"TO COMPARE THE EFFICACY OF RECOMBINANT HUMAN PLATELET DERIVED GROWTH FACTOR DRESSING VERSUS NORMAL SALINE DRESSING IN WOUND REDUCTION IN PATIENTS WITH CHRONIC DIABETIC FOOT ULCER: A RANDOMIZED CONTROLLED TRIAL."

BY

Dr. BASAVARAJ G.VEERAPUR

DISSERTATION SUBMITTED TO THE KLE UNIVERSITY, BELGAUM, KARNATAKA

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UNDER THE GUIDANCE OF

Dr. I V. Uppin. MS.
PROFESSOR

DEPARTMENT OF GENERAL SURGERY.

JAWAHARLAL NEHRU MEDICAL COLLEGE, BELGAUM ,*KARNATAKA*

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KLE UNIVERSITY, BELGAUM

KARNATAKA

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I hereby declare that this dissertation entitled **"TO COMPARE THE EFFICACY OF RECOMBINANT HUMAN PLATELET DERIVED GROWTH FACTOR DRESSING VERSUS NORMAL SALINE DRESSING IN WOUND REDUCTION IN PATIENTS WITH CHRONIC DIABETIC FOOT ULCER: A RANDOMIZED CONTROLLED TRIAL"** *is a bonafide and genuine research work carried out by me under the guidance of* **Dr. I.V.UPPIN$_{MS}$**, *Professor, Department of General Surgery, Jawaharlal Nehru Medical College, Nehru Nagar, Belgaum-590010.*

Date:
Place: **Belgaum** **Dr. BASAVARAJ G.VEERAPUR**

KLE UNIVERSITY, BELGAUM

KARNATAKA

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"TO COMPARE THE EFFICACY OF RECOMBINANT HUMAN PLATELET DERIVED GROWTH FACTOR DRESSING VERSUS NORMAL SALINE DRESSING IN WOUND REDUCTION IN PATIENTS WITH CHRONIC DIABETIC FOOT ULCER: A RANDOMIZED CONTROLLED TRIAL"** *is a bonafide research work done by* **Dr. BASAVARAJ G.VEERAPUR** *in partial fulfillment of the requirement for the Degree of* **M.S. (GENERAL SURGERY)**, *examination to be held in May 2009.*

Date:

Place: **Belgaum**

Dr.I.V.UPPIN$_{MS.}$
Professor
Department of General Surgery.
J. N. Medical College,
KLE University, Belgaum- 590 010
Karnataka

KLE UNIVERSITY, BELGAUM
KARNATAKA

ENDORSEMENT BY THE HEAD OF THE DEPARTMENT/PRINCIPAL

This is to certify that the dissertation entitled **"TO COMPARE THE EFFICACY OF RECOMBINANT HUMAN PLATELET DERIVED GROWTH FACTOR DRESSING VERSUS NORMAL SALINE DRESSING IN WOUND REDUCTION IN PATIENTS WITH CHRONIC DIABETIC FOOT ULCER: A RANDOMIZED CONTROLLED TRIAL"** *is a bonafide research work done by* **Dr. BASAVARAJ G.VEERAPUR** *under the guidance of* ***Dr.I.V.Uppin.****MS, Professor, Department of General Surgery,, Jawaharlal Nehru Medical College, Nehru Nagar, Belgaum-590010.*

Dr.A.S.Godhi. **MS, FICS**
Professor & Head,
Department of General Surgery,
J. N. Medical College,
KLE University,
Nehru Nagar, Belgaum-590010
Date:
Place: Belgaum

Dr. V. D. PATIL **MD, DCH**
Principal,
J. N. Medical College,
KLE University,
Nehru Nagar,
Belgaum - 590010.
Date:
Place: Belgaum

KLE UNIVERSITY, BELGAUM

KARNATAKA

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Declaration by the Candidate

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Date:

Place: **Belgaum** **Dr. BASAVARAJ G.VEERAPUR**

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Place : Belgaum. ***Dr.Basavaraj G. Veerapur***

LIST OF ABBREVIATIONS

Hb : Haemoglobin

Vs : Versus

PR : Pulse Rate

BP : Blood Pressure

DM : Diabetes Mellitus

GF : Growth Factor

CBC : Complete blood count

PVD : Peripheral vascular disease

UKB : Urine ketone bodies

DBP : Diastolic Blood Pressure

SBP : Systolic Blood Pressure

FBS : Fasting blood sugar

PDGF :Platelet derived growth factor

rh-PDGF : Recombinant human Platelet derived growth factor

US-FDA : United States Food and Drugs Administration

ABSTRACT

Background

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. Chronic diabetic foot ulcer is the leading cause of amputations in these patients. These diabetic ulcers are known to be resistant to conventional treatment and may herald severe complications if not treated wisely. Platelet-derived growth factor (PDGF) is one of the numerous growth factors plays a significant role in angiogenesis.

Objective of the study

To compare the efficacy of topically applied recombinant human Platelet Derived Growth Factor (rh-PDGF) in chronic diabetic foot ulcer.

Methodology

The present study was a randomized controlled conducted at KLESH Prabakar Kore Hospital and MRC Belgaum. Total of 80 patients were assigned. They were grouped through computerized randomization into two. Control group patients were treated with conventional dressing and study group patients were treated with rh-PDGF dressing and observed for reduction in the wound size in a span of 15 days.

Results

The study group patients showed higher reduction in wound size of about 39.55% as against 11.79% of the control group with P value at 0.001.

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Conclusion

Topical application of 100µg/g of rh-PDGF once daily significantly increases incidence of wound healing in chronic diabetic foot ulcers**.**

Key words

rh-PGDF, Topical application, Diabetic foot ulcer, Diabetes mellitus.

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INTRODUCTION

"Diabetes Mellitus is characterized by chronic hyperglycaemia 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 Macloed demonstrated pancreatic extracts lower blood sugars. In 1936, Antanio discovered oral hypoglycaemic 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- 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).

PDGF was discovered as a protein released from the alpha granules of Platelets, it was purified from platelets. Recombinant human PDGF-BB has been prepared and purified for use in clinical studies of wound healing. The recombinant human platelet derived growth factor (rh-PDGF) is produced by recombinant DNA technology by insertion of the human gene for the B chain of PDGF in the yeast saccharomyces cevisiae.[7]

A series of studies in animals has shown that application of PDGF to a wound enhances the process of wound repair. PDGF has been demonstrated in preclinical studies to promote the formation of granulation tissue and thus stimulate cutaneous ulcer healing.[8] Various human cellular studieshave certainly established the fact that topically applied recombinant human platelet derived growth factor (rh-PDGF) is a new

pharmacologically active therapy for chronic neuropathic lower extremity diabetic ulcers, resistant to conventional mode of treatment.[9]

In phase II studies, recombinant human PDGF-BB(rhPDGF-BB) was shown to have a positive effect on healing pressure ulcers and lower extremity ulcers in patients with diabetes.[10,11] A phase III randomized placebo controlled double blind study on 382 patients with diabetic foot ulcers supported that becaplermin gel 100 µg/g, in conjunction with good wound care, significantly increased the incidence of complete wound closure and significantly reduced the time to complete closure of chronic diabetic neuropathic ulcers.[7]

Various phase II and phase III studies showed effectively the efficacy ,that is complete closure of the wound, and the reduction in the size of the wound. PDGF promote granulation tissue and stimulate cutaneous ulcer healing. It stimulates the proliferation of a variety of mesenchymal cells including fibroblasts.[12,13]

In view of further studies regarding the efficacy of rh-PDGF in chronic diabetic ulcers, we undertook this study to know whether rh-PDGF applied topically over the chronic diabetic foot ulcers reduces the size of the wound effectively compared to conventional treatment alone(Normal saline dressing).

AIM AND OBJECTIVE OF THE STUDY

To compare the efficacy of recombainant human platelet derived growth factor (rh-PDGF) dressing Vs normal saline dressing in wound reduction in patients with chronic diabetic foot ulcers, admitted tin KLES Prabhkar Kore hospital and MRC, Belgaum from January 2007 to December 2007.

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 Ambroise, 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 & egains 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 parenchymal 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

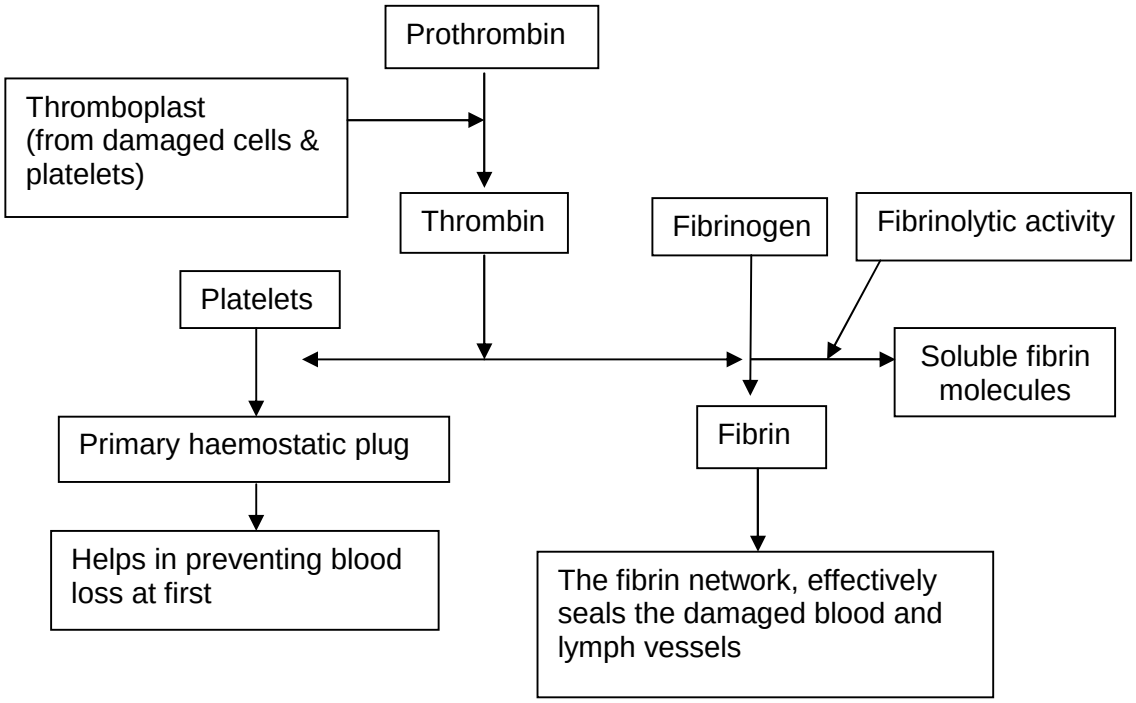
[5] Collagen Synthesis

[6] Wound contraction / Tissue Remodelling / 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 & organisation 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 remodelling 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?

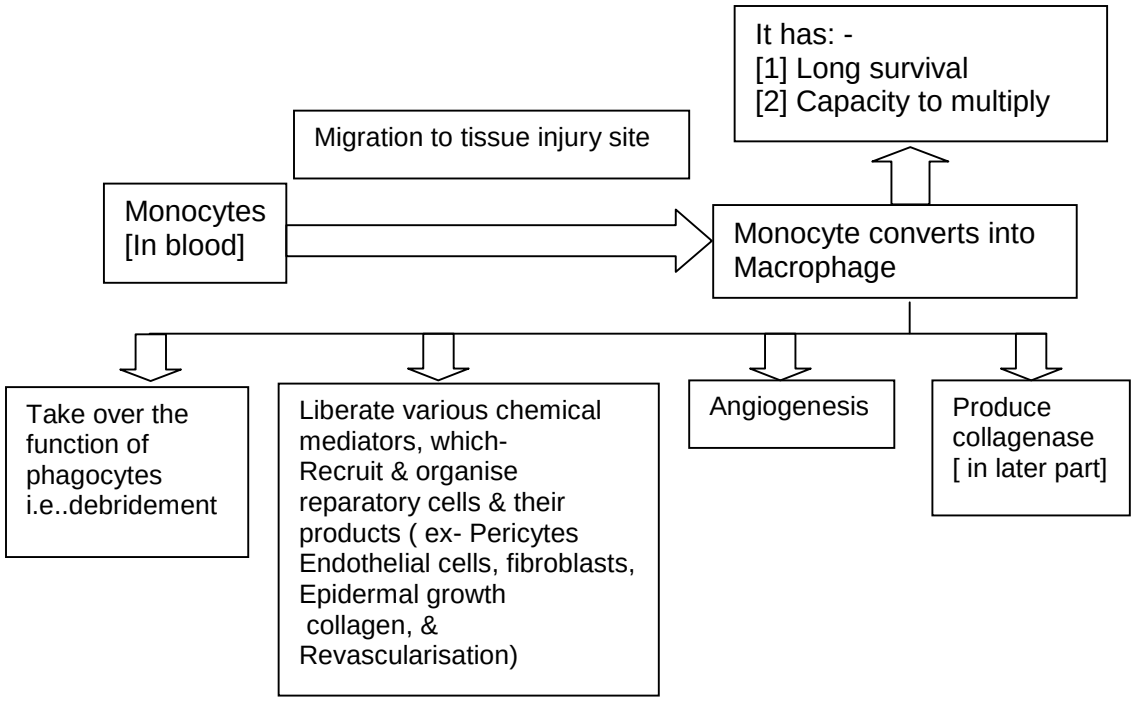
Macrophagia is

[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

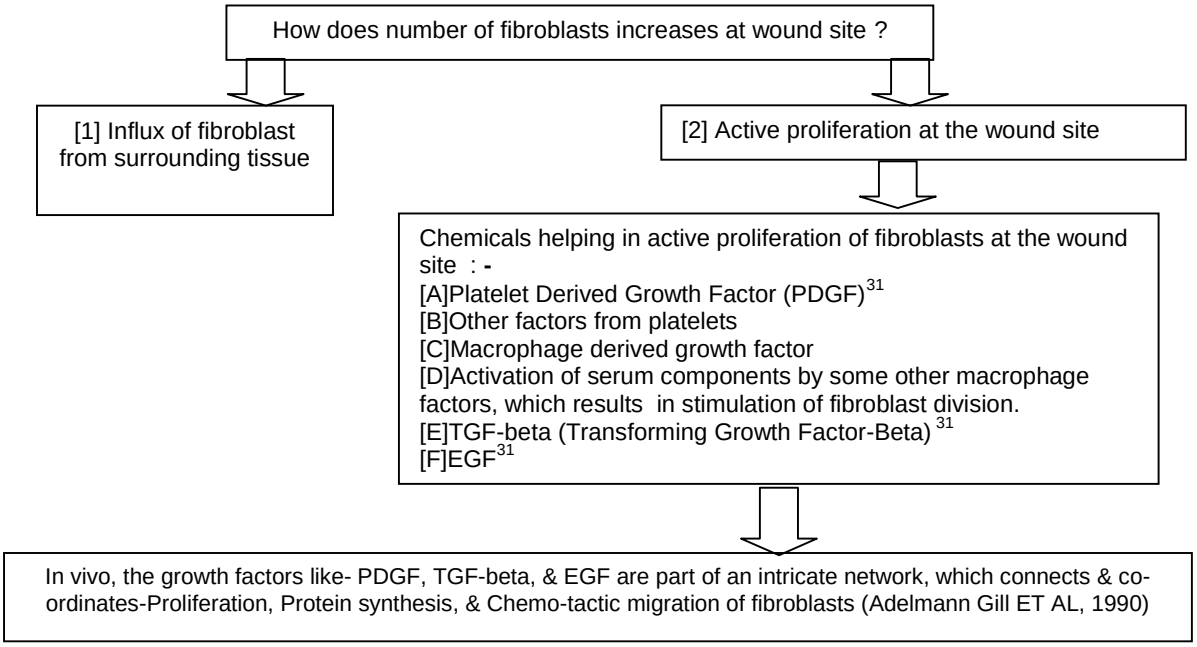
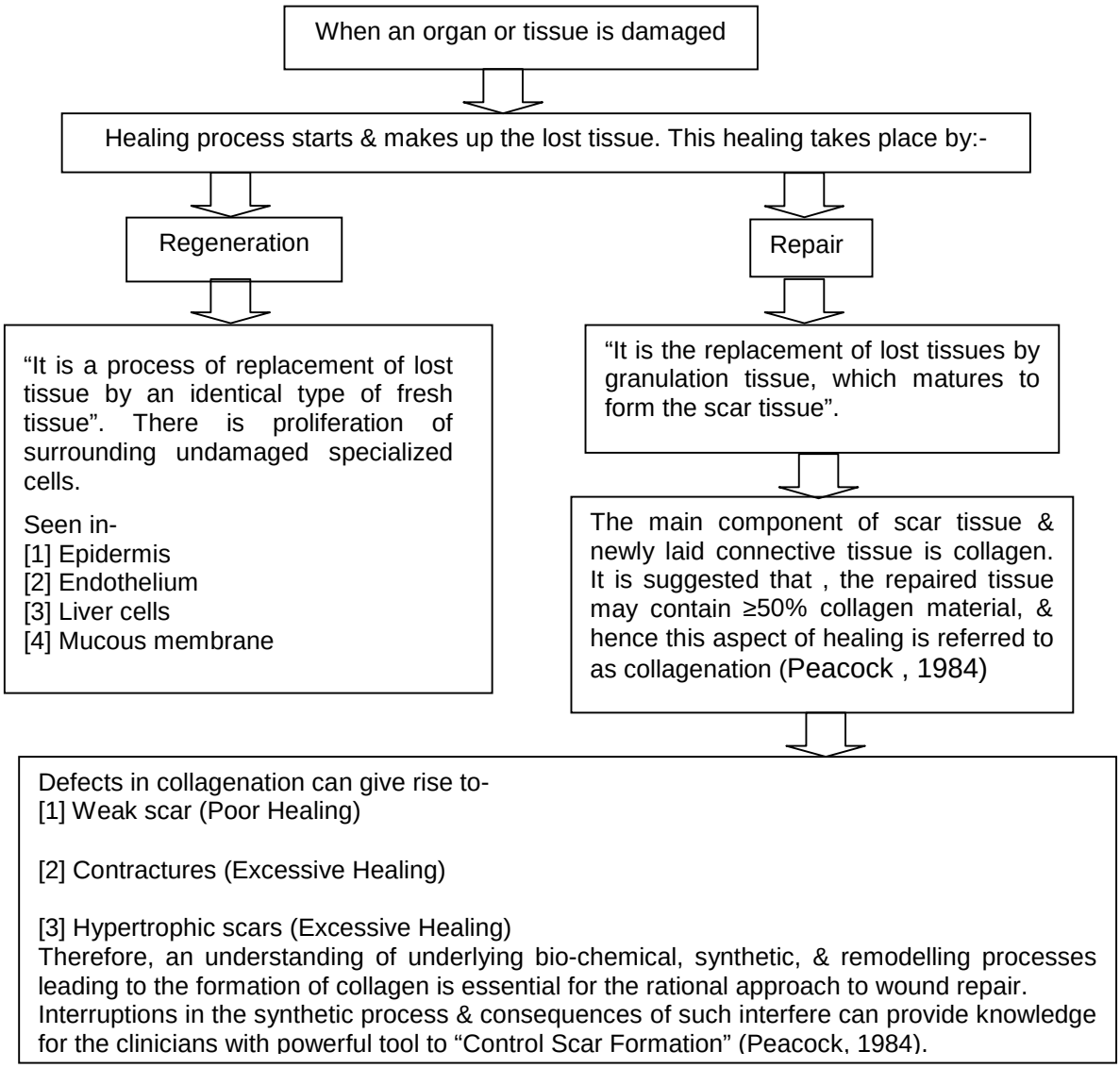


Fig 2.4 : Collagenation Mechanism



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 characterised 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]

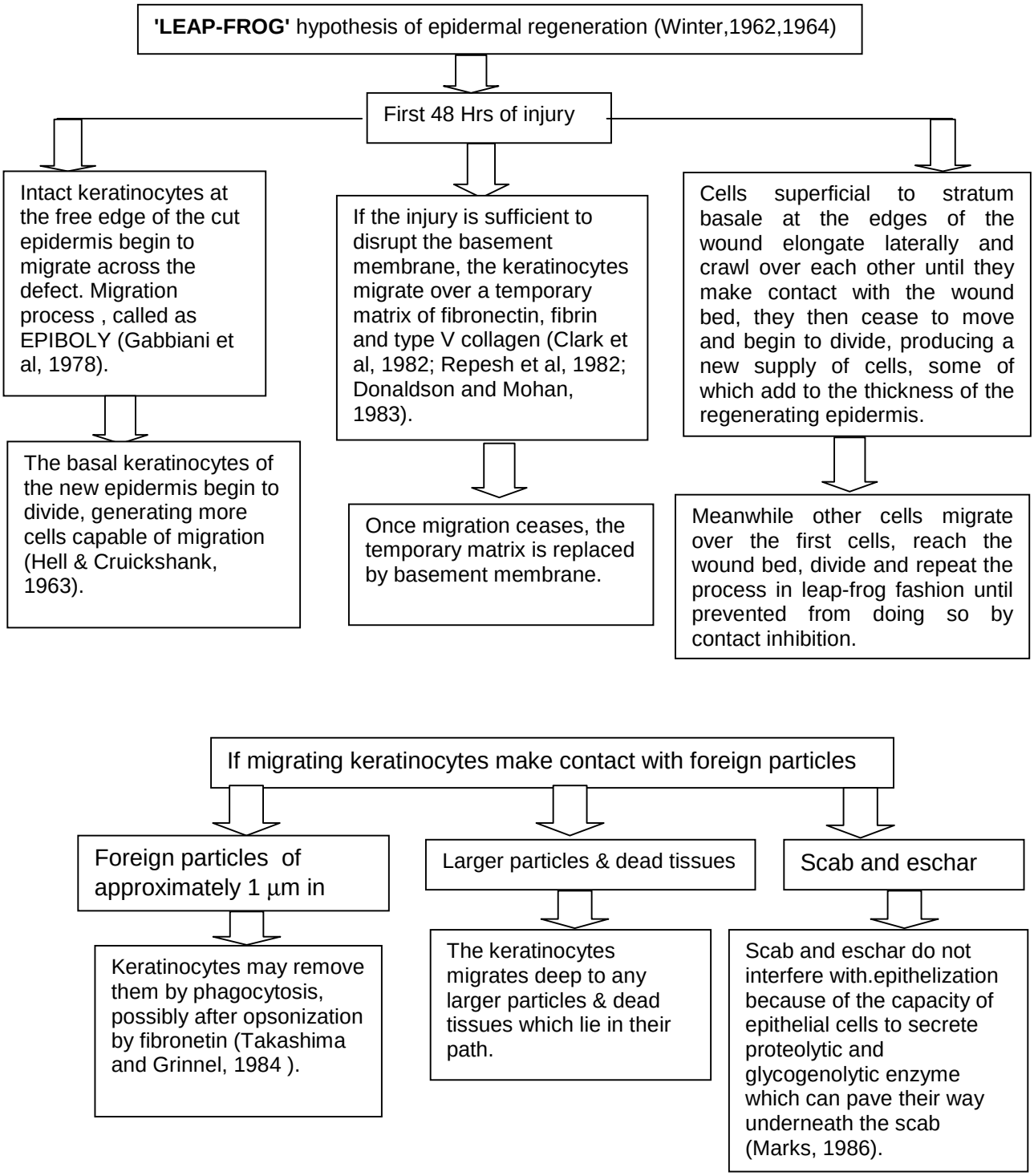
- **Definition**: Epithelization is a process of wound healing involving body surfaces.
- Unlike healing by fibroplasias where lost parenchymal cells are replaced by non-specific connective tissue, in epithelialization lost epithelial cells are replaced by epithelial cells only. It is an example of healing 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 Epithelisation



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.

HISTORICAL BACKGROUND OF GROWTH FACTORS

Over the past several decades, the discovery of growth factors has led to much hope and speculation about the use of these potent peptides in the treatment of difficult to heal wounds, particularly chronic wounds. In vitro experiments showed that growth factors were very effective in regulating cell proliferation, chemotaxis, and extracellular matrix formation. Animal experiments confirmed the that growth factors could accelerate wound repair, although most such experiments dealt with wounds created by acute injury.[23,24]

However, it was not until later, when further advances in recombinant technology made it possible to obtain large amounts of purified growth factors, that these agents could be tested in human clinical trials. Over the last 10 to 15 years, a large number of trials have been performed to evaluate the safety and effectiveness of growth factors in the healing of chronic wounds due to pressure (decubitus ulcers), diabetic neuropathy, and venous insufficiency. Platelet-derived growth factor (PDGF) is now approved for topical treatment of diabetic neuropathic ulcers. PDGF is the only growth factor approved by US-FDA.[23,25]

Growth factors are secreted proteins from many tissues in the body exert diverse effects on cell growth, metabolism, differentiation, and on the growth and development of organisms. 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 Autocrine, Paracrine , juxtacrine and Intracrine modes. Paracrine mode of action occurs when a growth factor that is secreted by one cell has an effect on adjacent cells. juxtacrine is similar as paracrine, although the growth

factor is bound to the cell membrane or extra cellular matrix. Autocrine actions are mediated by a growth factor on its cell of origin after its secretion in to the extcellular environment. 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 [23, 28]

Table 3.5 Partial list of growth factors used to accelerate the repair of chronic wounds in humans

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-ß	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

* EGF = epidermal growth factor; FGF = fibroblast growth factor; GMCSF = granulocyte-macrophage colony-stimulating factor; HGH = human growth hormone; IL-1 = interleukin-1; IGF-1 = insuling growth factor-1; PDGF = platelet-derived growth factor; TGF-ß = transforming growth factor-ß

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 factors and their receptors are expressed in a wide variety of cell and tissue types. PDGF-BB has been prepared and purified for use in clinical studies of wound healing. The recombinant human platelet derived growth factor (rh-PDGF) is produced by insertion of the human gene for the B chain of PDGF in the yeast saccharomyces cerisiae.[23]

STRUCTURE OF PLATELET-DERIVED GROWTH FACTOR

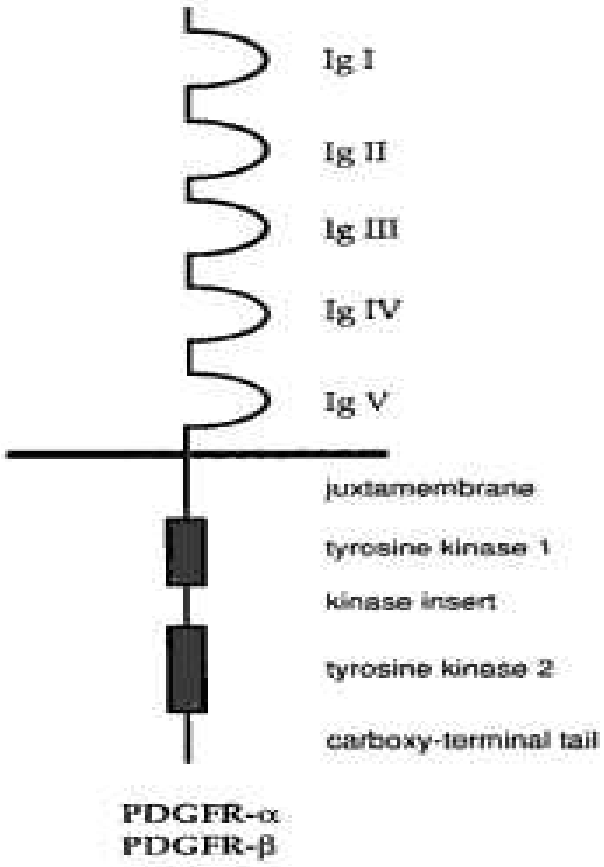
Mature PDGF-A and -B chains are 109 amino acids in length and are 60% identical. Both PDGF chains are synthesized as precursor proteins that undergo pro-cessing to yield mature glycoproteins. All three combinations of growth factor dimers have been isolated from tissues: AA, AB, BB in addition to platelet 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]



BIOLOGIC 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]

A series of studies in animals has shown that application of platelet-derived growth factor (PDGF) to a wound enhances the process of wound repair.[29, 30] Various human cellular studies have certainly established the fact that topically applied recombinantt human platelet derived growth factor(rh-PDGF) is a new pharmacologically active therapy for chronic ,neuropathic lower extremity diabetic ulcers, resistant to conventional mode of treatment.

In pahase II studies , recombinant human PDGF-BB(rhPDGF-BB) was shown to have a positive effect on healing pressure ulcers and lower extremity ulcers in patients with diabetes.[10,11] A phase III randomized placebo controlled double- blind study on 382 patients with diabetic foot ulcers supported that becaplermin gel 100 µg/g, in conjunction with good wound care, significantly increased the incidence of complete wound closure and significantly reduced the time to complete closure of chronic diabetic neuropathic ulcers.[7]

Various phase II and phase III studies showed effectively the efficacy ,that is complete closure of the wound, and the reduction in the size of the wound. PDGF promote granulation tissue and stimulate cutaneous ulcer healing. It stimulates the proliferation of a variety of mesenchymal cells including fibroblasts.[12,13]

DIABETES MELLITUS

Definition :

"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"**.** [31-37]

Depending on the etiology of the DM, factors contributing to hyperglycemia may include:

- Reduced insulin secretion
- Decreased glucose utilization
- Increased glucose production

Or

"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

It is a heterogeneous group of disorders characterized by :-

- Impaired insulin secretion
- Variable degree of insulin resistance
- Increased glucose production

Distinct genetic & metabolic defects in insulin action &/or secretion give rise to the common phenotype of hyperglycaemia in type-2 DM.

Type-2 DM is preceded by a period of abnormal glucose haemostasis classified 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.

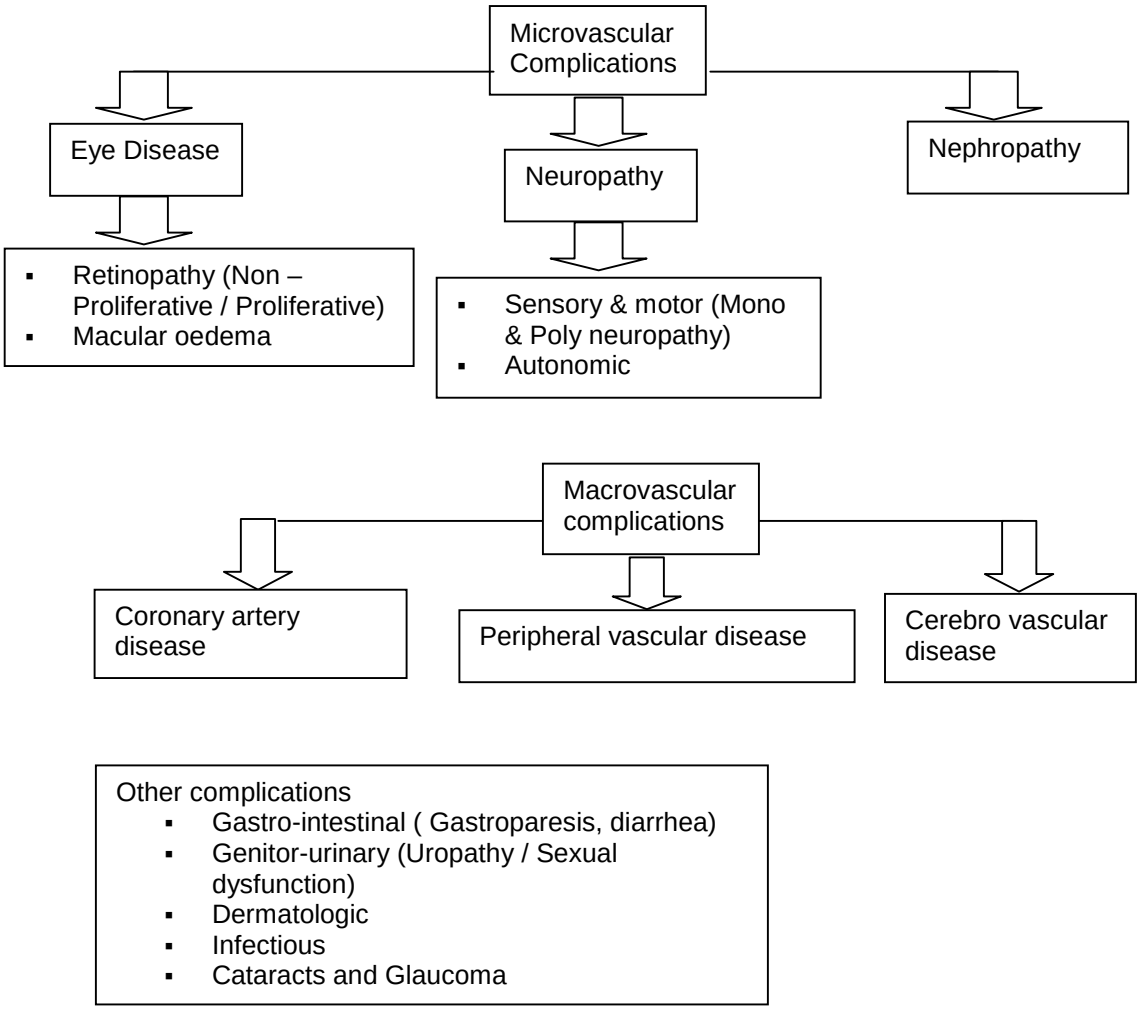
Table 3.6: Diagnosis of Diabetes Mellitus

Terms	Definition
Random Blood Glucose (RBS)	Blood Glucose levels without regard to time since last meal
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]

The chronic complications of DM affect many organ systems and are responsible for the majority of morbidity and mortality associated with the disease.

Fig. 2.7: Chronic Complications of DM



- The risk of chronic complications increases as a function of the duration of hyperglycemia. They usually become apparent in the second decade of hyperglycemia.
- Since type 2 DM often has a long asymptomatic period of hyperglycemia, many individuals with type 2 DM have complications at the time of diagnosis.
- The microvascular complications of both type 1 and type 2 DM result from chronic hyperglycemia.

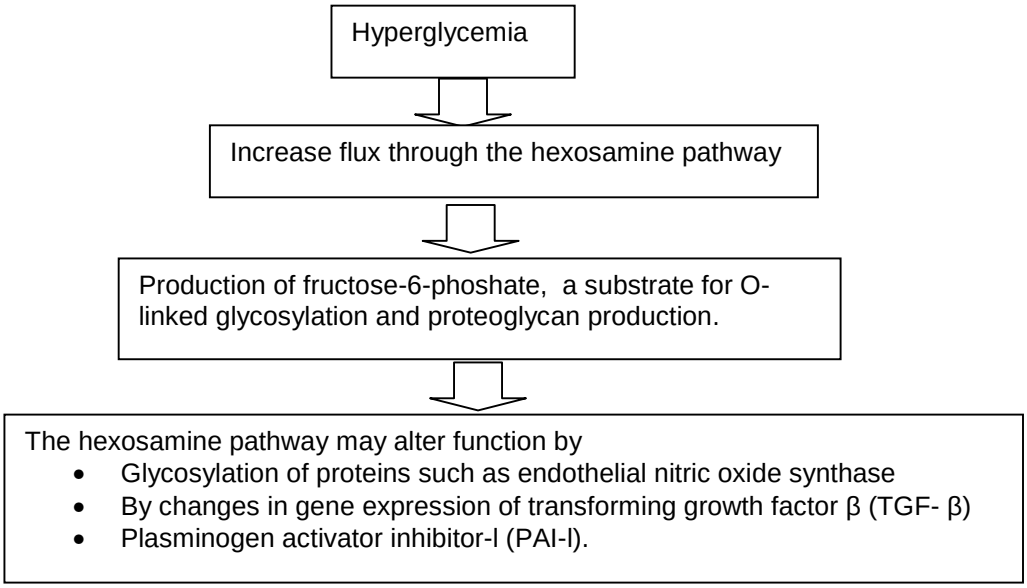
- Large, randomized clinical trials of individuals with type 1 or type 2 DM have conclusively demonstrated that a reduction in chronic hyperglycemia prevents or delays retinopathy, neuropathy, and nephropathy.
- However, coronary heart disease events and mortality are two to four times greater in patients with type 2 DM.
- These events correlate with fasting and postprandial plasma glucose levels as well as with the Hb A 1 C.
- Other factors (dyslipidemia and hypertension) also play important roles in macrovascular complications.

Mechanisms of complications [31-37]

Theory which is not mutually exclusive, has been proposed to explain how hyperglycemia might lead to the chronic complications of DM (Fig. 2.8).

A hypothesis proposes that leading to :

Fig. 2.8: Mechanisms of Complication of Diabetes Mellitus



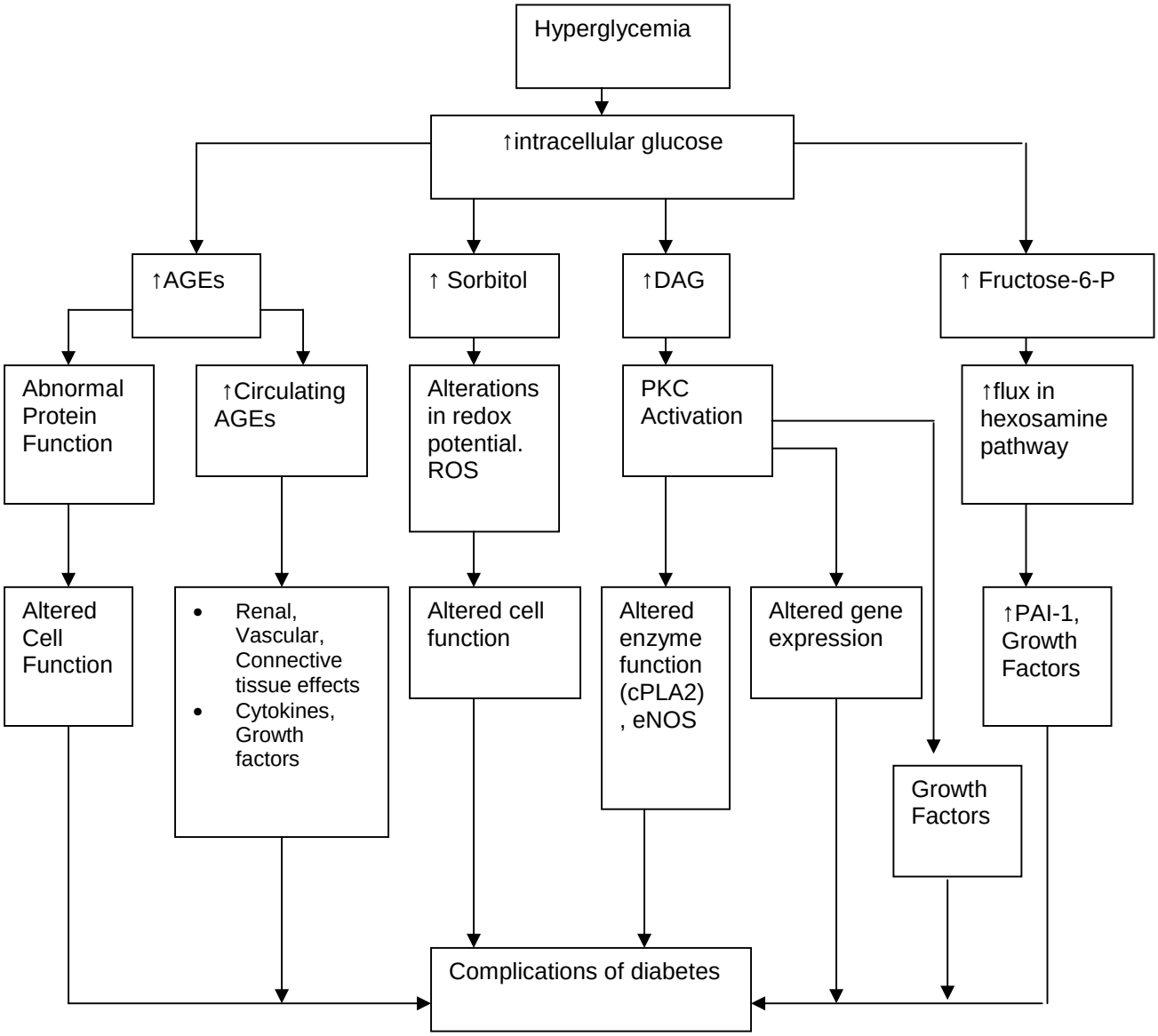


Figure showing - Possible molecular mechanism of diabetes-related complications

AGEs : Advanced glycosylation end products

PKC : Protein kinase – C

DAG : Diacylglycerol

c PLA 2 : Phospholipase A2

Enos : Endothelial Nitric Oxide Synthase

ROS : Reactive oxygen species

PAI-l : Plasminogen activator inhibitor-l

Neuropathy And Diabetes Mellitus [31-37]

- The prevalence of diabetic neuropathy in patients with type 2 diabetes is 32 percent overall and more than 50 percent in patients over 60 years of age.[1,2]
- Diabetic neuropathy correlates with the duration of diabetes and glycemic control) type1 & 2 DM.
- May manifest as
    1. Polyneuropathy
    2. Mono-neuropathy
    3. Autonomic Neuropathy
- Both myelinated and unmyelinated nerve fibers are affected.
- Because the c/f of diabetic neuropathy are similar to those of other neuropathies, the diagnosis of diabetic neuropathy should be made only after other possible etiologies are excluded.

Poly-neuropathy / Mono-neuropathy :

- The most common form of diabetic neuropathy is distal symmetric polyneuropathy.
- 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.

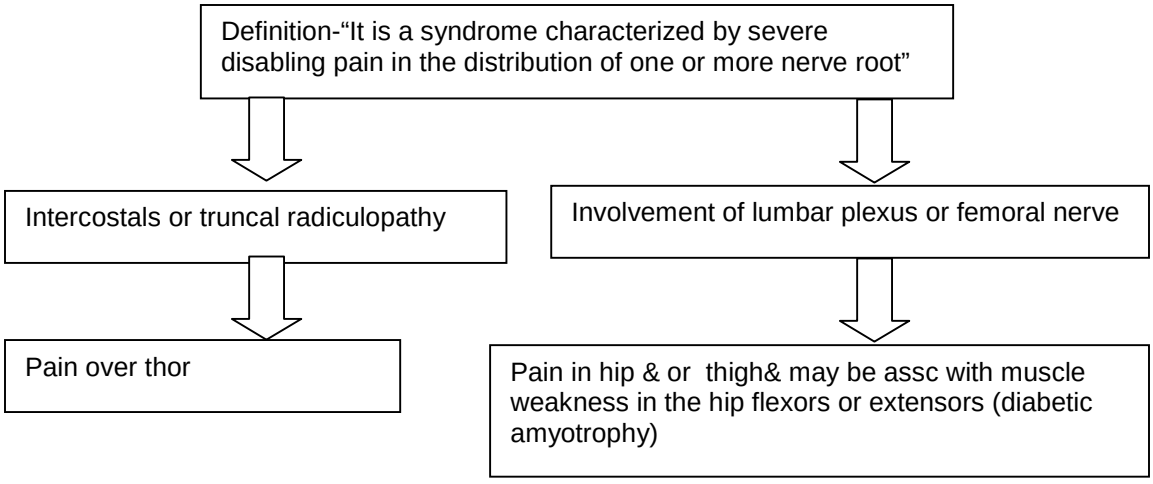
1. Numbness,
2. Tingling
3. Sharpness
4. Burning

Any combination of these symptoms may develop as neuropathy progresses

- Physical examination reveals
    1. Sensory loss
    2. Loss of ankle reflexes
    3. Abnormal position sense.
- Pain typically involves lower extremities, is usually present at rest, and worsen at night.
- Both an acute (lasting <12 months) and a Chronic form of painful diabetic neuropathy have been described.
- As diabetic neuropathy progresses, the pain subsides & eventually disappears, but a sensory deficit in the lower extremities persists.
- Neuropathic pain develops in some of these individuals, occasionally preceded by improvement in their glycemic control.

Diabetic Neuropathy :

It may be accompanied by - Motor weakness



Treatment of diabetic neuropathy :

- Improved glycaemic control should be pursued and will improve nerve conduction velocity, but the symptoms of diabetic europathy may not necessarily improve.
- Avoidance of neurotoxins (alcohol), supplementation with vitamins for possible deficiencies (BI2, B6, folate).
- 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.

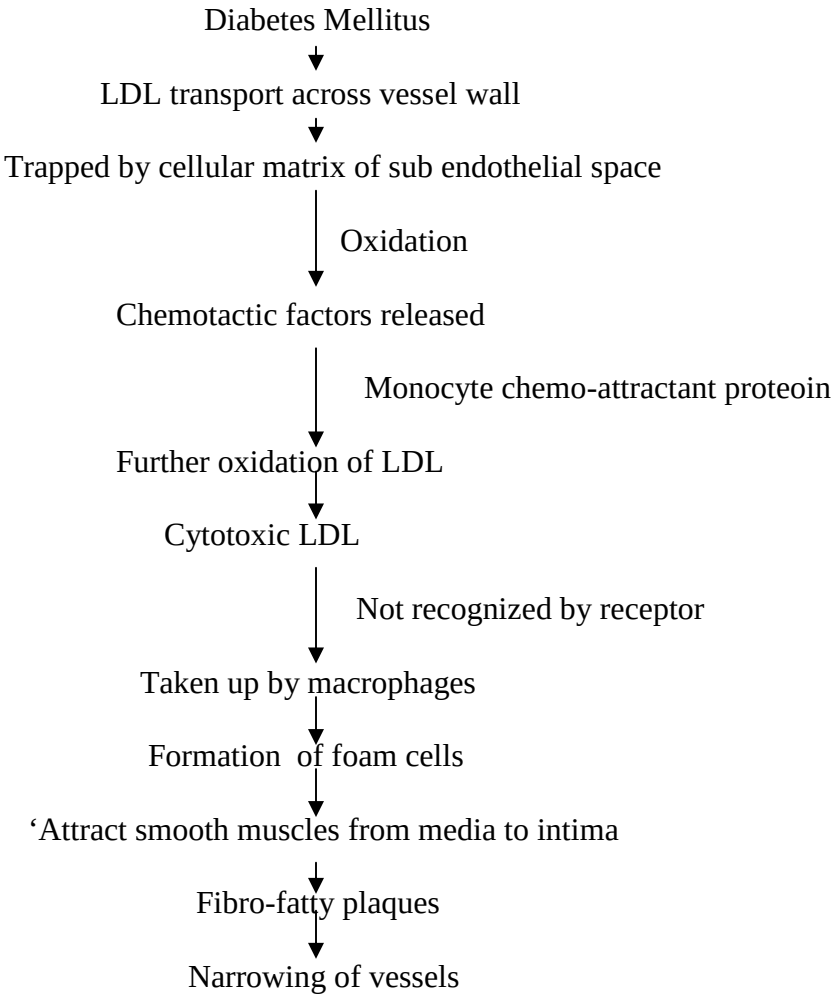
VASCULAR CHANGES IN DIABETES

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

. **I Lipoproteins pathogenesis:**[40,41]

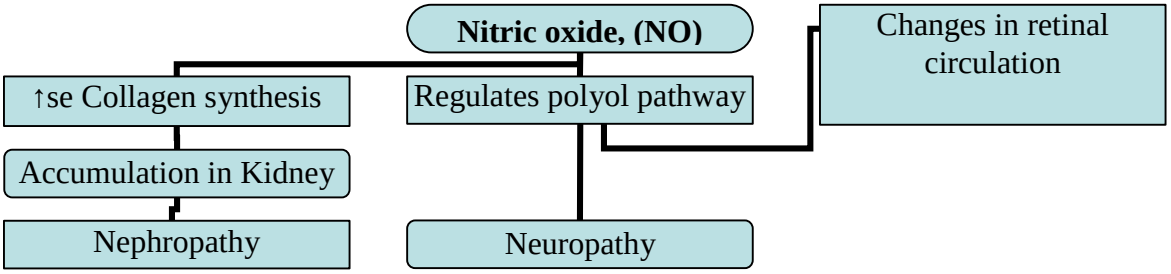
Fig 2.9 pathophysiology diabetic vasculopathy



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

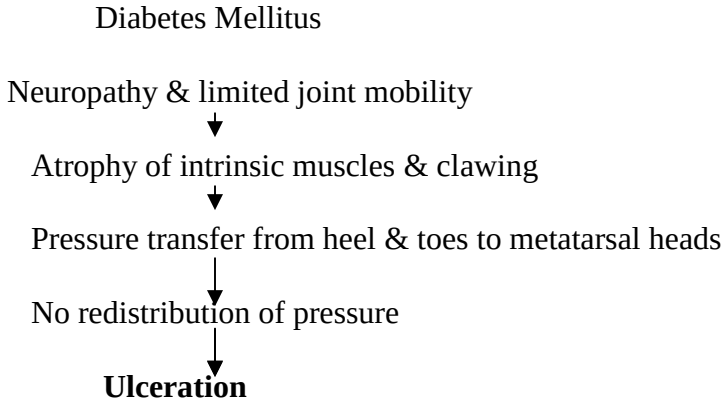
c Thromboxane A_2($TX\text{-}A_2$):>>>>Vasoconstrictor- Conteracts effect of N.O

↑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]



Predisposing factors for ulceration: [47]

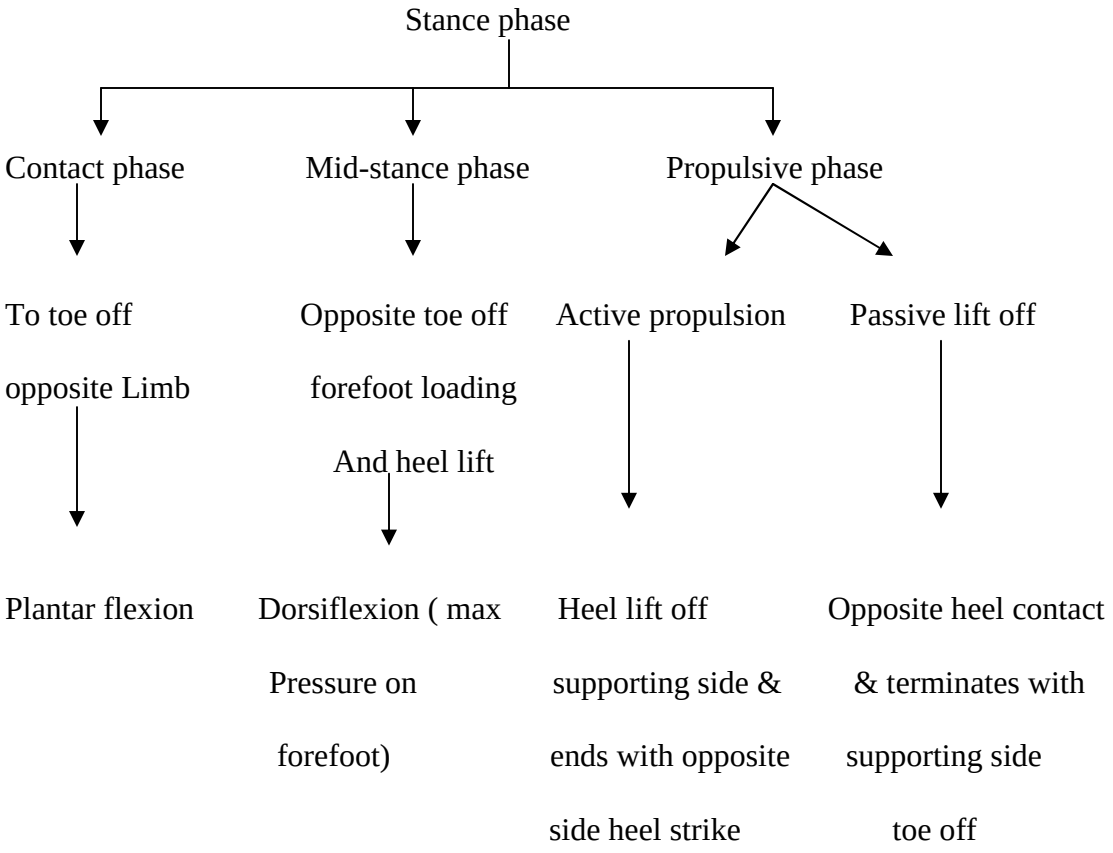
1) Limited joint mobility.
2) Peripheral neuropathy.
3) High plantar pressure.
4) Vascular diseases.

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 ofskin tendons & joint capsules

↓

Decreased tissue flexibility

↓

Increased plantar pressure

4 Trauma [50,51]

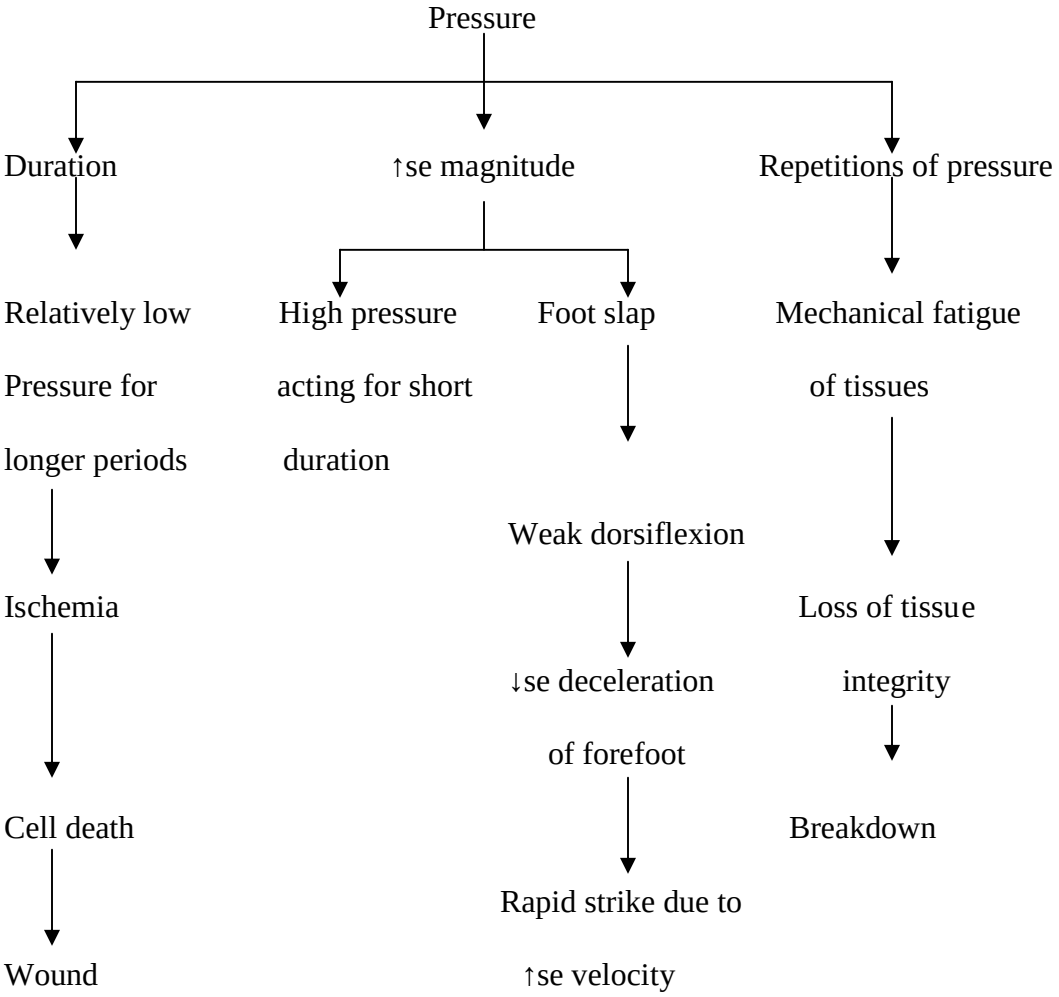
Trauma

↓

Intrinsic → Repetitive stress from high pressure / Callus

Extrinsic → Ill-fitting footwear

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

ABOUT THE DRESSING MATERIAL USED IN THIS STUDY

Over the fast few years recombinant human platelet derived growth factor(rh-PDGF-BB) is being used for treatment of chronic non healing ulcers.

Preparation of the material:

Dr Reddys laboratories LTD, India, supplied a recombinant human platelet derived growth factor-BB(rh-PDGF-BB) under the trade name PLERMIN 0.001% gel which is manufactured under proprietary method. It is available in 15 gm and 30 gram gel form for topical application.

METHODOLOGY

Study design : Randomised controlled trial

Source of Data : Patients with diabetic foot ulcers admitted in surgery wards at K.L.E.S Prabhakar Kore Hospital and MRC, Belgaum over a period of one year from January 2007 to December 2007

Sample Size :

80 patients

40 patients -in the study group

40-patients -in the control group

Inclusion criteria

1. Type I and II Diabetes mellitus.
2. Diabetics between 18 to 65 years of age
3. If female must be practicing birth control
4. Have documented wound etiology resulting from complications of Diabetes mellitus.
5. Duration of the ulcer more than 4 weeks.
6. Size of ulcer less than 10x10 cm
7. Fasting blood glucose levels measured in two occasions 24 hours
8. 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 Be a pregnant female or a nursing mother.

8 Have exposed tendon or bone or presence of charcot joint.

9 Diabetic gangrene toe.

Method

The present study was carried out at Jawaharlal Nehru Medical college and K.L.E.S Hospital and M.R.C Belgaum for a period of one year, where 80 patients with diabetic foot ulcers participated in the present study. Using a pretested and predesigned proforma the study population was randomized into either study group or control group using a computerized randomization chart. Out of 80 patients, 40 took treatment in the form of conventional normal saline dressings and 40 took treatment with rh-PDGF dressing. Off-loading of pressure from the affected area and adequate control of infection was maintained in both the groups. If culture grows organism, both control and study group cases would be treated with antibiotics as per culture sensitivity report. The initial wound area was recorded after sharp debridement by Measuring length x width (ulcer should be less

than 10x10 cm). The outcome, that is the area of the target ulcer was measured by Plannimetry using a transparent graph sheet .Results were calculated by using student 't' test.

DRESSING TECHNIQUE

After allotting the dressing with the help of Random number table

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.

For rh-PDGF dressing

The infected ulcer was cleaned with Normal Saline. rh-PDGF-BB gel (PLERMIN 0.01%) was applied on the gauze piece and put on the ulcer. It was then covered with pad and roller bandage.

The dressings were changed daily morning in both control and study groups for 15 days and appearance of healthy granulation tissue is observed and the final area is measured on 15^{th} day by planimetry using a transparent graph sheet and subjected to statistical analysis.

OBSERVATIONS AND RESULTS

Table 4.1: Age Distribution

Age (Years)	No. of Cases	Percentage
18-30	01	01.25%
31-40	09	11.25%
41-50	16	20.00%
51-60	48	60.00%
> 60	06	07.50%
Total	80	100

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

Table 4.2 : Mean Age of Groups.

Group	*Age of Population*	
	Mean	**SD**
Study	53.67	9.89
Control	51.57	11.31

Mean age groups in the groups, that is study and controls were 53.67 yrs and 51.57 yrs respectively which were statistically not significant.

Table 4.3 : Sex Distribution

Sex	No of Cases.	Percentage.
Male	49	61.25%
Female.	31	38.75%
Total.	80	100.

In our study in was observed that Diabetic foot was more common in the males (61.25%) as compared to females (38.75%)

Table4. 4: Site of ulcer in the study.

Site	No. of Cases	Percentage
Plantar	45	56.25%
Dorsum	35	43.75%
Total	80	100%

In our study it was observed that diabetic foot more commonly occurs on the plantar aspect (56.25%) of the foot as compared to the dorsal aspect (44.12%)

Table4.5 : Onset of Diabetic Foot Ulcers.

Type of onset	No of Patients.	Percentage
Traumatic	55	68.75%
Spontaneous	25	31.25%
Total	80	100

Trauma is the most common cause of diabetic foot ulcer (68.75%) while only 31.25% were spontaneous in origin.

Table4.6 : Anti Diabetic Agents

Anti Diabetic	No. of cases	Percentage
OHA	25	31.25%
Insulin	55	68.75%
Total	80	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	11.79**%**	2.55	9.81	
Study	39.55%	2.52	39.58	P<0.001

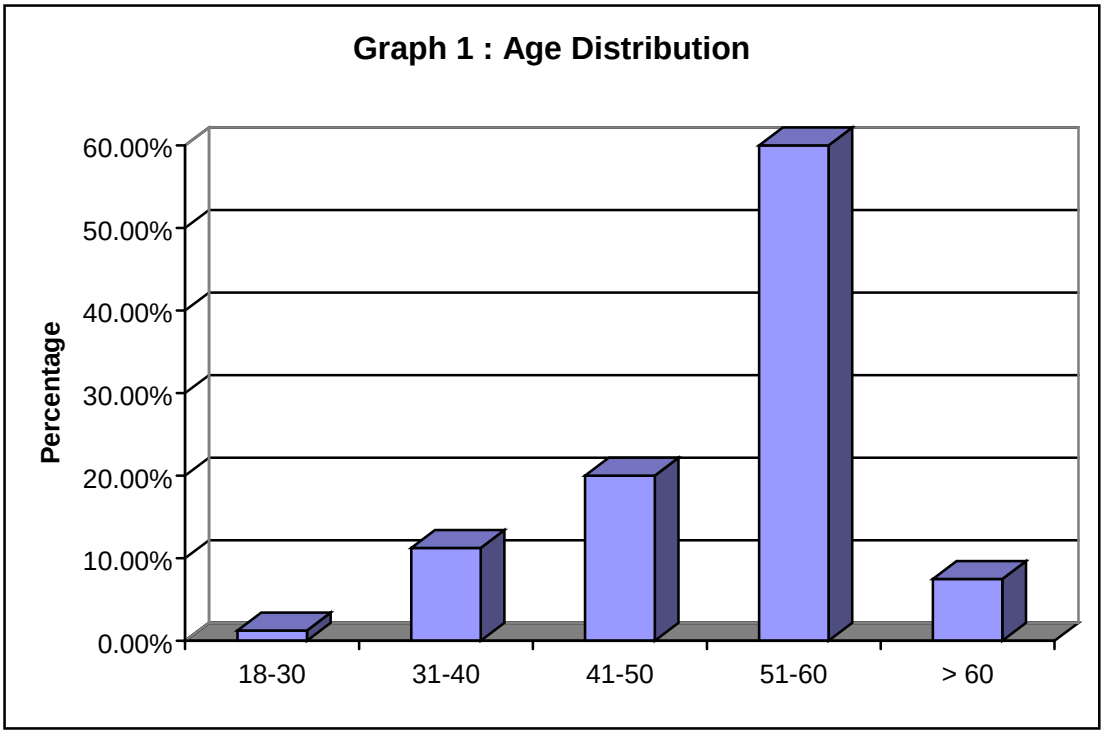
In our study it was observed that Mean % of area reduction was higher in study group (39.55%) as compared to the controls (11.79%).

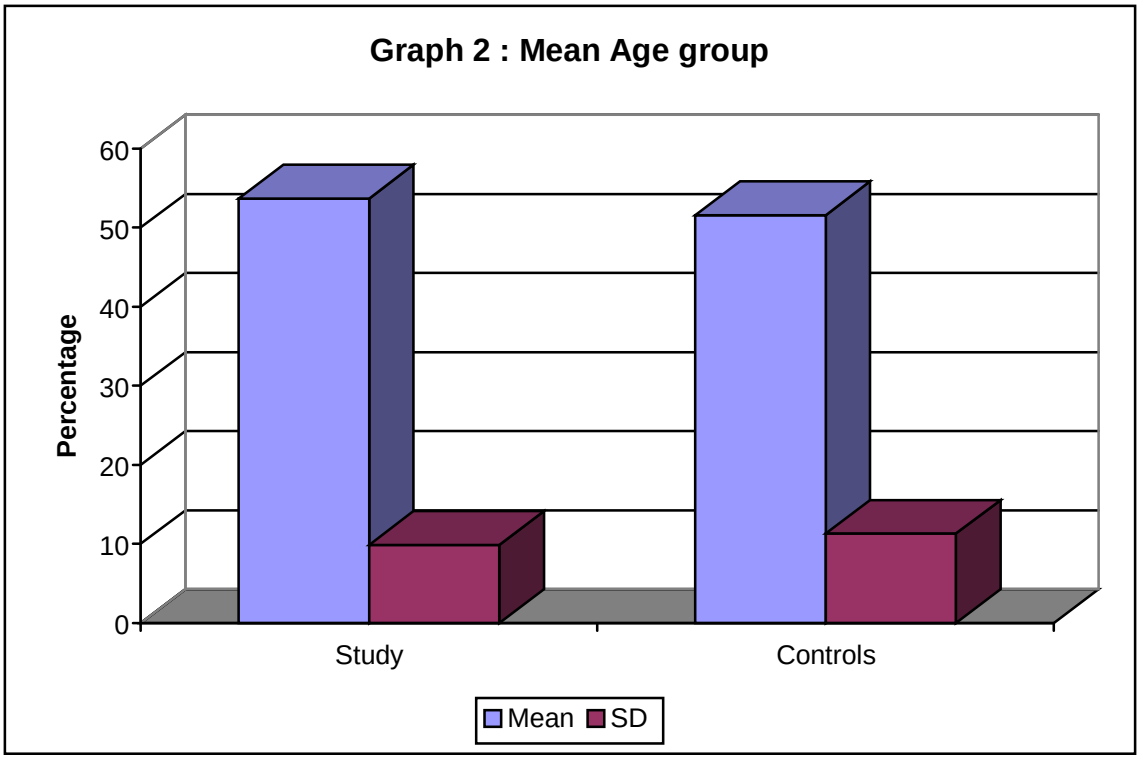
Table 4.8 : Wound Contraction Related to Site.

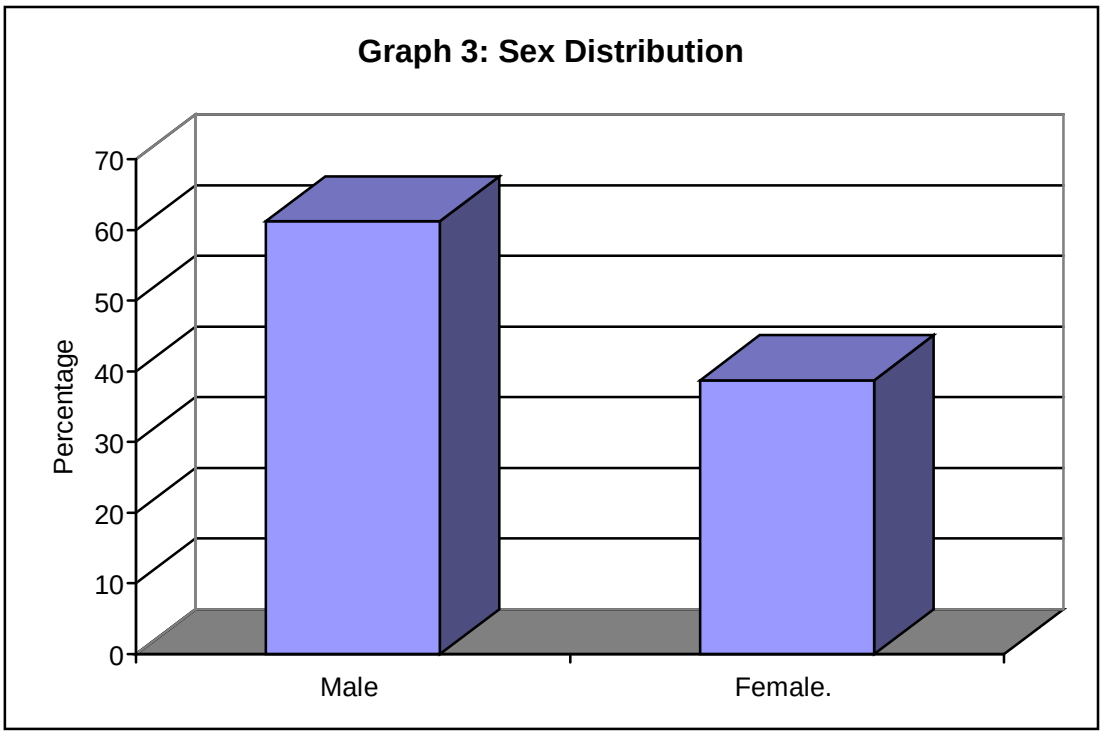
Group	Plantar			Dorsal		
	No	Mean % Red	S.D.	No.	Mean % Red	S.D.
Control	24	10.40%	1.61	16	13.87%	2.27
Study	22	40.31%	2.57	18	38.63%	2.20

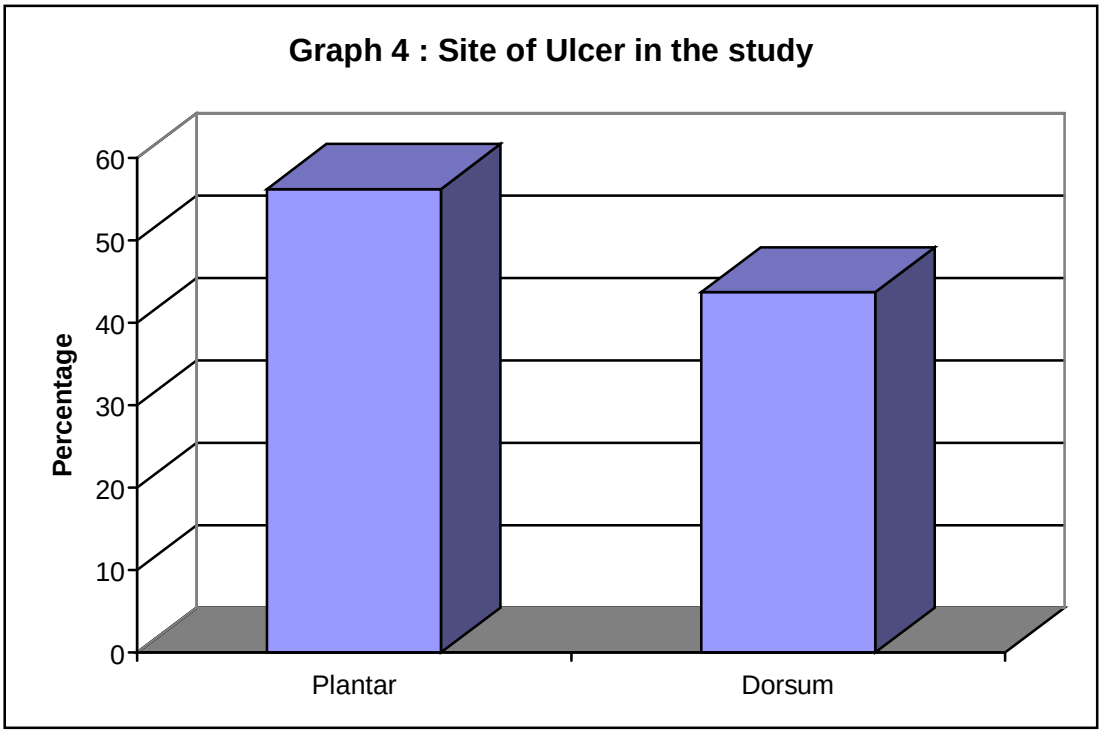
In our study it was observed that patients with Ulcers over the plantar aspect had lesser % of mean wound area reduction as compared to the participants with wound over the dorsal aspect when compared within the same group, that is, plantar wounds in the control group had lesser wound contraction (10.40%) as compared to participants who had ulcers over the dorsal aspect (13.87%) and likewise in the study group.

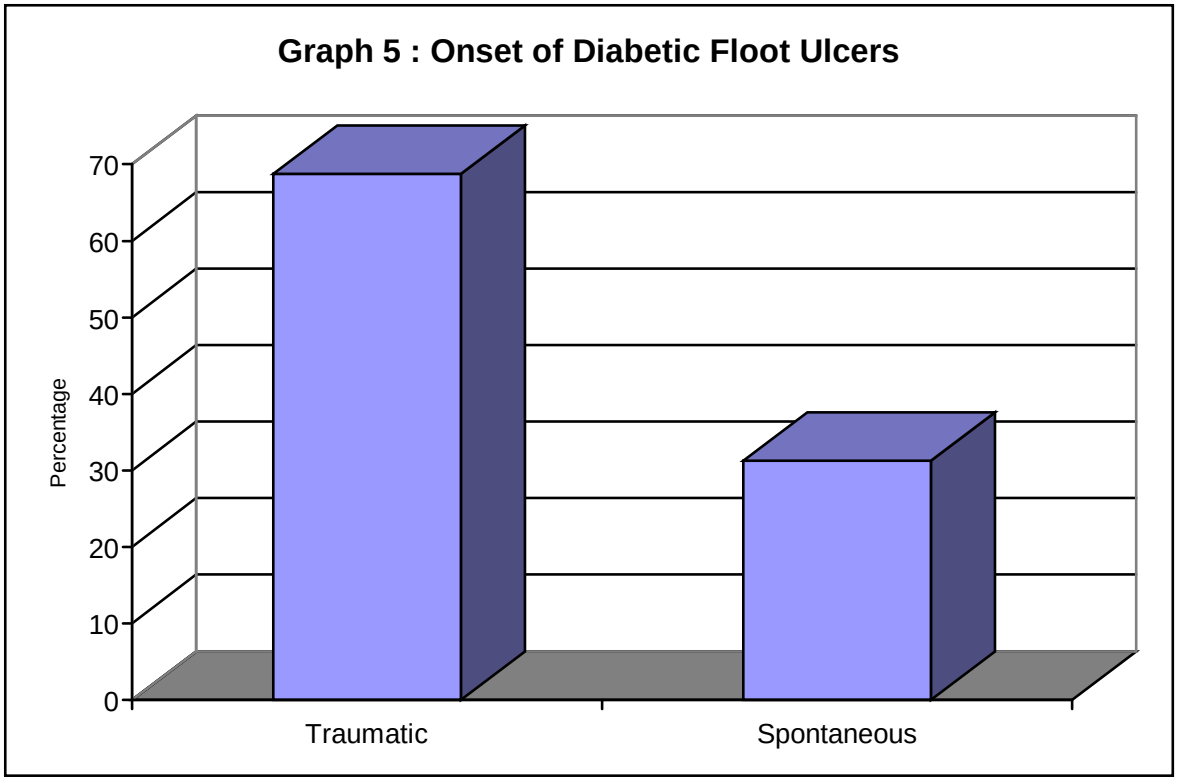
GRAPHS

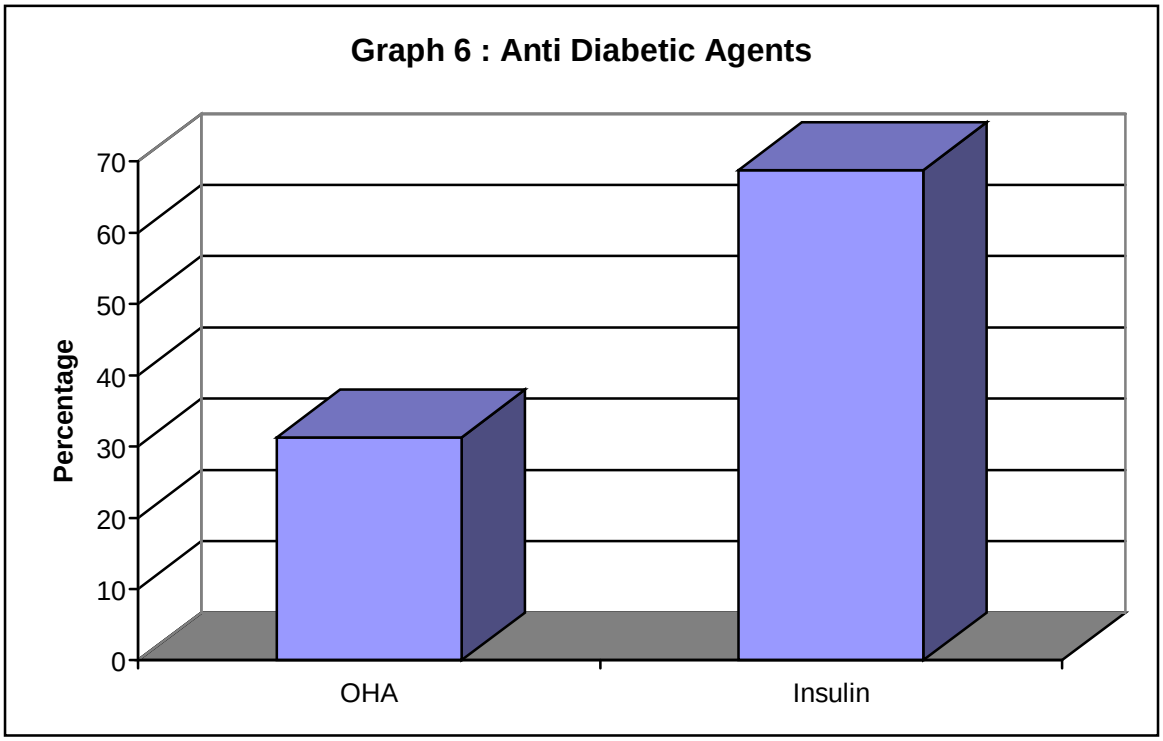

Graph 1 : Age Distribution
Percentage
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
18-30
31-40
41-50
51-60
> 60

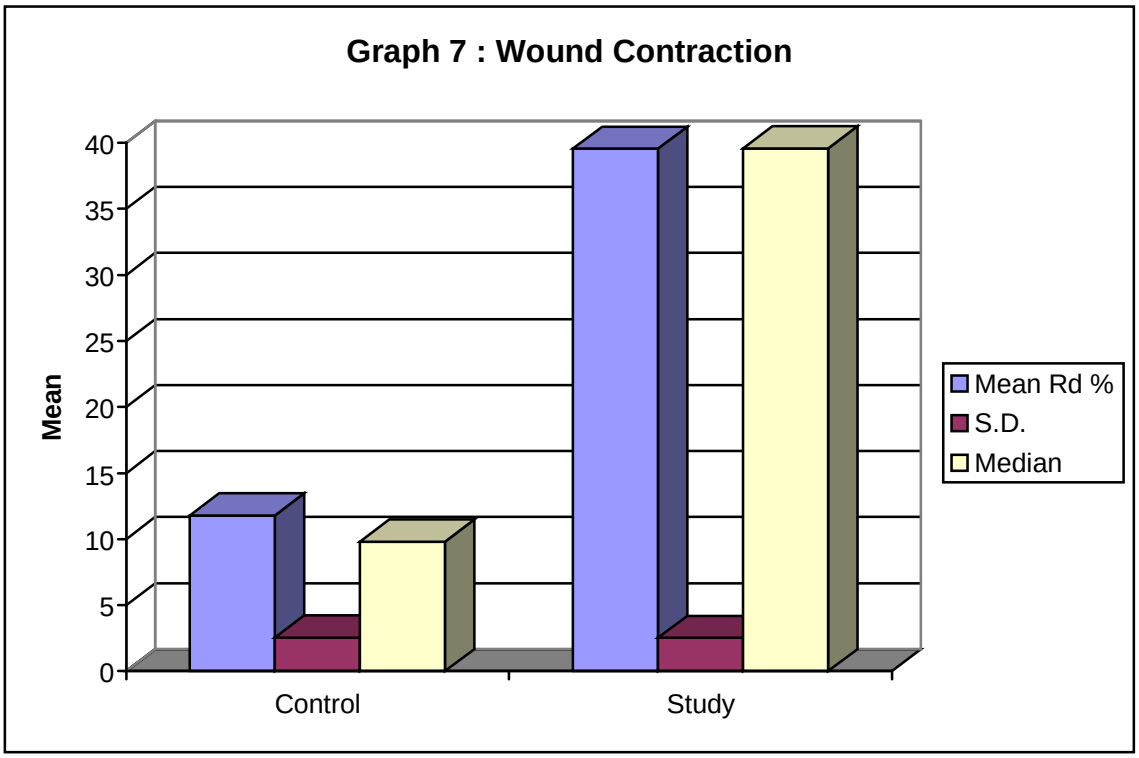

Graph 2 : Mean Age group
Percentage
60
50
40
30
20
10
0
Study
Controls
Mean
SD

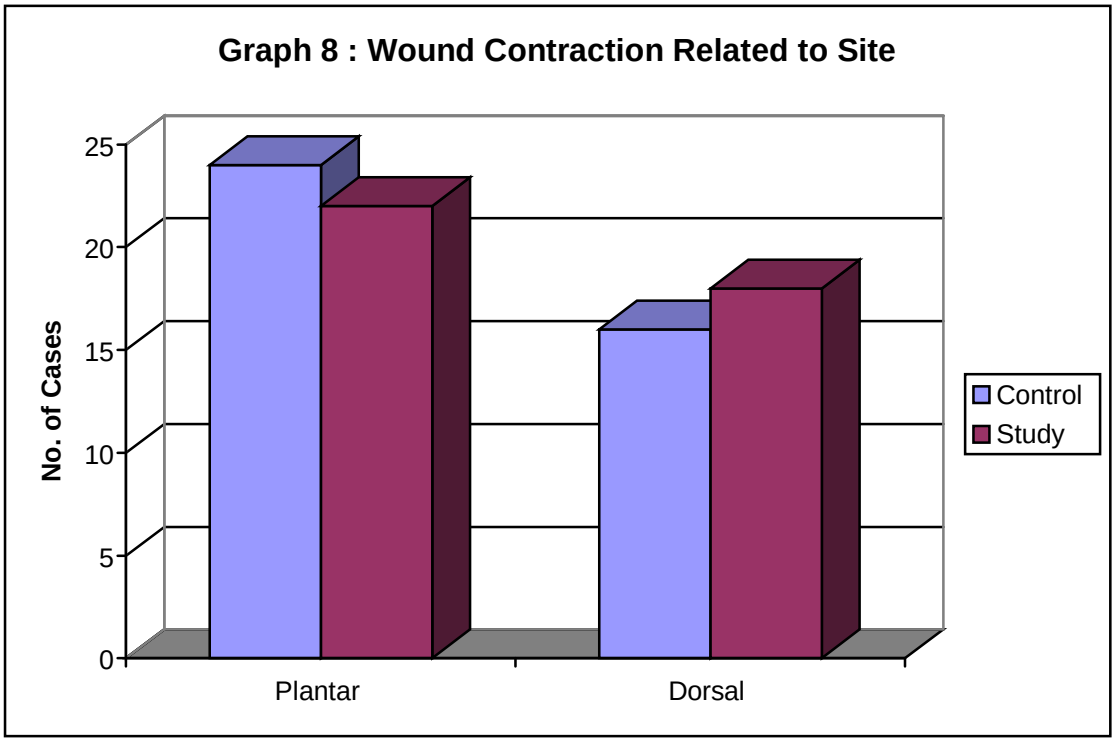

Graph 3: Sex Distribution
Percentage
70
60
50
40
30
20
10
0
Male
Female.


Graph 4 : Site of Ulcer in the study
Percentage
60
50
40
30
20
10
0
Plantar
Dorsum


Graph 5 : Onset of Diabetic Floot Ulcers
Percentage
70
60
50
40
30
20
10
0
Traumatic
Spontaneous


Graph 6 : Anti Diabetic Agents
Percentage
70
60
50
40
30
20
10
0
OHA
Insulin


Graph 7 : Wound Contraction
Mean
40
35
30
25
20
15
10
5
0
Control
Study
Mean Rd %
S.D.
Median


Graph 8 : Wound Contraction Related to Site
No. of Cases
25
20
15
10
5
0
Plantar
Dorsal
Control
Study

STATISTICAL ANALYSIS

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

Diabetic foot ulcers in the study group had better mean % of wound contraction of 39.55% (S.D; 2.52 : Median; 39.58) as compared to the control group which had mean % of wound contraction of 11.79 % (S.D; 2.55 : Median; 9.81), the difference in the mean 27.76% 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 Jawaharlal Nehru medical college and K.L.E.S Prabhakar Kore Hospital and MRC, Belgaum 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 (61.25%) as compared to females (38.75%).

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. **We** had only one patient in the third decade (1.25%). 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, 68.75% of the ulcers were traumatic in origin, trauma being the triggering factor secondary to neuropathy. 31.25% were spontaneous in origin secondary to blister rupture or unnoticed trivial trauma.

More than half (56.25%) of the patients had ulcer on the plantar surface of the forefoot and the remaining 43.75% 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.

It was also observed in our study that patients who had ulcers on the plantar aspect of the foot in the control group had mean wound contraction of 10.4% (SD; 1.61) as compared to the dorsal wounds of the same group which had mean wound contraction of 13.87% (SD; 2.27), similarly the mean plantar wound contraction in the study group was 40.31% (S.D; 2.57) as compared to the mean dorsal wound contraction of 38.63% (S.D; 2.20) suggesting different wound healing dynamics in the two regions of the foot.

Most of the patients (68.75%) were on insulin for control of sugar whereas only 31.25 % were on Oral Hypoglycaemic Agents.

In our study it was observed that participants receiving rh-PDGF (Plermin 0.01% gel) dressing had better wound contraction of 39.55% (S.D; 2.52 : Median; 39.58) As compared to the group receiving only conventional dressing (normal saline dressing) in whom the mean wound contraction was 11.79% (S.D; 2.55 , Median; 9.81), these were

found to be statistically significant on unpaired Student T test ($p<0.001$)suggesting that rh-PDGF dressing enhances wound healing in diabetic wounds.

Feasibility of this study:

In the present study we have taken 80 patients suffering from Diabetes Mellitus with foot ulcers. Patients were taken up for study based on inclusion and exclusion criteria. Out of 80 patients, 40 (25 males, 15 females) were cases and 40 (24 males and 16 females) were control. Participants included in the study group were treated with the rh-PDGF (Plermin 0.01% gel) dressing from day 01 to day 15. All 40 patients selected for rh-PDGF treatment complied for the fifteen days period of the study. The initial area measurement was taken on day 01 and final area measurement on day 15 was taken on transparent sheet.

All 40 patients selected as a control complied for the fifteen days duration period of the study. The initial area measurement on day 01 final area measurement on day 15 was taken on transparent sheet. The area measurement was done using planimetry.

We have applied the following formula to calculate % reduction in area of wound after 15 days period in both cases and control groups.

Rate of contraction of wound after 15 days of treatment =

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

We have found 11.79% (S.D; 2.55 : Median; 9.81) contraction of wounds in the control groups as compared to 39.55% (S.D; 2.52 , Median; 39.58) 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 rh-PDGF 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 rh-PDGF dressing contracted more than the wounds in the non treated group (39.55% Vs 11.79%; P = < 0.001→ Significant) which indicates rh-PDGF 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 debridment) 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.

80 patients of diabetic foot ulcers were studied. They were divided into two groups of 40 each.

One group received rh-PDGF 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. 61.25% males Vs 38.75% females. 68.75% of the ulcers were traumatic in onset. Plantar aspect (56.25%) was most common site.

Most of the patients were on insulin (68.75%) compared to the oral hypoglycaemic agents (31.25%).

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 rh-PDGF had better wound contraction of 39.55% as compared to the group receiving only conventional treatment in whom the mean wound contraction was 11.79%, these were found to be statistically significant on unpaired Student T test ($p<0.001$) suggesting that rh-PDGF enhances wound healing in diabetic wounds.

Thus, rh-PDGF 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.

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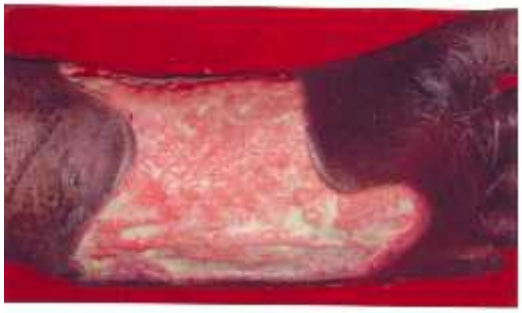
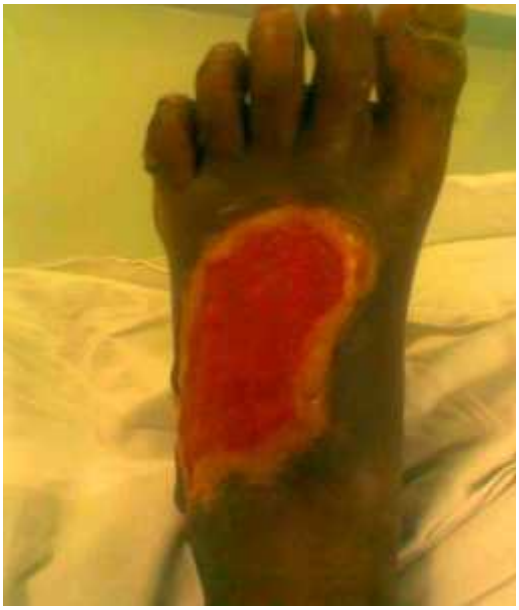
PLERMIN 0.01% gel (rh-PDGF)

Control group (normal saline dressing)

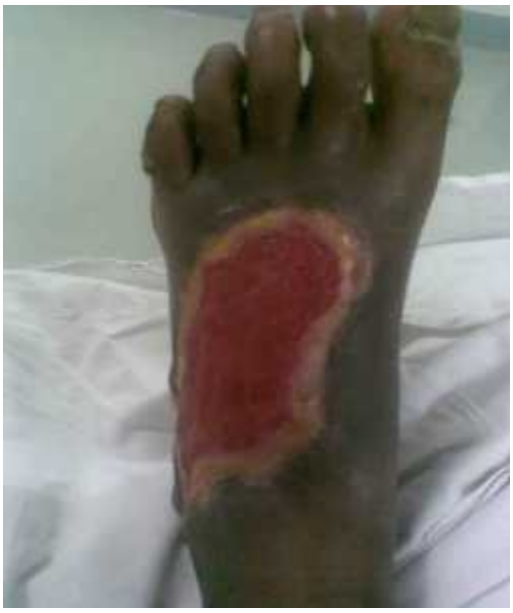


Before treatment

After treatment

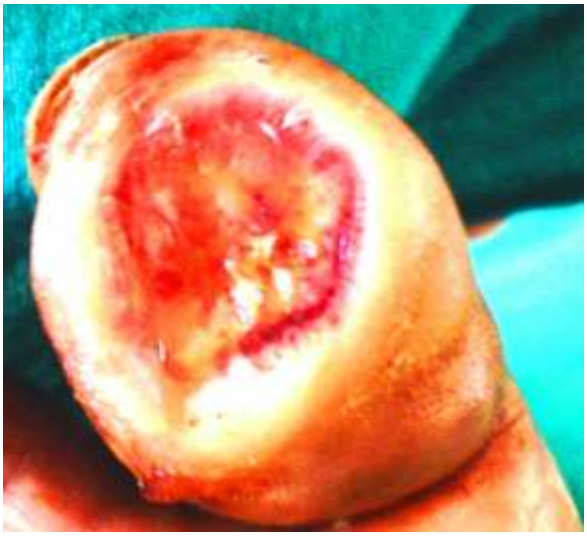


Before treatment

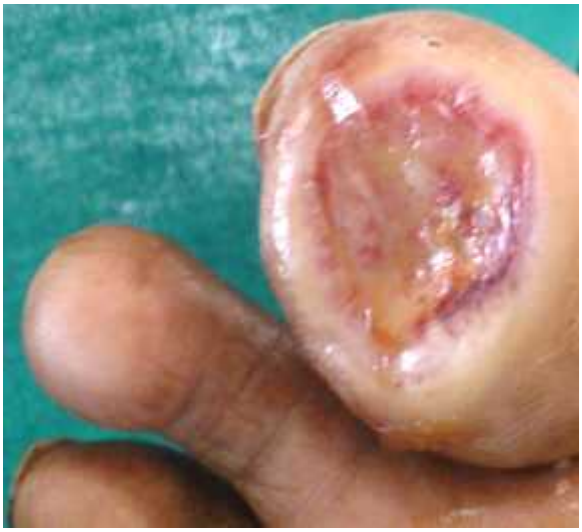


After treatment

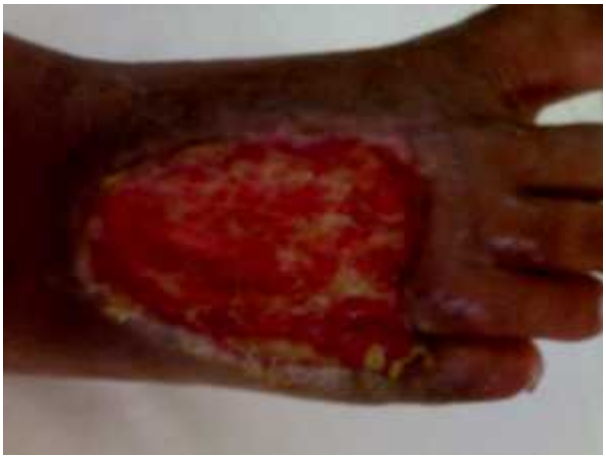
Study group (rh-PDGF dressing)



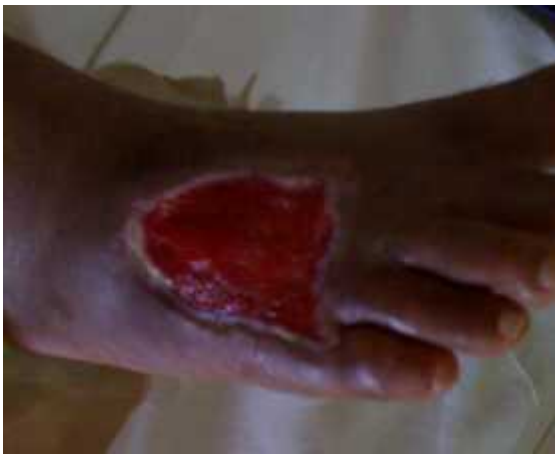
Before treatment



After treatment



Before treatment



After treatment

ANNEXURE-II

RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Mr. /Miss/ Mrs. _____

You are invited to participate in our research study that is **"TO COMPARE THE EFFICACY OF RECOMBINANT HUMAN PLATELET DERIVED GROWTH FACTOR DRESSING VERSUS NORMAL SALINE DRESSING IN WOUND REDUCTION IN PATIENTS WITH CHRONIC DIABETIC FOOT ULCER: A RANDOMISED CONTROLLED TRIAL"**

Since you are suffering from Diabetes and the foot ulcer, which is not healing since a long time and will be requiring treatment for the same, you are eligible to be part of the study and hence asked to participate. This research is about the beneficial effects of rh PDGF dressing therapy on your foot ulcer and the result of this research will help in a better treatment of similar participants in the future.

If you agree to be a part of this research, we would ask you some relevant clinical history. You are free to not to answer to whichever question u think are not relevant. A clinical examination will be done and then you will be treated with either rh PDGF dressing therapy or the normal saline dressing for 15 days. On the first day the area of the ulcer will be measured and this will be repeated at the end of the therapy that is 15th day.

There are chances you may have a speedy and better recovery with this therapy and it will also help in the treatment of participants with similar complaints in the future. Your decision of whether or not to participate in this study will not affect the quality of treatment you receive. Further you may withdraw from the study at any time.

All the new information collected about you during the course of this study will be kept confidential to the extent permitted by the law. Any information, which identifies you personally, will not be released without your written consent

This study does not have any damaging aspect and there are no chances of injury during the course of the study, but if injured the investigator is not responsible. There will be extra cost incurred by you. However you will have to pay for the routine investigations, which are a part of the existing management protocol for the treatment of diabetes. There is no commitment for any reimbursement or any compensation for the participant. The participation in this study is entirely voluntary and you may withdraw from the study at any time. At any time during or after the study, for any information you may contact the researcher.

Dr.Basavaraj G. Veerapur

Door No

Shivabasava Nagar

Belgaum, Karnataka

Or

Chairman,

Institutional Ethics Committee,

Dr. V.D. Patil,

Phone – 0831-2471350.

Signature of the participant or legally authorized representative:

Participant's name _____

Signature :_____

Experimenter/witness's Name :_____

Signature : _____

Date : _____

Place : _____

ANNEXURE-III

PROFORMA.

I) PATIENT IDENTIFICATION DATA :

NAME IP/OPD NO.

AGE DOA :

SEX DOD:

OCCUPATION

ADDRESS

II) CHIEF COMPLAINTS :

MEDICAL HISTORY :

Peripheral Neuropathy : ( )

Nephropathy ( )

Retinopathy ( )

PVD ( )

CVD ( )

DIABETIC STATUS :

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 ( )

MASTER CHART-STUDY GROUP

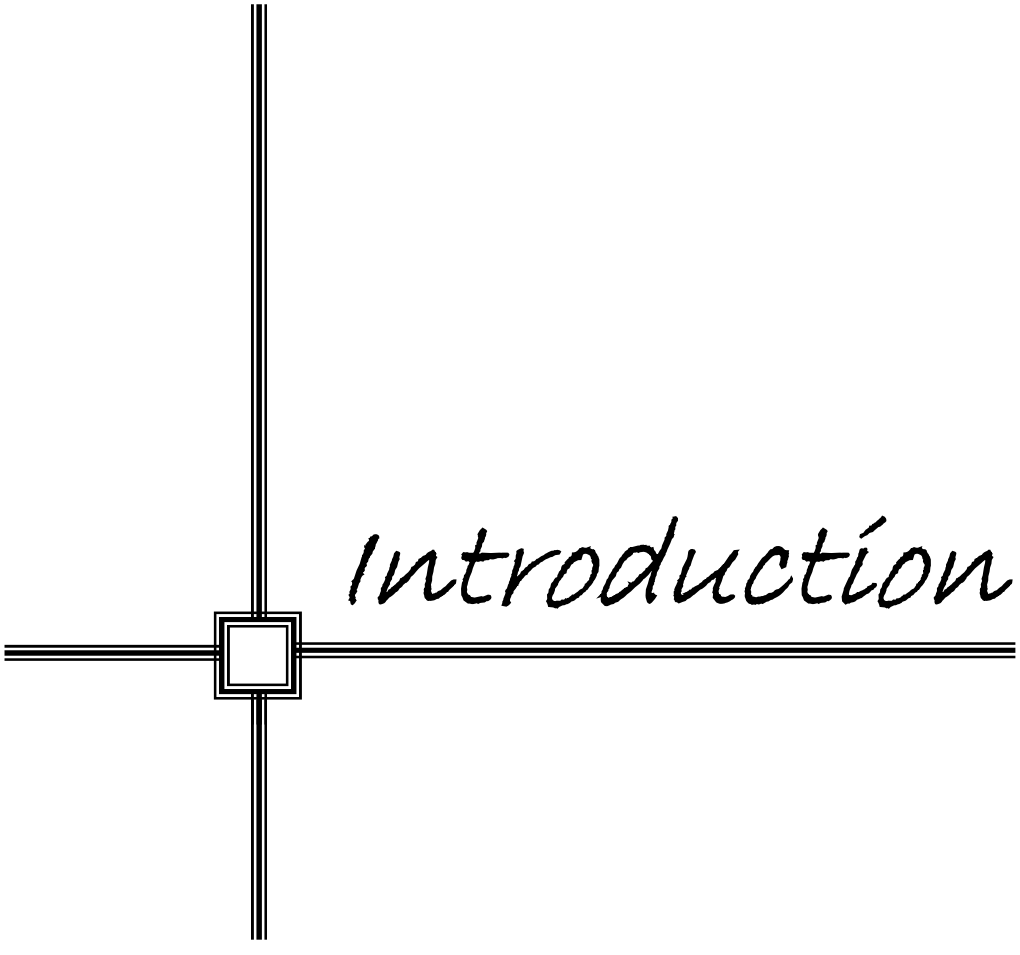
Sl. no	Ip.no	Age & sex	0nset	Site	Anti DM Rx	FBS	X ray	c/s	Initial Area in mm^2	Final Area in mm^2	IA-FA= CA	%Area Reduct-ion
1	219857	38/M	T	P	I	132	N	NOGC	2192.003	1322.8738	869.1292	39.65
2	222965	60/M	T	D	O	99	N	NOGC	3181.937	2060.9639	1120.9740	35.23
3	227159	52/M	S	P	I	123	N	NOGC	3563.038	2110.0311	1453.0060	40.78
4	229075	48/M	T	D	O	101	N	NOGC	1783.993	1090.5549	693.4390	38.87
5	230880	60/M	S	D	I	76	N	NOGC	2735.654	1656.4385	1097.2150	39.45
6	232708	58/F	T	P	I	122	N	NOGC	1563.836	942.3675	621.4690	39.74
7	233827	53/M	T	D	O	99	N	PM	2536.974	1625.6929	911.2811	35.92
8	241232	48/M	T	P	I	146	N	NOGC	1535.736	890.2661	645.4699	42.03
9	234504	56/M	T	P	O	111	N	NOGC	1432.388	881.6348	550.7532	38.45
10	236175	56/M	S	D	I	100	N	NOGC	2536.84	1592.3745	944.4700	37.23
11	236223	57/M	T	P	O	150	N	PA	1535.736	890.2661	645.4699	42.03
12	236258	60/F	T	D	I	97	N	NOGC	1432.388	881.6348	550.7532	38.45
13	234212	49/M	S	D	I	90	N	NOGC	3231.937	2010.9112	1221.0260	37.78
14	239592	53/F	T	P	O	88	N	NOGC	2435.836	1340.197	1095.6390	44.98
15	223132	40/M	T	P	I	98	N	NOGC	3216.073	1729.2825	1486.7900	46.23
16	248033	58/F	T	P	I	133	N	EC	2433.947	1498.0944	935.8520	38.45
17	253971	57/F	S	P	I	140	N	NOGC	2433.764	1517.9386	915.8250	37.63
18	208634	44/M	S	D	I	88	N	NOGC	3546.836	2282.7436	1264.0920	35.64
19	207649	52/M	T	D	I	129	N	NOGC	2435.635	1393.6703	1041.9600	42.78
20	260322	55/M	T	P	O	133	N	NOGC	2194.937	1241.0174	953.9205	43.46
21	232263	39/F	S	P	I	93	N	NOGC	3425.846	1971.5744	1454.2716	42.45
22	237616	60/M	S	P	I	133	N	PM	3487.833	2108.7438	1379.0900	39.54
23	238154	58/M	T	D	O	79	N	NOGC	2369.837	1378.2972	991.5398	41.84
24	260322	50/F	T	D	I	147	N	NOGC	3598.937	2273.4485	1325.4885	36.83
25	201249	51/M	T	P	I	111	N	NOGC	3563.038	2110.0311	1453.0060	40.78
26	242204	59/M	S	P	I	132	N	NOGC	1783.993	1090.5549	693.4390	38.87
27	260301	45/F	T	D	O	99	N	NOGC	2735.654	1656.4385	1097.2150	39.45
28	263488	56/F	T	D	I	100	N	NOGC	1563.836	942.3675	621.4690	39.74
29	251204	59/F	T	P	O	122	N	NOGC	2536.974	1625.6929	911.2811	35.92
30	242333	55/F	S	P	I	97	N	KP	3369.889	1956.8945	1412.9940	41.93
31	265166	70/M	T	P	I	176	N	NOGC	2713.354	1610.3756	1102.9840	40.65
32	263628	65/M	S	D	I	145	N	NOGC	2536.84	1592.3745	944.4700	37.23
33	183375	45/F	T	D	I	172	N	NOGC	1535.736	890.2661	645.4699	42.03
34	202324	62/M	T	D	O	187	N	NOGC	1432.388	881.6348	550.7532	38.45
35	269241	37/F	T	D	I	156	N	NOGC	3231.937	2010.9112	1221.0260	37.78
36	209826	45/M	S	P	O	167	N	NOGC	2365.992	1450.3531	915.6389	38.72
37	214729	63/M	T	P	O	180	N	NOGC	2345.874	1495.2601	850.6130	36.26
38	217855	52/M	S	D	I	178	N	SA	3563.038	2110.0311	1453.0060	40.78
39	219375	70/F	T	P	I	190	N	NOGC	1783.993	1090.5549	693.4390	38.87
40	219377	52/M	T	P	I	190	N	NOGC	2735.654	1656.4385	1097.2150	39.45

MASTER CHART-CONTROL GROUP

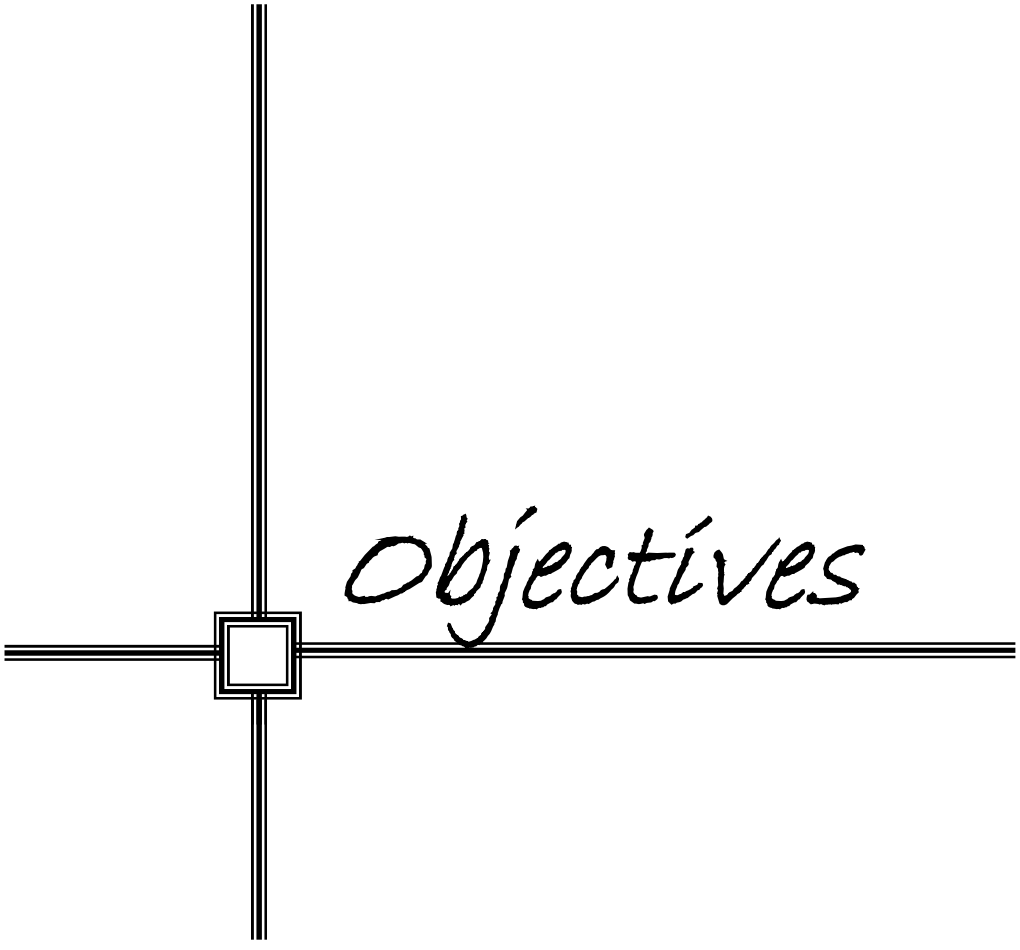
Sl No.	I P No.	Age/ Sex	Onset	Site	Anti-DM Rx	FBS	X-Ray	C/s	Initial Area mm^2	Final Area mm^2	IA - FA = CA	% Area Reduct-ion
1	268152	52/M	T	P	O	146	N	NOGC	3546.882	3186.164	360.718	10.17%
2	221849	39/M	T	P	I	111	N	NOGC	3134.45	2845.141	289.309	9.23%
3	227787	58/M	S	P	I	122	N	NOGC	2673.993	2360.066	313.927	11.74%
4	216120	60/F	S	D	I	123	N	NOGC	3547.273	3078.678	468.595	13.21%
5	232353	49/F	T	P	I	146	N	PA	2534.45	2271.881	262.569	10.36%
6	201208	27/M	T	D	I	133	N	NOGC	1344.994	1153.013	191.981	14.27%
7	241008	50/M	T	P	O	145	N	NOGC	3564.98	3217.038	347.942	9.76%
8	238546	45/F	T	P	I	186	N	NOGC	3365.45	2875.441	490.009	14.56%
9	239424	55/F	T	P	O	127	N	NOGC	2673.83	2402.703	271.127	10.14%
10	241106	56/M	T	D	I	144	N	PM	2893.003	2619.324	273.679	9.46%
11	229456	54/M	S	D	I	122	N	NOGC	2663.748	2228.491	435.748	16.34%
12	214484	50/M	T	P	O	134	N	NOGC	2513.734	2278.699	235.035	9.35%
13	241106	56/F	T	P	I	139	N	NOGC	2436.748	2163.101	273.647	11.23%
14	240975	48/M	T	P	I	98	N	NOGC	3226.56	2895.514	331.046	10.26%
13	210860	52/M	S	P	I	190	N	NOGC	3456.643	3123.768	332.875	9.63%
16	212891	59/M	T	D	I	95	N	EC	2263.744	1934.142	329.602	14.56%
17	209811	55/F	T	P	O	127	N	NOGC	2673.83	2402.703	271.127	10.14%
18	206782	58/M	T	P	I	144	N	NOGC	3428.923	2961.903	467.023	13.62%
19	201281	46/M	S	D	I	122	N	NOGC	2283.485	1887.528	395.957	17.34%
21	201287	58/M	T	P	I	111	N	NOGC	3546.875	3147.496	399.406	11.26%
22	192970	53/F	T	D	I	140	N	PA	3317.992	2941.731	376.261	11.34%
23	231432	57/M	T	P	I	119	N	NOGC	1945.644	1766.061	179.583	9.23%
24	192239	45/M	T	D	I	146	N	NOGC	1925.773	1662.905	262.868	13.65%
24	201266	55/F	S	D	O	150	N	NOGC	3187.093	2780.736	406.357	12.75%
25	212432	38/F	T	D	O	128	N	NOGC	1672.934	1391.379	281.545	16.83%
26	213553	56/F	T	P	I	139	N	KP	2436.748	2163.101	273.647	11.23%
27	215043	55/F	S	D	I	142	N	NOGC	2298.47	2019.205	279.265	12.15%
28	199874	59/F	T	P	I	134	N	NOGC	2778.385	2397.189	381.196	13.72%
29	212451	64/F	T	P	I	129	N	NOGC	1562.737	1416.777	145.96	9.34%
30	200928	53/F	T	D	I	140	N	NOGC	3317.992	2941.731	376.261	11.34%
31	233901	56/M	S	P	I	156	N	PM	3221.09	2939.566	281.524	8.74%
32	199464	45/F	S	D	O	172	N	NOGC	2812.802	2439.824	372.978	13.26%
33	198004	56/F	T	D	O	163	N	NOGC	2783.289	2426.193	357.096	12.83%
34	217855	36/M	S	P	O	154	N	NOGC	2436.118	2238.061	198.057	8.13%
35	234562	60/M	S	D	I	89	N	SA	2611.902	2185.639	426.263	16.32%
36	241226	58/M	T	P	I	183	N	NOGC	3085.87	2794.255	291.87	9.45%
37	219000	36/M	T	P	O	133	N	NOGC	2663.985	2411.972	252.013	9.46%
38	221122	60/M	S	D	I	106	N	NOGC	2611.902	2185.639	426.263	16.32%
39	241114	58/M	T	P	I	99	N	NOGC	3085.87	2794.255	291.87	9.45%
40	243007	36/M	T	P	O	133	N	NOGC	2663.985	2411.972	252.013	9.46%

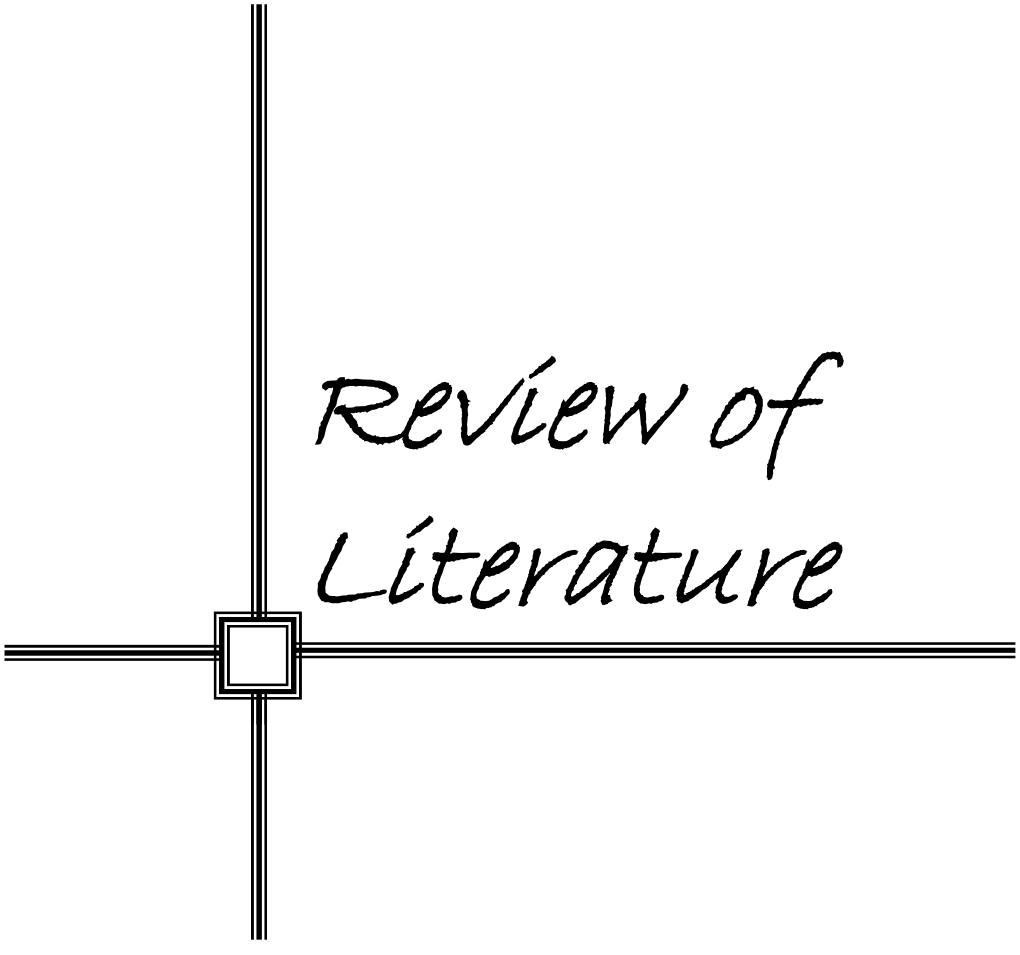
Key for using the master sheet

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



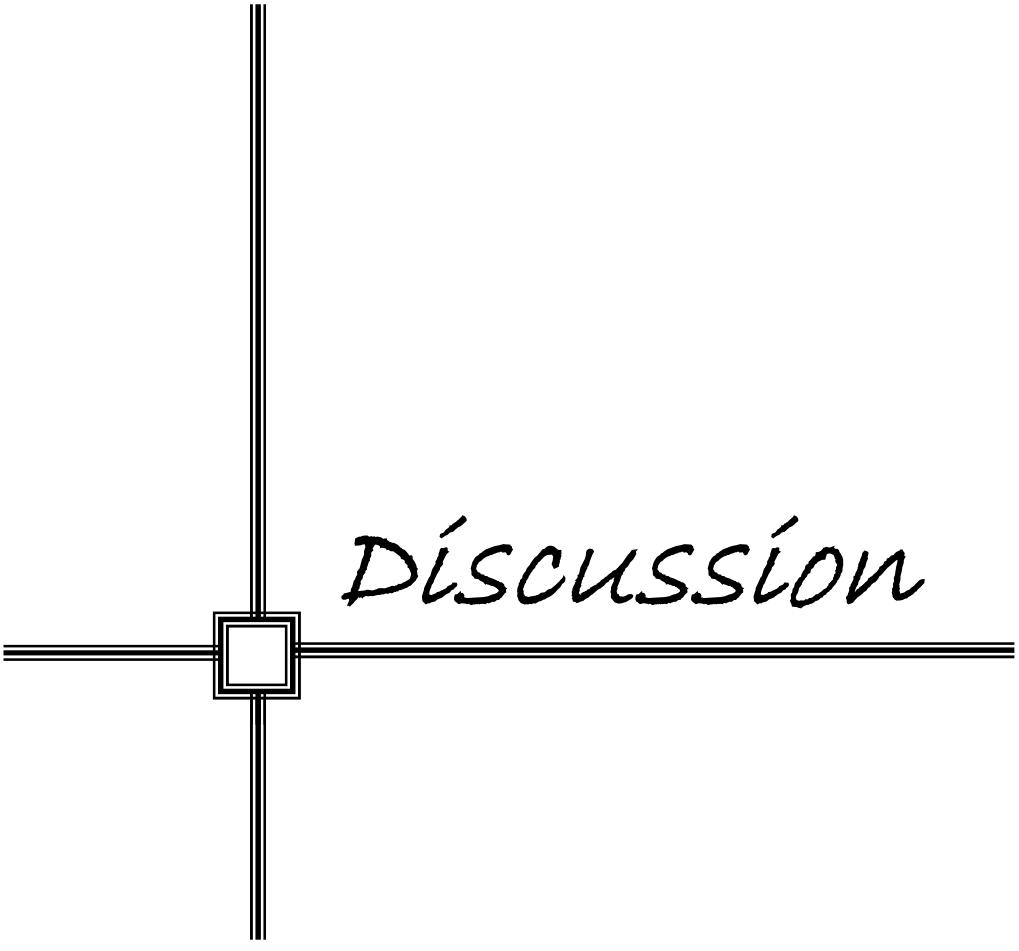
Introduction

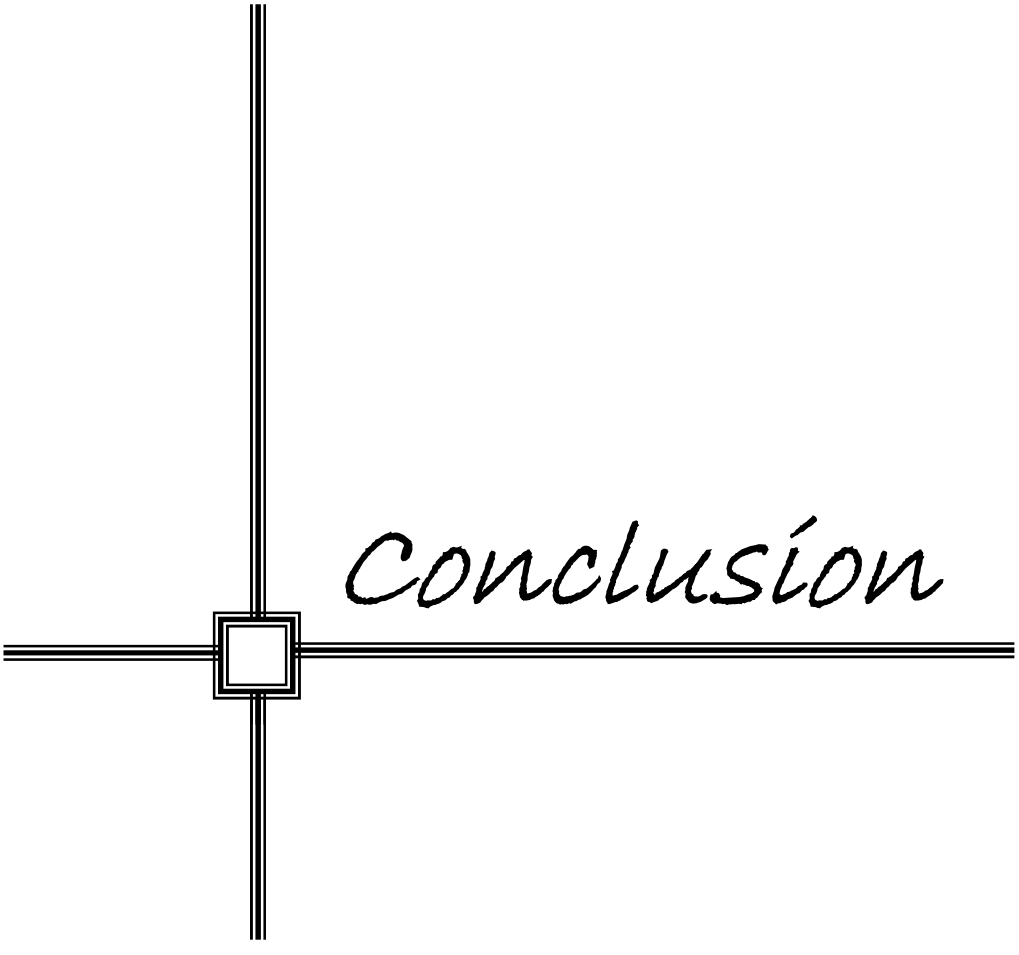

Objectives

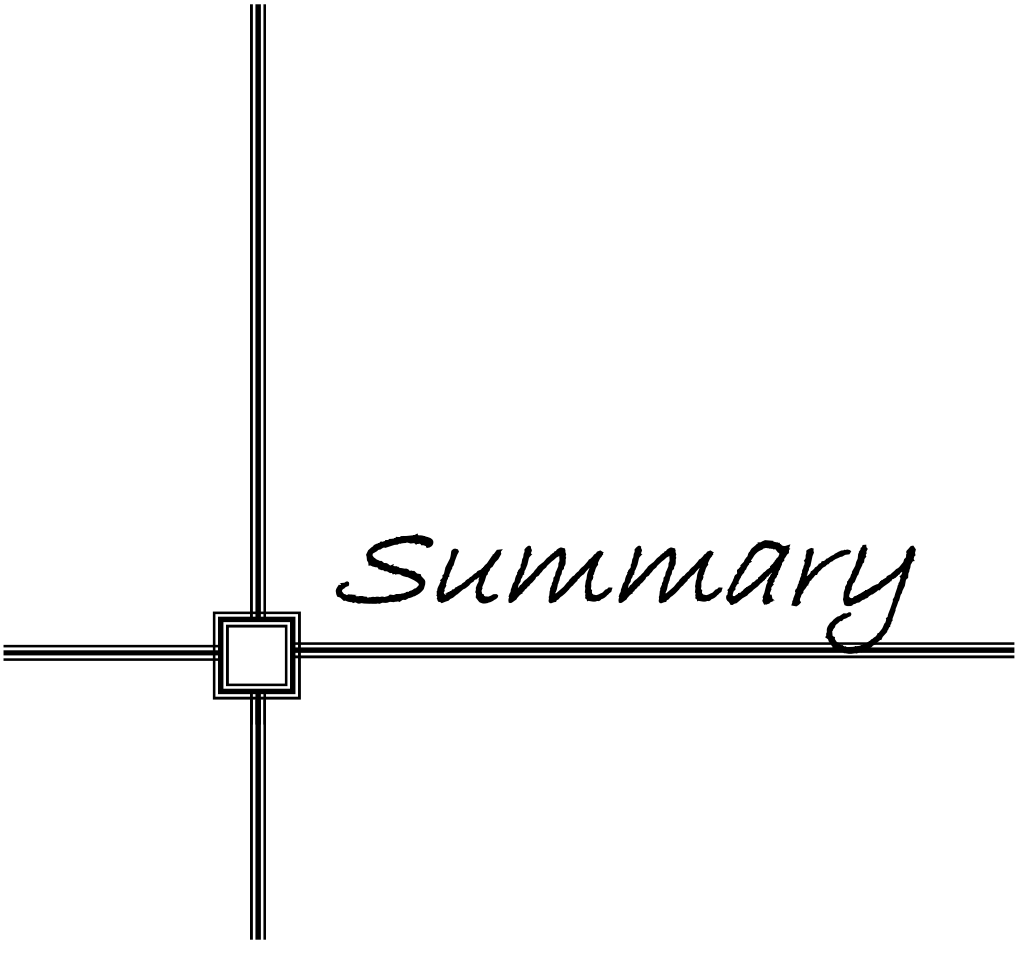

Review of
Literature

Methodology

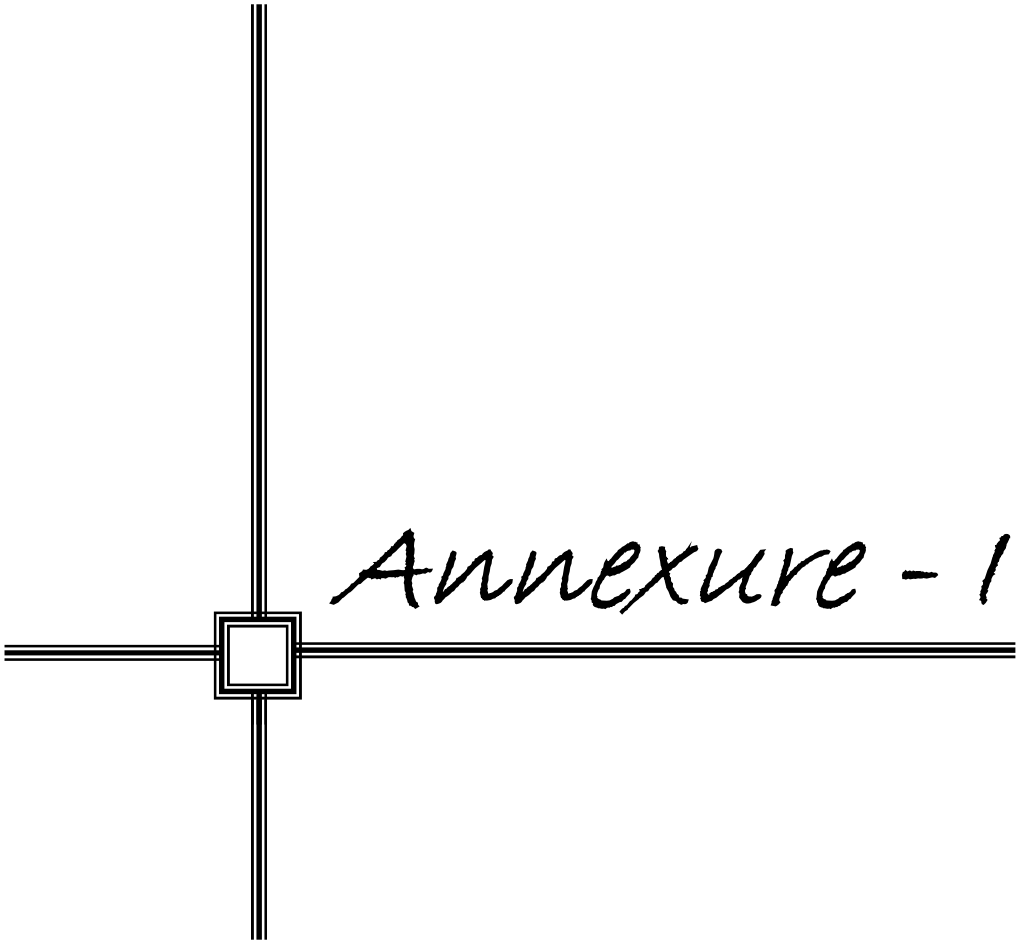
Results


Discussion

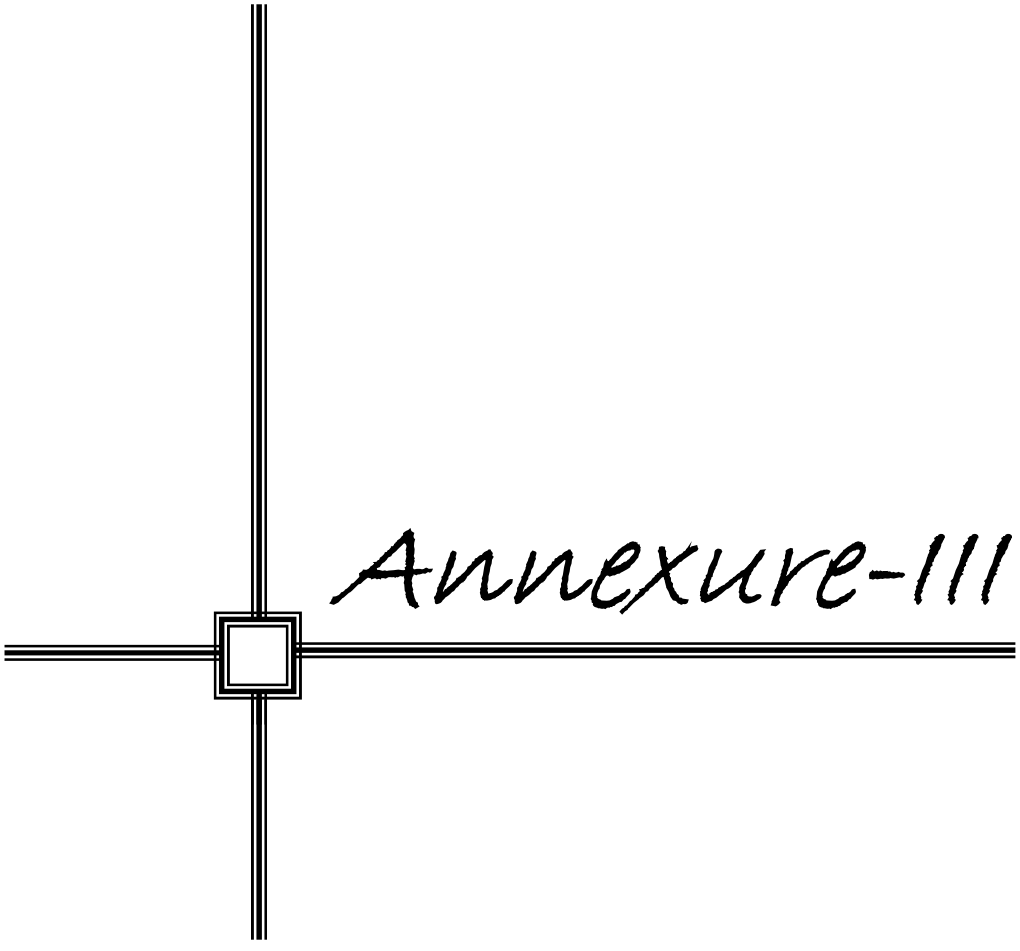

Conclusion

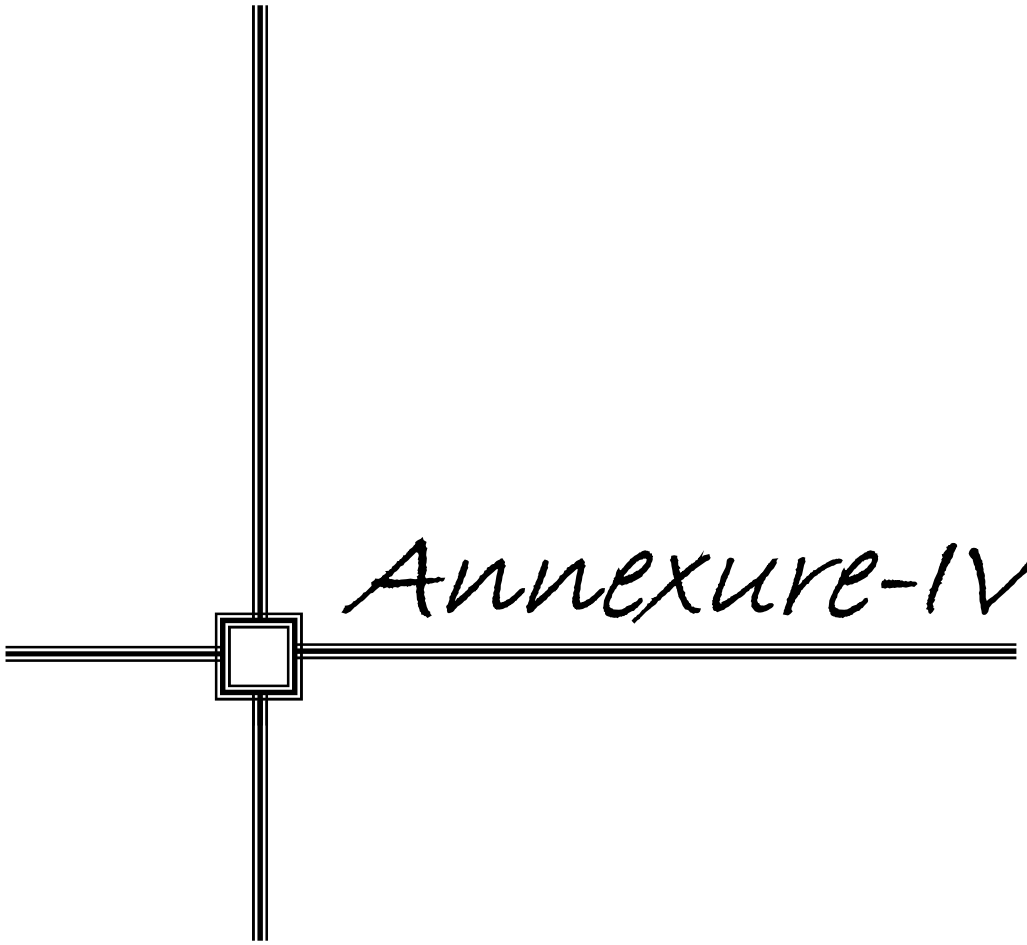

Summary

Bibliography


Annexure - I

Annexure - II


Annexure-III


Annexure-IV