknown etiology

↓

Collagen abnormality

↓

Thickening of skin tendons & joint capsules

↓

Decreased tissue flexibility

↓

Increased plantar pressure

4 Trauma [50,51]

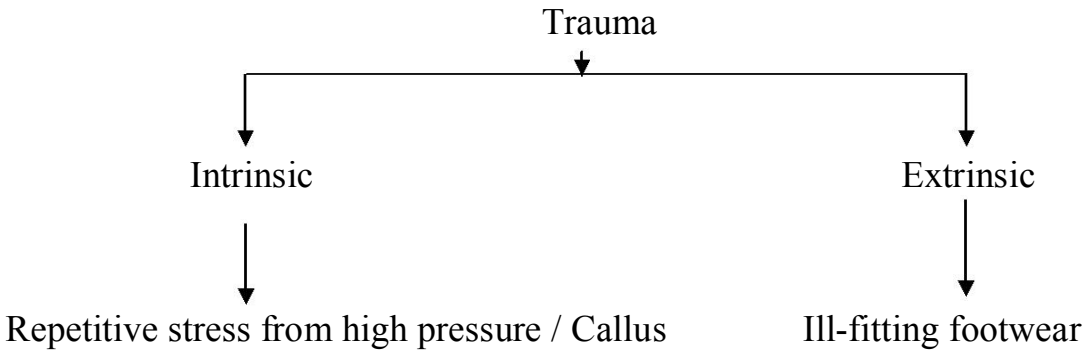
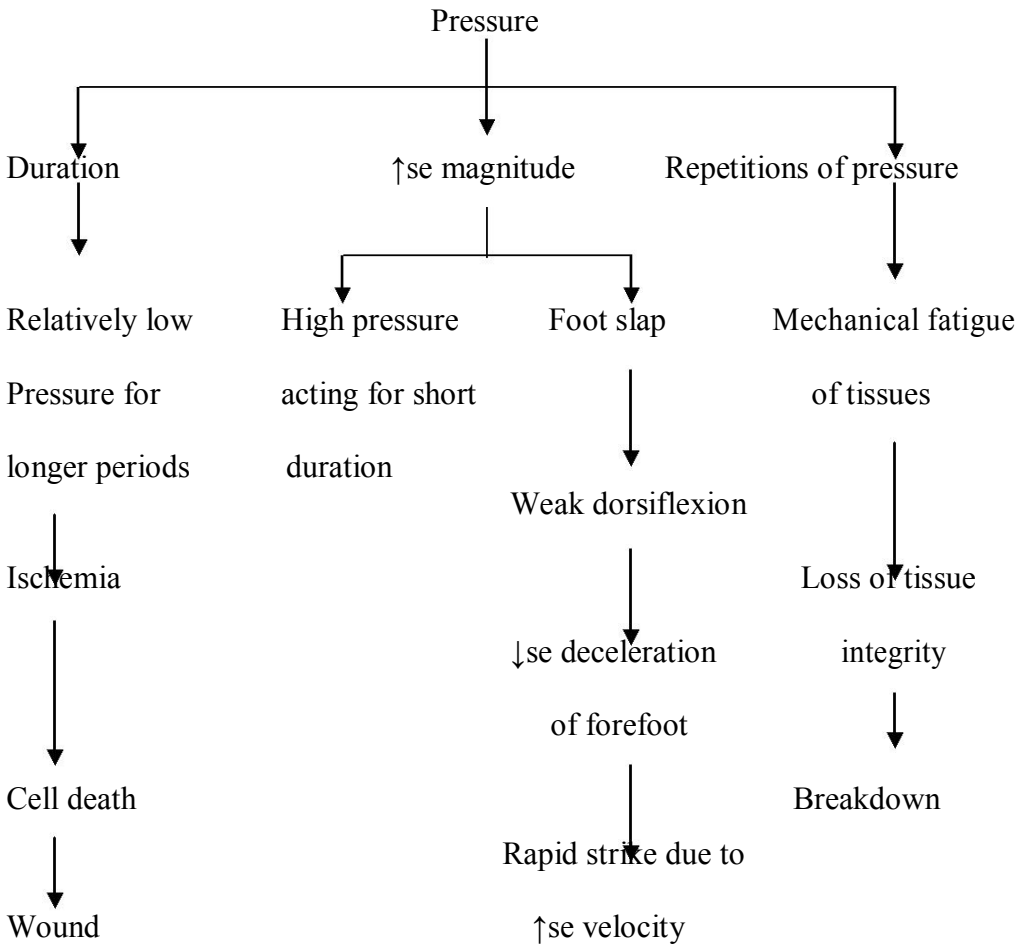


Fig 2.12: Causation of ulceration.[52,53]



CLASSIFICATION OF DIABETIC FOOT ULCERS.[54]

Several foot ulcer classification schemes have been proposed, but none is universally accepted. The six grade Wagne- Meggit classification, which has been used for decades, classifies wounds by the depth of ulceration and extent of gangrene. The International Working Group on Diabetic Foot has proposed the PEDIS classification, which grades the wound on the basis of five features:

- Perfusion (arterial supply)
- Extent (area)
- Depth
- Infection
- Sensation

WOUND DRESSING IN DIABETIC FOOT

The management of wound and wound dressing is an important aspect of diabetic foot management. Proper dressing with cost effective dressing material, done with scientifically correct method can help in salvaging diabetic foot. The various functions of the dressings are:

- Isolation of the wound from external environment.
- Limit/reduce tissue oedema.
- Reduce pain.
- Improve gas exchange between tissues and blood.
- Limit inflammation.
- Absorb exudate.
- Should not promote bacterial growth.
- Prevent dessication and contamination.

All the dressings can be classified as primary or secondary. Primary dressing is the one, which is in direct contact with the wound. Secondary dressing is of the material, which holds the primary dressing in place. It has function of compression, occlusion and additional protection.

VARIOUS TYPES OF DRESSINGS

A wide variety of dressing materials are available for dressing of infected diabetic foot ulcers.

1. **Eusol**: contains bleaching powder and boric acid. Acts by chemical desloughing of the wound.

2. **Collagenase dressing**: contains collagenase enzyme which helps in the break down of devitalized tissues

3. **PDGF gel**: contains platelet derived growth factor. Causes angiogenesis and leads to formation of healthy granulation tissue

4. **Comupimet** ointment: contains collagen crystals with Mupirocin and Metranidazole. Acts by enhancement of granulation tissue along with antibacterial action

5. **Aquacell**: contains silver ions, which has anti microbial action. Helps in cleansing the wound.

6. **Biological dressings**-
   a) APLIGRAFT- Bioengineered skin
   b) DERMA GRAFT- Human dermis

METHODOLOGY

Study design: Randomized controlled trial

Source of Data : Patients with diabetic foot ulcers admitted in surgery wards at Stanley medical college hospital, Chennai .

Sample Size :

50 patients

25-patients -in the study group

25-patients -in the control group

Inclusion criteria

1. Type I and II Diabetes mellitus.
2. Diabetics between 12 to 75 years of age.
3. Have documented wound etiology resulting from complications of DM
4. Duration of the ulcer more than 4 weeks.
5. Size of ulcer less than 10x10 cm
6. Fasting blood glucose levels measured in two occasions 24 hours apart between 140mg/dl- 200mg/dl

Exclusion criteria:

1 Pulseless limb

2 Immunocompromised patients

3 Associated osteomyelitis.

4 Skin malignancy

5 Cellulitis

6 Diabetic Ketoacidosis.

7 Exposed bone and tendon in ulcer.

Method

The present study was carried out in Stanley Medical College Hospital, Chennai. where 50 patients with diabetic foot ulcers participated in the present study. Using a pretested and predesigned proforma was randomized into either study group or control group population using randomization chart.

DRESSING TECHNIQUE

For conventional dressing.

The ulcer was cleaned with normal saline and saline soaked gauze piece was kept over the ulcer which was covered with pad and roller bandage.

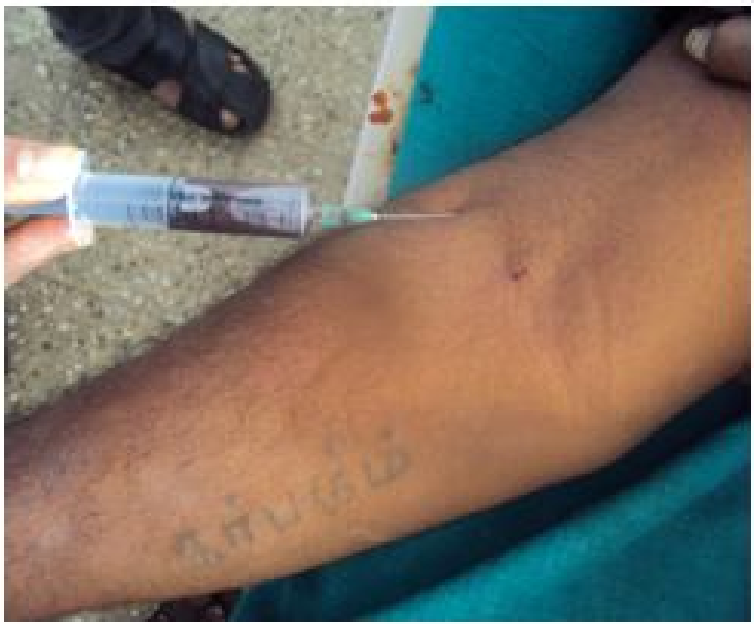
Study group:

Patient in study group is treated with PRP .Platelet rich plasma is made manually by drawing 10 ml of blood by venipuncture.5ml of blood is put in a two test tube each ,and adding anticoagulant citrate dextrose (ACD).centrifuge for 10 minutes at 2000 rotation per minute. Three layers obtained as, top layer plasma, middle layer buffy coat ,RBC at the bottom .Plasma and the buffy coat layer was separated by pippet, and put in test tube mixed with calcium chloride (cacl2) .Second centrifugation done for 10 minutes at 2000 rotation per minute. It resulted in three layers, as top platelet poor plasma (PPP),platelet rich plasma and at bottom , RBC.

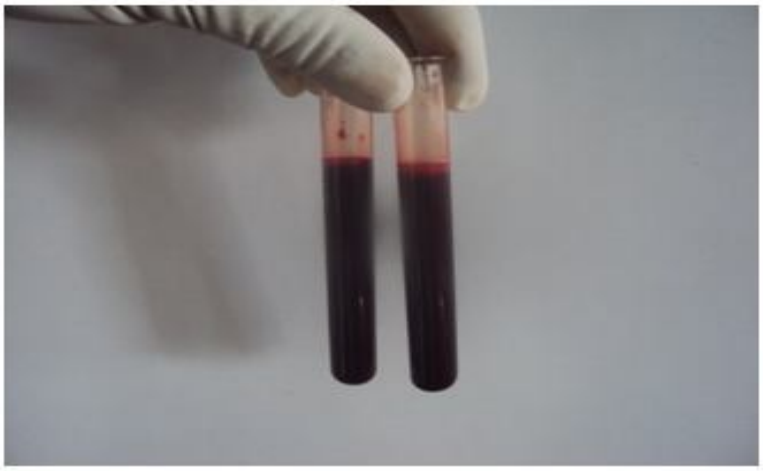
The platelet poor plasma is discarded and platelet rich plasma is separated and taken in syringe, which is injected in wound site. This platelet rich plasma dressing is done biweekly for four weeks and ascessed for wound contracture.

CENTRIFUGE

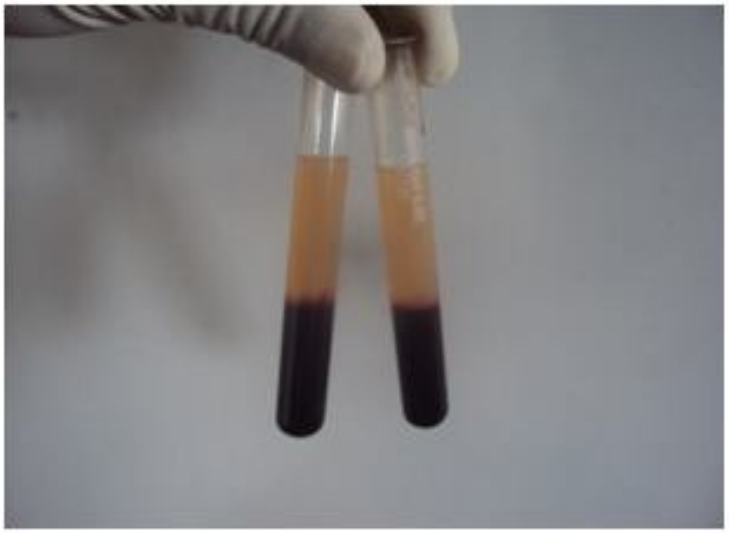
VENIPUNCTURE



BLOOD COLLECTED IN TEST TUBE WITH ACD

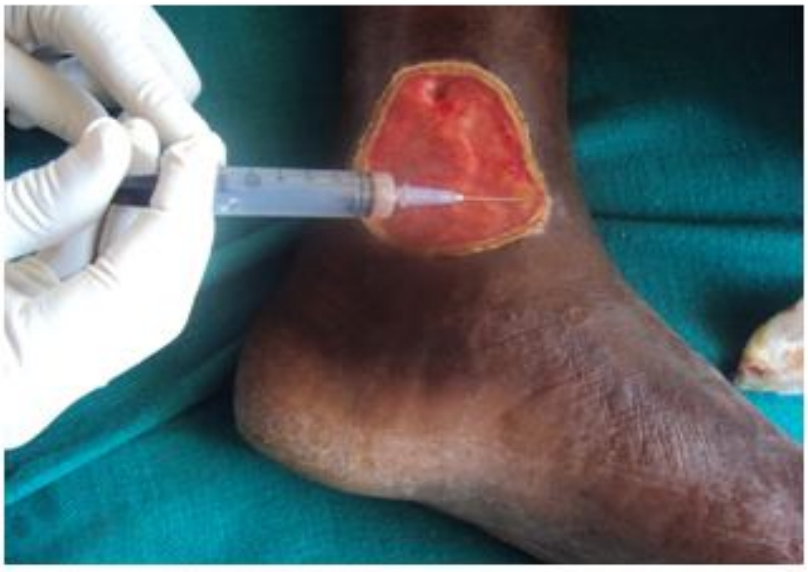


AFTER FIRST CENTRIFUGE

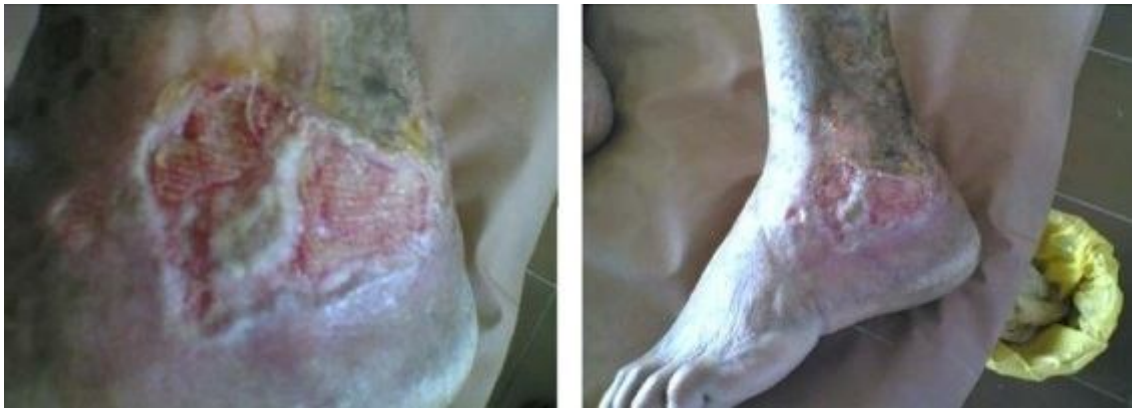


AFTER SECOND CENTRIFUGE

PRP INJECTED IN WOUND SITE



BEFORE AFTER



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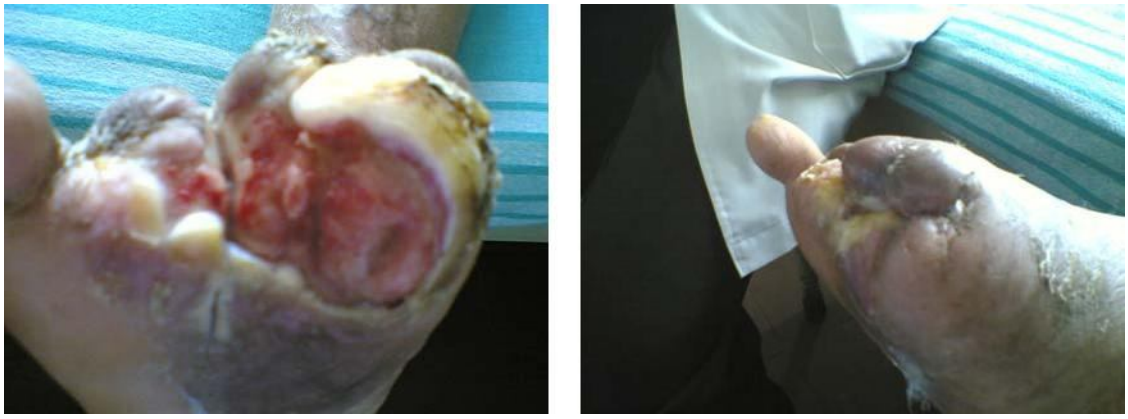

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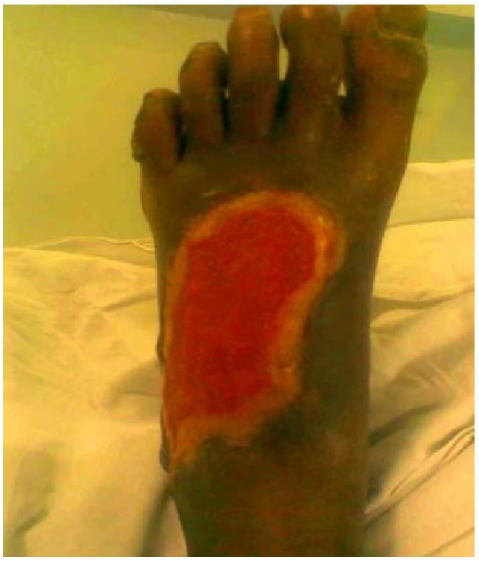

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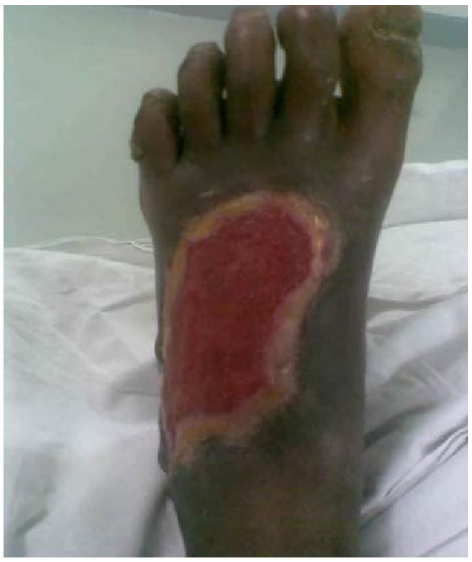

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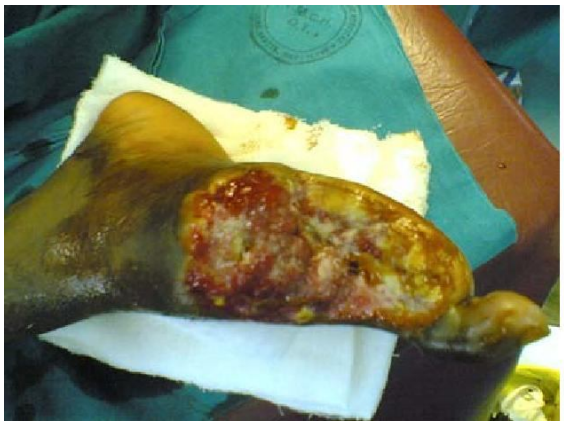

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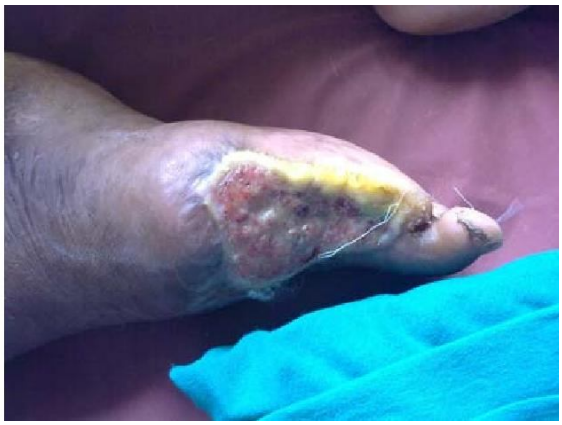

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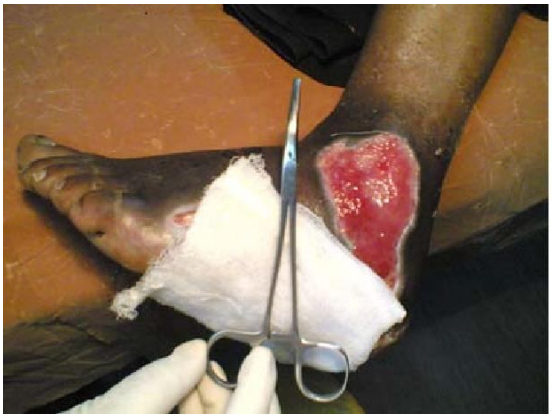
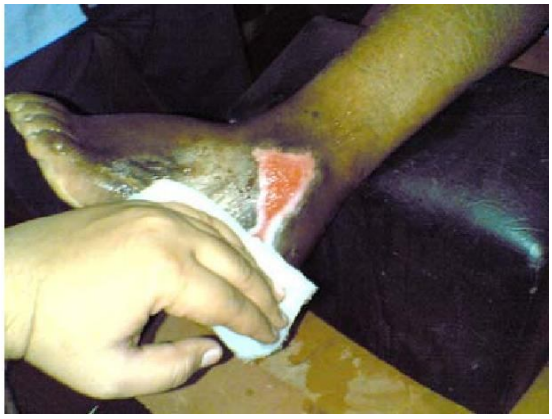




OBSERVATIONS AND RESULTS

Table 4.1: Age Distribution

Age (Years)	No. of Cases	Percentage
18-30	0	0%
31-40	06	12.00%
41-50	09	18.00%
51-60	23	46.00%
> 60	12	24.00%
Total	50	100

In our study it was observed that Diabetic foot was commonest in the age group between 51-60 yrs of age

Table 4.3 : Sex Distribution

Sex	No of Cases	Percentage
Male	33	66.00%
Female	17	34.00%
Total	50	100.

In our study in was observed that Diabetic foot was more common in the males (66.00%) as compared to females (34.00%)

Table 4.4: Site of ulcer in the study

Site	No. of Cases	Percentage
Plantar	31	62.00%
Dorsum	19	38.00%
Total	50	100%

In our study it was observed that diabetic foot more commonly occurs on the plantar aspect (62.00%) of the foot as compared to the dorsal aspect (38.00%)

Table 4.5: Onset of Diabetic Foot Ulcers

Type of Onset	No of Patients	Percentage
Traumatic	32	64.00%
Spontaneous	18	36.00%
Total	50	100

Trauma is the most common cause of diabetic foot ulcer (64.00%) while only 36.00%were spontaneous in origin.

Table 4.6 : Anti Diabetic Agents

Anti Diabetic	No. of cases	Percentage
OHA	11	22.00%
Insulin	39	78.00%
Total	50	100%

In our study most of the participants were taking Insulin for glycaemic control.

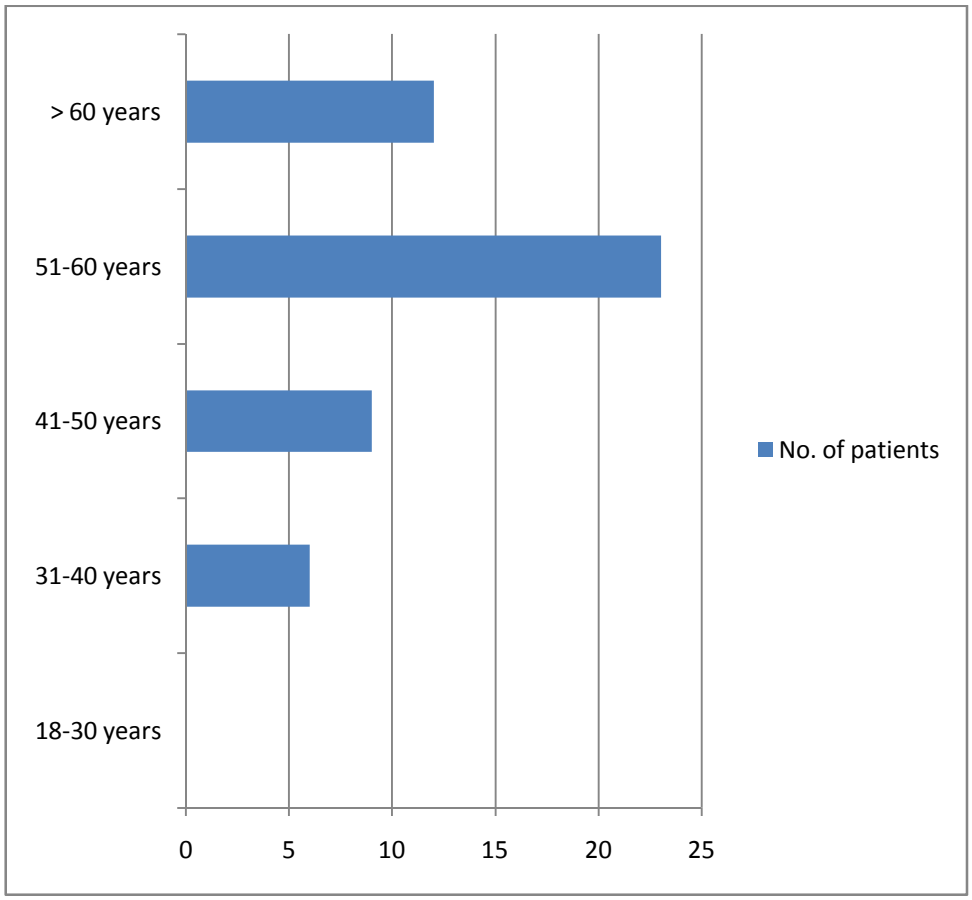
Table 4.7 : Wound Contraction

Group	Mean Red%	S.D.	Median	P Value
Control	13.52%	2.55	13.20	
Study	34.42%	2.52	34.58	P<0.001

In our study it was observed that Mean % of area reduction was higher in study group (34.42%) as compared to the controls (13.52%).

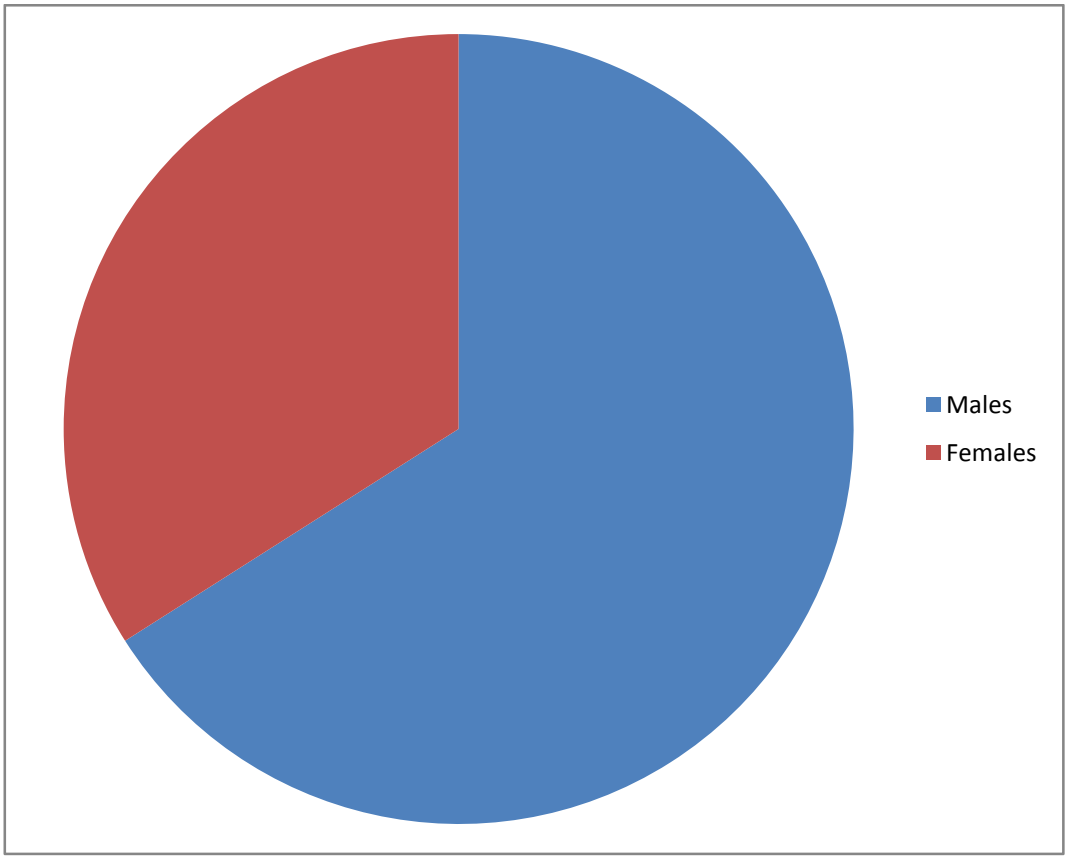
GRAPHS

AGE DISTRIBUTION

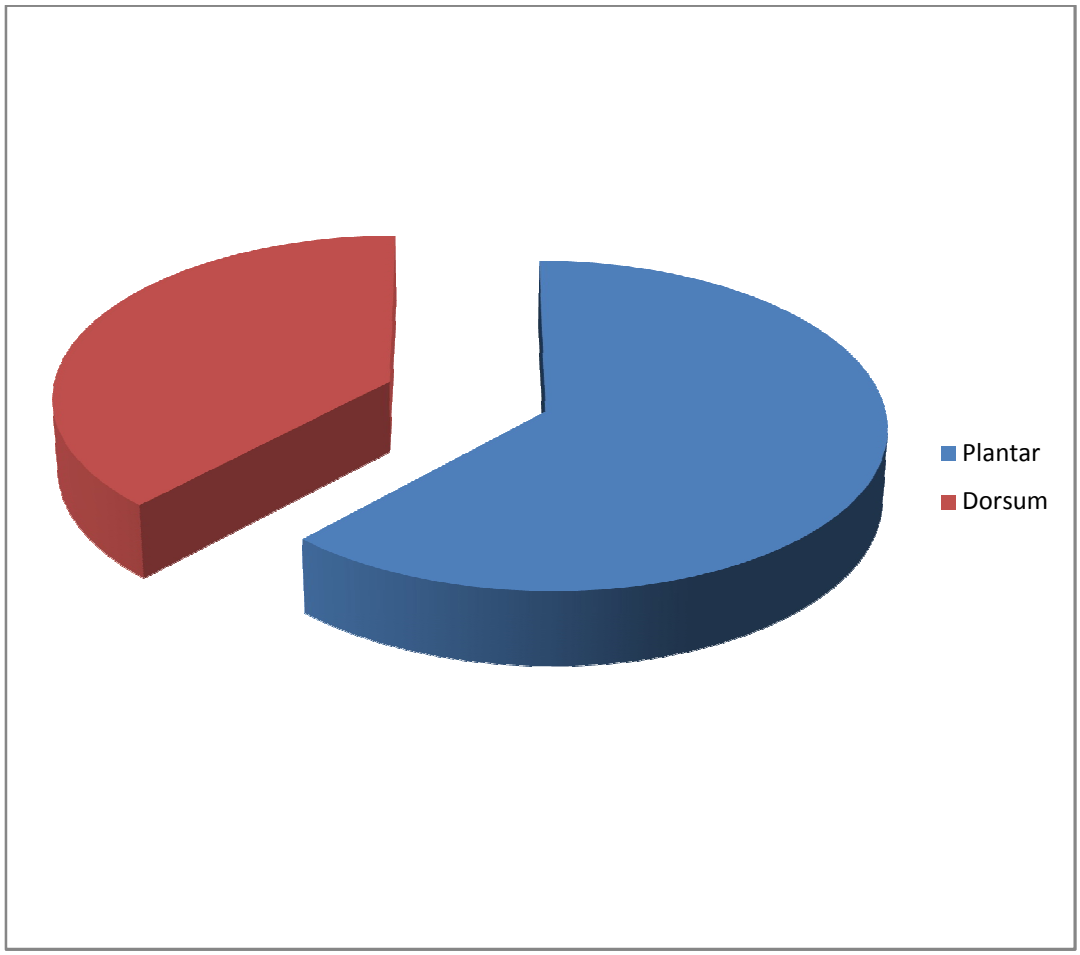


SEX DISTRIBUTION

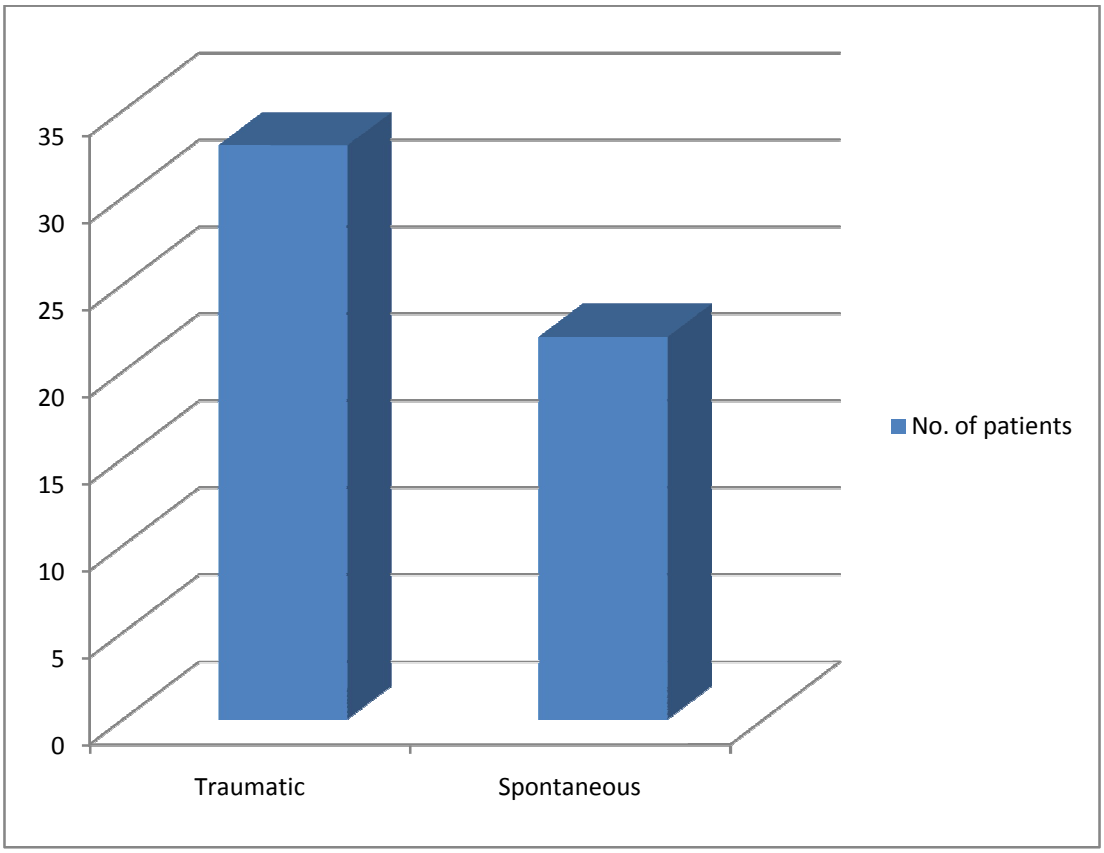
SEX DISTRUBUTION



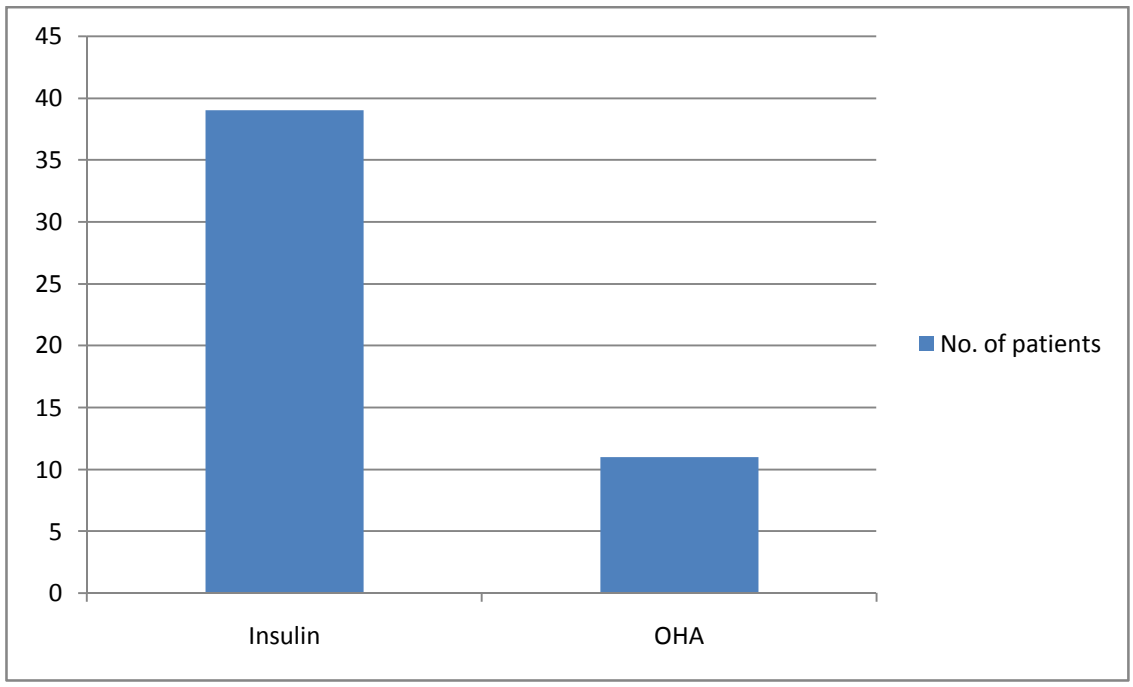
SITE OF DIABETIC ULCER



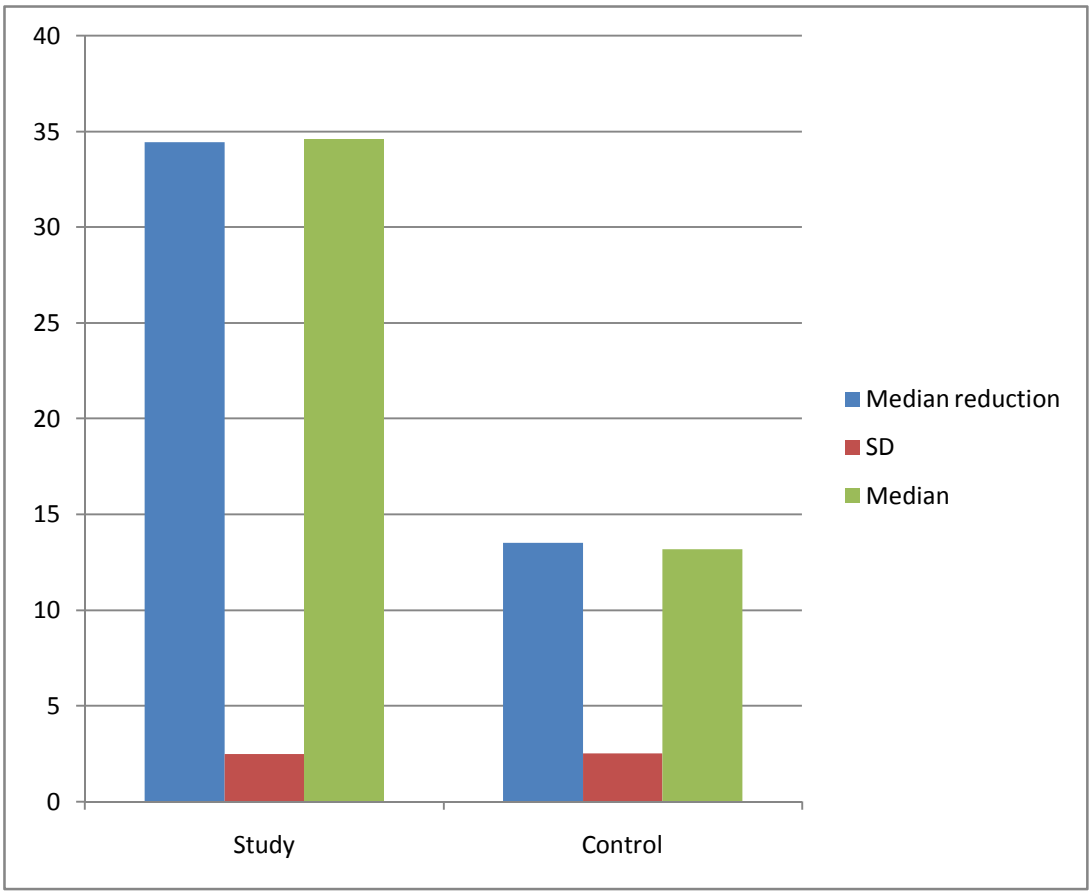
ONSET DIABETICS ULCER



ANTI DIABETICS



WOUND CONTRACTION



STATISTICAL ANALYSIS

Statistical analysis was done by using Microsoft EXCEL software and SPSS computer program.

Diabetic foot ulcers in the study group had better mean % of wound contraction of 34.42% (S.D; 2.52: Median; 34.58) as compared to the control group which had mean % of wound contraction of 13.52 % (S.D; 2.55: Median; 13.20), the difference in the mean 20.90% of area reduction of the two groups where studied using unpaired student t test was found to be significant ($p<0.001$).

DISCUSSION

It is every surgeon's desire that after dressing the wound, it should heal without any complications. Successful wound dressing should keep the wound moist and be devoid of any adverse reactions such as infection, maceration and allergy. Diabetic foot ulcers are chronic wounds, stuck in inflammation phase and shows cessation of epidermal growth

The present study was conducted at Stanley medical college hospital, chennai to study the effect on chronic diabetic wound healing dynamics

In the present study it was seen that the incidence of diabetic foot ulcers were more in males (66.00%) as compared to females (34.00%).

The second national data source, NHDS documented higher hospital rates in males suffering from diabetic foot ulcer.

Diabetic foot ulcers are most commonly seen in 6^{th} decade (60%), the next common being in the fifth decade (20%). While only 11.25% of the patients were in the fourth decade. Older the patient more the chances of having diabetic foot ulcer. The prevalence of diagnosed diabetics increases with age (the diabetic foot). In this study patients with vascular complications such as pulse less limb and the patients with osteomyelitis were excluded.

In this study, 64.00% of the ulcers were traumatic in origin, trauma being the triggering factor secondary to neuropathy. 36.00% were spontaneous in origin secondary to blister rupture or unnoticed trivial trauma.

More than half (62.00%) of the patients had ulcer on the plantar surface of the forefoot and the remaining (38.00%) had on the dorsum of foot. Study conducted by Edmonds et al in 1986, (Edmonds) showed more foot ulcers were on plantar and fore foot areas. Most of the diabetic foot ulcers are invariably shoe related and due to gait abnormalities. They can be prevented by appropriate sized footwear. However in our study the incidence of ulcers over the plantar aspect of the foot were not as high as postulated by Edmonds et al.

Most of the patients (78.00%) were on insulin for control of sugar whereas only 22.00 % were on Oral Hypoglycaemic Agents.

In our study it was observed that participants receiving PRP dressing had better wound contraction of 34.42% (S.D; 2.52: Median; 34.58) As compared to the group receiving only conventional dressing (normal saline dressing) in whom the mean wound contraction was 13.52% (S.D; 2.55,

Median; 13.20), these were found to be statistically significant on unpaired Student t test ($p<0.001$) suggesting that PRP dressing enhances wound healing in diabetic wounds.

Feasibility of this study:

In the present study we have taken 50 patients suffering from Diabetes Mellitus with foot ulcers. Patients were taken up for study based on inclusion and exclusion criteria. Out of 50 patients, 25 (18 males, 07 females) were study cases and 25 (15 males and 10 females) were control. Participants included in the study group were treated with the PRP dressing biweekly for four weeks. All 25 patients selected for PRP treatment complied for the four weeks period of the study. The initial area measurement was taken on first week and final area measurement on fourth week was taken on transparent sheet.

All 25 patients selected as a control complied for the four week duration period of the study. The initial area measurement on first week final area measurement on fourth week was taken on transparent sheet.

We have applied the following formula to calculate % reduction in area of wound after four weeks period in both cases and control groups.

Rate of contraction of wound after four weeks of treatment =

$$\frac{\text{(Initial area – Final Area)}}{\text{Initial area}} \text{ X } 100$$

We have found 13.52% (S.D; 2.55 : Median; 13.20) contraction of wounds in the control groups as compared to 34.42% (S.D; 2.52 , Median; 34.58) contraction of wounds in study group. Therefore, study groups are having % more wound contraction as compared to control group. On applying unpaired student t test $p<0.001$ which is significant.

From our study, we can say that PRP dressing therapy facilitates wound healing in patients suffering from diabetes mellitus.

Limitations of our study:

1. Follow up is short to derive conclusion on long term healing of the ulcers.
2. The cost involved was not analyzed in this study.

CONCLUSION

The wounds in subjects treated with PRP dressing contracted more than the wounds in the non treated group (34.42% Vs 13.52%; P = < 0.001 Significant) which indicates PRP dressing is an effective modality to **FACILITATE** wound contraction in patients suffering from diabetes and can be used as an adjunct to conventional mode of treatment (conventional dressings and debridement) for healing of diabetic wounds.

SUMMARY

The incidence of diabetes and complications are on rise. Diabetic foot being one of the most common complications, where 15% of all diabetics develop diabetic ulcers, the most common site being the foot. Diabetes has highest risk factor associated with limb threatening ischemia. Trivial trauma secondary to neuropathy and distorted pedal architecture causes ulcerations. 15% of all diabetics develop foot ulcer. 20% of admissions in diabetics are for foot problems.

Various modalities of treatment have been developed to aid faster healing of diabetic foot ulcers. Course of healing in diabetic foot patients is unpredictable and resistant to treatment.

50 patients of diabetic foot ulcers were studied. They were divided into two groups of 25 each.

One group received PRP and the control group received treatment in the form of conventional therapy. A comparative study was done between both groups regarding percentage area wound reduction.

Patients were between 51-60 years of age, Males were more affected than females. 66.00% males Vs 34.00% females. 64.00% of the ulcers were traumatic in onset. Plantar aspect (62.00%) was most common site.

Most of the patients were on insulin (78.00%) compared to the oral hypoglycaemic agents (22.00%)

All patients in the study underwent X-ray of the affected foot, patients with stress fractures and osteomyelitis were excluded.

In our study it was observed that participants receiving PRP had better wound contraction of 34.42% as compared to the group receiving only conventional treatment in whom the mean wound contraction was 13.52%, these were found to be statistically significant on unpaired Student T test ($p<0.001$) suggesting that PRP enhances wound healing in diabetic wounds.

Thus, PRP dressing therapy in the treatment of diabetic foot ulcers was found to be more effective, safe, promoter of wound healing, and hence can be recommended for the treatment of diabetic foot ulcers as an adjuvant to the conventional mode of treatment.

BIBLIOGRAPHY

1. Principles of Internal medicine- Harrison's 15th edn. Vol 2, Chapter 333, Pg. 2109-2111

2. Most RS, Sinnock P. Epidemiology of lower extremity amputation in diabetic individuals. Diabetes Care. 87(91) : 1983

3. Lehto ST, Ronnemaa T, Pyorala K. Risk factors predicting lower extremity amputation in patients with NIDDM. Diabetes Care 1996; 19:607.

4. Seshian V and Venkataraman S. Aetiopathogenesis and management of diabetic foot, current concepts in DM, Ed. Saini GS and Talwalkar P, Typographics, 1993; 88-97

5. King H. Global burden of diabetes 1995-2025, Diabetes care, 1999(Asian edition); 1:233-250

6. Mulder GD .Diabetic foot ulcers; old problems – new technologies Mulder 16(4):695.

7. Wieman TJ, MD, FACS, Janice MS, MD, Yachin Su. Efficacy and safety of a topical gel formulation of recombinant human platelet derived growth factor-BB (Becaplermin) in patients with chronic neuropathic diabetic ulcers. Diabetes care.1998 May;21(5):822.

8. Martin CR, Payne G W, Garner L W. Integrating the results of phase IV (post marketing) clinical trial with four previous trials reinforces the position that regranex (Becaplermin) gel 0.01% is an effective adjunct to the treatment of diabetic foot ulcers. The journal of applied research.2005;5(1):36

9. Embil J M, Papp K, Sibbald G, Tousigant Jr, Smiell J M, Wong B et al. wound repair and regeneration, vol 8 no 3 june 2000, pp 162-168(7).

10. Robson MC, Thompson A, Pierce GF , Phillips LG, Robson LE: Platelet derived growth factor BB for the treatment of chronic pressure ulcers. Lancet 339:23-25,1992

11. Steed DL, the diabetic ulcer study group: clinical evaluation of Re combinant human platelet derived growth factor for the treatment of lower extremity diabetic ulcers. J Vasc Surg21:71-81, 1995

12. Lynch SE, Colvin RB, Antiniades HN. Growth factors in wound healing: single and synergistic effects on the partial thickness porcine skin wounds. J Clin invest. 1989;84:640-646.

13. Pierce GF, Mustoe TA, Lingelback J. Platelet derived growth factor and transforming growth factor—B enhance tissue repair activities by unique mechanisms. J Cell Biol 1989;109:429- 440.

14. Watkins P J ; ABC of diabetes, The diabetic foot; British Medical Journal; May 2003 vol. 326, 977-979

15. Joslin's Diabetes Mellitus – 13th edition by Ronald Kahn, Gordon Weir- 1996

16. Pati S, Sauandal BK, Bhattacharyya AR, Bhattacharyya AK., P Saumandal; Clinical Evaluation of Effect of Dressing with Placental Extract in the treatment of Infected wounds; Indian journal of obgyn; may-june 2001,

17. Sabiston textbook of surgery, 17th edn vol.(1): 183

18. Maiya GA; Kumar P; Rao L; Photo Medicine and Laser Surgery; Effect of Low Intensity Helium-Neon (He-Ne) Laser Irradiation on Diabetic Wound Healing Dynamics; April 2005, Vol 23(2) : 187-190.

19. Cotran R, Kumar V, Robbins S, 1994 ; 5th (ed.), W. B. Saunders company.

20. Krieg T. Molecular defects of collagen metabolism in ehlers-danlos syndrome. Int. J. Dermatol. 20: 415, 1981.

21. Lingenmayer, T.F.: Collagen. *In* Hay, E. (ed.): Cell BLology of the Extracellular Matrix, 2nd ed. New York, Plenum Press. 1992, 7-44.

22. Matrisian, L.M.: The matrix-degrading metalioproteinases. Bioassays 14: 455, 1992

23. Rotwein P. Peptide growth factors other than Insulin like growth factors or cytokines, 675-690

24. Sporn MB, Roberts AB: Peptide growth factors are multifunctional. Nature 332;217-218, 1998

25. Keating MT, Williams LT: Autocrine stimulation of intracellular PDGF receptors inv-sis-transformed cells. Science 239: 914-916, 1998

26. Matsui T, Heidaran M, Miki T,Isolation of novel receptor cDNA

27. Dahn MS. The role of growth factors in wound management of diabetic foot ulcers.

28. Federal Practitioner 1998; July, 14-19.

29. Robson MC: Exogenous growth factor application effect on human wound healing. Progress in Dermatology. Editor Alan N.

30. Beer H S, Longakar M T, Wernar S. Reduced expression of PDGF and PDGF receptors during wound healing.

31. Greenhalgh D G, Sprugel K H, Murray M J, Ross R. PDGF and FGF stimulate wound healing in the genetically diabetic mouse.

32. American diabetes association: Clinical practice recommendations 2002. Diabetes Care 27: 51, 2004.

33. Clement S et al: Management of diabetes & hyperglycemia in hospitals. Diabetes Care 27: 553, 2004.

34. Kirpichnikov D et al: Metformin: An update, Ann intern med 137: 25, 2002

35. Knowler WC etal for the Diabetes prevention program research group: Reduction in the incidence of type-2 diabetes with lifestyle intervention of metformin. N Engl J Med 346: 393, 2002.

36. Saltiel AR, Kahn CR: Insulin signaling & the regulation of glucose & lipid metabolism, Nature 414: 799, 2001.

37. The writing team for the diabetes control & complications trial/ Epidemiology of the diabetes interventions & complications research group: Effect of intensive therapy on the microvascular complications of type-I Diabetes mellitus. JAMA 287: 2563, 2002.

38. UK Prospective diabetes study group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment & risk of complications in patients with type-2 diabetes (UKPDS 33). Lancet 352: 1998, 1998.

39. Levin and O'Neals – The Diabetic foot – VI Edition 4:65-106, 2001.

40. Berliner JA, et al : Atherosclerosis Basic mechanics, Oxidation, Inflammation and genetics, Circulation 91;2488-2496, 1995

41. Gisinger C, Vivella GT, Lopes, Vivella MF: Erythrocyte bound low density Lipo protein immune complexesniead to cholesterol accumulation in human monocyte derived macrophages. Clin Immunol- Immunopathol 59:37 – 52, 1991

42. Griffith RL, et al, LDL metabolism by macrophages activated with LDL immune complexes: A possible mechanism of foam cell formation J. Exp med 168: 1041-1059, 1998

43. Ignaro L J, et al : Endothelium derived relaxing factor produced and released from artery and vein is nitric oxide. Proc NaH Acad Sci USA 84: 9265, 1987

44. Moncada S: Biological importance of prostacyclin, Br. J. Pharmacol 76:3, 1982

45. Cavanagh PR, Simoneau GG, Ulbreech T JS: Ulceration, unsteadiness and uncertainity; The biomechanical consequences of DM. J Bio mechanical, 26 (suppl: 1); 23-40, 1993

46. Frykerg RG: Biomechanical considerations of diabetic foot Lower extremity 2:207-214, 1995

47. Frykerg RG, Lavery LA, Pham H, et al, Role of neuropathy and high foot pressures in diabetic foot ulceration; Diabetic care 21; 1714-1719, 1998

48. Payne CB: Biomechanics of the foot in DM: some theoretical considerations. J

Am Podiatr med Assoc 88: 285-289, 1998

49. Boulton AJM, Late sequelae of diabetic neuropathy, Marios Press, Carnferth, Lancashire, UK, 1997, PP.6376

50. Fernando DJS, Masson EA, Veves A, Boulton AJM: Relationship of limited joint mobility to abnormal foot pressures and diabetic foot ulceration. Diabetes care 1991; 14:8- 11

51. Tooke JE, Brash PD, Microvascular aspects of diabetic foot disease, Review diabetic med 13(suppl. 1) : 526-529, 1996

52. Delbridge L, Ctercteko G, Flower C et al – Etiology of diabetic neuropathic ulceration of the foot. Br. Journal of surgery 72: 1-16, 1985

53. Davis BL: Foot ulceration: Hypothesis concerning shear and vertical forces working on adjacent regions of skin; med Hypothesis 40:44-47, 1993

54. Cavanagh PR, Morag E, Boulton AJM et al. Relationship of static foot structure to dynamic foot function, J Biomech 30:243-250, 1997

55. Peter R Cavanagh, Benjamin A Lipsky, Andrew W Bradbury, Georgeanne Botek; Treatment for diabetic foot ulcers; The Lancet November 2005.

56. Everts PA, Knape JT, Weibrich G, et al. Platelet-rich plasma and platelet gel: a review. J Extra Corpor Technol 2006;38:174-187.

57. Marx RE. Platelet-rich plasma: Evidence to support its use. J Oral Maxillofac Surg 2004; 62:489-496.

58. Knighton DR, Hunt TK, Thrakral KK, Goodson WH. Role of plate- lets and fi brin in the healing sequence. Ann Surg 1982;196:379-388.

59. Landesberg R, Burke A, Pinsky D, et al. Activation of platelet-rich plasma using

thrombin receptor agonist peptide. J Oral Maxillofac Surg 2005;63:529-535.

60. Man D, Plosker H, Winland-Brown JE. The use of autologous plate- let-rich plasma (platelet gel) and autologous platelet-poor plasma (fi brin glue) in cosmetic surgery. Plast Reconstr Surg 2001;107:229-237.

61. Lind M. Growth factor stimulation of bone healing. Effects on osteoblasts, osteomies, and implants fi xation. ACTA Orthop Scand 1998; 283:2-37.

62. Marx RE. Platelet-Rich Plasma: A Source of Multiple Autologous Growth Factors for Bone Grafts. In: Lynch SE, Genco RJ, Marx RE, eds. Tissue Engineering: Applications in Maxillofacial Surgery and Peridontics. Chicago: Quintessence Publishing Co, Inc.; 1999; 71-82.

63. Kassolis JD, Reynolds MA. Evaluation of the adjunctive benefi ts of platelet-rich plasma in subantral sinus augmentation. J Craniofac Surg 2005;16:280-287.

64. Everts PA, Devilee RJ, Brown Mahoney C, et al. Platelet gel and fi brin sealant reduce allogeneic blood transfusions in total knee arthroplasty. Acta Anaesthesiol Scand 2006 May;50(5):593-9.

65. Trowbridge CC, Stammers AH, Woods E, et al. Use of platelet gel and its effects on infection in cardiac surgery. JECT 2005; 37:381-386.

66. Margolis DJ, Kantor J, Santanna J, et al. Effectiveness of platelet releasate for the treatment of diabetic neuropathic foot ulcers. Diabetes Care 2001;24:483-488.

67. Senet P, Bon FX, Benbunan M, et al. Randomized trial and local biological effect of autologous platelets used as adjuvant therapy for chronic venous leg ulcers. J Vasc Surg 2003;38:1342-1348.

PROFORMA

I) <u>PATIENT IDENTIFICATION DATA</u> :

NAME IP/OPD NO.

AGE DOA :

SEX DOD:

OCCUPATION

ADDRESS

II) <u>CHIEF COMPLAINTS</u> :

<u>MEDICAL HISTORY</u> :

Peripheral Neuropathy : ( )

Nephropathy ( )

Retinopathy ( )

PVD ( )

CVD ( )

<u>DIABETIC STATUS</u> :

TYPE :

DURATION :

MEDICATION : Oral Hypoglycemics Insulin

( ) ( )

COMPLICATION Neuropathy ( )

Vasculopathy ( )

ULCER DETAIL :

1. Mode of onset

Traumatic	(	)
Spontaneous	(	)
Pressure	(	)
Others	(	)

2. Duration
3. Progress

WOUND OBSERVATION:

1. Site
2. Size
3. Shape
4. Edge
5. Margin
6. Floor
7. Base
8. Discharge
9. Surrounding Skin
10. Contractor

NERUROLOGICAL EXAMINATION :

VASCULAR EXAMINATION	Left	Right
Popliteal a.	( )	( )
Ant . Tibial	( )	( )
Post Tibial	( )	( )
Dorsalis Pedis	( )	( )

ANY FOOT DEFORMITY PRESENT :

Toe deformity

Bunion

Charcots foot

Foot drop

IF AMPUTATION HAS BEEN DONE

SPECIFY : Date

: Side

: Level

: Cause for amputation

FOOT WEAR ASSESSMENT :

Does patient wear appropriate shoes

Does patient require contact cast immobilization.

INVESTIGATIONS.

CBC

FBS 1st _____ Date :_____ Time :_____

2nd (24 hr apart) _____ Date :_____ Time : _____

Sr. Creatinine

UKB

Urine : Routine

Microscopy

X-ray Foot

AP View

Lat. View

Wound C/s

WOUND AREA MEASUREMENT ON D_1 in cm^2

Type of Dressing – saline dressing ( )

- rh-PDGF dressing ( )

CONSENT FORM

FOR OPERATION/ANAESTHESIA/PROCEDURE

I _____ Hosp. No._____ in my full senses hereby give my complete consent for _____ or any other procedure deemed fit which is a / and diagnostic procedure / transfusion / operation to be performed on me / my son / my daughter / my ward _____ age _____ under any anesthesia deemed fit. The nature and risks involved in the procedure have been explained to me to my satisfaction. For academic and scientific purpose operation/procedure may be televised or photographed.

Date:

Signature/Thumb Impression

Of Patient/Guardian

Name:

Designation:

Guardian:

CONSENT

I am ready to participate in the study on **"A COMPARATIVE STUDY OF PLATELET RICH PLASMA VERSUS NORMAL SALINE DRESSINGIN DIABETIC FOOT"** conducted by Dr.sakthivel.v, post graduate student, Department of General Surgery, Government Stanley Medical college, chennai. I am ready to undergo investigations like, blood tests, urine test, radiological imaging etc. to confirm the diagnosis of my condition.

I understand that as a part of this study, I will not be subjected to any other treatment modalities.

I am assured that being a part of this study, there will not be any financial burden on me.

I know the fact that I can withdraw from the study at any time without showing reason and if at all I quit, there will not be any deprivation in the treatment I receive in the hospital.

Hereby, I willingly give consent to take part in this study.

Name of the patient

Date:

Place: Signature

Patients relative

MASTER CHART-STUDY GROUP

Sl. no	Ip.no	Age & sex	0nset	Site	Anti DM Rx	FBS	X ray	c/s	Initial Area in mm^2	Final Area in mm^2	IA-FA= CA	%Area Reduct-ion
1	817023	56/m	S	D	I	132	N	NOGC	37.52	23.94	13.58	36.2
2	5705759	51/f	S	P	I	98	N	NOGC	38.88	25.12	13.76	35.4
3	605162	48/f	T	D	0	123	N	NOGC	36.58	23.27	13.31	36.4
4	605267	52/m	S	P	I	101	N	NOGC	47.56	29.30	18.26	38.4
5	670355	46/m	T	D	0	76	N	NOGC	45.88	32.95	12.93	28.2
6	324971	32/m	T	P	I	122	N	NOGC	41.60	27.71	13.89	33.4
7	638973	62/m	S	D	I	99	N	PM	45.56	30.80	14.86	32.4
8	699474	58/m	T	P	I	145	N	NOGC	45.36	28.49	16.87	37.2
9	693325	54/f	T	D	O	111	N	NOGC	58.22	37.50	20.72	35.6
10	698534	48/f	S	P	I	100	N	NOGC	44.08	27.69	16.39	37.2
11	715816	64/f	S	D	I	150	N	PA	53.76	33.82	19.94	37.1
12	698134	36/m	T	P	I	97	N	NOGC	69.66	45.14	24.52	35.2
13	714977	52/m	T	P	I	90	N	NOGC	60.48	40.41	20.07	33.2
14	723449	57/m	T	D	I	88	N	NOGC	36.40	25.70	10.70	29.4
15	728805	63/f	T	P	I	98	N	NOGC	49.92	36.65	14.27	28.6
16	728796	65/f	S	P	0	133	N	EC	47.12	35.63	11.59	24.6
17	743353	45/m	T	P	I	140	N	NOGC	71.34	43.24	28.10	39.4
18	767126	51/m	T	D	0	88	N	NOGC	70.98	46.35	24.63	34.7
19	761547	38/m	T	P	I	129	N	NOGC	57.12	35.31	21.81	38.2
20	761125	61/f	S	D	I	133	N	NOGC	49.92	33.55	16.37	32.8
21	808391	52/m	T	P	I	93	N	NOGC	49.60	31.15	18.45	37.2
22	817023	54/f	T	P	I	133	N	PM	67.50	44.69	22.81	33.8
23	334017	35/m	S	D	O	79	N	NOGC	36.72	24.83	11.89	32.4
24	410663	64/f	S	P	I	148	N	NOGC	51.48	32.75	18.83	36.4
25	420071	58/m	T	P	I	111	N	NOGC	60.48	37.99	22.49	37.2

MASTER CHART-CONTROL GROUP

Sl No.	I P No.	Age/ Sex	Onset	Site	Anti-DM Rx	FBS	X-Ray	C/s	Initial Area mm^2	Final Area mm^2	IA - FA = CA	% Area Reduct-ion
1	832322	63/F	S	D	I	146	N	NOGC	46.08	40.46	5.62	12.2
2	847632	36/M	T	P	O	110	N	NOGC	36.54	31.65	4.89	13.4
3	865342	42/M	S	P	I	122	N	NOGC	43.20	35.99	7.21	16.7
4	432627	51/F	T	P	O	123	N	NOGC	53.36	46.32	7.04	13.2
5	324971	44/F	T	P	I	146	N	PA	43.68	37.05	6.63	15.2
6	259249	56/M	S	D	I	134	N	NOGC	47.12	40.43	6.69	14.2
7	538527	64/M	T	P	I	145	N	NOGC	38.86	34.90	3.96	10.2
8	560571	58/M	T	D	I	186	N	NOGC	64.08	55.82	8.26	12.9
9	582902	54/F	T	P	O	127	N	NOGC	34.17	29.66	4.51	13.2
10	587733	47/M	S	P	I	144	N	PM	49.14	43.20	5.94	12.1
11	633920	53/M	T	D	I	122	N	NOGC	52.70	44.22	8.48	16.1
12	644488	56/F	T	P	I	134	N	NOGC	54.28	48.48	5.80	10.7
13	119208	62/M	T	P	O	139	N	NOGC	48.36	42.27	6.09	12.6
14	644793	46/F	S	D	I	98	N	NOGC	64.08	55.63	8.45	13.2
15	668431	35/M	T	P	I	190	N	NOGC	36.72	30.78	5.94	16.2
16	597042	51/M	T	P	I	95	N	EC	57.40	50.69	6.71	11.7
17	714118	57/F	T	P	I	128	N	NOGC	38.94	34.35	4.89	11.8
18	746552	65/M	S	D	I	144	N	NOGC	69.92	59.02	10.90	15.6
19	515444	55/M	T	P	O	122	N	NOGC	48.72	41.76	6.96	14.3
20	768535	54/M	T	P	I	111	N	NOGC	36.72	30.34	6.38	17.4
21	824053	61/F	S	D	I	140	N	PA	42.24	34.44	4.88	10.8
22	674944	52/F	T	D	I	119	N	NOGC	40.12	33.26	6.86	17.1
23	832323	45/M	T	P	I	146	N	NOGC	56.16	39.26	6.90	12.3
24	535230	64/M	S	P	I	151	N	NOGC	48.38	41.52	6.86	14.2
25	668640	56/F	T	D	I	128	N	NOGC	49.68	44.32	5.36	10.8

KEY FOR USING THE MASTER SHEET

Sl. No.	:	Serial number
M	:	Male
F	:	Female
IP No	:	Inpatient Number
DM	:	Diabetes mellitus
FBS	:	Fasting Blood Sugar
C/s	:	Culture sensitivity report
mm^2	:	millimetre square
N	:	Normal
T	:	Traumatic
S	:	Spontaneous
D	:	Dorsal
P	:	Plantar
I	:	Insulin
O	:	Oral Hypoglycaemic Agents
NOGC	:	No Organisms Grown in Culture
SA	:	Staphylococcus Aureus
KP	:	Klebsiella Pneumonia
PM	:	Proteus Mirabilis
PA	:	Pseudomonas Aeruginosa
EC	:	Eischericia Coli