EFFICACY OF RECOMBINANT EPIDERMAL GROWTH FACTOR IN THE HEALING PROCESS OF DIABETIC ULCER

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ABSTRACT

OBJECTIVES

- To evaluate the efficacy of the Recombinant Epidermal Growth Factor in the healing of diabetic ulcer.
- To assess the tolerability of the Recombinant Epidermal Growth Factor in the diabetic ulcer management.

METHODS

Study Design: Randomized, interventional, prospective, comparative study.
Study Centre: Department of General Surgery and Department of Diabetology, Thanjavur Medical College Hospital, Thanjavur.
Study Period: February 2014-August 2015.
Duration of Study: 18 Months.
Sample Size: 60 (30+30), Study group A-30, Study group B-30.
Sample Drug: Recombinant Epidermal Growth Factor.

Selection Criteria.

Inclusion Criteria

1. Age - 20-70 years. 2. Both sexes. 3. Fasting blood sugar <130 mgs/dL and postprandial blood sugar >130 mgs/dL <180 mgs/dL with treatment. 4. Patients with Grade 2 diabetic ulcer. 5. Patients with diabetes along with comorbidities like hypertension with BP <140/90 mmHg with antihypertensives, anaemia with haemoglobin between 8-10 gms%, chronic renal failure Stage 1 and 2, CAHD patients on drugs with no symptoms at present. 6. Palpable peripheral pulses or Doppler showing flow in peripheral vessels. 7. Patients who give consent and are willing for regular followup.

Exclusion Criteria

1. Patients with extensive gangrenous changes. 2. Patients with vascular occlusion (Absent peripheral pulses or no flow in peripheral vessels in the Doppler study). 3. Patients with diabetic ketoacidosis or with fasting blood sugar >130 mgs/dL, post-prandial blood sugar >180 mgs/dL in spite of treatment. 4. Patients with diabetes with hypertension with BP >140/90 mmHg, anaemia with haemoglobin <8 gms%, chronic renal failure with stage 3, 4, 5 congestive heart failure and recent onset myocardial infarction. 5. Pregnant or breastfeeding women. 6. Not willing for the study or not willing for regular visits.

CONCLUSION

The conclusion of this study is that Recombinant Epidermal Growth Factor is effective in the healing of diabetic ulcers at significantly higher rate. No adverse reactions are encountered. It is tolerated well by all the patients.

KEYWORDS

Diabetic Ulcers, Recombinant Growth Factor, Lower Limb Ulcers.

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INTRODUCTION

Wound healing is a process that leads to tissue restoration and normal functioning. It is synonymous with an organism's defence response to injury, which leads to reformation of the organ's defect by the initiation of chain reactions of pathways.

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Once a Tissue is Injured, the response to Injury Occurs in Either of the Two Ways.

1. Formation of scar-different cellular matrix filling the defect, or
2. Regeneration of tissue - complete replacement of the original architecture of the tissue.

The Stages in Wound Healing are Sequenced as.[1]

1. Phase of inflammation.
2. Phase of cellular proliferation.
3. Phase of maturation and remodeling.

Inflammation is initiated by a constellation of factors such as growth factor, proteases, kinins, cytokines, eicosanoids and cellular metabolites. All these factors set in motion the wound stabilization, infection eradication and tissue replacement. All these process depend on the regulation of inflammatory mediators.

The topic of study, the growth factors or modulating factors, are peptides involved in signalling which are increasingly produced during inflammation and target non-haematopoietic cells. The factors of prime importance are Fibroblast Growth Factor (FGF), Insulin like Growth Factor (IGF), Platelet Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF), Epidermal Growth Factor (EGF) and Transforming Growth Factor beta. They act locally on the target site without systemic side effects.

These healing cycles in inflammation are extremely deranged in longstanding wounds like diabetic foot ulcer, which lead to failure of epithelialization and granulation tissue formation. These failures along with neuropathy and joint instability (Biomechanical) result in debilitating ulcer further exaggerated by arterial insufficiency (30-60%) and absence of strict glucose regulation.

The wide reaching negative social impact of non-healing chronic diabetic ulcers is emphasized by the estimate that 70 million of the world population might be affected with diabetes by 2025.[2] and these people will be physically and socially handicapped if they develop foot problems, which demands increased hospitalization and also the fact that chronic diabetic foot ulcers accounts for 20-25% of all hospital stays.

A novel approach for combating longstanding diabetic wounds is the external application of the growth factors, which hastens healing in impaired models of wound healing.

A vital cog in the wheel of wound healing, Epidermal Growth Factor has profound implication in fibroblast function, keratinocyte migration and formation of granulation tissue. Its earlier limitation of rapid degradation in chronic wound surroundings has been overcome by protein stabilizing drug delivery system.

Hence, this study has been done with the view to evaluate the efficacy of Epidermal Growth Factor in accelerating the healing of diabetic foot ulcers. This study also compares the Epidermal Growth factor vs the standard therapy of wash with antiseptic solution and moist saline dressing in healing of diabetic ulcer disease.

Risk Factors for Ulceration

The below table shows the risk factors in diabetic foot.

Relative Risk/Odds Ratio	Systemic Risk Factors
1.02 to 2.13	Hypertension
1.02 to 6.4	Hyperlipidaemia
1.3 to 3.2	Hyperglycaemia
2.6 to 5.2	Male sex
1.07 to 3.7	Retinopathy
2	Above 65 years
1.9	Poor vision
1.2	Obesity
2.4	Proteinuria

Relative Risk/Odds Ratio	Local Risk Factors
1.6 to 18	History of foot ulcer or amputation
3.3 to 3.5	Structural foot deformity
2.0 to 5.9	Abnormal foot pressures
2.2 to 18.4	Sensory neuropathy
2.4 to 3	Peripheral vascular disease

The Epidermal Growth Factor

It is discovered by Stanley Cohen and Rita Levi Montalcini.[3] They won the Nobel Prize in Medicine and Physiology for their discovery. It is a peptide with low molecular weight. It has 53 amino acids. Its molecular weight is 6045 Da. It lacks three amino acids namely - lysine, phenylalanine, alanine.[4] It was first isolated from the submandibular gland of mouse. The Epidermal Growth Factor belongs to a group of family called the EGF family. It also includes TGF-alpha, Betacellulin, Epigen, Epiregulin, Amphiregulin, Heparin binding EGF. The gene coding EGF is located in chromosome.[4]

Prepro EGF is a transmembrane precursor protein with a mature sequence in the extracellular domain. Cleavage of Arg/His and Arg/Asp dipeptide bonds at N and C terminal ends resulting in the formation of soluble peptide.[5] It has two domains.

- N terminal-major antiparallel beta sheet structures and two of the disulphide bonds.
- C terminal - double hairpin structure, a minor antiparallel beta sheet and the third disulphide bond.
- The gene coding EGF is located in chromosome.[4]

Sl. No.	Action of EGF	Clinical Uses	Trial
1.	Smooth muscles, epithelial cell and fibroblast proliferation	Wound healing.[6] in diabetic ulcer, venous ulcer, healing of burns wound	Human trial
2.	Maintains integrity of oesophageal and gastric mucosa, inhibits gastric acid secretion, stimulation of DNA synthesis, mucosal protection.[7]	Healing of duodenal ulcers, also being tried in Zollinger-Ellison syndrome	Human trial
3.	Increased proliferation of duodenal, jejunal cells and increased transport of nutrients across the small intestine.[7]	Intestinal atrophy, Crohn's, after massive enterectomy, also tried in necrotizing enterocolitis.	Animal trial
4.	Decreased bacterial translocation from the gut in acute pancreatitis.[7]	Preventing systemic infection	Animal trial
5.	EFGR is over-expressed in high-grade gliomas and muscle invasive urothelial cancer of bladder.[8]	Targeting tumour cells to decreases tumour load	Animal trial
6.	Epithelial regeneration in wounded cornea along with hyaluronic acid.[8]	Repair of eye damage	Animal trial
7.	Regeneration of hepatocytes.[7]	Repair of liver injury	Animal trial

8.	Closure of tympanic membrane perforation		Human trial
9.	Active sodium transport and clearance of lung fluid	Decreases pulmonary oedema	Animal trial
10.	Enhances the sensitivity of many cancer cells (Ovary, head and neck, cervix, colon, pancreas, prostate, lung) to drugs like cisplatin, 5-FU, melphalan.[8]	Potentiating anti-cancer agents	Human trial
11.	Inhibits enteropathogenic E. coli colonization	Traveller's diarrhoea	Human trial
12.	Tissue repair and regeneration, formation of collagen and elastic fibres.	Anti-ageing cream	

MECHANISM OF ACTION OF EGF.[9,10,11,1]

Mechanism of Action of Egf

Epidermal growth factor + Epidermal growth factor receptor

↓

Activation of tyrosine kinase activity

↓

Signal transduction cascade

↓

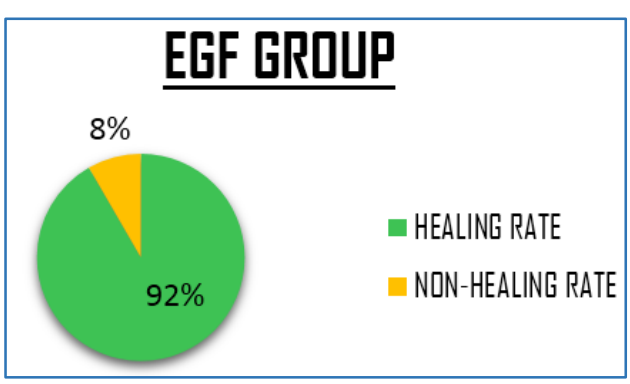
Results in increased intracellular calcium, increased glycolysis increased protein synthesis and increased expression of certain genes.

Biochemical Changes Occurring at the Cellular Level

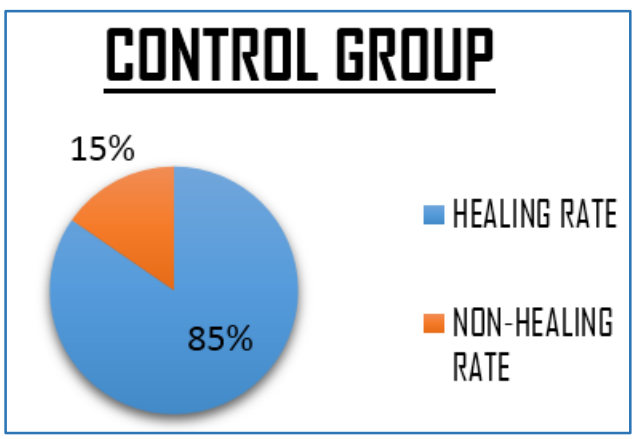
The Epidermal Growth Factor results in the increased transport of aminoisobutyric acid and uridine into cells. The incorporation of uridine into the RNA is also increased. The stimulation of RNA synthesis is also increased.

RESULTS

1. The rate of healing of ulcers <5 cm are compared between the EGF and the control groups. In EGF group is 91.67% and in the control group is 84.62%.
2. The rate of healing of ulcers >5 cm are compared between the two groups. In EGF group is 83.33% and in control group is 52.94%.
3. Overall, the rate of healing of ulcers in the EGF group are compared with the control group. Rate of healing in EGF group is 86.67% and control group is 66.67%. Next, the duration of healing of the ulcers are compared between the two groups. Those ulcers which are not healed by 15 weeks and those that are treated by skin grafting are considered as non-healing and are not considered in the T test.



Healing Rate and Non-Healing Rate in EGF Group



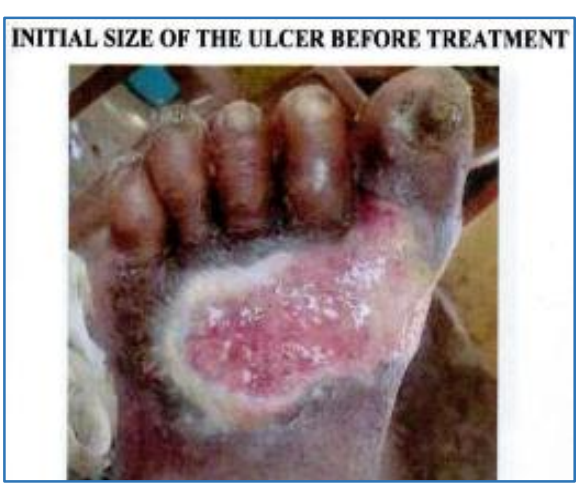
Healing Rate and Non-Healing Rate in Control Group

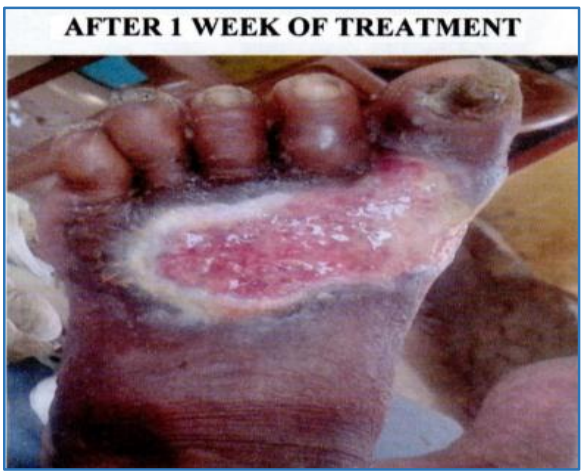
DISCUSSION

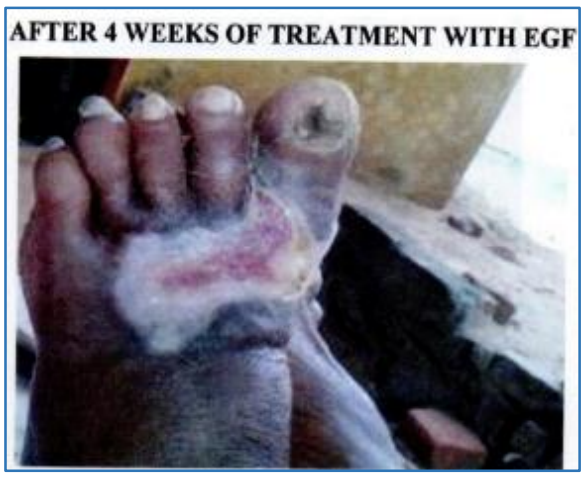
The rate of healing of ulcers <5 cm in the EGF treated group is significantly greater than in the control group. The rate of healing of ulcers >5 cm in the EGF treated group is significantly greater than in the control group. Overall, the rate of healing of ulcers in the EGF treated group is significantly greater than in the control group.

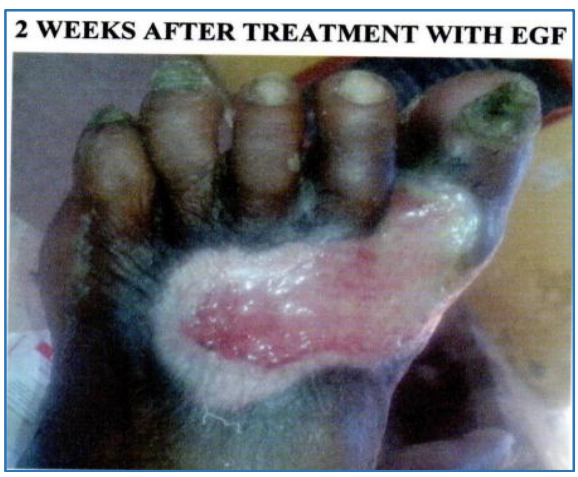
CONCLUSION

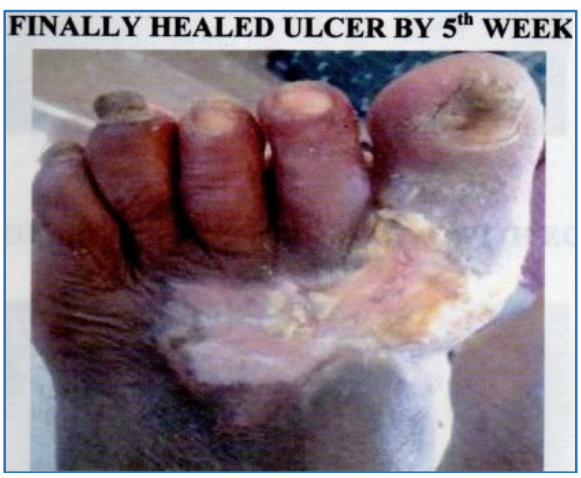
The conclusion of this study is that Recombinant Epidermal Growth Factor is effective in the healing of diabetic ulcers at a significantly higher rate. No adverse reactions are encountered. It is tolerated well by all the patients.

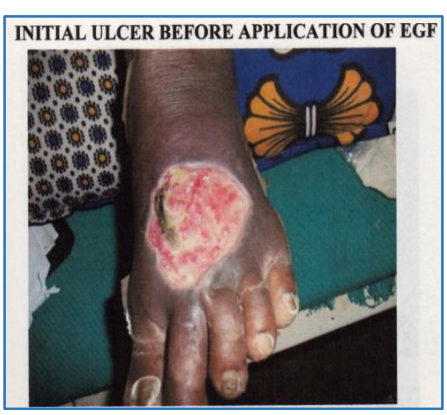


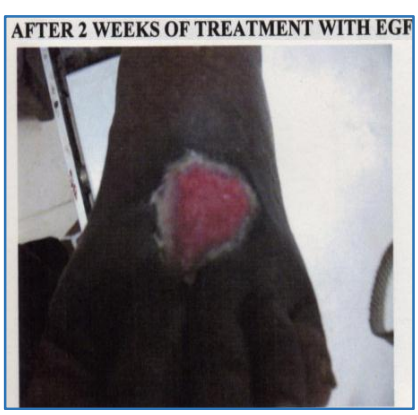

AFTER 1 WEEK OF TREATMENT

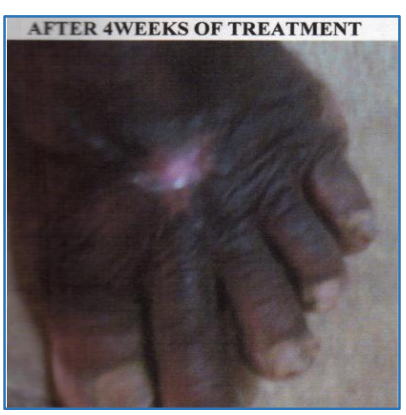

AFTER 4 WEEKS OF TREATMENT WITH EGF

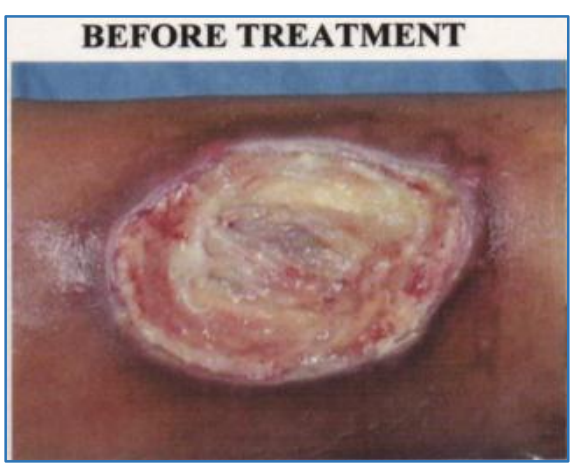

2 WEEKS AFTER TREATMENT WITH EGF

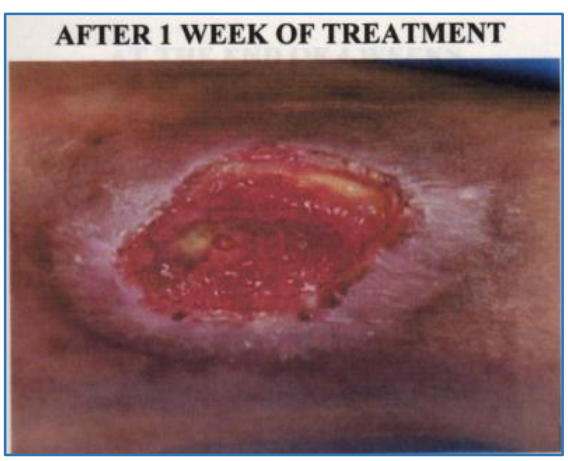

FINALLY HEALED ULCER BY 5th WEEK


INITIAL ULCER BEFORE APPLICATION OF EGF


AFTER 2 WEEKS OF TREATMENT WITH EGF


AFTER 4WEEKS OF TREATMENT


BEFORE TREATMENT


AFTER 1 WEEK OF TREATMENT

Sl. No.	AGE /SEX	IP NO.	ULCER SITE	DOPPLER	NEURO-PATHY	RISK FACTORS	PUS CULTURE	SENSITIVITY	HEALED OR NOT	DURATION
1.	53/M	1478345	DORSUM OF R FOOT	MONOPHASIC-PT, DP	YES	NO	E. COLI, KLE	AMI	NOT HEALED	-
2.	30/M	1458656	DORSUM OF R FOOT	MONOPHASIC FLOW-DP	NO	HT	STAPH	CIPRO	HEALED	9 WEEKS
3.	38/F	1383872	DORSUM OF L FOOT	BIPHASIC FLOW-PT, DP	NO	ANAEMIA	STAPH	CIPRO, AMP	HEALED	12 WEEKS
4.	54/M	1466846	DORSUM OF L FOOT	NORMAL	YES	NO	-	-	HEALED	5 WEEKS
5.	28/F	1463707	PLANTAR R FOOT	MONOPHASIC-PT, DP	NO	HT	ANAEROBE	METRO	HEALED	10 WEEKS
6.	60/F	1464852	DORSUM OF R FOOT	BIPHASIC-PA, PT, DP	NO	CRF/ ANAEMIA	-	-	HEALED	7 WEEKS
7.	38/M	1458577	DORSUM OF R FOOT	NORMAL	YES	NO	E. COLI	CE	HEALED	8 WEEKS
8.	55/M	1476472	DORSUM OF L FOOT	MONOPHASIC - PA,PT,DP	NO	HT/CAHD	STREP	P	GRAFTING	-
9.	60/M	1469248	PLANTAR R FOOT	MONOPHASIC-PT,DP	NO	CRF	ANAEROBE	METRO	NOT HEALED	-
10.	68/F	1496923	DORSUM OF L FOOT	NORMAL	YES	NO	-	-		
11.	59/M	1478281	DORSUM OF R FOOT	NORMAL	NO	CAHD	STREP	CE, AMP	HEALED	9 WEEKS
12.	55/M	1410363	DORSUM OF R FOOT	BIPHASIC FLOW-PT,DP	NO	CRF/ANAEMIA	STREP	CE/CIPRO	GRAFTING	-
13.	52/M	1481042	DORSUM OF L FOOT	NORMAL	YES	NO	KLEB	AMI, GM	HEALED	9 WEEKS
14.	57/F	1448401	DORSUM OF R FOOT	MONOPHASIC-PT,DP	NO	CAHD	-	-	HEALED	12 WEEKS
15.	45/M	1464058	DORSUM OF L FOOT	BIPHASIC FLOW-PT,DP	YES	CRF	FUSIFORM	CIPRO, AM	HEALED	7 WEEKS
16.	37/F	1447369	DORSUM OF L FOOT	NORMAL	YES	NO	E. COLI, STREP	CE, SEPTRAN	HEALED	6 WEEKS
17.	68/M	1473251	DORSUM OF R FOOT	MONOPHASIC-PT,DP	YES	HT/CAHD	-	-	HEALED	8 WEEKS
18.	30/F	1465940	ANT R LEG	BIPHASIC-PT, DP	NO	ANAEMIA	E. COLI	CIPRO, AMP	HEALED	12 WEEKS
19.	23/F	1379606	R ANKLE DORSUM	NORMAL	YES	NO	-	-	HEALED	8 WEEKS
20.	40/M	1460953	DORSUM OF R FOOT	MONOPHASIC-PT,DP	NO	HT	KLEB	CE, DOXY	HEALED	6 WEEKS
21.	50/F	1416321	PLANTAR L FOOT	BIPHASIC FLOW-PT,DP	NO	CRF	KLEB	P, DOXY	HEALED	8 WEEKS
22.	37/F	1396855	DORSUM R FOOT	BIPHASIC FLOW-PT,DP	NO	HT/ANAEMIA	E. COLI	GM,AMI	HEALED	12 WEEKS
23.	31/F	145948	DORSUM R FOOT	MONOPHASIC-DP	NO	NO	PSEUDO	CE,P	HEALED	5 WEEKS
24.	50/F	1475622	PLANTAR L FOOT	BIPHASIC-DP	YES	CAHD	E. COLI	CIPRO	HEALED	12 WEEKS
25.	40/F	1474135	DORSUM L FOOT	BIPHASIC FLOW-PT,DP	NO	HT	-	-	HEALED	9 WEEKS
26.	49/M	1410359	LAT R LEG	MONOPHASIC-PT,DP	YES	CAHD/ANAEMIA	STAPH	CE,AMP	HEALED	5 WEEKS
27.	65/M	1459538	DORSUM R FOOT	BIPHASIC-PT,DP	NO	HT/CRF	E. COLI	GM,AMI	HEALED	7 WEEKS
28.	55/M	1465923	ANT L LEG	BIPHASIC FLOW-DP	NO	CAHD/CRF	STREP	CE,P,CIPRO	HEALED	10 WEEKS
29.	40/F	1484503	DORSUM OF L FOOT	NORMAL	NO	NO	-	-	HEALED	10 WEEKS
30.	68/M	1461280	DORSUM OF R FOOT	MONOPHASIC -PT,DP	NO	NO	-	-	HEALED	4 WEEKS

Sl. No.	AGE /SEX	IP NO.	ULCER SITE	DOPPLER STUDY	NEURO-PATHY	RISK FACTORS	PUS CULTURE	SENSITIVITY	HEALED/ NOT	DURA-TION
1.	65/F	1448391	DORSUM L FOOT	BIPHASIC FLOW-PA,PT,DP	YES	NO	ANAEROBE	METRO	HEALED	10 WEEKS
2.	60/F	1388533	DORSUM OF R FOOT	MONOPHASIC FLOW-PT,DP	NO	CAHD/CRF	-	-	HEALED	12 WEEKS
3.	57/M	1471602	PLANTAR R FOOT	MONOPHASIC FLOW-DP	NO	CRF/HT	KLEB	GM,AMI	NOT HEALED	
4.	35/F	1433179	PLANTAR R FOOT	MONOPHASIC FLOW-PT,DP	NO	HT	-	-	HEALED	8 WEEKS
5.	65/M	1475211	DORSUM R FOOT	NORMAL	YES	NO	E. COLI	CIPRO	NOTHEALED	-
6.	35/M	1465412	PLANTAR R FOOT	NORMAL	YES	CAHD/CRF	PSEUDO	AMI	HEALED	9 WEEKS
7.	49/M	1459617	DORSUM L FOOT	MONOPHASIC FLOW-DP,PT	YES	NO	STREP	CE	HEALED	12 WEEKS
8.	57/F	1444514	DORSUM R FOOT	NORMAL	NO	NO	STREP	CIPRO,DOXY	HEALED	11 WEEKS
9.	20/M	1466657	DORSUM L FOOT	BIPHASIC FLOW-DP	YES	NO	E. COLI	AMI	HEALED	12 WEEKS
10.	50/M	1476512	DORSUM R FOOT	MONOPHASIC FLOW-PA,DP,PT	NO	CAHD/HT	KLEB	CE	HEALED	10 WEEKS
11.	53/M	1461159	DORSUM L FOOT	NORMAL	YES	HT	-	-	HEALED	10 WEEKS
12.	46/M	1482650	DORSUM OF L FOOT	BIPHASIC FLOW-PA.PT,DP	NO	ANAEMIA/ HT	FUSIFORM	GM	NOTHEALED	-
13.	46/F	1422813	DORSUM L FOOT	MONOPHASIC FLOW-PA,DP	NO	CRF	E. COLI,STAPH	CE,CIPRO	HEALED	10 WEEKS
14.	30/F	1462334	PLANTAR L FOOT	MONOPHASIC FLOW-PT,DP	YES	HT	KLEB	P	HEALED	13 WEEKS
15.	29/F	1422837	DORSUM R FOOT	BIPHASIC FLOW-PT,DP	NO	ANAEMIA	E. COLI	GM,AMI	HEALED	7 WEEKS
16.	45/M	1464625	L THIGH	NORMAL	YES	NO	-	-	HEALED	6 WEEKS
17.	61/M	1476330	DORSUM OF L FOOT	MONOPHASIC FLOW-DP	NO	CRF/HT/CAHD	ANAEROBE	METRO	HEALED	9 WEEKS
18.	54/M	1483944	DORSUM L FOOT	MONOPHASIC FLOW- PT,DP	NO	CRF	E. COLI	CE,CIPRO	NONHEALING	
19.	55/M	14675164	PLANTAR L FOOT	BIPHASIC FLOW-DP	NO	CRF/CAHD	STREP	CE,AMI	NON HEALING	
20.	46/F	1385758	DORSUM OF R FOOT	MONOPHASIC FLOW-PT,DP	YES	ANAEMIA	-	-	HEALED	14 WEEKS
21.	40/F	1479293	DORSUM OF L FOOT	NORMAL	YES	NO	PSEUDO	AMI,AMP	HEALED	7 WEEKS
22.	35/F	1479293	DORSUM OF L FOOT	NORMAL	YES	NO	FUSIFORM	GM,SEPTRAN	NON-HEALING	-
23.	40/M	1466951	DORSUM R FOOT	MONOPHASIC FLOW-PT,DP	NO	HT	STREP	P	HEALED	7 WEEKS
24.	60/M	1469012	ANT R LEG	NORMAL	YES	NO	-	-	HEALED	8 WEEKS
25.	50/F	1444341	DORSUM OF R FOOT	MONOPHASIC FLOW-PA, PT, DP	NO	CAHD/ANAEMIA	E. COLI,KLEB	CE,CIPRO	NON-HEALING	-
26.	65/M	1470373	DORSUM OF R FOOT	MONOPHASIC FLOW-PT, DP	NO	ANAEMIA	E. COLI	P,GM	NON-HEALING	
27.	42/M	14722118	DORSUM OF L FOOT	BIPHASIC FLOW-PA, PT, DP	NO	ANAEMIA	ANAEROBE	METRO	NON-HEALING	-
28.	57/M	1468143	DORSUM OF L FOOT	MONOPHASIC FLOW-PT,DP	YES	CAHD,HT	STAPH	CIPRO,AMI	NON-HEALING	
29.	55/F	1479601	DORSUM OF R FOOT	NORMAL	YES	NO	PSEUDO	CIPRO	HEALED	8 WEEKS
30.	35/M	1462911	DORSUM OF R FOOT	BIPHASIC FLOW-PT, DP	NO	NO	KLEB	AMP, GM	HEALED	7 WEEKS

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