“A COMPARATIVE STUDY ON THE EFFICACY OF TOPICAL AUTOHEMOTHERAPY VERSUS TOPICAL AUTOLOGOUS PLATELET RICH PLASMA IN CHRONIC VENOUS LEG ULCERS”

Dissertation Submitted in
Partial fulfillment of the University regulations for

MD DEGREE IN

DERMATOLOGY, VENEREOLOGY AND LEPROSY

(BRANCH XX)

MADRAS MEDICAL COLLEGE

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

INDIA.

APRIL 2017

CERTIFICATE

Certified that this dissertation titled **"A COMPARATIVE STUDY ON THE EFFICACY OF TOPICAL AUTOHEMOTHERAPY Vs TOPICAL AUTOLOGOUS PLATELET RICH PLASMA IN CHRONIC VENOUS LEG ULCERS"** is a bonafide work done by **Dr. BHUVANESWARI.V,** Post graduate student of the Department of Dermatology, Venereology and Leprosy, Madras Medical College, Chennai – 3, during the academic year 2013 – 2017. This work has not previously formed the basis for the award of any degree.

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DECLARATION

The dissertation entitled **"A COMPARATIVE STUDY ON THE EFFICACY OF TOPICAL AUTOHEMOTHERAPY Vs TOPICAL AUTOLOGOUS PLATELET RICH PLASMA IN CHRONIC VENOUS LEG ULCERS"** is a bonafide work done by **Dr. BHUVANESWARI.V** at Department of Dermatology, Venereology and Leprosy, Madras Medical College, Chennai – 3, during the academic year 2013 – 2017 under the guidance of **Professor Dr. R.PRIYAVATHANI, M.D., D.D., D.N.B., M.N.A.M.S.,** Professor, Department of Dermatology, Madras Medical College, Chennai -3.

This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai towards partial fulfillment of the rules and regulations for the award of M.D Degree in Dermatology, Venereology and Leprosy (BRANCH – XX)

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I, **Dr. BHUVANESWARI.V** solemnly declare that this dissertation titled **"A COMPARATIVE STUDY ON THE EFFICACY OF TOPICAL AUTOHEMOTHERAPY Vs TOPICAL AUTOLOGOUS PLATELET RICH PLASMA IN CHRONIC VENOUS LEG ULCERS"** is a bonafide work done by me at Madras Medical College during 2013-2017 under the guidance and supervision of **Prof. U. R. DHANALAKSHMI, M.D., D.D., D.N.B.,** Professor and Head, Department of Dermatology, Madras Medical College, Chennai-600003.

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DATE: 28.09.2016

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SPECIAL ACKNOWLEDGEMENT

My sincere thanks to **Dr. K. MURALITHARAN M.S., M.C.H.,** Dean, Madras Medical College, Chennai-3 for allowing me to do this dissertation and utilize the Institutional facilities.

ACKNOWLEDGEMENT

I am gratefully indebted to the Professor and Head of the Department of Dermatology, **Prof. Dr. U.R. DHANALAKSHMI, M.D., D.D., D.N.B.,** for her advice, guidance and encouragement for my study.

I would like to express my sincere and heartfelt gratitude to **Prof. Dr. S. KALAIVANI, M.D., D.V.,** Director and Professor, Institute of Venereology, for her kindness and support throughout the study.

I sincerely thank **Prof. Dr. R. PRIYAVATHANI, M.D., D.D., D.N.B., M.N.A.M.S.,** Professor of Dermatology for her priceless support. She has been a source of constant motivation and encouragement throughout the study. I am extremely grateful to her for guiding me throughout the study.

I thank my Professor and Head of the Department of Occupational and Contact Dermatitis, **Prof. Dr. S. NIRMALA M.D.,** for her help and support.

I thank **Prof. Dr. S. KUMARAVEL, M.D., D.D.,** Professor of Dermatology for his advice and encouragement.

I thank **Prof. Dr. V. SAMPATH M.D., D.D.,** Professor of Dermatology for his advice and encouragement.

I thank **Prof. Dr. A. RAMESH M.D., D.D., D.N.B.,** Professor of Dermatology for his advice and encouragement.

I am grateful to **Prof. Dr. J. MANJULA, M.D., D.N.B.,** Professor, Department of Dermatology for her invaluable guidance, help and encouragement.

I also extend my sincere thanks to my former Professor and Head of Department **Dr. K. MANOHARAN, M.D., D.D.,** for his advice and encouragement.

I humbly thank my Co-Guide **Dr. K.DEEPA, M.D.D.V.L.** and former Co-Guide **Dr. N.SARAVANAN, M.D.D.V.L., D.C.H.,** Assistant professors, Department of Dermatology for their valuable guidance throughout my work. I would like to express my sincere and heartfelt gratitude for the time which they devoted for my research project.

I extend my gratitude to **Dr.R.MADHU,M.D.,(DERM).,D.C.H., Dr.SAMUEL JAYARAJ DANIEL,M.D.D.V.L., Dr.V.N.S.AHAMED SHARIFF,M.D.D.V.L., Dr.B.VIJAYALAKHSMI,M.D.D.V.L., Dr.K.UMAMAHESWARI,M.D.D.V.L., Dr.R.MANIPRIYA,M.D.D.V.L., D.C.H., and Dr.C.L.CHITRA,M.D.DV.L** Assistant professors, Department of Dermatology for their kind support and encouragement.

I also thank my Assistant Professors **Dr. P. PRABHAKAR,M.D.D.V.L., Dr. C. VIDHYA,M.D.DVL., Dr.R.HEMAMALINI,M.D.D.V.L., Dr.H.DHANASELVI,M.D.D.V.L., Dr.K.GAYATHRI,M.D.D.V.L., Dr.E.BALASUBRAMANIAN,M.D.D.V.L** and **Dr.R.SNEHAVALLI M.D.D.V.L.,** Institute of Venereology for their able guidance.

I am thankful to my colleagues for their support throughout the study. I am also grateful to all paramedical staffs for rendering timely help to complete my study. I am also extremely thankful to my family for their motivation and encouragement. Last but not the least I am profoundly grateful to all patients for their co-operation and participation in this study. They have been the principal source of knowledge which I have gained during the course of my clinical research.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

VLU - Venous leg ulcers

CVU - Chronic venous ulcers

CVI - Chronic venous sufficiency

GSV - Great saphenous vein

SSV - Small saphenous vein

SFJ - Saphenofemoral junction

SPJ - Saphenopopliteal junction

PRP - Platelet rich plasma

PDGF- Platelet derived growth factor

TGF β- Transforming growth factor β

FGF - Fibroblast growth factor

VEGF- Vascular endothelial growth factor

EGF - Epidermal growth factor

KGF - Keratinocyte growth factor

CTGF- Connective tissue growth factor

ABPI - Ankle brachial pressure index

MPFF- Micronized purified flavonoid fraction

PRFM- Platelet rich fibrin matrix

A0- Baseline area of the ulcer

A1- Area of the ulcer at 1st week

A2- Area of the ulcer at 2nd week

A3- Area of the ulcer at 3rd week

A4- Area of the ulcer at 4th week

A5- Area of the ulcer at 5th week

A6- Area of the ulcer at 6th week

V0- Baseline volume of the ulcer

V1- Volume of the ulcer at 1st week

V2- Volume of the ulcer at 2nd week

V3- Volume of the ulcer at 3rd week

V4- Volume of the ulcer at 4th week

V5- Volume of the ulcer at 5th week

V6- Volume of the ulcer at 6th week

PA1- Percentage of improvement in the area of ulcer at 1st week

PA2- Percentage of improvement in the area of ulcer at 2nd week

PA3- Percentage of improvement in the area of ulcer at 3rd week

PA4- Percentage of improvement in the area of ulcer at 4th week

PA5- Percentage of improvement in the area of ulcer at 5th week

PA6- Percentage of improvement in the area of ulcer at 6th week

PV1- Percentage of improvement in the volume of ulcer at 1st week

PV2- Percentage of improvement in the volume of ulcer at 2nd week

PV3- Percentage of improvement in the volume of ulcer at 3rd week

PV4- Percentage of improvement in the volume of ulcer at 4th week

PV5- Percentage of improvement in the volume of ulcer at 5^{th} week

PV6- Percentage of improvement in the volume of ulcer at 6^{th} week

INTRODUCTION

INTRODUCTION

Venous ulcerations of the lower limb are one of the leading causes of leg ulcers. Around 60 -70 % of leg ulcers are due to venous origin[1].The prevalence of venous leg ulcers (VLU) ranges between 0.18% and 1%[2]. Its prevalence increases with age, accounting for about 4% in elderly patients over 65 years[3]. Venous ulcers persisting beyond 6 weeks are known as **chronic venous ulcers**[4] (CVU).They are prone for recurrence with an annual recurrence rate of 6 to 15%.

Venous ulcers are usually situated in the gaiters area and are associated with features of stasis dermatitis, pigmentation, induration, lipodermatosclerosis, atrophie blanche, inverted champagne bottle appearance of the leg. Less commonly they are located in the lateral retro malleolar area when occurring secondary to deep and short saphenous venous reflux.

Chronic venous insufficiency (CVI) resulting in chronic venous ulcer is due to increased venous hypertension over the microcirculation of dermis. Chronic peripheral venous hypertension or chronic ambulatory venous hypertension results from venous reflux in the lower limbs including perforator veins or by neuromusculoskeletal dysfunction of the leg.

The management of CVU is challenging. The majority of patients can be effectively treated and healed, and those with refractory lesions can be helped

with palliative measures. The key factor in the management of chronic ulcers remain proper patient evaluation and correction of the underlying cause once identified. Hence a comprehensive diagnostic evaluation is essential .They are primarily managed by limb elevation, compression bandage, oral drugs like pentoxifylline and diosmin. This is followed by vascular surgery once the ulcer has healed. However in significant number of patients, these methods do not provide satisfactory healing as they do not provide the necessary growth factors that can modulate wound healing.

Hence this study was aimed at comparing the efficacy of the use of topical Autohemotherapy and topical autologous Platelet Rich Plasma in patients with chronic venous ulcerations of the lower limbs.

REVIEW OF LITERATURE

ANATOMY -VEINS OF THE LOWER LIMB[5]

There are two main venous systems draining the lower limb namely the superficial and deep venous system.

The superficial veins are located within the subcutaneous tissue and the deep veins are found deep to the deep fascia.

Superficial Veins of the Lower Limb:

The Great saphenous vein (GSV) and Small saphenous vein (SSV) are two important veins of superficial system (fig.1)

The Great Saphenous Vein:

The Great Saphenous Vein is the largest superficial vein of lower limb. It is formed from medial marginal vein and moves upwards in the leg behind the medial malleolus. In the thigh its courses in the medial aspect before opening into the femoral vein at the saphenous femoral junction. GSV has communication with SSV through perforators.

The Short Saphenous Vein:

The short saphenous vein arises as a continuity of lateral marginal vein and ascends between the superficial and deep fascia in the distal third of the leg. At the midline of the leg, it penetrates the deep fascia and becomes superficial

to the gastrocnemius muscle. It finally terminates in the popliteal vein within the popliteal fossa. It has numerous valves and many communicating veins with GSV.

Deep veins of the lower limb:

The deep veins of the lower limb usually have their course along the arteries. The plantar digital veins and dorsal digital veins join to form four metatarsal veins which again unite to form the plantar venous arch. The medial and lateral plantar veins originate from plantar arch and join to form posterior tibial vein. The anterior tibial veins unite with the posterior tibial vein to form the popliteal vein. The popliteal vein emerges from the adductor magnus opening to become the femoral vein. It receives the SSV and other tributaries. It usually contain four valves. The femoral vein accompanies the femoral artery and is joined by the Profunda femoris vein. Near its termination into the external iliac vein, it is joined by the GSV. The femoral vein contains three valves.

Perforator Veins:

Perforator veins allow communication between the superficial and deep venous system of the legs. It perforates the aponeurosis of the muscle, getting its name. Normally the blood flows from the superficial to the deep veins of the legs through the perforator veins. Perforator veins typically have one to three

valves which are numerous below the knees. These are bicuspid valves and allows unidirectional flow of blood from the superficial to the deep veins. The unidirectional blood flow is also maintained by the oblique course of the perforator veins through the muscle and aponeurosis. When the valves in the perforators become incompetent blood flows from the deep into the superficial veins resulting in venous hypertension and varicosities of the lower extremities.

The clinically important perforators are the Hunterian perforators in the proximal thigh, Dodd perforators in the distal thigh, Boyd perforators situated below the knee, Cockett perforators – upper, middle and lower in the lower leg and May and Kuster perforators in the ankle.(Fig.2)

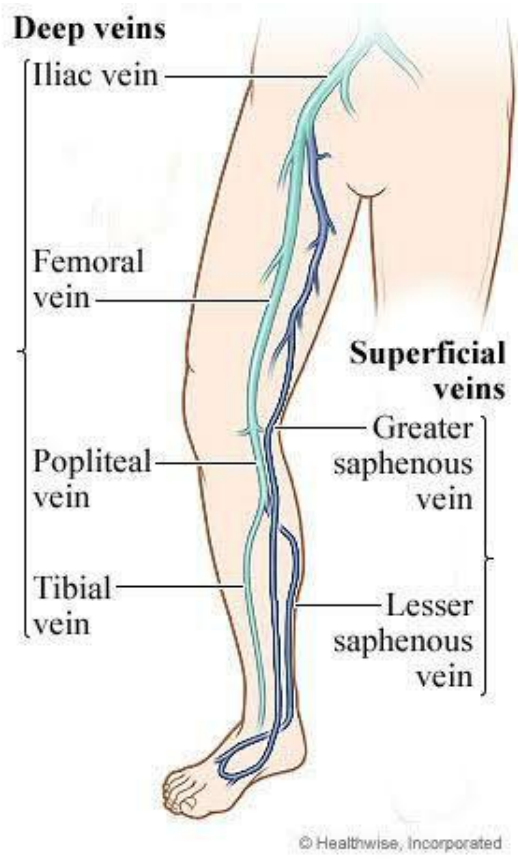


Figure 1: Veins of Lower limb

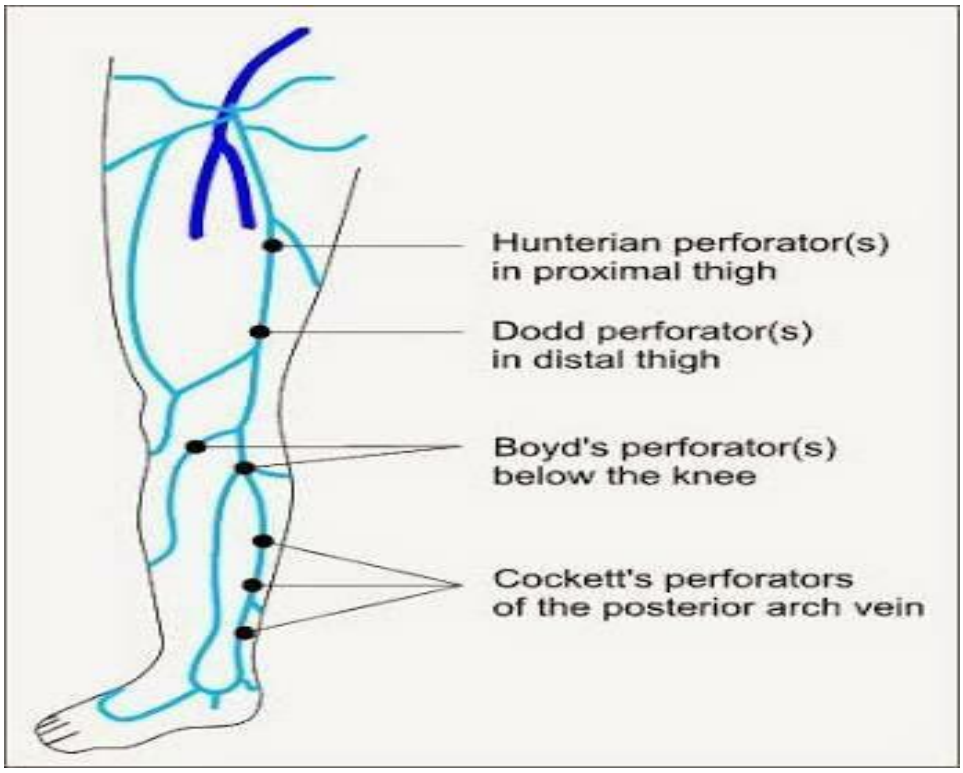


Figure 2: Perforators of the lower limb

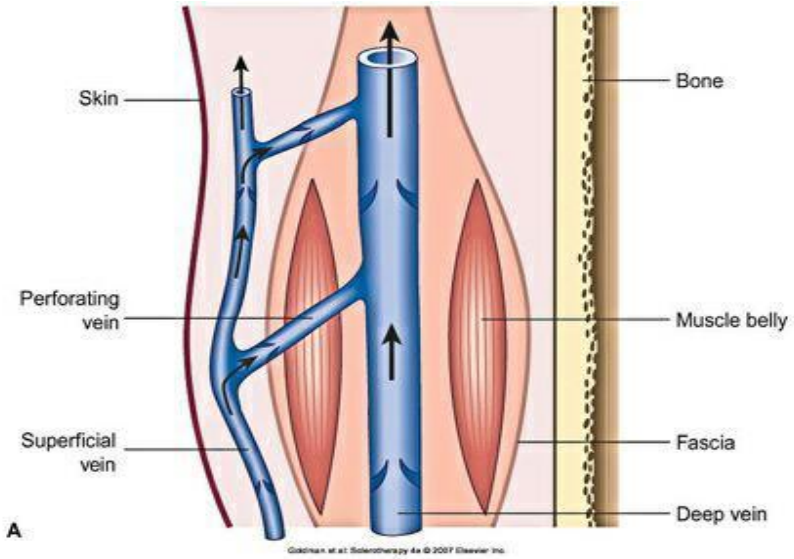


Figure 3: Calf muscle pump

NOMENCLATURE

Leg Ulcer:

It is defined as full thickness loss of skin in the leg or foot due to any cause[6].

Chronic venous ulceration is defined as a break in the skin, present for more than 6 weeks, between the malleoli and tibial tuberosity that is presumed to be wholly or partly due to venous disease[7].

Chronic venous insufficiency refers to those patients with irreversible skin damage due to sustained ambulatory venous hypertension which occurs when the blood no longer flows in the correct path from the superficial system into the deep venous system and thence back to the heart[7].

Chronic venous insufficiency may be classified anatomically into three categories:

Superficial vein insufficiency: It occurs due to failure of primary valve secondary to distension of superficial veins which further leads to secondary valvular incompetence and is characterized by the presence of visible, tortuous, truncal varicose veins.

Perforating vein insufficiency, which is rare and caused by primary valve insufficiency in isolation. It usually occurs secondarily in combination with deep vein insufficiency (post-thrombotic limb).

Deep-vein insufficiency which occurs most commonly due to reflux. 10% of cases are associated with post-thrombotic syndrome. Avalvulosis is rare and is associated with FOXC2 gene mutation.

CAUSES OF LEG ULCERS:

Venous hypertension	Varicose veins, deep-vein thrombosis, venous obstruction, congenital vascular malformations, Klippel–Trenaunay syndrome
Arterial disease	Atherosclerosis, diabetes, hypertension, embolism, calcific uraemic arteriolopathy (calciphylaxis)
Skin cancer	Basal and squamous cell carcinoma, melanoma or any fungating tumour
Vasculitis	Connective tissue diseases, e.g. systemic lupus erythematosus, rheumatoid disease, progressive systemic sclerosis, necrotizing leukocytoclastic vasculitis, ANCA-associated disorders, polyarteritis nodosa
	Livedoid vasculopathy, cryoglobulinaemia
Haematological disorders	Coagulation states, e.g. factor V Leiden, protein S or C deficiency, hyperhomocysteinaemia, antithrombin III deficiency, sickle cell disease, thalassaemia, spherocytosis, antiphospholipid syndrome, myeloproliferative disorders, e.g. polycythaemia rubra vera, thrombocythaemia
Emboli	Cholesterol, platelets, tumour cells, infective
Loss of sensation	Leprosy, diabetes, spina bifida
Infections	Diabetes, osteomyelitis, Buruli ulcer, fungal infections, syphilis, mycobacteria, leishmaniasis, acute 'desert' sore, necrotizing fasciitis and cellulitis
Trauma	Physical, e.g. pressure sore, chemical or thermal (e.g. burns or cold injury)
	Self-harm
	Intravenous drug use
Drugs/therapy	Radiation
	Hydroxycarbamide, ergotamine
	Iododerma
Skin conditions	Pyoderma gangrenosum
	Necrobiosis lipoidica
	Scleroderma
	Graft-versus-host disease
	Blistering disorders (e.g. pemphigoid)
	Bites and stings: insects, snake, scorpion
Genetic	Prolidase deficiency
	Klinefelter's syndrome

Table 1: Causes of leg ulcers

Of the various causes of leg ulcers, 70% are venous ulcers, 20% mixed venous and arterial ulcers and 10% due to other causes.

COMPARISON OF CLINICAL FINDINGS IN THE THREE MAJOR TYPES OF LEG ULCERS			
	Venous	**Arterial**	**Neuropathic/mal perforans***
Location	Medial malleolar region	Pressure sites Distal points (toes)	Pressure sites
Morphology	Irregular borders Yellow fibrinous base	Dry, necrotic base Well-demarcated ("punched out")	"Punched out"
Surrounding skin	Yellow–brown to brown discoloration due to hemosiderin deposits Pinpoint petechiae ("stasis purpura") Lipodermatosclerosis	Shiny atrophic skin with hair loss	Thick callus
Other physical examination findings	Varicosities Leg/ankle edema ± Stasis dermatitis ± Lymphedema	Weak/absent peripheral pulses Prolonged capillary refill time (>3–4 seconds) Pallor on leg elevation (45° for 1 min) Dependent rubor	Peripheral neuropathy with decreased sensation ± Foot deformities

Table 2: Comparison of clinical findings in the three major types of leg ulcers

EPIDEMIOLOGY:

Incidence and prevalence:

Venous ulcerations of the lower limb are one of the leading cause of leg ulcers. Around 60 -70 % of leg ulcers are due to venous origin[1].The lifetime incidence of venous leg ulcers is 0.5% and has a prevalence of 0.05% at any point of time[6]. The prevalence of VLUs ranges between 0.18% and 1%. It increases to 4% in patients over 65 years. Healed and active ulcers of the lower limb affect 1% of the adults of which 3.6% are above 65 years of age. About 50% of CVU heal within 4 months but approximately 20% have not healed after 1 year and about 8% have not healed after 5 years. The annual recurrence rate is 6 to 15% and most ulcers tend to recur more than once.

Age:

Although VLUs can occur in younger adults, the incidence increases with every decade. The prevalence increases with age, accounting to 3.6% in patients more than 65 years.

Gender:

Though frequently stated that it is commoner in females, once prevalence is corrected for age, both sexes are affected almost equally.

Genetics:

FOXC2 plays a critical role in valve development and function and mutations in FOXC2 may promote varicose veins.

Social class:

CVU is common in lower socio economic class.

Occupation:

Occupations that involve prolonged standing are more frequently associated with varicose veins.

Environmental factors:

A sedentary lifestyle reduces the efficiency of the muscle pump and leads to reduced venous return.

Smoking:

It is related to the presence of concomitant arterial disease which determines the prognosis and treatment of CVU.

CLASSIFICATION OF CHRONIC VENOUS INSUFFICIENCY[8]

CEAP CLASSIFICATION:

C Clinical status	E Etiology	A Anatomy	P Pathophysiology
C0 no visible disorder			
C1 telangiectases or reticular veins	Ec congenital	As superficial	Pr reflux
C2 varicose veins	Ep primary	Ap perforating	Po obstruction
C3 oedema	Es secondary (post-thrombotic)	Ad deep	Pr,o combination
C4a pigmentation, oedema	En no known venous etiology	An no known venous location	Pn no known venous pathophysiology
C4b lipo- et dermatosclerosis, white atrophy			
C5 healed ulcer			
C6 active, venous ulcer			
S symptomatic			
A asymptomatic			

Table 3: CEAP classification

The Basle classification (Widmer):

CVI I – corona phlebectatica (venous or malleolar, flare)

CVI II – hyper or depigmented areas (lipodermatosclerosis, atrophie blanche)

CVI III – open or healed ulceration

PATHOPHYSIOLOGY OF VENOUS MICROCIRCULATION IN VENOUS ULCER[7]:

Venous return from the leg is maintained by the pumping function of the muscles and by the competent valves that prevent its backflow. In a healthy standing subject the venous system of the legs contains a volume of 300 to 350 ml. Most of the blood from the lower limbs drains via superficial venous system through perforating veins into the deep system, and only 10% passes through the saphenofemoral junction (SFJ). Calf muscle contraction empties the deep veins thereby propelling the blood column towards the heart against gravity. During muscle relaxation, backflow of blood is prevented by the venous valves. This mechanism is called the 'calf muscle pump'. Other muscle groups also aid in venous return, but the calf muscle pump is the most important.

Venous reflux:

Reflux refers to the retrograde flow in a vein due to incompetent valves in response calf muscle contraction. It can occur in the superficial, deep and perforating veins. In normal individuals there is fall in the venous pressure during calf muscle contraction which is known as normal lowering of ambulatory venous pressure. An elevated and sustained ambulatory venous pressure indicates chronic venous insufficiency (CVI). This occurs due to valvular incompetence or venous outflow obstruction after deep-vein thrombosis (DVT) known as the post-thrombotic leg.

The venous microcirculation:

In chronic venous insufficiency, the raised ambulatory venous pressure is transmitted directly to the venular side of the capillary bed causing capillary hypertension, and eventually leading to destruction of the nutritive capillaries. The capillaries in the skin of the lower leg are most affected and the clinical signs results from the microcirculatory disturbances in the veins.

The following theories account for the microcirculatory disturbances in venous diseases.

1. Capillary stasis:

Homans postulated that ‘stasis’ of venous blood in patients with post-thrombotic syndrome lead to the development of anoxia which caused venous ulcers. Blalock reported that the percentage of oxygen in the venous blood in varicose vein patients was higher than in healthy persons and the blood flow in patients with venous ulcer was higher than that of healthy persons. Despite the increase in venous flow, decreased flow velocity in the capillary beds is responsible for focal hypoxia. Morphological changes develop in the microcirculation in response to prolonged CVI which also plays a role in the lower tcPo2 value. Bollinger et al first reported microangiopathy, a prominent sign of CVI. Reduced diffusion was present in the skin with decreased capillary density in chronic venous insufficiency.

2 .Fibrin cuff-theory:

It was observed that following venous ulceration, there is deposition of fibrin in the pericapillary region. It also causes leakage of fibrinogen which in turn polymerizes to fibrin leading to fibrin cuff around the capillaries which acts as a barrier for oxygen diffusion.

3. White cell trapping:

Coleridge Smith et al, suggested that leukocytes get trapped in the capillaries, which obstructs flow leading to ischemic ulceration. Over expression of the adhesion molecules play an important role by causing continuous leukocyte migration to the dermis.

4. Trapping of growth factors:

Falanga and Eaglestein postulated that the pericapillary fibrin cuffs with α2-macroglobulin, interfere with the transport of growth factors, making them biologically unavailable in patients with severe CVI resulting in delayed wound healing.

5 .Multicausal model: the Maastricht model:

Researchers at the University Hospital of Maastricht in the Netherlands suggested the raised microcirculatory pressure at the venular end of the capillaries is a consequence of elevated ambulatory venous pressure. This leads to broadening of the interendothelial space, disintegration of collagen IV,

thickening of the capillary wall which interfere with capillary exchange. The increase in the capillary filtration fraction affects water diffusion initially resulting in edema; later larger molecules escape into the tissues and accumulate leading to halo formation. The fibrin/ fibrinogen cuffs not only act as a diffusion barrier for oxygen; but also stimulates collagen formation. Collagen IV is laid down around the capillaries and collagens I, III in the dermis. α2-macroglobulin laid down binds with molecules such as TGF-β, leading to decreased biological availability of various growth factors.

White cells marginate and adhere to the widened capillary walls, sometimes blocking the circulation and leading to micro thrombi formation. The plasma proteins escaping into the tissues enhance inflammatory response, and leukocytes releases proteolytic enzymes causing free radical damage. This is seen clinically as the changes of lipodermatosclerosis (LDS). Increased action of proteolytically active matrix metalloproteinase-1 (MMP-1) and matrix metalloproteinase-2 (MMP-2) results in continuous degradation of collagen. The elevated proteolytic MMP-2 expression is a consequence of plasminogen activation in LDS which causes an elevated matrix turnover causing structural changes in the dermis and subcutaneous tissue leading to ulceration.

WOUND HEALING

The primary goal of the wound healing response is to re-establish the barrier functions of the skin at the earliest.

Types of wound healing:

The three types of wound healing are

1. Healing by primary intention
2. Healing by secondary intention and
3. Healing by tertiary intention.

In ***primary intention healing***, wound closure is achieved by approximation of the borders e.g. Closures, flaps and grafts.

In ***secondary intention***, healing occurs on its own e.g. acute wounds. Here time taken depends on factors like wound depth, shape, anatomic location, presence of secondary infection, vascular supply etc.

Tertiary intention healing are wounds in which closure was initially done by primary intention healing, but later is allowed to heal by second intention due to dehiscence.

Phases of normal wound healing[9, 10]:

Healing of wounds occurs through the following four phases: Hemostasis, inflammation phase, proliferation phase, remodeling or maturation phase.

Acute wounds pass through all the phases of wound healing in a linear pathway. Chronic wounds results when there is arrest in any of the stages.

Hemostasis phase: Following tissue injury, there is recruitment of platelets to the site of injury which initiates the coagulation cascade and cause hemostasis. The platelets are also a rich source of growth factors (e.g. PDGF, TGF, FGF, VEGF,) and proinflammatory cytokines which mediate wound healing

Inflammation phase: It ranges from one day to approximately 2 weeks. It starts with neutrophils margination and adherence to endothelial cells and migrates for phagocytosis. TNF-αand IL-1causes recruitment of fibroblasts and epithelial cells. Macrophages in addition to phagocytosis releases growth factors and cytokines involved in the proliferative phase of healing. In the end lymphocytes infiltrate and produce IL-2, which helps in fibroblast recruitment.

Proliferation phase: It consists of fibroplasia, granulation, and epithelialization. Growth factors involved in this step are

- Platelet derived growth factor (PDGF) - helps in haemostasis and fibroblast proliferation.
- Connective tissue growth factor(CTGF)- laying down of collagen,
- Fibroblast growth factor (FGF β), transforming growth factorβ, vascular endothelial growth factor (VEGF) helps in angiogenesis.

- Epidermal growth factor (EGF) and keratinocyte growth factor (KGF) - helps in epithelial proliferation and migration.

Remodelling phase: In this phase, the fibroblasts continue to lay down collagen with covalent cross linking of collagen resulting in scar formation and the inflammatory cells reduce.

Figure 4: Wound healing-Stages

Factors affecting wound healing:

Local factors such as poor perfusion, increased bacterial activity, toxins, mechanical stress etc.

Systemic factors like venous insufficiency, advanced age, ischemia, diabetes mellitus-associated microangiopathy, less commonly vasculitis, hypercoagulability, malnutrition, and concomitant therapies (e.g. hydroxyurea, immunosuppressives).

CLINICAL FEATURES OF CHRONIC VENOUS ULCERS:

Risk factors:

The major factors contributing to venous ulceration of leg are increased age, family history of venous diseases, obesity, pregnancy, prolonged standing, trauma, reduced physical activity, DVT and factor V Leiden mutation.

Poor prognostic factors[11]:

Long standing ulcers, large size, excess fibrin in the wound, ankle-brachial pressure index (ABPI) <0.8are associated with poor prognosis. VLU are usually preceded by patchy erythema or intense bluish discoloration due to capillary congestion, leading to ischemia and necrosis of the skin following minor trauma.

Most common site of leg ulcer is on the medial side of the leg just above the ankle, the gaiter area. Less commonly it may also be present over the lateral malleolus when the short saphenous system is involved. When ulcers occurs at other sites, especially just below the knee or over the foot, other causes of leg ulceration must be considered.

A typical healing venous ulcer is shallow in appearance with sloping edges, with healthy granulation tissue at the base with yellowish exudate. Non healing ulcers are boggy in appearance with undermined edges and base of the ulcer appearing white and fibrous.

Irritation of the surrounding skin due to exudates, inflamed varicose veins and medicament dermatitis may lead to increase in ulcer size and prevent healing.

It is usually associated with other signs of chronic venous insufficiency such as

- Ankle flare or Corona phlebectatica paraplantaris- Presence of abnormally visible cutaneous blood vessels at the ankle
- Lipodermatosclerosis - Pathognomonic of venous hypertension and associated with an increased risk of leg ulceration. In the early stages, skin appears inflamed erythematous and slightly indurated associated with pain and tenderness. In longstanding cases, it becomes woody hard

in nature with hyperpigmentation due to increased matrix turnover caused by a chronic inflammatory reaction.

- Atrophie blanche- Multiple asymptomatic atrophic ivory-white depressed areas 0.5-15cm in diameter are present over the lower legs. It may also be seen in association with other disorders including lupus erythematosus, scleroderma, vasculitis, cryoglobulinaemia, polycythaemia and leukaemia.
- Inverted "champagne bottle" appearance of the leg
- Varicose veins, stasis eczema or oedema.

Differential diagnosis[6]:

Mixed venous and arterial leg ulcers, arterial leg ulcers, hypertensive and ischemic leg ulcers, vasculitic leg ulcers.

Complications and co-morbidities:

These include chronic pain, impairment of quality of life, local wound infection, systemic infection and sepsis, infestation with maggots (fly larvae), secondary squamous cell carcinoma and secondary lymphoedema (peri ulcer lymphoedema and/or foot and toe lymphoedema).

Disease course and prognosis:

VLU are chronic and recurrent unless patients receive an accurate diagnostic assessment, appropriate treatment and secondary prevention.

APPROACH TO A PATIENT WITH VENOUS LEG ULCER[7,12]:

A detailed and comprehensive assessment of all patients with venous ulcers for the first time or recurrent ulcers is essential for timely and appropriate treatment.

Clinical history:

1. Full clinical history which includes the duration of ulcer, pain, recurrence, previous treatment modalities.

2. Detailed history which provides clues to venous etiology

a. documented deep vein thrombosis

b. positive family history

c. history of varicose veins

d. situation leading to valve damage like fracture of lower limb.

e. history of prolonged immobilization (standing/sitting)

2. Arterial disease which are indicated by the following has to be ruled out:

a. history of intermittent claudication

b. absence of pedal pulses

c. abnormal blood pressure

d. previous history of cardiovascular diseases, stroke

3. Co morbid factors – increasing age, DVT, obesity, chronic varicosities.

4. Co-existing medical illness like type 2 diabetes, peripheral arterial diseases, rheumatoid arthritis, systemic vasculitis

5. Complications like severe infections, osteomyelitis and malignant changes should be ruled out.

Clinical examination:

Examination of ulcer:

a. Site of the ulcer
b. Size: the length, width and depth of the ulcer has to be measured. Disposable ruler, photography and computerised calculation with digital photography may be used. Serial measurements has prognostic valve.
c. Characteristics of the ulcer: The depth, margins and edges of the ulcer and appearance of the ulcer bed, exudates if any must be noted. Presence of red viable granulation tissue is seen in healing ulcers, thick slough or eschar indicates arterial insufficiency.
d. Clues of infection such as increase in local warmth, increase in pain, presence of new ulcers, extension of pre-existing ulcers, malodour, dull black or brick red discoloration, friable granulation tissue which easily bleeds predisposes to delayed healing despite appropriate therapy

Assessment of the leg:

1. Associated skin changes due to chronic venous insufficiency such as firm ("brawny") edema, warmth, reddish brown pigmentation due to hemosiderin deposition, dilated and tortuous superficial veins, stasis eczema, ankle flare, lipodermatosclerosis, atrophie blanche, inverted "champagne bottle" appearance of the leg.

Frequent assessments during the management will help in prognosis and healing.

INVESTIGATIONS

The investigations performed should ideally be rapid, inexpensive and of minimal inconvenience to the patient.

Non-invasive tests:

1. **Ankle Brachial Pressure Index-**This procedure is done with the help of handheld Doppler ultrasound for identifying arterial insufficiency in the lower limb.

   ABPI = highest systolic foot pressure (dorsalis pedis/ posterior tibial artery)/highest systolic brachial BP

   a. ABPI: 0.8-1.2: Indicative of good arterial flow. Suggests venous etiology if an ulcer is present

b. ABPI: <0.8 with the clinical picture of arterial disease – suggests arterial insufficiency

c. ABPI: >1.2: Suggestive of possible arterial calcification

ABPI value of less than 0.8 is a contraindication for compression therapy.

2. Handheld Doppler study:

Handheld Doppler (HHD) also known as continuous wave Doppler (CWD) is increasingly replacing the use of tourniquet (Trendelenburg, Perthes) tests. It is quick, easy to learn, non-invasive and inexpensive.

3. Duplex ultrasonography:

This is a recent test incorporating both ultrasound and Doppler. By duplex ultrasonography veins can be directly visualized and flow of blood through valves in both superficial and deep venous can be identified. Duplex has virtually replaced all other tests in day-to- day practice.

4. Ascending and descending phlebography:

It provides information about structure of the deep veins of leg and helps in identification of thrombi and presence of collateral circulation

5. Varicography:

This is done by injecting contrast medium directly into the varices. It is useful in patients with recurrent varicose veins for delineating the anatomy of previously operated SFJ and SPJ.

Invasive:

All patients should undergo baseline biochemical and hematological assessment such as complete hemogram, blood glucose, liver function tests, urea and electrolytes. Serum albumin, transferrin, fasting lipid profile, rheumatoid factor, auto antibodies and thrombophilia screening if clinically required.

Bacteriological examination:

Most venous ulcers are colonized with bacteria rather than infected. Swab for culture is necessary if the ulcer has an arterial component, diabetic patient or ulcer appears infected or is slow to heal.

Biopsy:

It is indicated in ulcers which fail to heal with conventional therapy, tendency to bleed, or long standing ulcers to exclude malignancy. The biopsy specimen should include both the margin and base of the ulcer.

MANAGEMENT OF CHRONIC VENOUS ULCERS:

Normal wound healing requires adequate perfusion, good nutritional and immune status and avoidance of deleterious mechanical forces.

Cleansing:

The ulcer should be cleaned using normal saline or tap water[14].

Debridement:

It consists of removal of nonviable necrotic and infected tissue from the ulcer so as to promote healing. By debridement a chronic wound is converted to an acute wound, and it undergoes a normal healing process[15,16].

Wound dressings:

Characteristics of an ideal wound dressing:

1. Should provide moist environment
2. Should absorb excess exudate
3. Prevent infection
4. Less irritating
5. Should be cheap

Moist retentive wound dressings stimulate collagen synthesis, create a hypoxic environment and promotes angiogenesis, causes re-epithelization and decreases pain. It decreases wound infection rates by acting as a barrier to external contamination and decreasing wound pH.

Management of wound infection:

Topical antibiotics and antiseptics:

Chronic venous ulcers are colonised with poly microbial flora. Topical antibiotics are indicated only in cases of infected wounds[17,18]. Various agents like iodine[19], silver[20], mupirocin[21] were tried in the management of chronic venous ulcers but did not show promising results on randomized trials.

Adjunct therapies:

Compression therapy[7,12]: Compression therapy is the essential adjunct in the management of CVI. It facilitates venous return by improving calf muscle pump function and lymph drainage thereby reducing edema, stimulating healthier granulation tissue, and reducing discomfort and improving the quality of life. It is contraindicated in patients with congestive heart failure and arterial insufficiency as it leads to worsening of ulcer, gangrene, and even amputation of the limb.

The initial management of chronic venous ulcers is by multilayered compression bandages.

A comparative study shows that medical elastic compression stocking is more effective in daily practice than standard compression therapy with bandages.

Types of Compression	Function	Examples
High elastic, high compression bandage	Sustained high levels of compression at work and at rest	Tenopress, Setopress, Surepress
Light compression support bandage	Light support with low pressure	Tubigrip
Multi-layer high compression bandage	Graduated sustained compression at ankle and below knee	Profore, Dynaflex, 3M 2 layer Coban
Inelastic Compression Bandage	Graduated static compression Remains rigid for effective edema control	Unna's Boot, Duke Boot Used with a layer of zinc or calamine impregnated gauze

Table 4: Compression Therapies

Compression-Hosiery Classification and Indications[a]

Class	Pressure	Indications for Use
	8-15 mmHg (support)[b]	Tired/aching feet and legs, slight edema Spider veins, early varicose veins
1	14-17 mmHg[c] 15-20 mmHg[b]	Varicose veins, mild edema DVT prevention
2	18-24 mmHg[c] 20-30 mmHg[b]	Moderate varicose veins, mild edema Prevention of venous-ulcer recurrence
3	25-35 mmHg[c] 30-40 mmHg[b]	Severe varicose veins Prevention and treatment of venous ulcers Lymphedema Postphlebitic limb Chronic venous insufficiency

[a] Choice of type and level of compression is patient-specific. Patient considerations include arterial status and ability to tolerate and put on the stocking.
[b] U.S. recommendation.
[c] British standard.
DVT: deep vein thrombosis.
Source: References 5, 7, 27, 28.

Table 5: Compression-Hosiery classification

Pain management:

Appropriate and adequate pain relief should be provided to chronic venous ulcer patients.

ROLE OF SYSTEMIC THERAPY:

Systemic therapy is a valuable alternative in cases failing compression, patients with poor compliance and whose refuse surgery[22].

ANTIBIOTICS:

Role of antibiotics in venous ulcer has been studied extensively by Cochrane peer group and found to be useful only in the presence of infection[1].

Few antibacterial agents which were studied in the treatment of venous ulcers are

- **Levamisole** – studies on levamisole showed promising results in the management of CVU[23].
- **Doxycycline** -Doxycycline inhibits proinflammatory cytokines like TNF-alpha and matrix metalloproteinases, and promotes healing in VLU[24,25].
- **Pentoxifylline:** Pentoxifylline (400 mg three-times daily) has a beneficial effect when used along with compression[26,27].

- **Aspirin:** Aspirin causes inhibition of platelet activation and has anti-inflammatory and analgesic properties[28,29]. RCT on aspirin showed improvement in size of ulcer when compared to placebo group[30].
- **Zinc:** Studies on zinc as adjuvant therapy showed no beneficial effect[31,32].
- **Flavonoids:** Micronized purified flavonoid fraction (MPFF) reduces pain, edema and cramps associated in VLU[33]. It is widely used and is free of major side-effects. Coleridge et al. showed beneficial effects of MPFF in long standing venous ulcer[34,35].

Phlebotonics:

They act by improving venous tone and decreasing capillary hyper permeability in venous diseases[36]. Cochrane analysis does not recommend phlebotonics in CVI[37].

Fibrinolytics:

Due to the fibrinolytic property, drugs like stanozolol and defibrotide were studied under trials in CVU. Results were promising with defibrotide[38] whereas stanozolol showed no beneficial effect[39].

Mesoglycans:

Mesoglycans are glycosaminoglycan with profibrinolytic action, microrheologic, and macro rheologic benefits. It inhibits neutrophil adhesion and activation, and enhances wound healing[40].

Care of surrounding skin:

Gentle care of the surrounding skin with application of emollients will help in restoring barrier function and early healing.

Supportive measures[41]:

Exercise to calf muscle increases the pumping functions thereby promotes healing. Other supportive measures like limb elevation, weight control, adequate intake of proteins, vitamins and minerals, cessation of smoking and alcohol consumption are recommended.

Other treatments:

Hyperbaric oxygen therapy:

Increase in the oxygen helps in neutrophil dependent microbial killing, collagen cross-linking and neovascularization[42]. The efficacy of hyperbaric oxygen therapy in venous ulcers is still not proven.

Topical negative pressure therapy:

It promotes wound healing by creating mechanical forces which stimulate a biological response and maintaining a moist environment. It also removes exudates, decreases edema, improves local perfusion, decreases bacterial load,

and enhances granulation tissue formation[43,44].

Laser therapy:

The possible mechanisms of action of laser in venous ulcers are improved metabolism of the tissue, stimulates tissue repair and increases collagen synthesis[45]. Their role in the treatment of leg ulcers needs more studies.

PLATELET RICH PLASMA

Platelet rich plasma (PRP) refers to large quantity of platelets concentrated in a small volume of plasma[46].

Alternative names of PRP:

- autologous platelet gel
- plasma rich growth factors
- platelet concentrated plasma

Working definition: Platelet count of 10 lakh/ml in 5 mL of PRP[47].

Autologous PRP: PRP derived from the patient's own blood.

PRP contains an effective concentration of growth factors which help in wound healing and the plasma proteins in PRP act as a scaffold for bone, connective tissue and epithelial migration[48].

Growth factors released from platelets and their biological action[49]:

Growth factor	Source cells	Target	Biologic action
Platelet-derived growth factor	Platelets, macrophages, monocytes, endothelial cells, smooth muscle cells	Fibroblasts, smooth muscle cells, glial cells, macrophages, neutrophils	Stimulates DNA and protein synthesis in osseous tissues; mitogenic effects on mesenchymal cells; angiogenic effect on endothelial cells
Transforming growth factor β	Platelets, T-lymphocytes, macrophages/monocytes, neutrophils	Fibroblasts, marrow stem cells, endothelial cells, epithelial cells, preosteoblasts	Stimulates angiogenesis; enhanced woven bone formation; stimulate matrix synthesis in most culture systems; chemotactic effect on osteoblastic cells; stimulates endothelial chemotaxis; stimulates bone formation by inhibitory effect on osteoclasts
Platelet-derived angiogenesis factor	Platelets, endothelial cells	Endothelial cells	Mitogenic effect on endothelial cells; increased angiogenesis and vessel permeability
Insulin-like growth factor 1	Osteoblasts, macrophages, monocytes, chondrocytes	Fibroblasts, osteoblasts, chondroblasts	Stimulates proliferation of osteoblasts and matrix synthesis; increases expression of bone matrix proteins, such as osteocalcin; in combination with PDGF it enhances the rate and quality of wound healing
Platelet factor 4	Platelets	Fibroblasts, neutrophils	Chemoattractant for neutrophils and fibroblasts

PDGF - platelet-derived growth factor, TGF-β - transforming growth factor β, PDAF - platelet-derived angiogenesis factor, IGF-1 - insulin-like growth factor -1, PF-4 - platelet factor - 4

Megakaryocytes in bone marrow

⇩

Platelets

⇩

α granule of platelets containing bioactive proteins

⇩

Activated platelets releases growth factors

⇩

Helps in wound healing and repair

In general, the activated PRP should be used within ten minutes of activation[47].

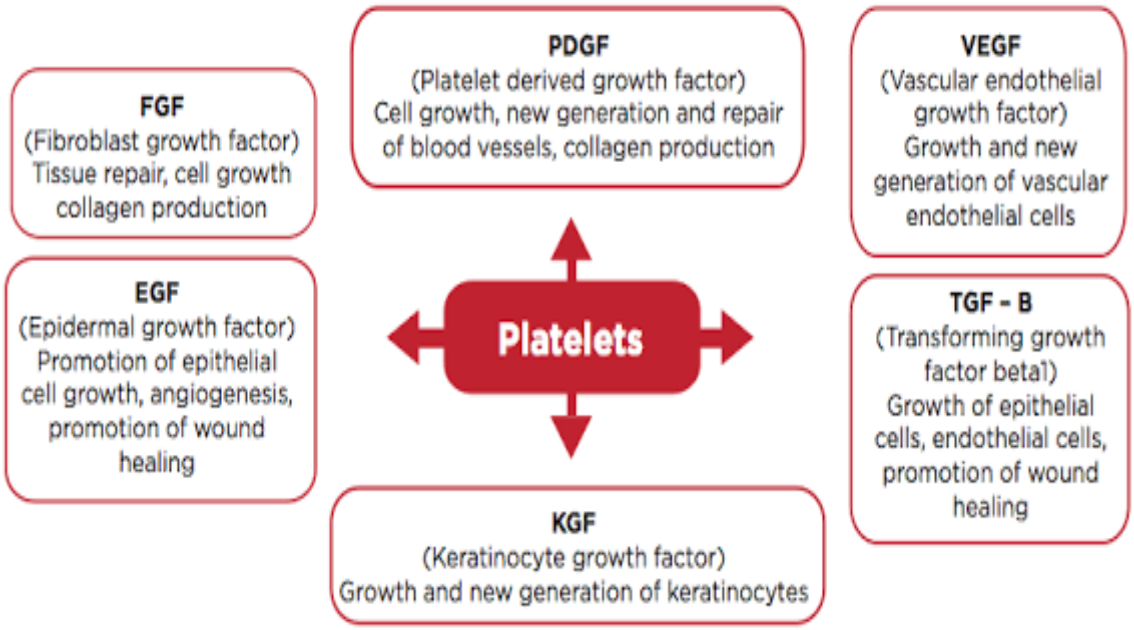


Figure 5: Growth factors in platelet rich plasma

These growth factors interact with the transmembrane receptors and activate intracellular signaling pathways which leads to synthesis of collagen[50] and helps in early repair and healing[51].

Classification of platelet concentrates:

Ehrenfest et al. (2009) proposed 4 main classification of PRP[52]

1. Pure Platelet-Rich Plasma (P-PRP) or leucocyte poor PRP contains a low-density fibrin network without leucocytes after activation.

2. Leucocyte- and PRP (L-PRP) contains a low-density fibrin network and leucocytes after activation.

3. Pure platelet-rich fibrin (P-PRF) or leucocyte poor platelet-rich fibrin contains a high-density fibrin network without leucocytes.

4. Leucocyte- and platelet-rich fibrin (L-PRF) or second-generation PRP contain high-density fibrin network and leucocytes.

Efficiency of PRP[53]:

Efficiency of PRP on wound healing depends on the following

- Concentration of platelets
- Volume of platelet rich plasma
- Size of the wound

More than one million per micro litre is regarded as therapeutic effective concentration[54,55].

PREPARATION OF PRP:

Blood sample obtained from the patient is mixed with an anticoagulant like citrate dextrose solution A or sodium citrate.

PRP can be prepared in a day care setting by 2 methods[56]:

- Manual double spin method
- Using automated devices.

The procedure is carried out at an optimum temperature of 20-22°C under aseptic conditions.

Manual double spin method:

In this method the initial light spin centrifugation separates the PRP from whole blood which is concentrated by the heavy spin centrifugation subsequently with removal of supernatant plasma.

COMMERCIALLY AVAILABLE PRP KITS

PRP devices can be of 2 types: Lower concentration systems (2.5-3 times baseline) and higher concentration systems (5-9 times baseline).

Factors influencing PRP yield:

Draw of blood: Large bore needles >22G are used to avoid unintentional activation of platelets[57]. Waters and Roberts et al found a decrease in platelet counts with longer draw time[58].

Temperature: for best results centrifugation is done at low temperatures below16°C[59].

Indications[56]:

- Androgenetic alopecia
- Alopecia areata
- Skin rejuvenation
- Acne scars and contour defects

- Wound ulcers
- Connective tissue disease associated ulcers
- Striae distensae
- Lipodermatosclerosis
- Lichen sclerosus

Contraindications for use of PRP:

These include critical thrombocytopenia, hypofibrinogenemia, acute and chronic infections, chronic liver disease and patients on anticoagulants.

PRP in acute and chronic ulcers:

PRP can be used as topical application or perilesional injection.

PRFM when applied topically to non healing ulcers enhances re-epithelialization.

Kim *et al*. observed that topical application of PRP accelerated re epithelialisation in 16 patients with acute and chronic ulcers of various causes including stasis ulcers[60].

Safety: Being autologous it is safe, free from risk of transmissible infections like HIV, hepatitis B, C and also immunogenic reactions due to allograft or xenograft[61].

AUTOHEMOTHERAPY

Autohemotherapy refers to the injection of autologous whole blood or serum typically into the vein or muscle. It was first described in 1913 by French physician Paul Ravaut[62] and Spiethoff[63]for various dermatologic conditions. It was a standard dermatologic treatment in the early 1900s including urticaria and eczema in the Europe, North America, and Japan[64-66]. Later, however autohemotherapy was not used widely because of a lack of rigorous supporting evidence[67] and no formal attempts to appraise the therapy systematically. In recent years, it has been re-discovered and evaluated for several dermatological conditions like urticaria[68,69], eczema, atopic dermatitis[70], chronic limb ischemia, PUPPP, herpes zoster and also in the field of sports medicine.

Autohemotherapy involves withdrawal of blood from a peripheral vein followed by injection of the same blood back into a vein or through the skin or muscle. It is commonly pretreated with ozone prior to reintroduction in the body to achieve upregulation of the anti-oxidant mechanisms in the body[71-74]. Modified topical autohemotherapy does not involve any pretreatment with ozone as the purpose of the therapy is to provide nutrients and growth factors for the healing process, lacking in chronic venous ulcer. It correlates with the process of healing by secondary intention where a blood clot formed locally enhances the ability of tissue to heal by various mechanisms. It provides barrier

to desiccation of wound and provides fibrin which acts as a scaffold for cellular infiltrate. It also provides high concentration of growth factors like PDGF, EGF, TGF-β, FGF and VEGF aiding epithelial regeneration, collagen synthesis and angiogenesis[75-77]. 90% of the growth factors are released due to platelet degranulation during coagulation of blood within1st hour of therapy. Autohemotherapy also provides a high concentration of leucocytes locally providing a potent antimicrobial effect negating the need for topical or systemic antibiotics with therapy.

Procedure:

Under aseptic precautions blood is drawn from a peripheral vein distant to the site of ulceration and uniformly applied all over the ulcer with a syringe after surgical debridement. The amount of blood applied depends on the size and depth of the ulcer. Blood is allowed to clot over the ulcer after which a sterile dressing is applied and covered with roller bandage. Patient is instructed to keep the dressing in situ for two days after which it is removed. Patient is also asked to maintain local hygiene. The procedure is repeated at weekly intervals until healing occurs.

Safety: It is autologous and does not carry any risk of transmission of infections or allergic reactions.

Advantages: Modified topical autohemotherapy is simple, inexpensive and less invasive modality for treating recalcitrant leg ulcers due to venous stasis.

PREVENTION OF ULCER RECURRENCE:

Recurrence rate in venous ulcers is about 37% after healing with conservative management or surgery[78].

Preventive Measures:

- Calf muscle exercise[79]
- Limb elevation
- Avoidance of prolonged standing[80]
- Diet and lifestyle modifications[81]

Specific measures:

- Continued use of compression therapy even after complete healing with conservative or surgical treatment[78].
- Venous surgeries like Sub-facial endoscopic venous surgery, Ultrasound Guided Foam sclerotherapy for incompetent perforators.

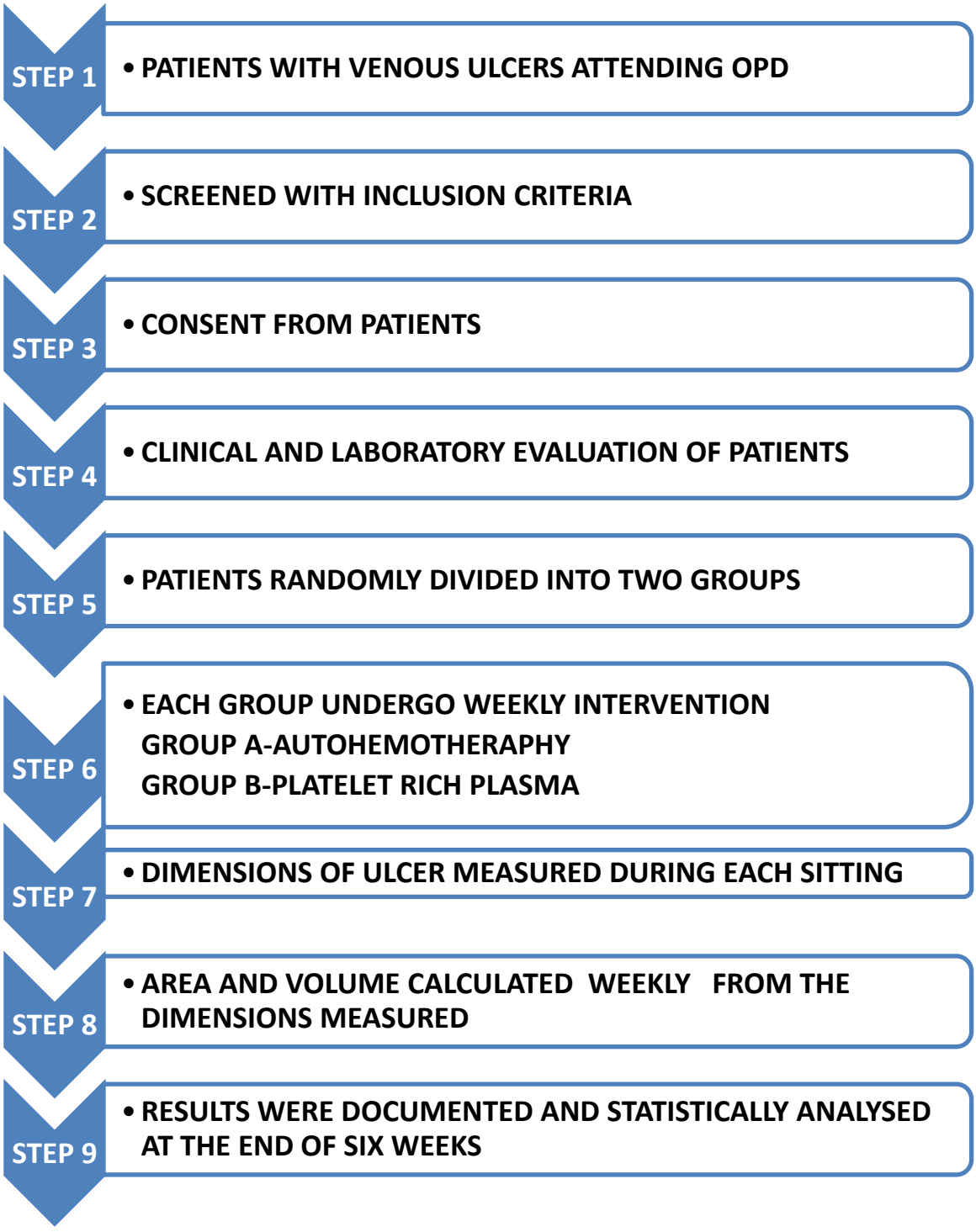
AIMS & OBJECTIVES

AIMS AND OBJECTIVES

1. To assess the efficacy of topical autohemotherapy in the healing of chronic leg ulcers due to venous stasis by estimating the change in the ulcer area and volume after treatment.
2. To assess the efficacy of topical autologous platelet rich plasma in the healing of chronic leg ulcers due to venous stasis by estimating the change in the ulcer area and volume after treatment.
3. To compare the efficacy of topical autohemotherapy and topical autologous platelet rich plasma in the healing of chronic leg ulcers due to venous stasis by estimating the change in the ulcer area and volume after treatment.

MATERIALS AND METHODS

STUDY DESIGN



MATERIALS AND METHODS

Type of study:

Prospective, interventional & comparative study

Study approval:

Institutional Ethical Committee of Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai had approved the thesis protocol.

Place of study:

Department of Dermatology, Madras Medical College &Rajiv Gandhi Government General Hospital, Chennai.

Period of study:

From November 2014 to February 2015 and January 2016 to July 2016.

Sample size: 36 cases

Selection of patients:

a) **Inclusion criteria :** all patients fulfilling the following criteria were

i) Patients with venous ulcers for more than 6 weeks

ii) Patients who have received conventional therapy like compression therapy for at least 6 weeks

iii) Normal blood investigations in terms of haemoglobin, haematocrit and platelet counts.

iv)Venous Doppler study of both lower limbs consistent with saphenous and perforator venous incompetence.

e) Absence of active infection with proven negative wound pus culture & sensitivity.

- Informed consent from the patient

b) Exclusion criteria:

Patients with the following conditions are excluded

(1) Diabetes mellitus

(2) Bleeding disorders

(3) Chronic venous ulcers with active infection

(4) Leg ulcers due to other causes

Study procedure:

All patients with chronic leg ulcers attending the outpatient clinic in the Department of Dermatology, Madras Medical College and RGGGH during the study period were analyzed and those patients fulfilling the inclusion criteria were taken up for the study. All these patients were explained about the nature of the disease, course of the disease and the benefits & possible side effects of the procedure proposed. After obtaining written consent patients were evaluated with the following

1) General and systemic examination.

2) Dermatological examination including measurement of ulcer size (length, width and depth).

3) Investigations namely complete haemogram, random blood sugar, liver function tests, renal function tests, bleeding time, clotting time.

4) Wound swab culture & sensitivity to rule out infection.

5) Vascular surgery opinion and Venous Doppler of both lower limbs.

Patients were randomized into two treatment groups (A & B).

Group A received topical autohemotherapy weekly and Group B received topical autologous Platelet Rich Plasma weekly.

In addition to these, all patients in both the groups were advised limb elevation and compression bandage during the treatment period.

METHODOLOGY

Group A - Topical Autohemotherapy:

Under aseptic precautions venous blood was drawn from a peripheral vein distant to the site of ulceration and was applied all over the ulcer with a syringe after surgical debridement. The amount of blood withdrawn depended on the size and depth of the ulcer at each sitting. The blood was allowed to clot over the ulcer after which a sterile dressing was applied and covered with roller bandage. Patient was instructed to keep the dressing in situ for two days after which it was removed. Patient was also asked to maintain local hygiene. The procedure was repeated once a week until the ulcer healed or for a period of 6 weeks whichever was the earliest.

Group B- Autologous Platelet Rich Plasma:

Separation of Platelet rich plasma involves the following steps:

- blood collection,
- double centrifugation,
- separation of PRP,
- activation of the coagulation process using 10%calcium chloride and
- topical application of Platelet Rich Plasma over the ulcer and
- application of dressing

Around 10- 15 ml of venous blood was collected in a vacutainer containing anticoagulant. RBCs are separated from platelets and plasma by centrifuging at 2000 rpm for 10 minutes. The supernatant containing the plasma and platelets is aspirated and centrifuged at 5000 rpm for 5-10 minutes again[82]. The lowermost layer containing around 1.5 ml was taken and activated by adding 10% calcium chloride (0.3 ml for 1 ml of Platelet Rich Plasma)[83]. Following surgical debridement of the wound, the activated PRP obtained by the above technique was applied immediately over the ulcer followed by non-absorbent dressing. The procedure was repeated once a week for 6 weeks or until the ulcer healed whichever was earliest.

As most of the ulcers are irregular in shape, the wound shape was considered to be elliptical and the area was calculated with the formula[83]:

$$\text{Area} = \text{length} \times \text{width} \times 0.7854$$

Similarly volume was calculated with the formula:

$$\text{Volume} = (\text{length} \times \text{width} \times 0.7854) \times \text{depth}$$

During the entire period, patients were advised limb elevation and compression bandage in addition to the procedure.

Follow Up Assessment:

All patients were reviewed every week for any complaints and assessing clinical improvement till completion of treatment.

The area and volume of the ulcer was calculated at each visit and serial photographs were taken.

- The percentage of improvement in the area of the ulcer at each sitting was calculated using the formula: (initial area of the ulcer – area of the ulcer at day of assessment)/ initial area of the ulcer[83].
- The percentage of improvement in the volume of the ulcer at each sitting was calculated using the formula: (initial volume of the ulcer – volume of the ulcer at day of assessment) / initial volume of the ulcer[83].

Variables analysed:

- Age
- Sex
- Presence of comorbidities if any
- Smoking
- Alcohol consumption
- Duration of ulcers
- Site of venous ulcer
- Side of venous ulcer

- Organisms isolated in wound swab culture and their senstivity pattern
- Baseline area and volume of the ulcer
- Area and volume of the ulcer at end of 6 weeks
- Percentage of improvement in the area and volume of the ulcer at each sitting.

Ethical consideration:

The study was approved priorly by the Institutional Ethical Committee. All the patients were explained about the study and the procedures, its merits and demerits, possible complications and outcome. They were selected for the study if he/she was willing to undergo the treatment and informed written consent was obtained. The study did not cause any economic burden to the patients.

Data collection:

Data were collected in the data collection form for all patients.

Data analysis:

The data were obtained and organized systematically into tables and figures The results derived from the above data were analysed statistically.

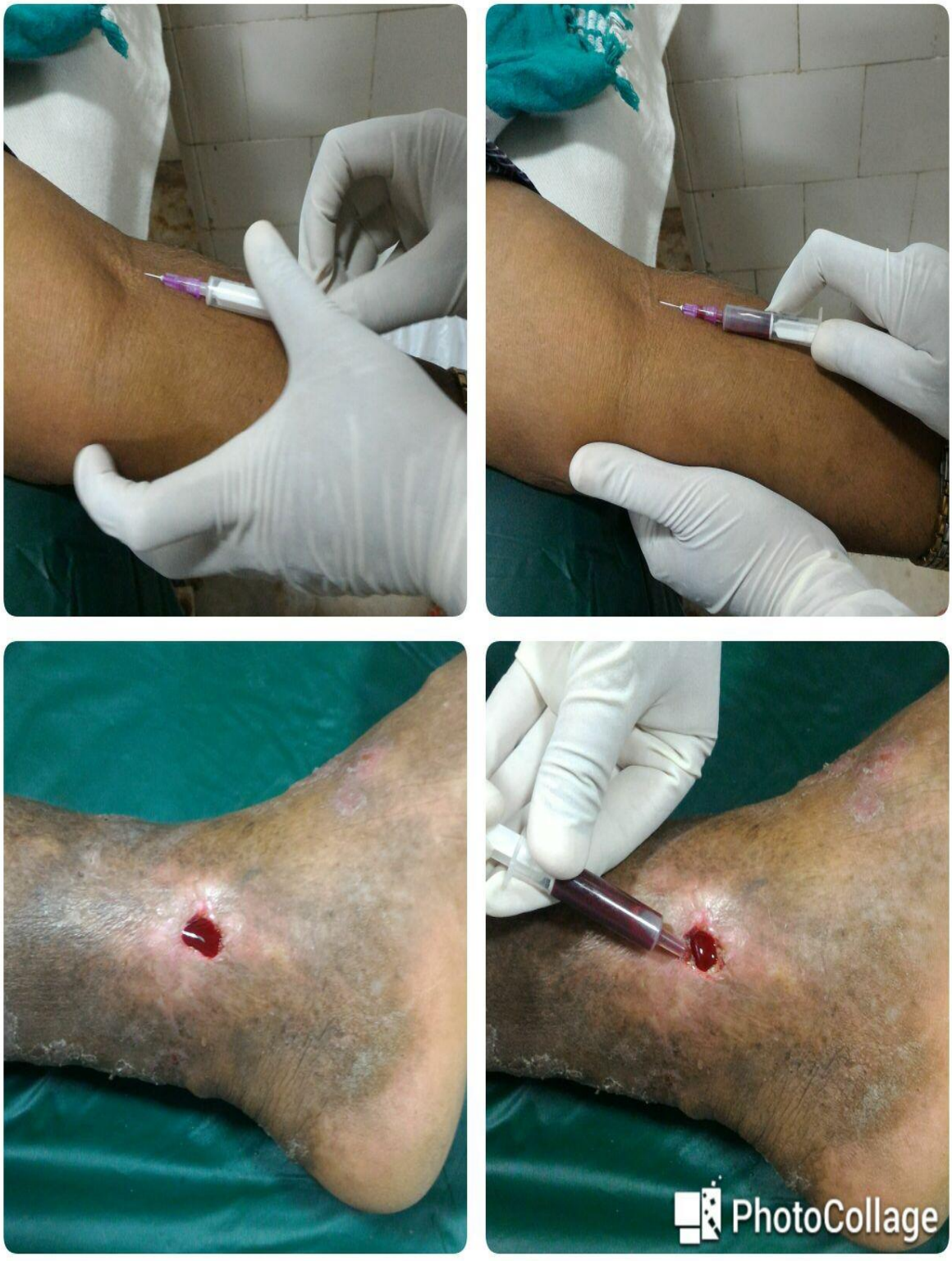


Figure 6: Topical Autohemotherapy –procedure in clockwise direction

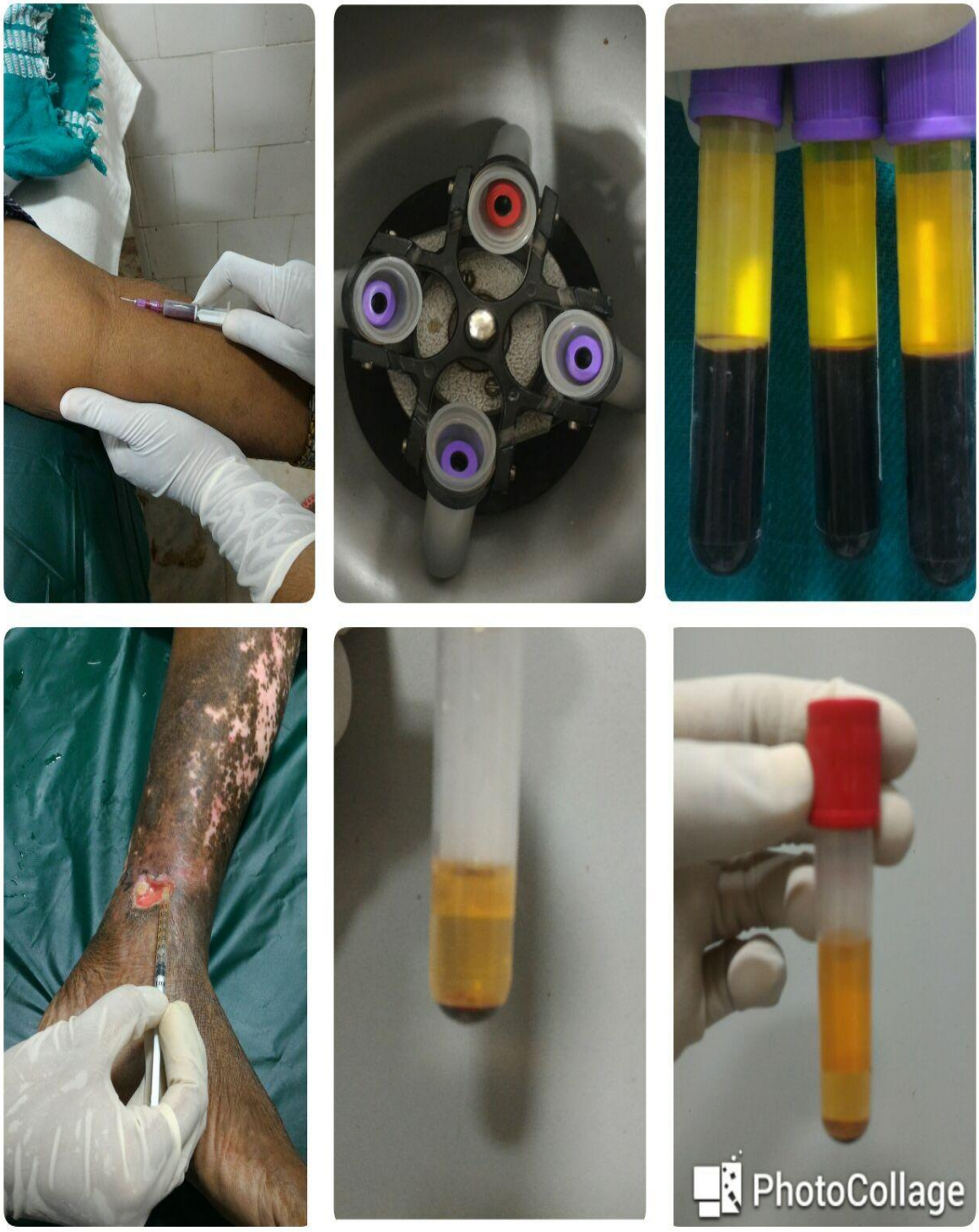
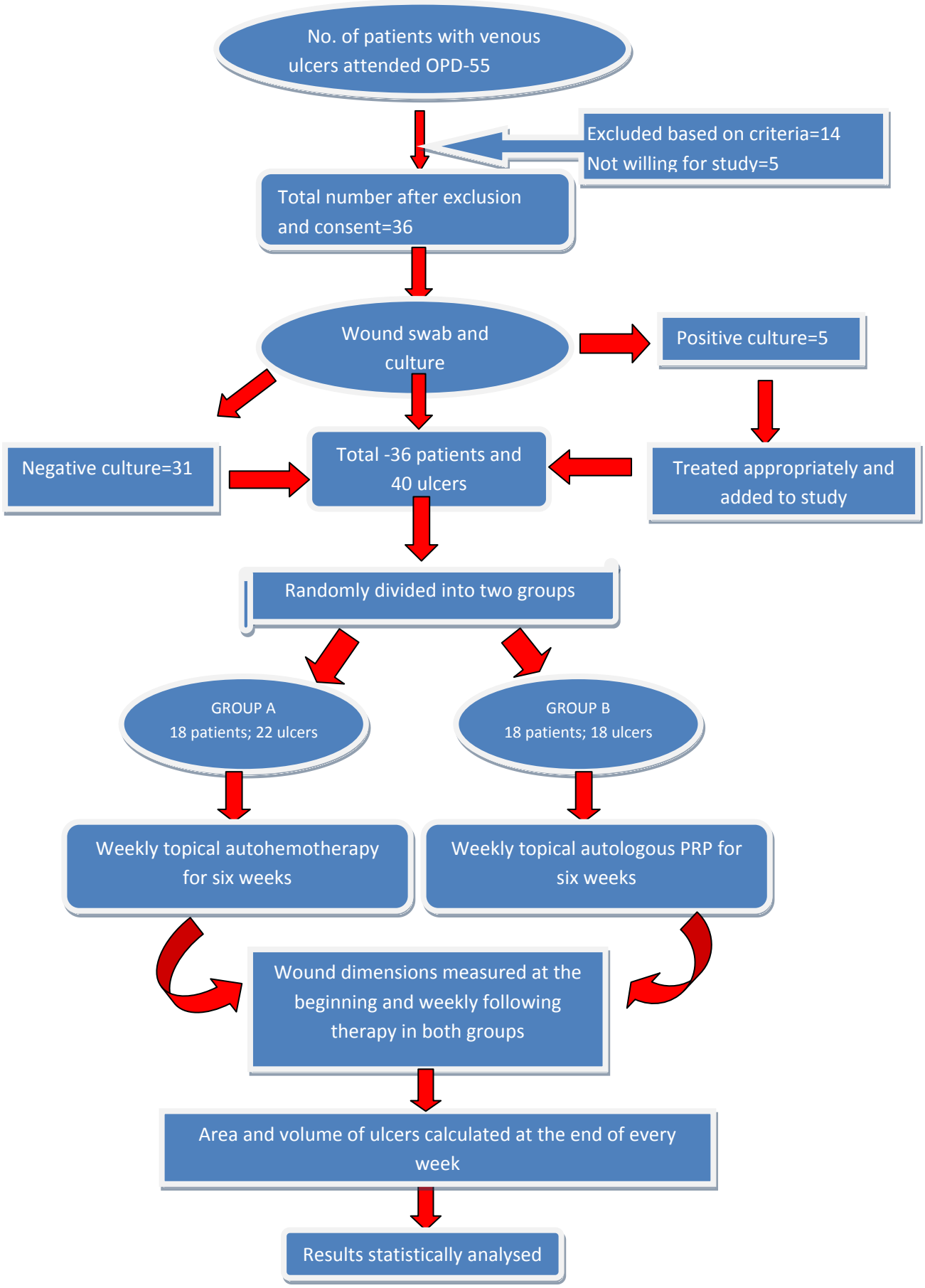


Figure 7: Topical autologous platelet rich plasma-procedure in clockwise direction

STUDY OUTLINE



OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

This prospective, interventional and comparative study was carried out to compare the effectiveness and the difference in the outcome of two different treatment modalities in patients with chronic venous ulcers. Thirty six patients with chronic venous ulcers visiting the Department of Dermatology of Madras Medical College and Rajiv Gandhi Government General Hospital during the period November 2014 to February 2015 and from January 2016 to July 2016 and satisfying the inclusion criteria were selected. All cases were assessed clinically and the necessary investigations required for diagnosis were carried out. Patients were then randomly divided into two treatment groups – topical autohemotherapy and topical autologous platelet rich plasma. The response to treatment in terms of healing of ulcer in the patients were assessed with serial measurements of the ulcer during the study period. All the data were documented, analysed and the results were derived.

Out the 36 eligible patients included in the study, 18 were randomly selected as INTERVENTION GROUP A and the rest 18 patients were grouped under INTERVENTION GROUP B.

As 3 patients had more than one ulcer, for statistical purposes, each ulcer was considered as a single entity and hence in intervention group A, total numbers of ulcers were 22 and in intervention group B total number of ulcers were 18.

Group	Number of patients	Percentage	Number of ulcers
A	18	50	22
B	18	50	18
Total	36	100	40

Table 6: Number of patients and ulcers in each group

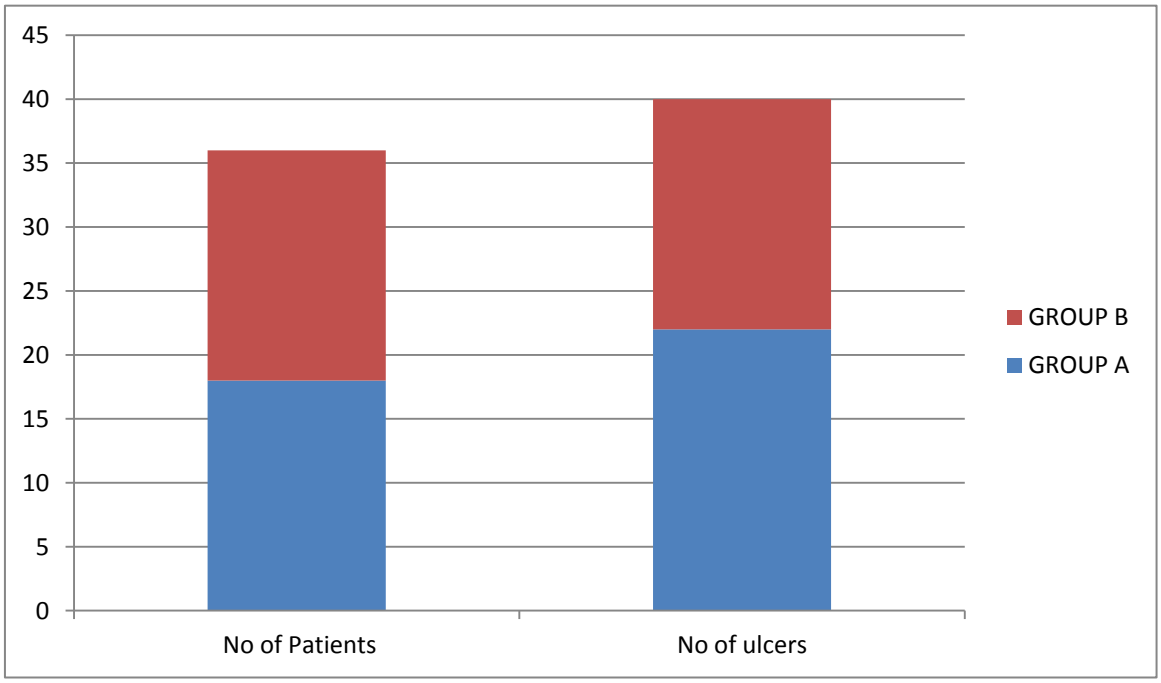


Figure 8: Number of patients and ulcers in each group

AGE DISTRIBUTION

The lowest age was 27 and the highest age was 72. Median age was 49.5 years.

AGE	No. OF PATIENTS
LESS THAN 20	0
21-30	1
31-40	6
41-50	12
51-60	8
61-70	8
MORE THAN 70	1
TOTAL	36

Table 7: Age distribution

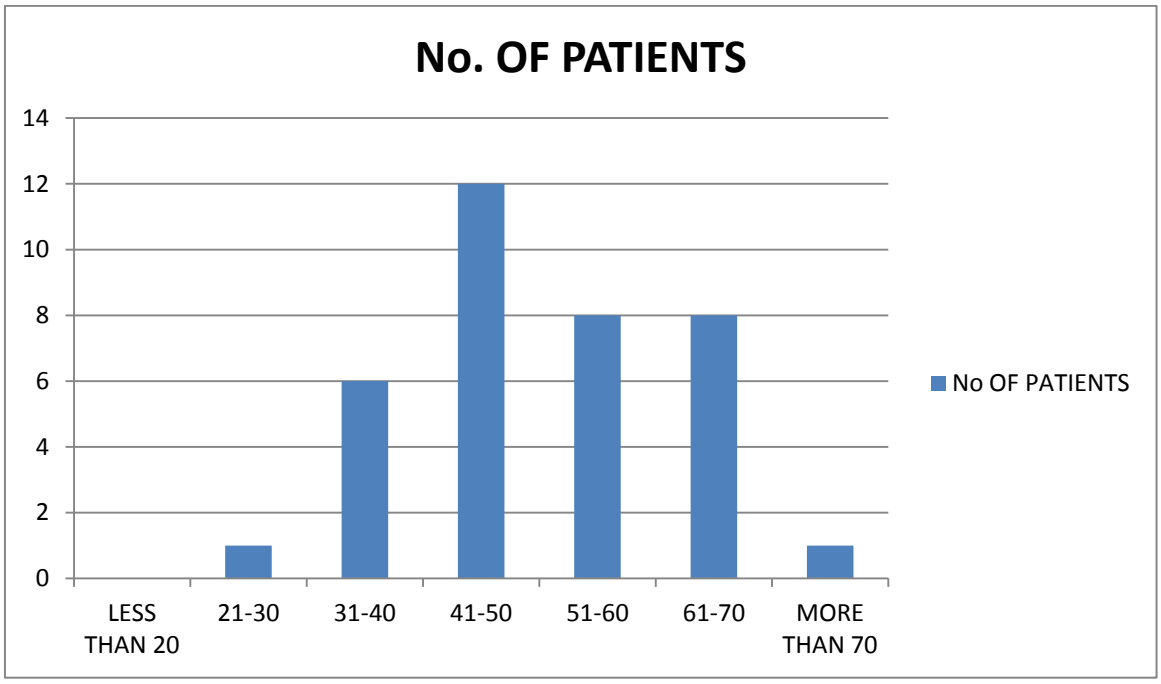


Figure 9: Age distribution

GENDER DISTRIBUTION

Totally 36 patients with venous leg ulcers, who fulfilled the inclusion criteria were taken into the study, of which 34 were male accounting for 94.4% of the total study population.

Out of the total 18 patients in intervention group A, all were males(100%) whereas in intervention group B, out of total 18 patients , 16 were male(88.8%) and 2 were female(11.2%).

SEX	NUMBER	PERCENTAGE
Females	2	5.6
Males	34	94.4
Total	36	100.0

Table 8: Gender Distribution

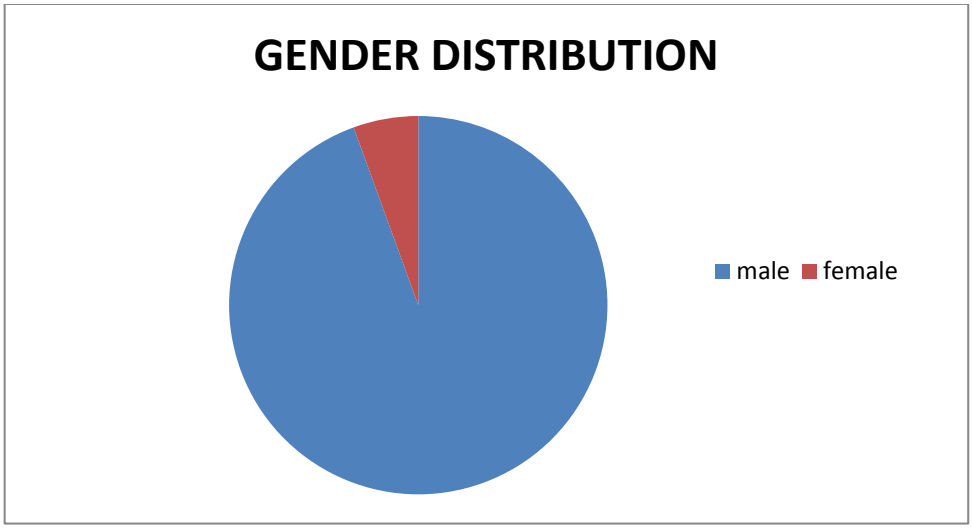


Figure 10: Gender Distribution

COMORBIDITIES

Out of 36 patients, 28 patients had no comorbid illness and 8 had hypertension.

Of the total 8 patients 3 patients were in group A and 5 were in group B.

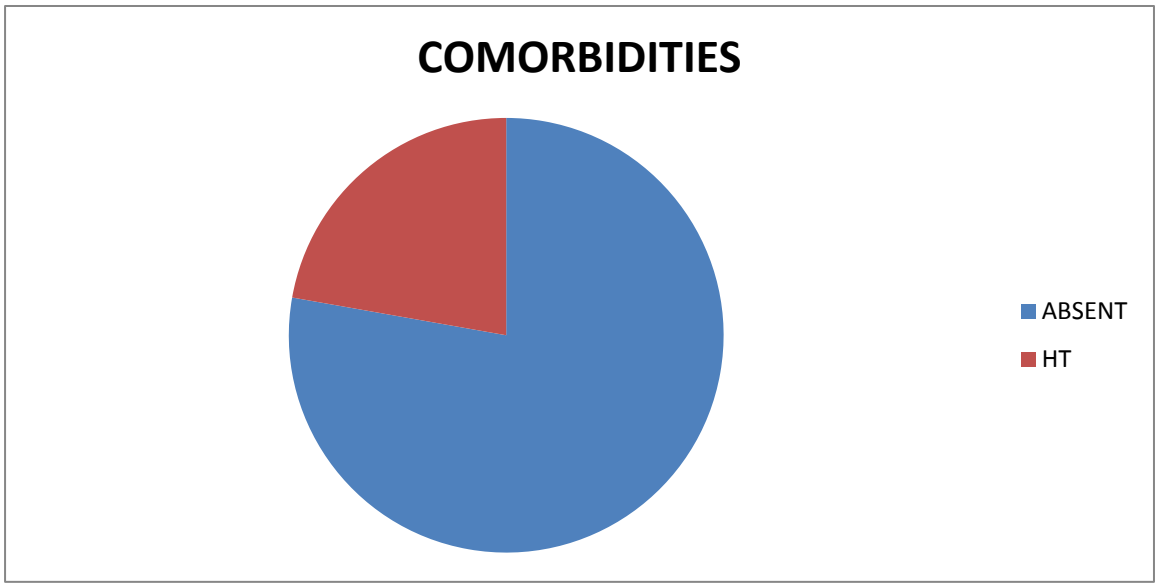


Figure 11: Comorbidities in the sample

Percentage Of Improvement In Ulcer Area	P Value	Percentage Of Improvement In Ulcer Volume	P Value
PA1	0.4	PV1	0.7
PA2	0.5	PV2	0.8
PA3	0.9	PV3	0.8
PA4	0.8	PV4	0.9
PA5	0.8	PV5	0.8
PA6	0.5	PV6	0.9

Table 9: Impact of hypertension on the percentage of improvement in area and volume of ulcers in two treatment groups

SMOKING

Out of total 36 patients in the study, 21 individuals gave positive history of smoking (58.3%).

Smoking	Number	Percentage
No	15	41.7
Yes	21	58.3
Total	36	100.0

Table 10: Presence of Smoking

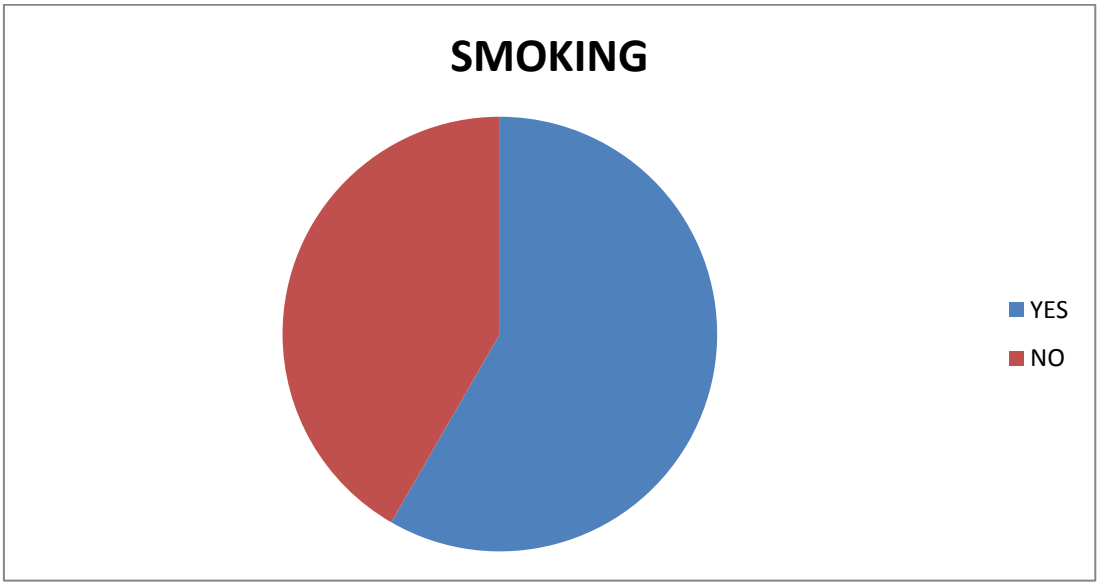


Figure12: Presence of smoking

ALCOHOL CONSUMPTION

Out of the total 36 patients included in the study 12 patients gave history of alcohol consumption (33.3%).

Alcohol Consumption	Number	Percentage
No	24	66.7
Yes	12	33.3
Total	36	100

Table 11: Alcohol consumption

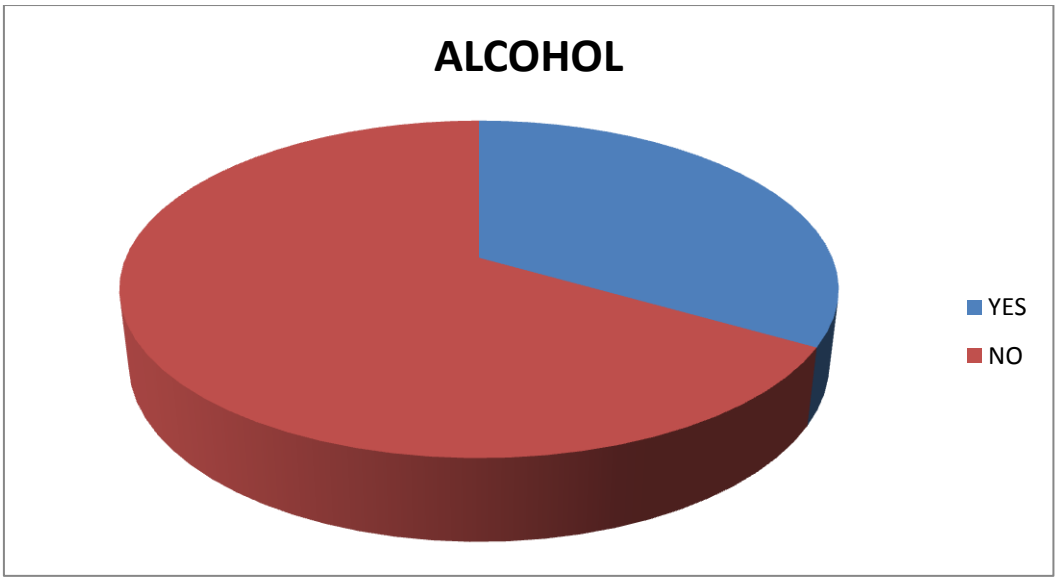


Figure 13: Alcohol consumption

DURATION OF ULCERS

The mean duration of ulcer was 4.23 months.

DURATION OF ULCER (months)	No. OF ULCERS (GROUP A)	No. OF ULCERS (GROUP B)
<3	8	3
3-6	8	8
6-9	6	7
9-12	0	0
TOTAL	22	18

Table 12: Duration of ulcers

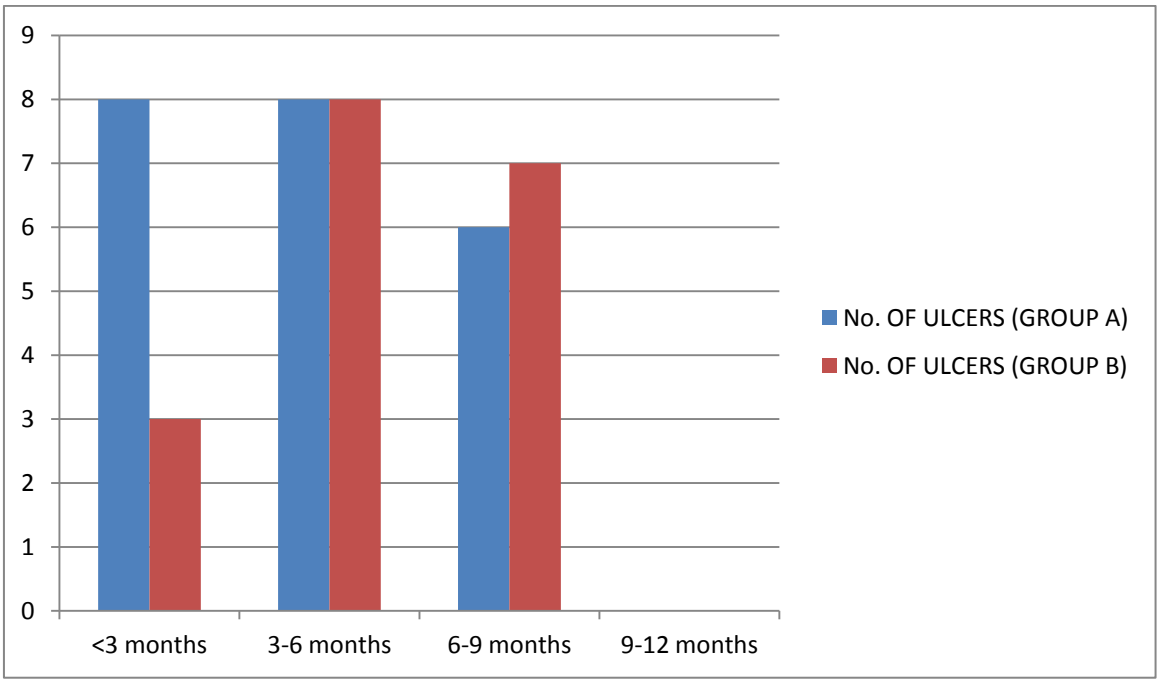


Figure 14: Duration of ulcers

SITE OF ULCER

Out of 40 ulcers from 36 patients taken into study, the ulcers were predominantly in the lower one third of the leg (92.5%), followed by middle one third accounting for 7.5% of the total.

SITE	GROUP A	GROUP B	TOTAL
LOWER 1/3rd	19	18	37
MIDDLE 1/3rd	3	0	3
TOTAL	22	18	40

Table 13: Site of ulcer distribution.

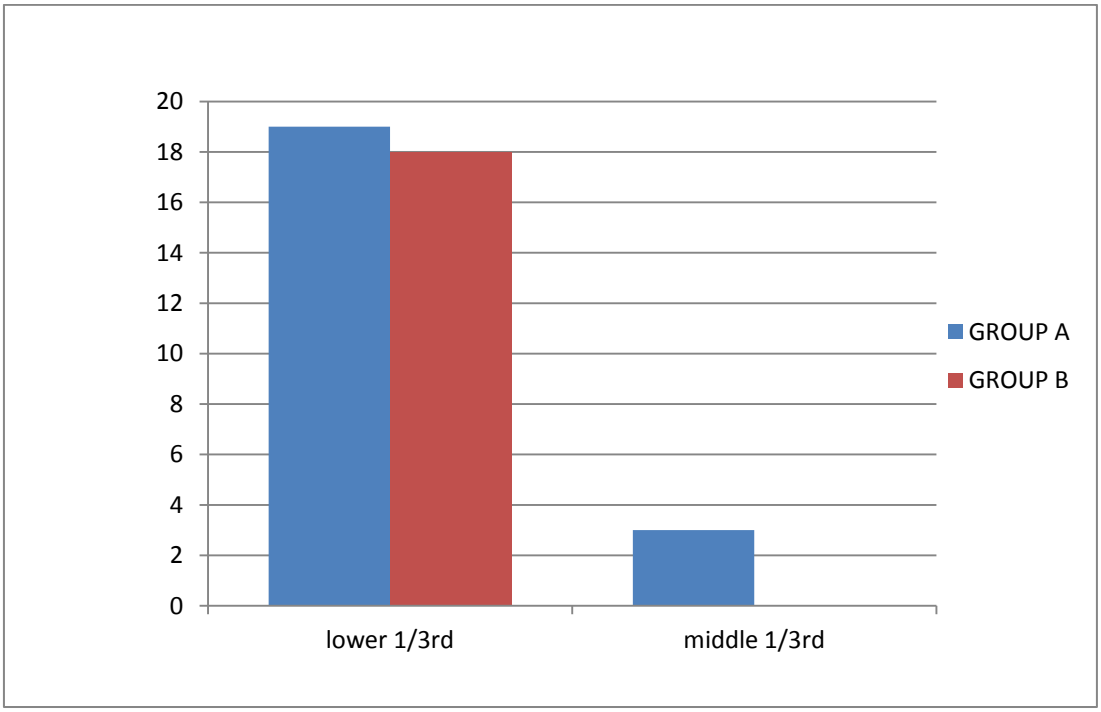


Figure 15: Site of ulcer distribution

SIDE OF ULCER

Out of the 40 ulcers in the study, most of the ulcers were in the medial aspect (72.5%) of the leg, followed by lateral aspect (22.5%) and anterior aspect of the leg (5%).

SIDE OF ULCER	GROUP A	GROUP B	TOTAL	PERCENTAGE
MEDIAL	15	14	29	72.5%
LATERAL	5	4	9	22.5%
ANTERIOR	2	0	2	5%
TOTAL	22	18	40	100%

Table 14: Side of ulcer distribution

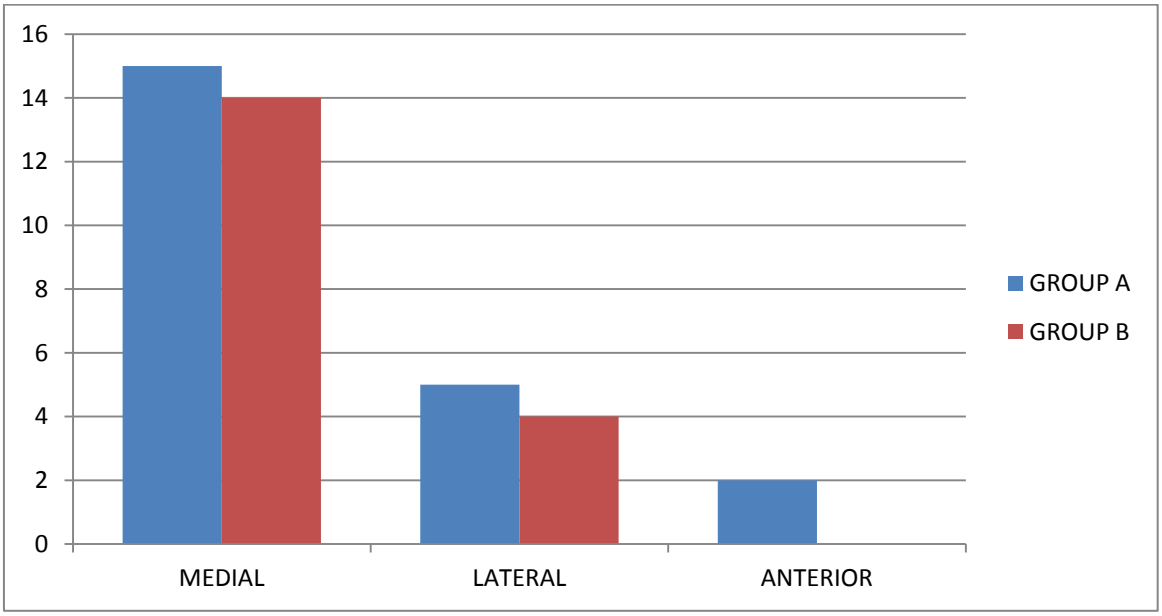


Figure 16: Side of ulcer distribution

WOUND SWAB CULTURE

In all the 36 patients, swabs were obtained and were sent for culture and sensitivity. Cultures were positive in 5 specimens (12.5%) with Klebsiella species (60%) as the most common organism isolated followed by Staphylococcus aureus (40%). Patients with positive growth in culture were appropriately treated with antibiotics and added to the study after completion of the antibiotic course and negative culture report**.**

CULTURE	No. OF ULCERS
No Growth	31
Klebsiella	3
Staph. Aureus	2
Total	36

Table 15: Wound swab culture

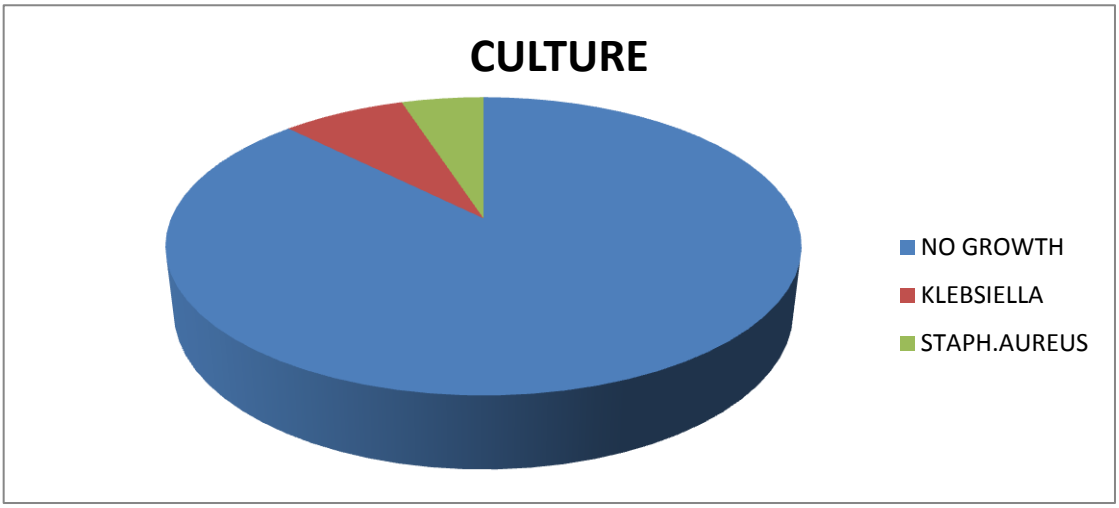


Figure 17: Wound swab culture

DIFFERENCE IN THE MEAN AREA FROM 1st WEEK TO 6thWEEK IN TWO TREATMENT GROUPS

At the end of each sitting the improvement in the area of the ulcer from the baseline value was calculated in both the groups.

In group A, the mean baseline area of the ulcer was 8.3 cm^2 and at the end of six sittings it was reduced to 1.1 cm^2.

In group B, the mean baseline area of the ulcer was 11.9 cm^2 and at the end of six sittings it was reduced to 2.2 cm^2.

Area	**A0**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**
Group A	8.3	7.8	6.3	4.9	3.3	2.2	1.1
Group B	11.9	11.01	8.8	6.9	5.0	3.5	2.2
P value	***.03***	***.05***	***.12***	***.2***	***.1***	***.2***	***.2***

Table 16: Difference in the mean area from 1st week to 6th week in two treatment groups

DIFFERENCE IN THE MEAN VOLUME FROM 1st WEEK TO 6th WEEK IN TWO TREATMENT GROUPS

At the end of each sitting the improvement in the volume of the ulcer from the baseline value was calculated in both the groups.

In group A, the mean baseline volume of the ulcer was 2.2 cm^3 and at the end of six weeks it was reduced to 0.06 cm^3.

In group B, the mean baseline volume of the ulcer was 3.4 cm^3 and at the end of six weeks it was reduced to 0.78 cm^3.

Volume	**V0**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Group A	2.2	1.8	1.0	.63	.29	.29	.06
Group B	3.4	2.6	1.6	.8	.47	.30	.78
P value	***.03***	***.1***	***.2***	***.5***	***.4***	***.9***	***.3***

Table17: Difference in the mean volume from 1st week to 6th week in two treatment groups

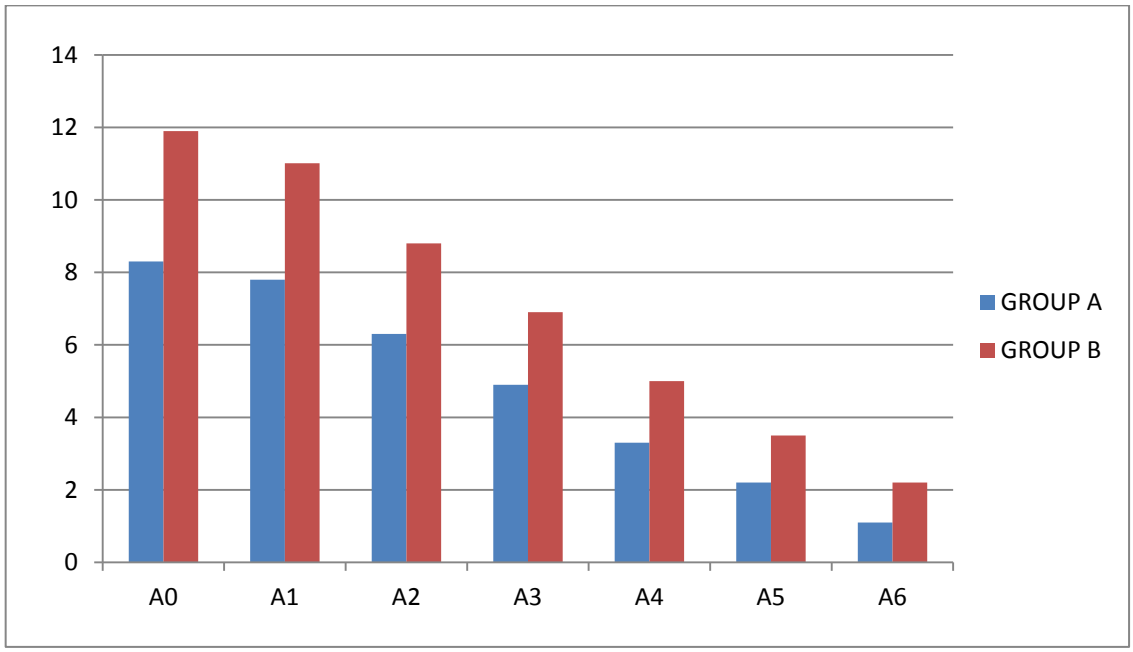


Figure 18: Difference in the mean area from 1st week to 6th week in two treatment groups

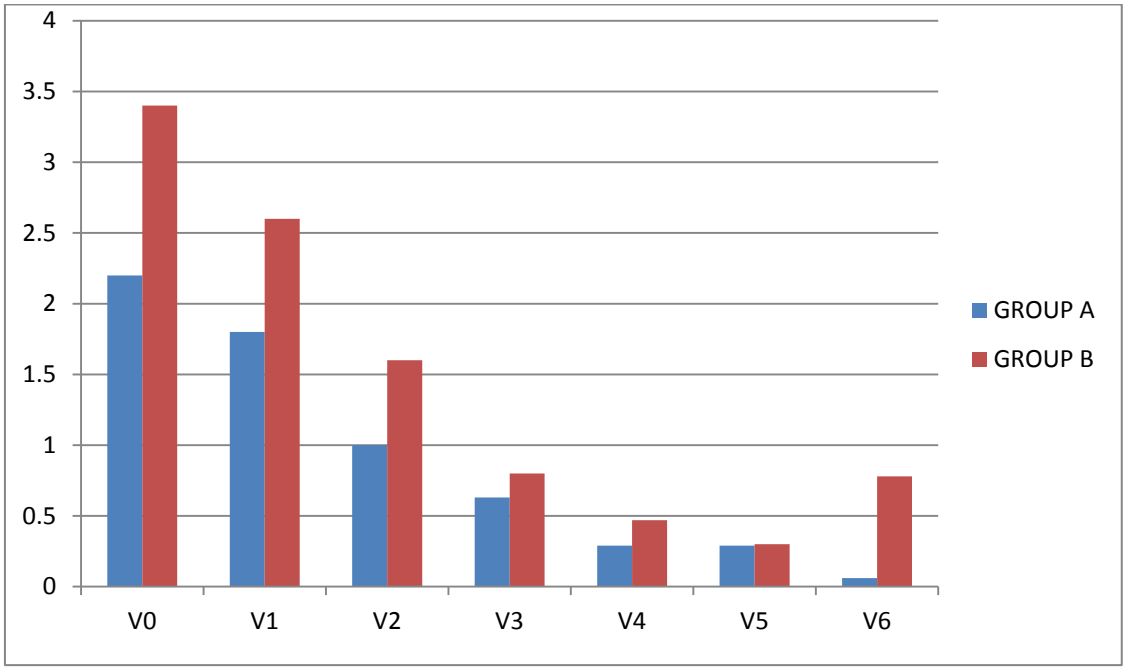


Figure 19: Difference in the mean volume from 1st week to 6th week in two treatment groups

DIFFERENCE IN THE MEAN PERCENTAGE OFIMPROVEMENT IN AREA FROM 1st WEEK TO 6th WEEK IN TWO TREATMENT GROUPS

After every sitting of intervention in both the groups, the percentage of improvement in the area of the ulcer was calculated. In group A, at the end of six weeks, the percentage of improvement in the area was88.4% whereas in group B, the percentage of improvement in the area was 85%.

% improvement in area	**PA1**	**PA2**	**PA3**	**PA4**	**PA5**	**PA6**
Group A	8.8	31.2	50	68.3	79.4	88.4
Group B	8.7	28	45.5	62	75.3	85
P value	***.9***	***.6***	***.5***	***.4***	***.4***	***.45***

Table 18: Difference in the mean percentage of improvement in area from 1st week to 6th week in two treatment groups

DIFFERENCE IN THE MEAN PERCENTAGE OF IMPROVEMENT IN VOLUME FROM 1st WEEK TO 6th WEEK IN TWO TREATMENT GROUPS

After each sitting, the percentage of improvement in the volume of the ulcer was calculated in both the groups.

In group A at the end of six weeks, the mean volume of the ulcer improved by 97.9% whereas in group B, the percentage of improvement in the mean volume was 88.4%.

% improvement in volume	**PV1**	**PV2**	**PV3**	**PV4**	**PV5**	**PV6**
Group A	25.7	63.6	79.3	85.2	90.8	97.9
Group B	25.4	59.8	80.6	85.6	95.3	88.4
P value	***.9***	***.6***	***.84***	***.95***	***.34***	***.1***

Table 19: Difference in mean percentage of improvement in volume from 1st week to 6th week in two treatment groups

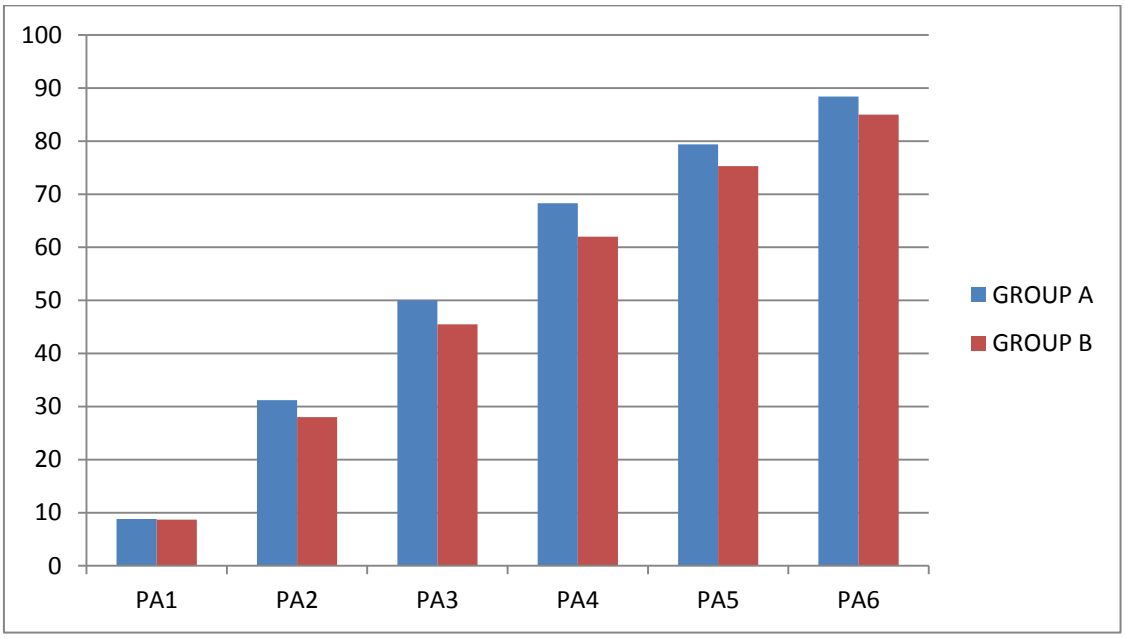


Figure20: Difference in the mean percentage of improvement in area from 1st week to 6th week in two treatment groups

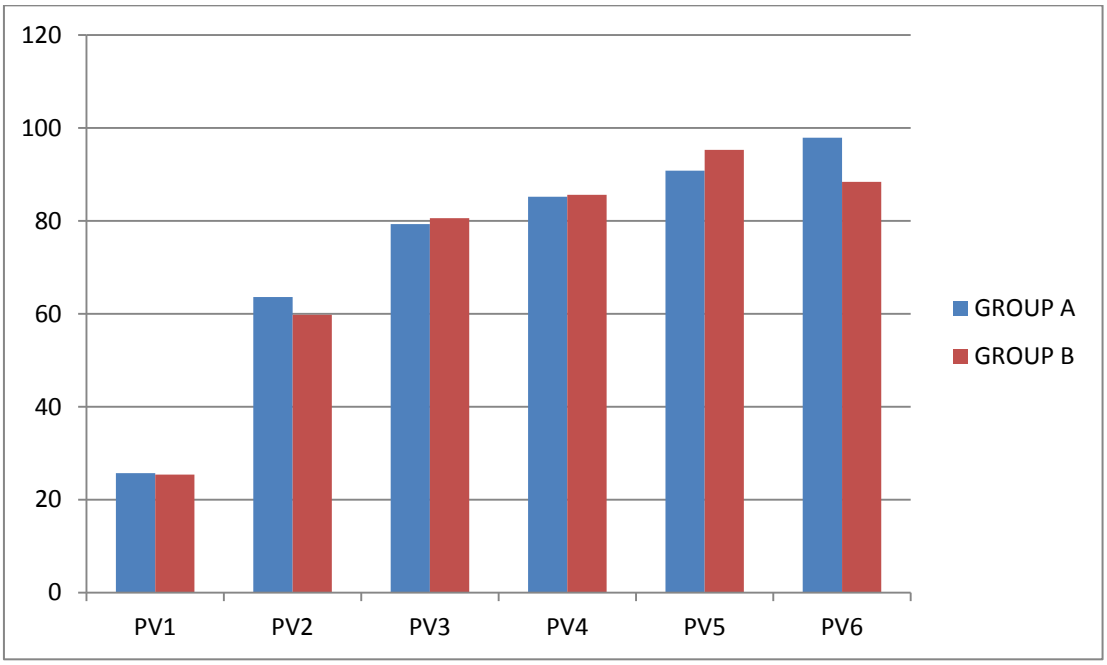


Figure21: Difference in mean percentage of improvement in volume from 1st week to 6th week in two treatment groups

PERCENTAGE OF IMPROVEMENT IN AREA OF ULCER AT THE END OF 6WEEKS:

In our study at end of 6 weeks of therapy, more than 90% improvement in ulcer area was noticed in 10 ulcers (45.5%) in group A and 8 ulcers (44.4%) in group B.

%improvement in area at the end of 6weeks	Group A	Group B
<60	0	2
61-70	3	2
71-80	3	1
81-90	6	5
91-100	10	8
Total	22	18

Table 20: Percentage of improvement in area of the ulcer at the end of 6 weeks

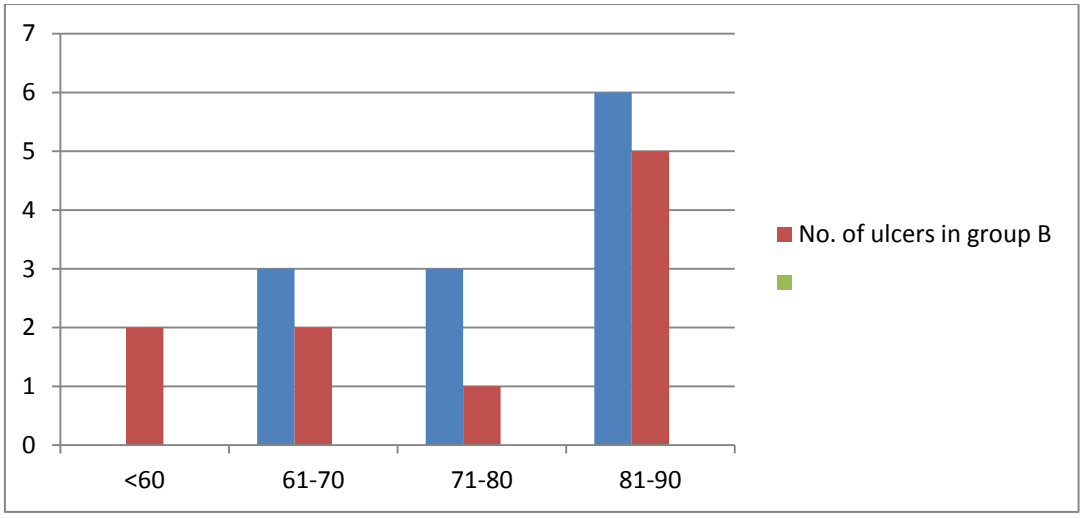


Figure22: Percentage of improvement in area of ulcer at the end of 6weeks

PERCENTAGE OF IMPROVEMENT IN VOLUME OF ULCER AT THE END OF 6WEEKS:

21 ulcers (95.45%) in group A showed more than 90% improvement in the volume of ulcer at six weeks and in group B it was seen in 17 ulcers (94.4%).

%improvement in volume at the end of 6 weeks	Group A	Group B
<60	0	0
61-70	0	0
71-80	0	0
81-90	1	1
91-100	21	17
Total	22	18

Table 21: Percentage of improvement in volume of ulcer at the end of 6 weeks

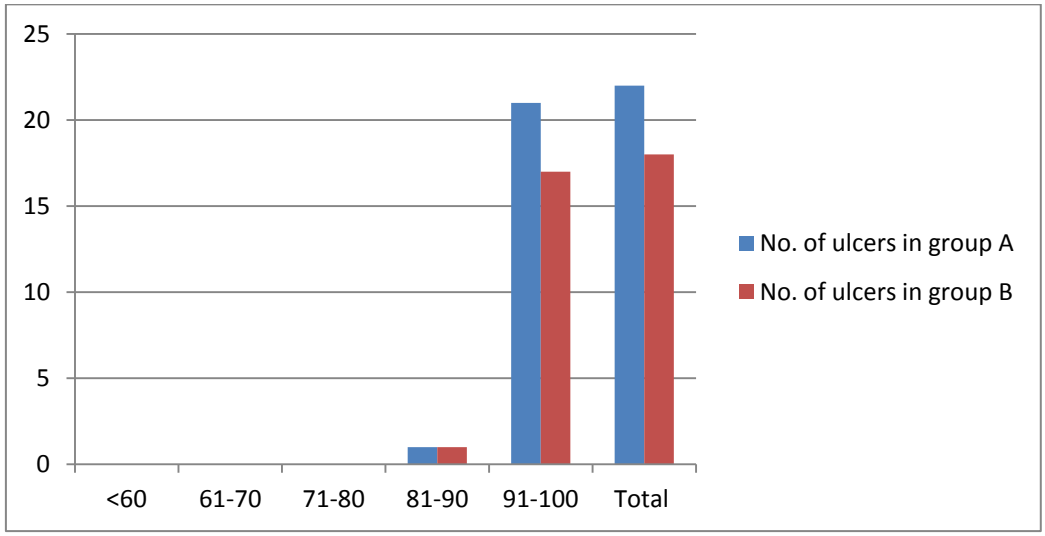


Figure 23: Percentage of Improvement in volume of ulcer at the end of 6 weeks

100% IMPROVEMENT IN AREA OF ULCER:

100% improvement in the area at the end of six weeks was noticed in 9 ulcers (41%) in group A and 3 ulcers (16.6%) in group B.

No. of weeks	Group A	Group B
2	0	0
3	1	0
4	3	0
5	1	0
6	4	3
TOTAL	9	3

Table 22: Number of weeks at which 100% improvement in area was observed

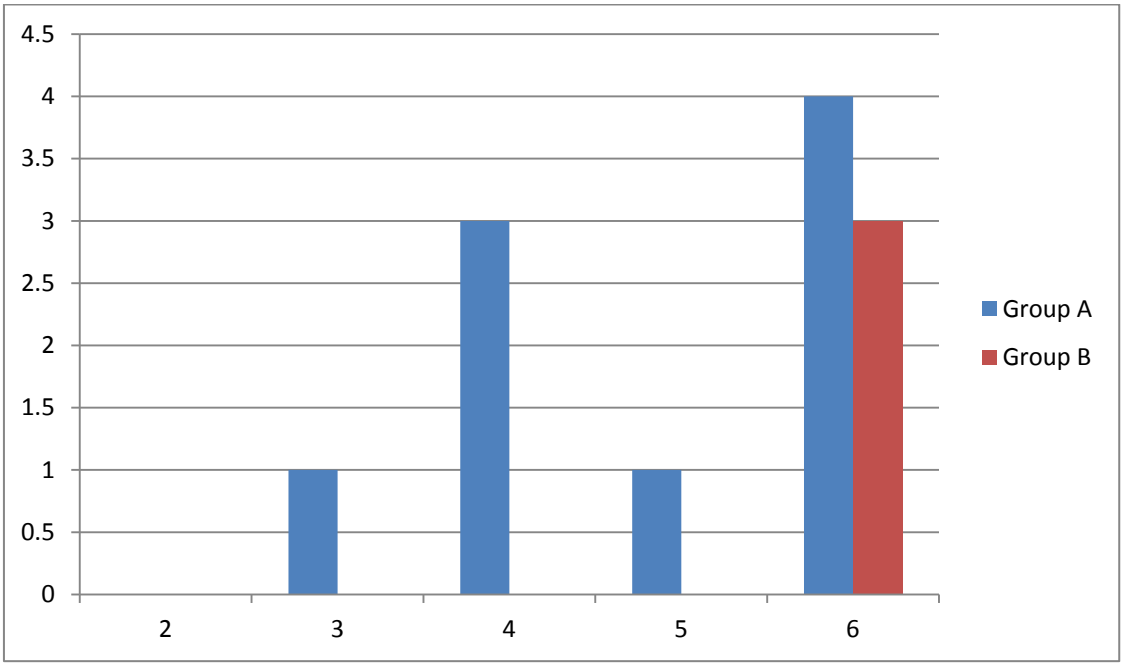


Figure 24: Number of weeks at which 100% improvement in area was observed

100% IMPROVEMENT IN VOLUME OF THE ULCER:

100% improvement in volume was noticed in 21 ulcers (95.45%) in group A and 16 ulcers (88.9%) in group B.

No. of weeks	Group A	Group B
2	2	2
3	7	4
4	5	4
5	3	6
6	4	0
TOTAL	21	16

Table 23: Number of weeks at which 100% improvement in volume was observed

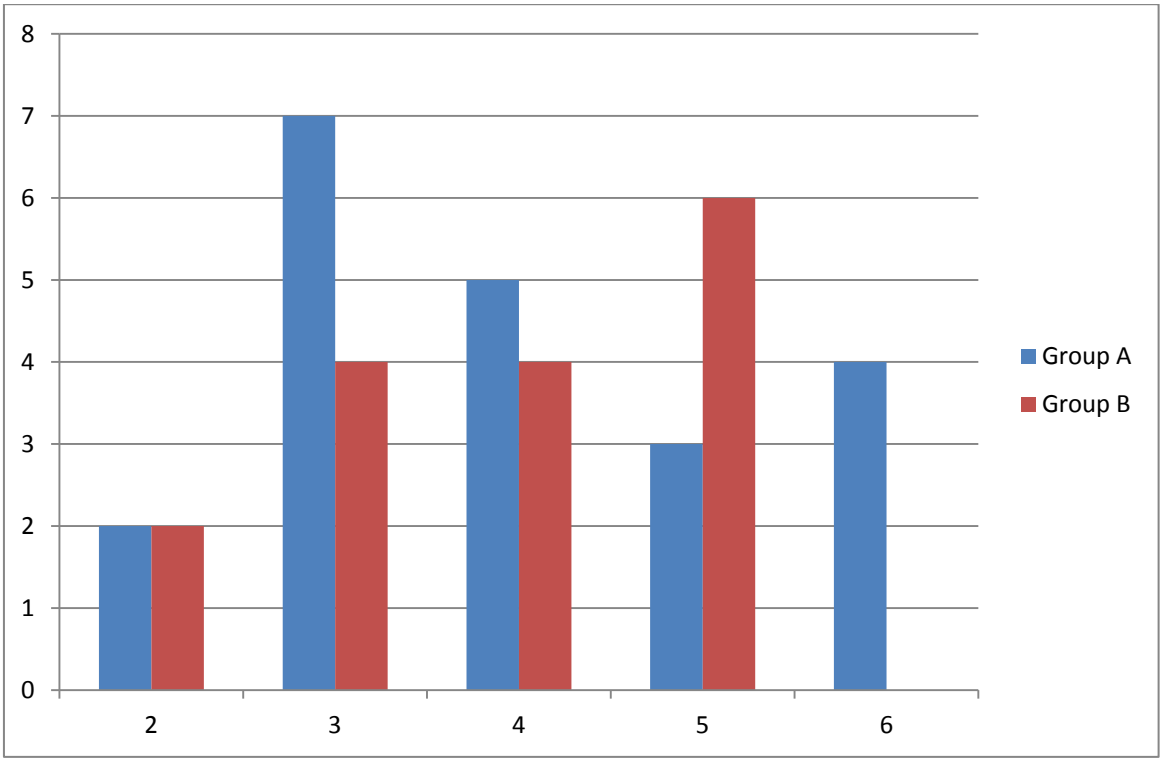


Figure 25: Number of weeks at which 100% improvement in volume was observed

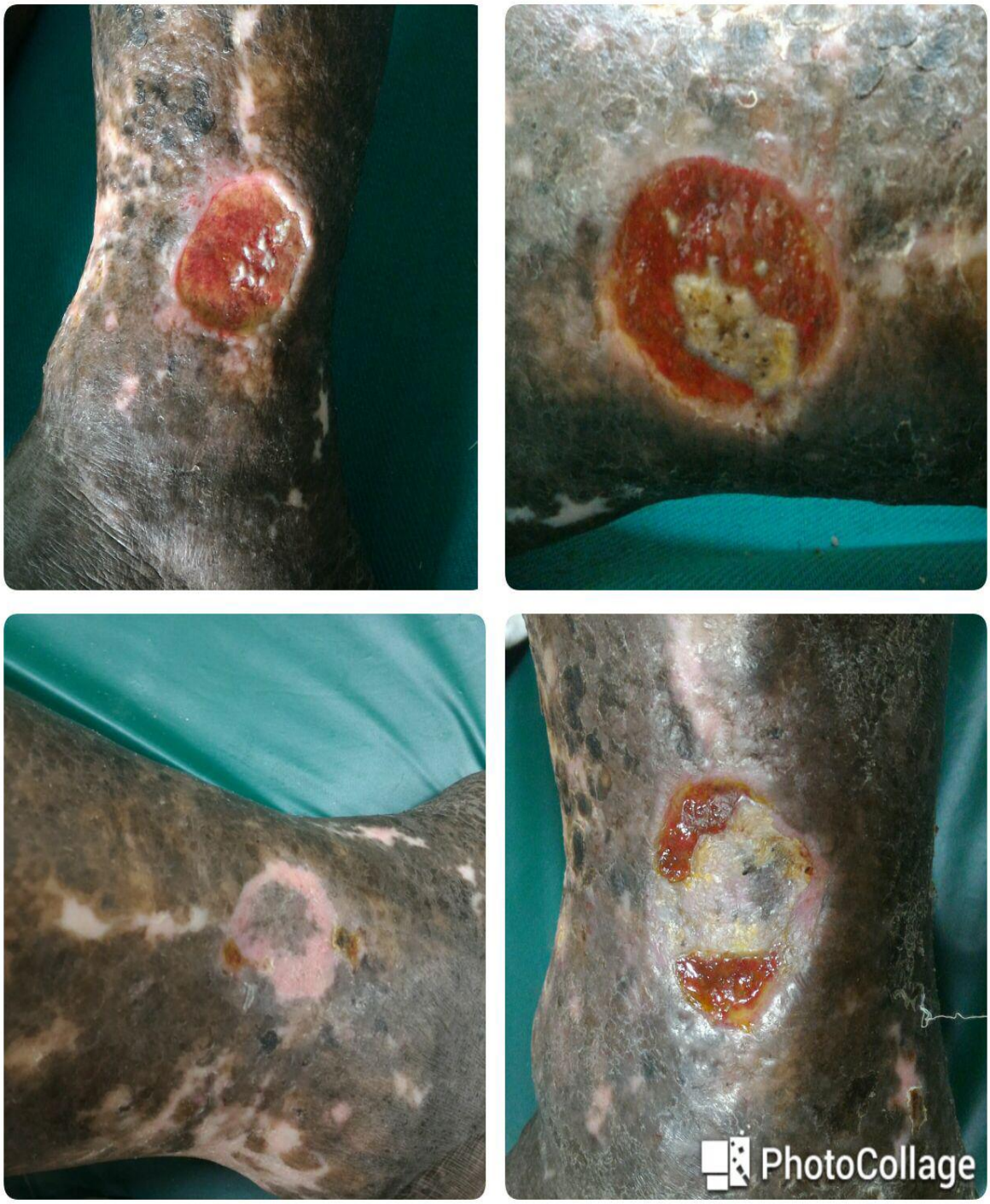


Figure 26: Ulcer healing following topical autohemotherapy

Clockwise-at week 0- week 2-week 4-week 6

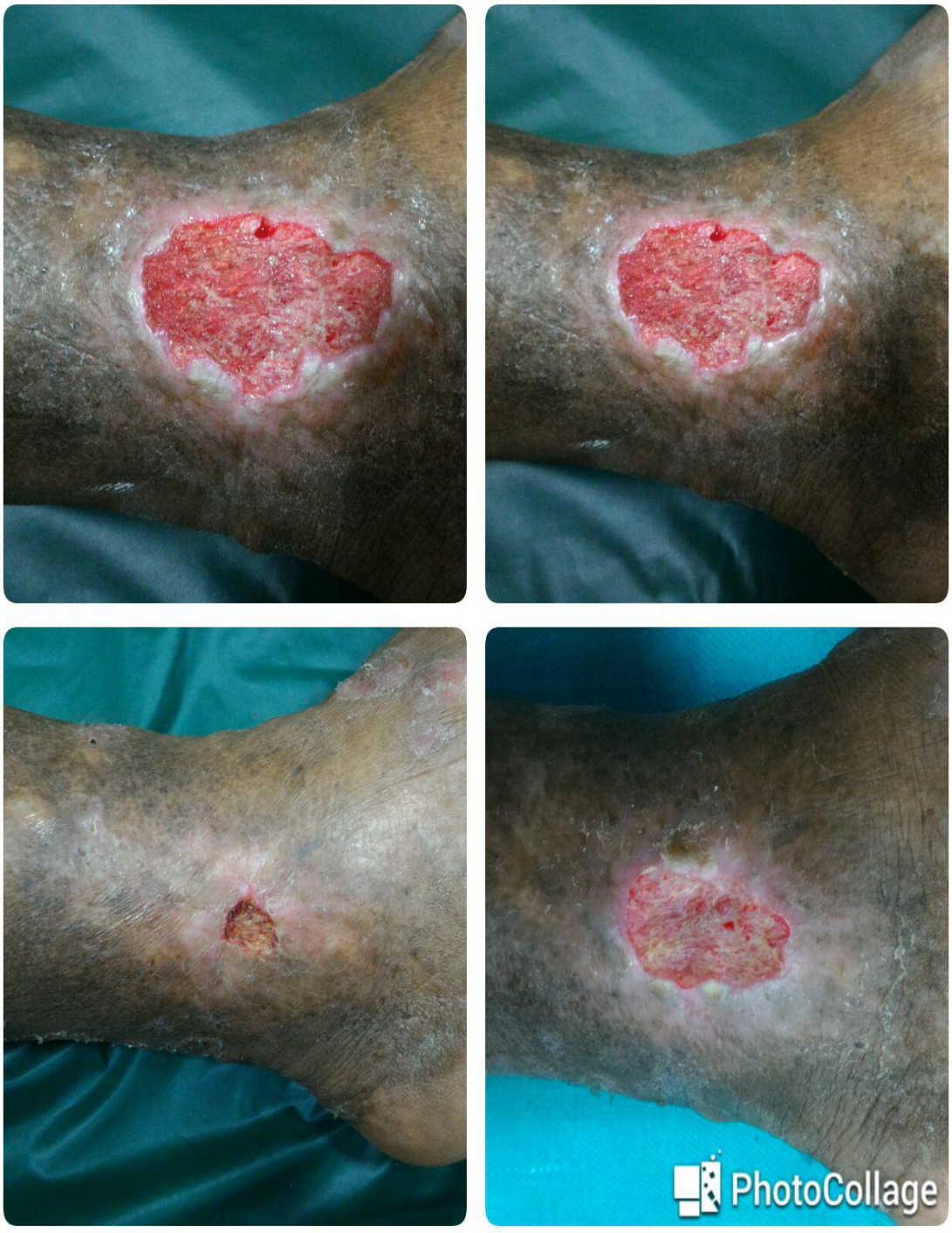


Figure 27: Ulcer healing following topical autohemotherapy

Clockwise-at week 0- week 2-week 4-week 6

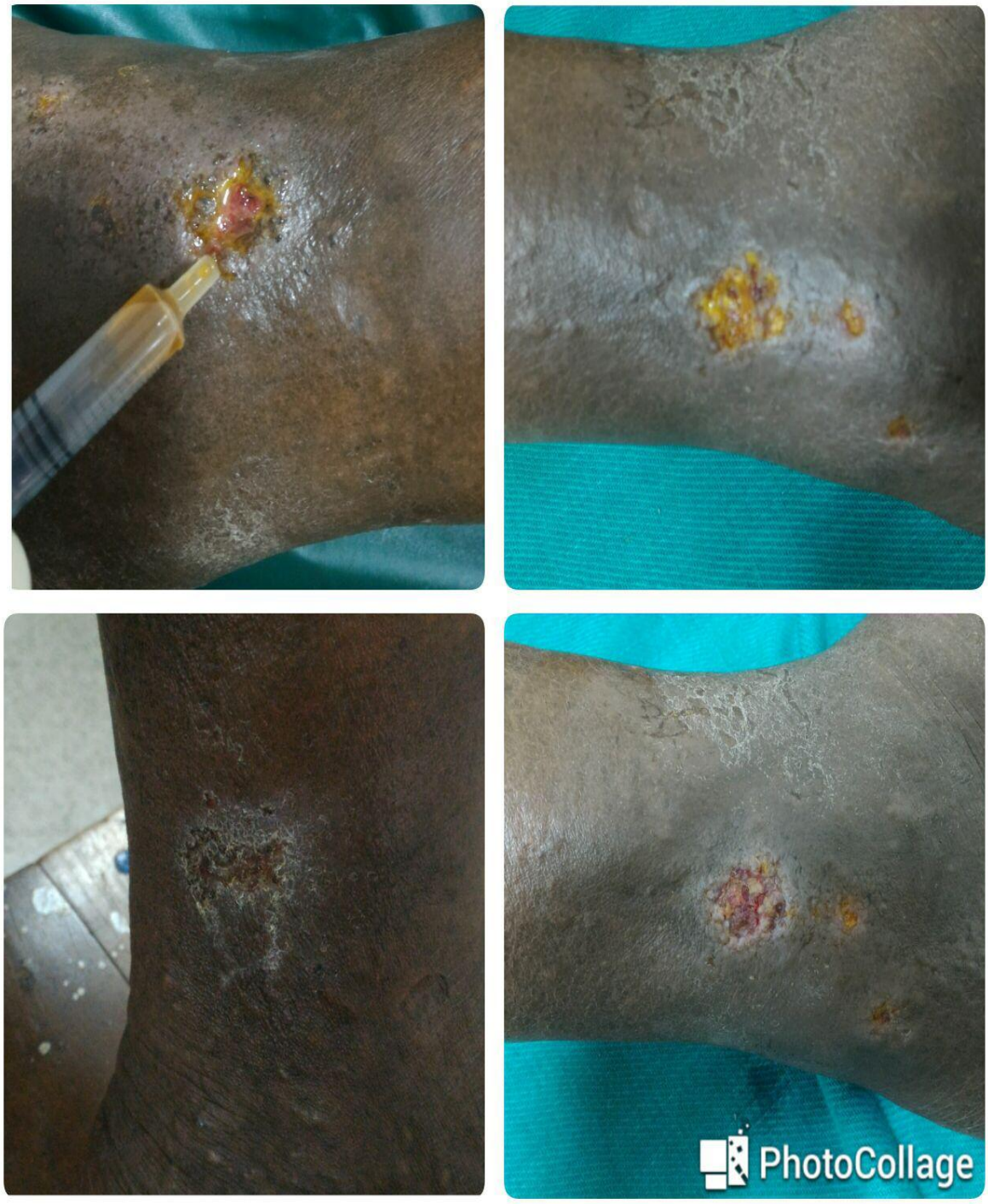


Figure 28: Ulcer healing following topical autologous PRP

Clockwise-at week 0- week 2-week 4-week 6

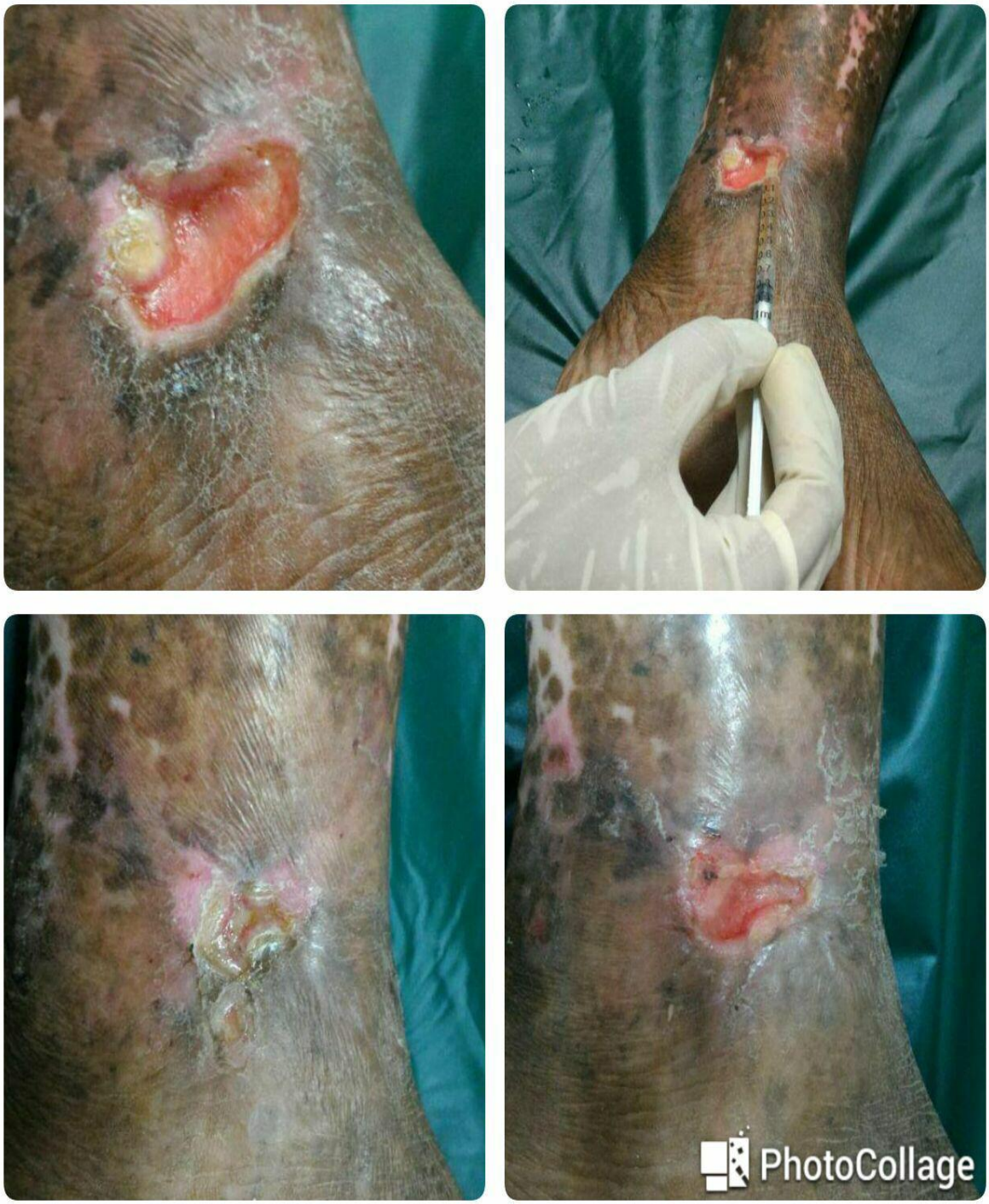


Figure 29: Ulcer healing following topical autologous PRP

Clockwise-at week 0- week 2-week 4-week 6

DISCUSSION

DISCUSSION

This prospective, interventional and comparative study was conducted to determine the effectiveness of topical autohemotherapy and topical autologous platelet rich plasma as a treatment modality in patients with chronic venous ulcers not responding to conventional therapies and also to compare their response in both the treatment groups. Thirty six patients with forty ulcers attending the Department of Dermatology, Madras Medical College and Rajiv Gandhi Government General Hospital during the period November 2014 to February 2015 and January 2016 to July 2016 were selected. They were assessed clinically and necessary investigations for diagnosis were carried out. Patients were then randomly divided into two treatment groups namely those receiving topical autohemotherapy and topical autologous platelet rich plasma. All patients were reviewed every week clinically with serial photographs and wound measurements to assess the response. All data were documented and analysed and the results were obtained.

36 were patients included in the study of which four patients had saphenofemoral incompetence, eight patients had saphenofemoral and perforator incompetence and twenty four patients had perforator incompetence.

Age:

Of the total 36 patients in the study, the maximum age was 72 years and the minimum age was 27.Median age in the study group was 49.5 years. Most of the patients were in the age group of 41 to 50 years (30.5%).

Sex:

Though literature states venous ulcers are more common in females, it was observed in our study that majority of patients were male (94.4%) and this observation was similar to the studies done by Sacchidanand et al[83],Salaar alvarez et al[85] and Yimaz et al[86].

Comorbidities and other risk factors:

In our study it was noted that hypertension was present in about 22.2% of the patients. But the impact of hypertension on the percentage of improvement in area and volume of ulcers in both the groups was not statistically significant. Similarly 58.3% of patients gave positive history of smoking and33.3 % of patients gave history of alcohol consumption.

Mean duration of ulcer:

In our study the mean duration of ulcer was 4.23 months. Mean duration of ulcer in Autohemotherapy group was 3.95 months and in PRP group, the mean duration was 4.59 months. Overall the minimum and maximum duration of ulcer at the time of presentation was 2 months and 6 months respectively.

The mean ulcer duration was comparable with other similar studies on venous ulcers. Studies done by Suryanarayan et al[84] and Sarvajanmurthy et al[83] have observed a mean duration of ulcer as 5.35 months and 4.75 months respectively.

Site and side of ulcer:

Almost 92.5 % of the ulcers were in the lower one third of leg. Majority of the ulcers were in the medial aspect (72.5%) of the leg, followed by lateral aspect (22.5%). Only 5% of the ulcers were present in the anterior aspect of leg. In a study done by Salaar alvarez et al, similar observations were noted with majority of the ulcers in medial aspect (66.6%).

Wound swab and culture:

In all the 40 ulcers, swabs were obtained and sent for culture and sensitivity. Cultures were positive in 5 specimens (12.5%) with Klebsiella species (60%) as the most common organism followed by Staphylococcus aureus (40%).

Area and volume of the ulcers:

In both the treatment groups, area and volume of the ulcers were measured prior to the initiation of treatment and at the end of each sitting for six consecutive weeks.

It was observed that the mean baseline area of the ulcer in Autohemotherapy group was 8.3 cm^2 and in PRP group it was 11.9cm^2. At the end six weeks the mean area of the ulcer in autohemotherapy group and PRP group were 1.1cm^2 and 2.2 cm^2respectively. Similarly the mean baseline volume of ulcer in Autohemotherapy group was 2.2cm^3 and in PRP group was 3.4 cm^3. At the end of six weeks the mean volume of ulcer in autohemotherapy group and PRP group were 0.06 cm^3 and 0.78 cm^3 respectively.

These observations show that there is significant improvement in area and volume of the ulcer in both the groups at the end of 6 weeks. But this observation of difference in the improvement in mean area (p=0.2)and mean volume (p=0.3) in both groups was not statistically significant .In terms of percentage of improvement in mean area in autohemotherapy group it was 88.4% where as in PRP group it was 85%, which is also statistically not significant (p=0.45). Similarly the percentage of improvement in mean volume at six weeks in autohemotherapy group was 97.9% and PRP group was 88.4%, which is also not statistically significant(p=0.1).

The improvement in area and volume of the ulcer after treatment with PRP is comparable with various studies on PRP treatment for ulcers.

In a study done by Suryanarayanan et al on effect of autologous PRP on non healing leg ulcers where a total of 33 ulcers were taken into study and at the

end of 6 weeks it was observed that 91.7% reduction in area and 95 % reduction in volume of the ulcers[84].

In another study by Sarvajnamurthy et al on efficacy of autologous PRP on chronic venous ulcers, 17 ulcers were treated with PRP and at the end of 6weeks it was observed a reduction in mean area and volume by 94.7% and 95.6% respectively[83].

Waniczek et al studied the effect of PRP on chronic ulcers in 10 patients and found that 54% reduction in ulcer area by 4 weeks and 93% reduction in ulcer area by 8 weeks of therapy[87].

In our study at end of 6 weeks of therapy, more than 90% improvement in ulcer area was noticed in 10 ulcers (45.5%) in autohemotherapy group and 8 ulcers (44.4%) in a PRP group. Similarly 21 ulcers (95.45%) in autohemotherapy group showed more than 90% improvement in ulcer volume at six weeks and in PRP group it was seen in 17 ulcers (94.4%).

It was also noticed that 100% improvement in area at the end of six weeks was noticed in 9 ulcers (41%) in the autohemotherapy group and 3 ulcers (16.6%) in PRP group.

Similarly 100% improvement in volume was noticed in 21 ulcers (95.45%) in the autohemotherapy group and 16 ulcers (88.9%) in PRP group.

Similar results were observed in other studies on autologous PRP on chronic venous ulcers.

In a study done by Suryanarayanan et al, it was shown that 100% reduction in area and volume in 76.5% and 82.3% of ulcers respectively[84].

In a similar study by Sarvajnamurthy et al, it was observed that 100% improvement in volume and area in 75.7% and 72.7% respectively[83].

Many studies have shown significant improvement in the area of the ulcer following treatment with autologous platelet rich plasma.

Study	% of improvement in ulcer area after treatment
Salazar et al[85]	45.5%
Yimaz et al[86]	94%
Fykberg et al[88]	96.9%
Kakudo et al[89]	60%
Ayman et al[90]	65%
Connel et al[91]	67.7%

Table 24: Comparative studies on improvement of ulcer area following platelet rich plasma therapy

SUMMARY

SUMMARY

Based on our study, the following interpretations were made:

- Most common age group of patients with chronic venous ulcer was 41 to 50 years (30.5%).
- The median age was 49.5 years.
- Males (94.4%) were more commonly affected than females (5.6%).
- Smoking was present in 58.3% of study population.
- Alcohol consumption was seen in 33.3 % of study population
- Hypertension was present in about 22.2% of the patients. But the impact of hypertension on the percentage of improvement in area and volume of ulcers in both the groups is not statistically significant.
- Mean duration of the ulcer was 4.23 months.
- Most common site of ulcer was in the lower one third of the leg (92.5 %).
- Most common side of the ulcer was the medial aspect (72.5%), followed by lateral aspect (22.5%).
- Wound swab cultures were positive in 12.5%.
- Most common organism isolated was Klebsiella species (60%) followed by Staphylococcus aureus (40%).

- There was significant improvement in mean area of the ulcer following autohemotherapy from 8.3 cm^2 to 1.1cm^2 at the end of 6 weeks.
- Similarly there was improvement in the volume of ulcer from 2.2 cm^3 to 0.6cm^3.
- It was also noted that there was significant improvement in mean area of the ulcer following topical autologous PRP from 11.9 cm^2 to 2.2cm^2 at the end of 6 weeks.
- Similarly there was improvement in the volume of ulcer from 3.4 cm^3 to 0.78cm^3.
- But the difference in improvement in mean area and volume at the end of 6 weeks between the two groups was statistically not significant - mean area (p=0.2) and mean volume (p=0.3).
- The percentage of improvement in the mean area at the end of six weeks following treatment with autohemotherapy and autologous platelet rich plasma was88.4% and 85% respectively (p=0.45).
- The percentage of improvement in the mean volume at the end of six weeks following treatment with autohemotherapy and autologous platelet rich plasma was97.9 % and 88.4% respectively (p=0.1).

- 45.5 % of ulcers in autohemotherapy group and 44.4% ulcers in autologous platelet rich plasma group showed more than 90% improvement in the area at the end of 6 weeks.
- 95.45 % of ulcers in autohemotherapy group and 90% ulcers in autologous platelet rich plasma group showed more than 90% improvement in the volume at the end of 6 weeks.

CONCLUSION

CONCLUSION

- Chronic venous ulcers, the most common cause of lower limb ulceration is emerging as a major public health challenge with morbidity and significant impact on the quality of life. Wound healing in these patients is a complex process which is regulated by the interaction between various cell types, extracellular matrix, cytokines and growth factors.
- Our study demonstrated that weekly treatments with topical autohemotherapy and topical autologous platelet rich plasma are equally efficacious in the management of chronic venous ulcers.
- Though both topical autohemotherapy and topical autologous PRP are safe, bio compatible, office based procedures, topical autohemotherapy is a simpler, cost effective, less time consuming procedure and does not require sophisticated equipment and hence may be considered as a primary treatment modality in the management of chronic venous ulcers in resource poor settings.
- From our study strong conclusions could not be made out regarding the efficacy of autohemotherapy and PRP due to small sample size. We need high quality data on comparative effectiveness of both treatment options to develop efficient algorithms for guiding therapy.
- In future, randomized controlled studies with sufficient sample sizes, focusing on outcomes as well as variations in platelet counts, growth

factor concentrations, applicability and cost effectiveness are necessary for the effective management of chronic venous ulcers.

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BIBILIOGRAPHY

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ANNEXURES

ANNEXURE- I

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. Bhuvaneswari V
Postgraduate M.D.(DVL)
Madras Medical College
Chennai – 600 003.

Dear Dr. Bhuvaneswari V,

The Institutional Ethics Committee has considered your request and approved your study titled **"A comparative study on the efficacy of topical autohaemotherapy Vs autologous platelet rich plasma in chronic venous leg ulcers". No.01122014.**

The following members of Ethics Committee were present in the meeting held on 02.12.2014 conducted at Madras Medical College, Chennai-3.

1. Dr.C.Rajendran, M.D.,	: Chairperson
2. Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3. Prof.B.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4. Prof.R.Nandini, M.D., Inst.of Pharmacology, MMC	: Member
5. Prof.P.Ragumani, M.S., Professor, Inst.of Surgery, MMC	: Member
6. Prof.Md.Ali, M.D., D.M., Prof. & HOD of Medl.G.E., MMC	: Member
7. Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC	: Member
8. Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3	: Member
9. Prof.S.G.Sivachidambaram, M.D., Director i/c, Inst.of Internal Medicine, MMC	: Member
10.Thiru S.Rameshkumar, Administrative Officer	: Lay Person
11.Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
12.Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

8/12/14

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

Member Secretary, Ethics Committee

ANNEXURE-II

PROFORMA

Name: Age: Sex:

OP No. :

Occupation:

Address with phone number:

CLINICAL HISTORY:

Main Complaints:

Duration:

H/o pain in the ulcer:

H/o discharge:

H/o fever:

H/o prolonged immobilization:

H/o varicose veins

H/o claudication pain

H/o trauma

Past history of similar illness and treatment details:

Other co morbid diseases: diabetes/ hypertension/ heart disease/ DVT/ stroke/ others

Personal history:

Smoker / consumes alcohol

Family history of similar illness:

Treatment history:

CLINICAL EXAMINATION:

GENERAL EXAMINATION:

Pallor:

VITALS: PULSE: BP:

Peripheral pulses:

Systemic examination:

CVS:

RS:

EXAMINATION OF THE ULCER:

Number of ulcer:

Limb involved: right/ left

Side of ulcer:

Site of ulcer:

Size:

Characteristics of ulcer:

EXAMINATION OF THE LEG:

INVESTIGATIONS:

CBC:

RBS:

LFT: RFT:

OTHERS:

WOUND SWAB CULTURE & SENSITIVITY:

VENOUS DOPPLER OF BOTH LOWER LIMBS:

INTERVENTION GROUP: **PROCEDURE DONE:**

FOLLOW UP:

No. of weeks/ sitting	Size of ulcer			Area of ulcer	Volume of ulcer	% Imp. in area of ulcer	%Imp. in volume of ulcer	Remarks
	Length	Width	Depth					
1.								
2.								
3.								
4.								
5.								
6.								
7.								

ANNEXURE- III

INFORMATION SHEET

TITLE: "A comparative study on the efficacy of topical autohemotherapy Vs topical autologous platelet rich plasma in chronic venous leg ulcers"

Name of Investigator: Dr. Bhuvaneswari. V

Name of Participant:

Purpose of Research:

The purpose of the study is to compare the efficacy of topical autohemotherapy and topical autologous platelet rich plasma for the treatment of chronic venous ulcers.

Study Design:

Prospective interventional and comparative study

Study Procedure:

Patient will be subjected to routine investigations, Venous Doppler of both lower limbs. The patients are then randomly grouped into 2 groups and treatment started. We will obtain a venous blood sample which will be used for treatment of the wound locally. The procedure will be repeated at weekly intervals and the treatment outcome will be assessed every week.

Possible Risks:

No risks to the patient

Possible benefits:

To patient:

Patient is provided an alternative and cost effective modality of treatment for their disease.

To doctor & to other people:

The results of this study will help to determine a better alternative, effective and cheaper modality of treatment to patients with chronic venous leg ulcers not responding to conventional therapies in the future.

Confidentiality of the information obtained from you:

The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

Can you decide to stop participating in the study:

Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time.

How will your decision to not participate in the study affect you:

Your decision will not result in any loss of benefits to which you are otherwise entitled.

Signature of Investigator Signature of Participant

Date :

Place :

PATIENT CONSENT FORM

Study Detail : **"A COMPARATIVE STUDY ON THE EFFICACY OF TOPICAL AUTOHEMOTHERAPY Vs TOPICAL AUTOLOGOUS PLATELET RICH PLASMA IN CHRONIC VENOUS LEG ULCERS"**

Study Centre : Rajiv Gandhi Government General Hospital, Chennai.

Patient's Name :

Patient's Age :

Op Number :

Patient may check (☑) these boxes

I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask question and all my questions and doubts have been answered to my complete satisfaction. ☐

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

I understand that sponsor of the clinical study, others working on the sponsor's behalf, the ethical committee and the regulatory authorities will not need my permission to look at my health records, both in respect of current study and any further research that may be conducted ☐

in relation to it, even if I withdraw from the study I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study.

I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperate with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or wellbeing or any unexpected or unusual symptoms. ☐

I hereby consent to participate in this study ☐

I I hereby give permission to undergo complete clinical examination and diagnostic tests including hematological, biochemical, radiological tests and to undergo treatment ☐

Signature/thumb impression	Signature of Investigator
Patient's Name and Address:	Investigator's Name:
	Dr. BHUVANESWARI.V

ஆய்வில் பங்கேற்பவர்களுக்கான தகவல் அறிக்கை

ஆய்வின் தலைப்பு :

சென்னை ராஜீவ்காந்தி அரசு பொது மருத்துவமனையின் தோல்நோய் பகுதிக்கு நாள்பட்ட இரத்த ஓட்ட தேக்கத்தினால் ஏற்படும் கால் புண்களுடன் வரும் நோயாளிகளுக்கு ஆட்டோஹீமோதெரபி (Autohemotherapy) மற்றும் ஆட்டோலாகஸ் பிளேட்லெட் ரிச் பிளாஸ்மா (Autologous Platelet Rich Plasma) எனப்படும் இரத்தவகை சிகிச்சை முறைகளின் திறன்களை கண்டறிவதற்கான ஒப்பீட்டு ஆய்வு.

ஆய்வாளரின் பெயர் : மருத்துவர் வே.புவனேஸ்வரி

பங்கேற்பாளரின் பெயர் :

வயது : **பால் :** **தேதி :**

ஆய்வு சேர்க்கை எண் : **நோயாளி எண் :**

இந்த ஆய்வில் தங்களை பங்கேற்க அழைக்கிறோம். இந்த தகவல் அறிக்கையில் கூறப்பட்டிருக்கும் தகவல்கள் தாங்கள் இந்த ஆய்வில் பங்கேற்கலாமா அல்லது வேண்டாமா என்பதை முடிவு செய்ய உதவியாக இருக்கும். இந்த படிவத்தில் உள்ள தகவல்கள் பற்றி உள்ள சந்தேகங்களை நீங்கள் தயங்காமல் கேட்கலாம்.

இந்த ஆய்வின் நோக்கம் என்ன?

நாள் இரத்த ஓட்ட தேக்கத்தினால் ஏற்படும் கால் புண்கள் நோயாளிகளின் உடல்நலம், வாழ்க்கைத்தரம் மற்றும் உற்பத்தித் திறனை குறிப்பிடத்தக்க வகையில் பாதிக்கிறது. இவ்வகை புண்களை குணப்படுத்த பலவகை சிகிச்சை முறைகள் மற்றும் அறுவை சிகிச்சை முறைகள் இருப்பினும் அவை அனைவருக்கும் முழுமையாக புண்களை குணப்படுத்துவதில்லை.

ஆட்டோஹீமோதெரபி (Autohemotherapy) மற்றும் ஆட்டோலாகஸ் பிளேட்லெட் ரிச் பிளாஸ்மா (Autologous Platelet Rich Plasma) போன்ற இரத்த சிகிச்சைகள் நோயாளிகளுக்கு ஓர் பயனுள்ள மற்றும் மலிவான சிகிச்சை முறையாக அமைந்துள்ளது. இவ்விரு முறைகளின் திறன் குறித்து ஒப்பீடு செய்வதே இந்த ஆய்வின் நோக்கமாகும்.

ஆய்வு முறைகள் :

இந்த ஆய்வில் நாள்பட்ட இரத்த ஓட்ட தேக்கத்தினால் ஏற்படும் கால் புண்கள் உள்ள நோயாளிகளுக்கு ஆட்டோ ஹீமோதெரபி அல்லது ஆட்டோலகாஸ் பிளேட்லெட் ரிச் பிளாஸ்மா எனப்படும் இரத்த சிகிச்சை முறைகள் அளிக்கப்படும். இவ்விரண்டு சிகிச்சை முறைகளிலும் நோயாளிகளிடம் தேவையான சிறிதளவு இரத்தம் எடுத்து புண்களின் மேல் அளிக்கப்படும். சிகிச்சைக்கு உட்படுத்தப்படுவதற்கு முன் நோயாளிகளுக்கு நோயின் தன்மை மற்றும் சிகிச்சை முறைகள் முழுமையாக எடுத்துரைக்கப்படும். மேலும் தேவையான இரத்தப் பரிசோதனைகளும் கால் இரத்த நாளங்களுக்கான வீனஸ் டாப்ளர் ஸ்கேன் (Venous Doppler Scan) செய்யப்படும்.

சிகிச்சையின் போது புண்களில் ஏற்படும் மாற்றங்கள் மற்றும் ஆறிவரும் தன்மை ஆகியவை தொடர்ந்து கண்காணிக்கப்படும்.

ஆய்வினால் மக்களுக்கு ஏற்படும் நன்மைகள் :

இந்த ஆய்வின் முடிவில் கிடைக்கும் தகவல்கள் சமுதாயத்திற்கு பயனுள்ளதாகவும் எதிர்காலத்தில் நோயாளிகளுக்கு மருத்துவ தீர்வாகவும் அமையும்.

தங்களிடமிருந்து பெறப்படும் தகவல்களின் நம்பகத்தன்மை :

தங்களிடமிருந்து பெறப்படும் தகவல்கள் பாதுகாக்கப்படும் அதற்கான முழு உரிமையும் தங்களுக்கு உண்டு.

இந்த படிவத்தில் கையொப்பமிடுவதன் மூலம் தாங்கள், தங்களை பற்றிய விவரங்களையும், ஆய்வு விவரங்களையும், ஆய்வாளர், ஆய்வு நடத்தும் ஏனையோர், வரைமுறை ஒழுங்கு குழுவினர் மற்றும் சட்டத்திற்கு உட்பட்ட மருந்து கட்டுப்பாடு இயக்குனர் ஆகியோர் பார்வையிட அனுமதிக்கின்றீர்கள்.

இந்த ஆய்வில் காட்டப்படும் தகவல்கள் அறிவியல் நாளேடுகளிலோ அறிவியல் கூட்டங்களிலோ சமர்பிக்கப்படும் பட்சத்தில் தங்களது அடையாளம் வெளிப்படுத்தப்படமாட்டாது.

இந்த ஆய்வில் பங்கேற்காமல் இருப்பதினால் ஏற்படும் பாதிப்பு :

இந்த ஆய்வில் தாங்கள் பங்கேற்க விருப்பம் தெரிவிக்காத நிலையில் தங்களின் மருத்துவரிடம் மற்றும் மருத்துவமனையில் தங்களுக்கு உள்ள உறவில் எந்த பாதிப்பும் ஏற்படாது. தாங்கள் எப்பொழுதும் சிறப்பாக கவனிக்கப்படுவீர்கள். மேலும் இதனால் தங்களுக்கு இழப்பு ஏதும் ஏற்படாது.

ஆய்வின் நடுவில் அதிலிருந்து விலகிக் கொள்ள நினைத்தால் :

இந்த ஆய்வில் பங்கேற்பது தங்களின் சொந்த விருப்பமே மேலும் ஆய்வின் நடுவில் எந்த நேரத்திலும் எக்காரணமும் கூறாமல் விலகிக் கொள்ள தங்களுக்கு முழு உரிமையும் உள்ளது. இருப்பினும் ஆய்வில் இருந்து விலகுவதற்கு முன் ஆய்வு குழுவுடன் கலந்து ஆலோசிப்பது உகந்தது என பரிந்துரைக்கப்படுகின்றது.

ஆய்வாளர் கையொப்பம்

பங்கேற்பாளர் கையொப்பம் :

ஆய்வாளரின் பெயர்

பங்கேற்பாளர் பெயர் :

இடம் :

தேதி :

இடம் :

தேதி :

ஆய்வு ஒப்புதல் படிவம்

ஆய்வின் தலைப்பு :

சென்னை ராஜீவ்காந்தி அரசு பொது மருத்துவமனையின் தோல்நோய் பகுதிக்கு நாள்பட்ட இரத்த ஓட்ட தேக்கத்தினால் ஏற்படும் கால் புண்களுடன் வரும் நோயாளிகளுக்கு ஆட்டோஸிமோதெரபி (Autohaemotherapy) மற்றும் ஆட்டோலாகஸ் பிளேட்லெட் ரிச் பிளாஸ்மா (Autologous Platelet Rich Plasma) எனப்படும் இரத்தவகை சிகிச்சை முறைகளின் திறன்களை கண்டறிவதற்கான ஒப்பீட்டு ஆய்வு.

ஆய்வாளரின் பெயர் : மருத்துவர்.வே.புவனேஸ்வரி

ஆய்வு மையம் : ராஜீவ் காந்தி அரசு பொது மருத்துவமனை,
சென்னை-600003.

---------- எனும் நான் எனக்கு கொடுத்துள்ள தகவல் தாளைப் படித்து புரிந்து கொண்டேன். நான் பதினெட்டு வயதைக் கடந்துள்ளதால், என்னுடைய சுயநினைவுடனும் முழு சுதந்திரத்துடனும் இந்த ஆய்வில் என்னை சேர்த்துக் கொள்ள சம்மதிக்கிறேன்.

1. நான் எனக்கு அளிக்கப்பட்ட ஒப்புதல் படிவத்தையும் தகவல்களையும் படித்து புரிந்து கொண்டேன்.
2.. ஒப்புதல் படிவத்தில் உள்ள தகவல்கள் எனக்கு விளக்கிக் கூறப்பட்டன.
3. ஆய்வின் தன்மை பற்றி எனக்கு விளக்கப்பட்டது.
4. என்னுடைய உரிமைகளையும் பொறுப்புகளையும் ஆய்வாளர் விளக்கிக் கூறினார்.
5. நான் இதுவரை எடுத்துள்ள, எடுத்து கொண்டிருக்கும் அனைத்து விதமான சிகிச்சை முறைகளையும் ஆய்வாளரிடம் கூறியுள்ளேன்.
6. இந்த ஆய்வினால் ஏற்படும் தீமைகள் பற்றி விளக்கப்பட்டன.
7. நான் ஆய்வாளருடன் ஒத்துழைப்பேன் என்றும் எனக்கு ஏற்படக்கூடிய அசாதாரணமான நிகழ்வுகள் பற்றியும் உடனடியாக ஆய்வாளரிடம் தெரிவிப்பேன் என்றும் உறுதி கூறுகிறேன்.
8. நான் கடந்த ---------- மாதங்களாக எந்தவிதமான ஆய்வுகளிலும் பங்கேற்கவில்லை.

9. எனக்கு செய்யப்படும் அனைத்து பரிசோதனைகளும் (உதாரணம் : இரத்தம் எடுத்தல்) என் நோயின் தன்மையை அறிவதற்காக செய்யப்படுபவை என்பதை அறிகிறேன்.

10. இந்த ஆய்விலிருந்து எப்போது வேண்டுமானாலும் எக்காரணமும் கூறாமல் நான் என்னை விடுவிடுத்து கொள்ளலாம் என்பதை அறிவேன் மற்றும் இதனால் எனக்குத் தரப்படும் சிகிச்சைக்கு எந்த பாதிப்பும் வராது என்பதை அறிவேன்.

11. ஆய்வாளர்கள் இந்த ஆய்வில் எனது பங்களிப்பை எந்த நேரத்திலும் எக்காரணமும் கூறாமல் என் சம்மதம் இல்லாமலும் என்னை விலக்கிவிட முடியும் என்பதை அறிவேன்.

12. என்னிடம் இருந்து பெறப்படும் தகவல்களை அரசு வரைமுறை அதிகாரிகள் அகியோர்களுடன் பகிர்ந்து கொள்ள ஆய்வாளர்களுக்கு அனுமதி அளிக்கிறேன். என்னுடைய தஸ்தாவேஜுகளை பார்வையிட அவர்களுக்கு உரிமை உண்டு.

13. என்னிடம் பெறப்படும் தகவல்களை பொதுவாக பரிசுரிக்கப்பட்டாலும், என்னுடைய அடையாளம் இரகசியமாக வைக்கப்படும் என்பதை அறிவேன்.

14. எனக்கு திருப்தி அளிக்கும் வகையில் என்னிடம் கேட்கப்பட்ட கேள்விகளுக்கு நான் பதில் அளித்துள்ளேன்.

15. இந்த ஆய்வில் பங்கேற்க தன்னிச்சையாக முழுமனுதடன் நான் சம்மதிக்கிறேன்.

இந்த ஆய்வின் போது எனக்கு என்ன சந்தேகம் ஏற்பட்டாலும் ஆய்வாளரை தொடர்பு கொள்ளலாம் என்பதை அறிவேன். இந்த ஒப்புதல் படிவத்தில் கையெழுத்துடுவதன் மூலம் இங்கு தரப்பட்டிருக்கும் அனைத்து தகவல்களும் தெளிவாகக் கூறப்பட்டு என்னால் முழுமையாக புரிந்து கொள்ளப்பட்டது என்பதை சான்றளிக்கிறேன். இந்த ஒப்புதல் படிவத்தின் நகல் என்னால் பெற்றுக் கொள்ளப்பட்டது.

பங்கேற்பவரின் கையொப்பம் / கட்டைவிரல்ரேகை :

இடம் :

தேதி :

பங்கேற்பவரின் பெயர் :

விலாசம் :

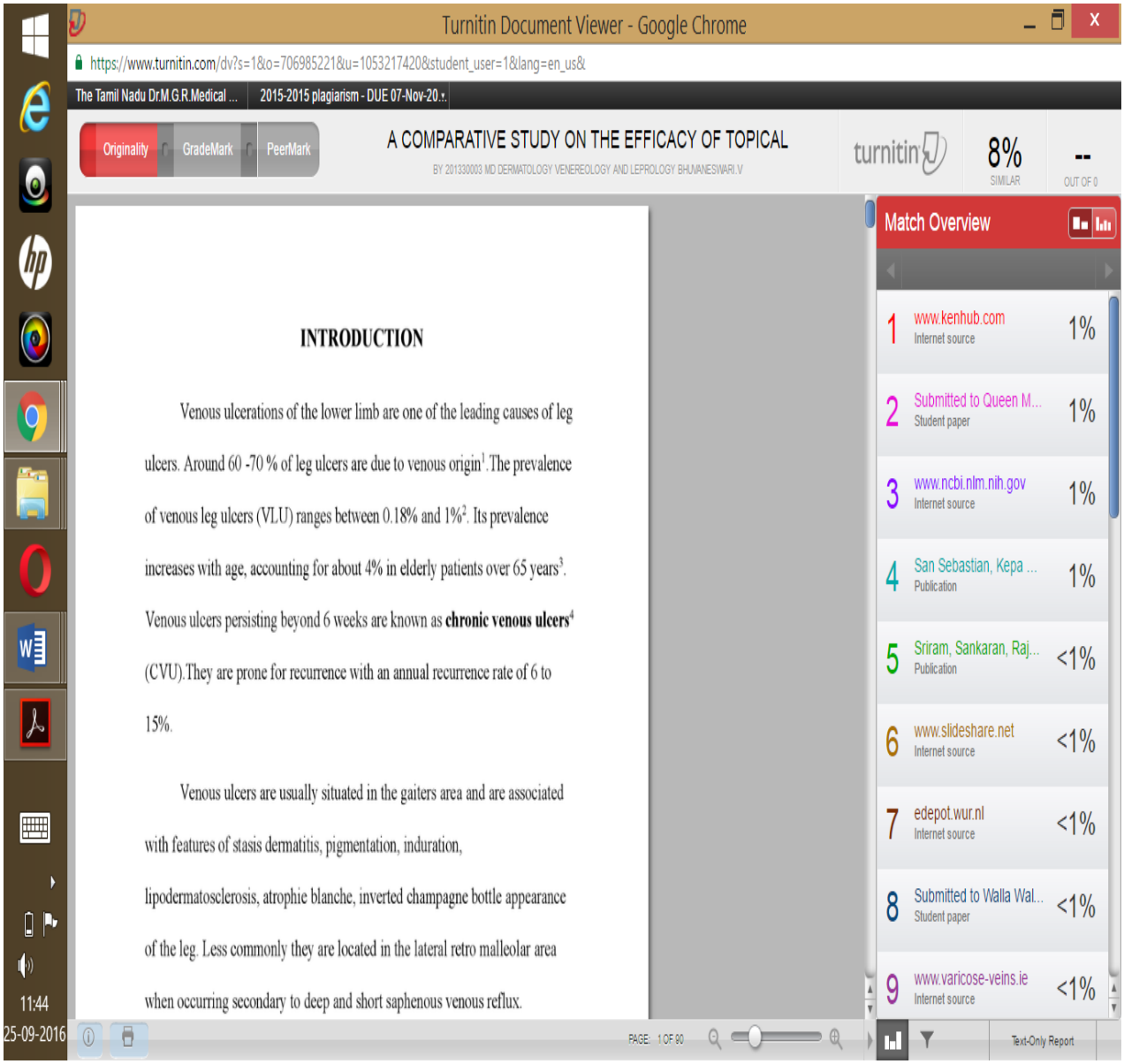
ஆய்வாளரின் பெயர் :

இடம் :

தேதி :

ANNEXURE- IV

PLAGIARISM



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INTRODUCTION

Venous ulceration of the lower limb are one of the leading causes of leg ulcers. Around 60 -70 % of leg ulcers are due to venous origin[1].The prevalence of venous leg ulcers (VLU) ranges between 0.18% and 1%[2]. Its prevalence increases with age, ascending for about 4% in elderly patients over 65 years[3]. Venous ulcers persisting beyond 6 weeks are known as **chronic venous ulcers**[4] (CVU).They are prone for recurrence with an annual recurrence rate of 6 to 14%.

Venous ulcers are usually situated in the pattern area and are associated with features of stasis dermatitis, pigmentation, induration, lipodermatosclerosis, atrophie blanche, inverted champagne bottle appearance of the leg. Less commonly they are located in the lateral inter malleolar area where coexisting secondary to deep and short saphenous venous reflux.

Chronic venous insufficiency (CVI) resulting in chronic venous ulcer is due to increased venous dynamic pressure over the microcirculation of dermis. Chronic peripheral venous hypertension or chronic ambulatory venous hypertension results from venous reflux in the lower limbs including perforator veins or by recanalized or occluded deep vein of the leg.

The management of CVU is challenging. The majority of patients can be effectively treated and healed, and those with refractory lesions can be helped

1

MASTER CHART

PT INDEX	Age	Sex	Co morbidities	Smoker	Alcoholic	Site of ulcer	Side of ulcer	No. of ulcer	Duration	PUS C/S	TREATMENT	Intervention group	A 0	V0	A 1	V1	PA1	PV1
1	69	M		N	N	lower 1/3	lateral	3	3 months	Klebsiella	Amikacin	A	15.708	4.7124	12.5664	2.51328	20	46.66667
						lower 1/3	anterior		2 months	NG		A	3.1416	0.62832	3.1416	0.31416	0	50
						mid 1/3	medial		2 months	NG		A	1.1781	0.11781	0.7854	0.07854	33.33333	33.33333
2	40	M		N	N	lower 1/3	medial	1	4 months	NG		B	23.562	7.0686	21.5985	4.3197	8.33333	38.88889
3	48	M		N	N	lower 1/3	medial	1	7 months	staph. Aureus	ciproflox	A	6.2832	1.25664	6.2832	0.62832	0	50
4	72	M	HT	N	N	lower 1/3	medial	1	2 months	staph. Aureus	oflox	B	11.781	2.3562	11.781	1.1781	0	50
5	40	M		Y	N	lower 1/3	medial	2	3 months	NG		A	9.4248	2.82744	9.4248	1.88496	0	33.33333
						lower 1/3	medial		2 months	NG		A	1.5708	0.31416	1.1781	0.11781	25	62.5
6	54	M	HT	Y	N	lower 1/3	lateral	1	5 months	kleb, p.aeroginosa	amikacin	B	9.4248	2.82744	7.854	1.5708	16.66667	44.44444
7	44	M		Y	Y	mid 1/3	medial	1	2 months	NG		A	3.1416	0.62832	3.1416	0.31416	0	50
8	56	M		N	Y	lower 1/3	lateral	1	3 months	klebsiella	oflox	B	4.7124	0.94248	4.7124	0.47124	0	50
9	63	M		Y	Y	lower 1/3	lateral	1	3 months	NG		A	1.76715	0.35343	1.1781	0.11781	33.33333	66.66667
10	60	M		N	N	lower 1/3	lateral	1	6 months	NG		B	4.7124	1.41372	3.5343	0.70686	25	50
11	55	M		Y	Y	lower 1/3	medial	1	5 months	NG		A	12.5664	3.76992	12.5664	2.51328	0	33.33333
12	69	M	HT	N	N	lower 1/3	medial	1	2 months	NG		B	13.7445	4.12335	12.9591	3.88773	5.714286	5.714286
13	45	M		Y	N	lower 1/3	medial	2	5 months	NG		A	7.0686	2.12058	7.0686	2.12058	0	0
						lower 1/3	medial		3 months	NG		A	1.5708	0.31416	1.1781	0.23562	25	25
14	70	M	HT	N	N	lower 1/3	medial	1	8months	NG		B	19.635	5.8905	19.635	5.8905	0	0
15	68	M		Y	Y	lower 1/3	medial	1	6 months	NG		A	12.5664	3.76992	12.5664	3.76992	0	0
16	34	F		N	N	lower 1/3	medial	1	5 months	NG		B	9.4248	2.82744	9.4248	2.82744	0	0
17	46	M		Y	Y	lower 1/3	medial	1	8 months	NG		A	7.0686	1.41372	7.0686	1.41372	0	0
18	38	F		N	N	lower 1/3	medial	1	3 months	NG		B	12.5664	3.76992	10.9956	2.19912	12.5	41.66667
19	46	M		Y	N	mid 1/3	medial	1	2 months	NG		A	15.708	4.7124	15.708	4.7124	0	0
20	48	M		Y	Y	lower 1/3	medial	1	5 months	NG		B	15.708	4.7124	14.1372	2.82744	10	40
21	64	M	HT	Y	Y	lower 1/3	lateral	2	4 months	NG		A	8.2467	1.64934	8.2467	1.64934	0	0
22	27	M		Y	N	lower 1/3	medial	1	3 months	NG		B	9.4248	2.82744	9.4248	2.82744	0	0
23	50	M	HT	Y	N	lower 1/3	lateral	1	6 months	NG		A	9.4248	2.82744	8.2467	1.64934	12.5	41.66667
24	67	M		N	N	lower 1/3	medial	1	6 months	NG		B	7.0686	2.12058	5.8905	1.1781	16.66667	44.44444
25	47	M		Y	Y	lower 1/3	medial	1	2 months	NG		A	12.5664	3.76992	10.9956	2.19912	12.5	41.66667
26	41	M		N	N	lower 1/3	medial	1	6 months	NG		B	9.4248	2.82744	8.95356	2.686068	5	5
27	37	M		Y	N	lower 1/3	medial	1	8 months	NG		A	15.708	4.7124	15.07968	4.523904	4	4
28	44	M		N	Y	lower 1/3	lateral	1	2 months	NG		B	7.0686	2.12058	7.0686	2.12058	0	0
29	38	M		Y	N	lower 1/3	medial	1	3 months	NG		A	10.9956	3.29868	9.62115	2.886345	12.5	12.5
30	57	M	HT	Y	N	lower 1/3	medial	1	7 months	NG		B	12.5664	3.76992	10.9956	2.19912	12.5	41.66667
31	48	M		Y	Y	lower 1/3	medial	1	2 months	NG		A	7.0686	1.41372	5.8905	1.1781	16.66667	16.66667
32	45	M		N	N	lower 1/3	medial	1	8 months	NG		B	23.562	7.0686	23.562	7.0686	0	0
33	60	M	HT	Y	N	lower 1/3	anterior	1	7 months	NG		A	9.4248	2.82744	9.4248	2.82744	0	0
34	59	M		Y	N	lower 1/3	medial	1	3 months	NG		B	12.5664	3.76992	10.9956	3.29868	12.5	12.5
35	70	M		N	N	lower 1/3	lateral	1	2 months	NG		A	9.4248	2.82744	9.4248	2.82744	0	0
36	58	M		Y	Y	lower 1/3	medial	1	6 months	NG		B	7.0686	1.41372	4.7124	0.94248	33.33333	33.33333

MASTER CHART

PT INDEX	Intervention group	A 2	V2	PA2	PV2	A 3	V3	PA3	PV3	A 4	V4	PA4	PV4	A 5	V5	PA5	PV5	A 6	V6	PA6	PV6
1	A	7.0686	0.70686	55	85	5.8905	0	62.5	100	3.927	0	75	100	1.5708	0	90	100	0.7854	0	95	100
	A	1.5708	0	50	100	0.7854	0	75	100	0.19635	0	93.75	100	0	0	100	100	0	0	100	100
	A	0.19635	0.019635	83.33333	83.33333	0	0	100	100	0	0	100	100	0	0	100	100	0	0	100	100
2	B	19.43865	1.943865	17.5	72.5	17.6715	0	25	100	15.708	0	33.33333	100	12.37005	0	47.5	100	9.4248	0	60	100
3	A	4.7124	0	25	100	3.927	0	37.5	100	2.3562	0	62.5	100	1.1781	0	81.25	100	0.7854	0	87.5	100
4	B	9.4248	0	20	100	6.2832	0	46.66667	100	1.5708	0	86.66667	100	0.7854	0	93.33333	100	0.19635	0	98.33333	100
5	A	9.4248	0.94248	0	66.66667	8.2467	0.82467	12.5	70.83333	7.0686	0.70686	25	75	4.90875	0	47.91667	100	2.3562	0	75	100
	A	0.7854	0	50	100	0.19635	0	87.5	100	0	0	100	100	0	0	100	100	0	0	100	100
6	B	5.8905	0.58905	37.5	79.16667	3.927	0	58.33333	100	2.3562	0	75	100	1.1781	0	87.5	100	0.3927	0	95.83333	100
7	A	1.5708	0	50	100	1.1781	0	62.5	100	0.7854	0	75	100	0.19635	0	93.75	100	0	0	100	100
8	B	3.927	0	16.66667	100	3.927	0	16.66667	100	2.3562	0	50	100	1.1781	0	75	100	1.1781	0	75	100
9	A	0.7854	0	55.55556	100	0.19635	0	88.88889	100	0	0	100	100	0	0	100	100	0	0	100	100
10	B	2.94525	0.294525	37.5	79.16667	2.3562	0	50	100	1.1781	0	75	100	0.3927	0	91.66667	100	0	0	100	100
11	A	12.5664	1.25664	0	66.66667	12.5664	0	0	100	8.2467	0	34.375	100	7.0686	0	43.75	100	3.927	0	68.75	100
12	B	7.854	1.5708	42.85714	61.90476	5.4978	1.09956	60	73.33333	5.4978	0.54978	60	86.66667	3.5343	0	74.28571	100	1.9635	0	85.71429	100
13	A	7.0686	2.12058	0	0	5.8905	1.1781	16.66667	44.44444	3.927	0.7854	44.44444	62.96296	2.3562	0.23562	66.66667	88.88889	1.5708	0	77.77778	100
	A	0.3927	0.03927	75	87.5	0.11781	0	92.5	100	0	0	100	100	0	0	100	100	0	0	100	100
14	B	17.6715	5.30145	10	10	15.708	3.1416	20	46.66667	10.9956	2.19912	44	62.66667	7.0686	0.70686	64	88	3.1416	0.31416	84	94.66667
15	A	9.62115	2.886345	23.4375	23.4375	8.2467	1.64934	34.375	56.25	7.0686	1.41372	43.75	62.5	4.90875	0.98175	60.9375	73.95833	3.927	0.3927	68.75	89.58333
16	B	9.4248	1.88496	0	33.33333	6.87225	1.37445	27.08333	51.38889	4.7124	0.47124	50	83.33333	3.927	0	58.33333	100	3.1416	0	66.66667	100
17	A	4.90875	0.490875	30.55556	65.27778	3.927	0.3927	44.44444	72.22222	1.76715	0	75	100	1.1781	0	83.33333	100	0.7854	0	88.88889	100
18	B	8.2467	1.64934	34.375	56.25	7.0686	0.70686	43.75	81.25	3.927	0	68.75	100	2.3562	0	81.25	100	0.7854	0	93.75	100
19	A	14.1372	2.82744	10	40	10.9956	2.19912	30	53.33333	7.0686	1.41372	55	70	3.927	0.3927	75	91.66667	1.76715	0	88.75	100
20	B	7.0686	1.41372	55	70	4.90875	0.490875	68.75	89.58333	3.1416	0	80	100	1.76715	0	88.75	100	0.19635	0	98.75	100
21	A	7.0686	0.70686	14.28571	57.14286	3.927	0.3927	52.38095	76.19048	1.5708	0	80.95238	100	1.1781	0	85.71429	100	0.7854	0	90.47619	100
22	B	8.2467	1.64934	12.5	41.66667	5.8905	1.1781	37.5	58.33333	4.7124	0.47124	50	83.33333	3.927	0	58.33333	100	3.1416	0	66.66667	100
23	A	7.0686	1.41372	25	50	3.927	0.3927	58.33333	86.11111	1.1781	0	87.5	100	0.7854	0	91.66667	100	0	0	100	100
24	B	5.8905	1.1781	16.66667	44.44444	3.927	0.3927	44.44444	81.48148	2.3562	0.23562	66.66667	88.88889	1.5708	0	77.77778	100	1.1781	0	83.33333	100
25	A	8.2467	1.64934	34.375	56.25	6.59736	0.659736	47.5	82.5	4.90875	0	60.9375	100	3.1416	0	75	100	2.12058	0	83.125	100
26	B	6.87225	1.37445	27.08333	51.38889	4.7124	0.94248	50	66.66667	3.927	0.3927	58.33333	86.11111	2.3562	0	75	100	1.1781	0	87.5	100
27	A	14.1372	2.82744	10	40	10.9956	2.19912	30	53.33333	8.2467	0.82467	47.5	82.5	7.0686	0	55	100	5.8905	0	62.5	100
28	B	4.90875	0.98175	30.55556	53.7037	3.1416	0.31416	55.55556	85.18519	1.76715	0	75	100	0.7854	0	88.88889	100	0.19635	0	97.22222	100
29	A	7.0686	1.41372	35.71429	57.14286	4.90875	0.98175	55.35714	70.2381	2.3562	0.23562	78.57143	92.85714	0.7854	0	92.85714	100	0	0	100	100
30	B	8.2467	0.82467	34.375	78.125	4.90875	0	60.9375	100	3.927	0	68.75	100	2.3562	0	81.25	100	1.1781	0	90.625	100
31	A	3.927	0.7854	44.44444	44.44444	2.3562	0.23562	66.66667	83.33333	1.5708	0	77.77778	100	0.7854	0	88.88889	100	0	0	100	100
32	B	23.562	7.0686	0	0	21.5985	4.3197	8.333333	38.88889	19.635	3.927	16.66667	44.44444	17.6715	1.76715	25	75	12.5664	1.25664	46.66667	82.22222
33	A	7.854	1.5708	16.66667	44.44444	7.69692	1.539384	18.33333	45.55556	5.8905	0.58905	37.5	79.16667	3.927	0.3927	58.33333	86.11111	2.3562	0	75	100
34	B	8.2467	1.64934	34.375	56.25	4.7124	0.47124	62.5	87.5	2.94525	0.294525	76.5625	92.1875	1.1781	0	90.625	100	0	0	100	100
35	A	9.4248	1.88496	0	33.33333	6.87225	1.37445	27.08333	51.38889	4.7124	0.47124	50	83.33333	3.927	0.3927	58.33333	86.11111	1.5708	0	83.33333	100
36	B	1.5708	0.15708	77.77778	88.88889	1.1781	0.11781	83.33333	91.66667	0.7854	0	88.88889	100	0.19635	0	97.22222	100	0	0	100	100

KEY TO MASTER CHART:

A0- Baseline area of the ulcer

V0- Baseline volume of the ulcer

A1- Area of the ulcer at 1st week

V1- Volume of the ulcer at 1st week

PA1- Percentage of improvement in the area of ulcer at 1st week

PV1- Percentage of improvement in the volume of ulcer at 1st week

A2- Area of the ulcer at 2nd week

V2- Volume of the ulcer at 2nd week

PA2- Percentage of improvement in the area of ulcer at 2nd week

PV2- Percentage of improvement in the volume of ulcer at 2nd week

A3- Area of the ulcer at 3rd week

V3- Volume of the ulcer at 3rd week

PA3- Percentage of improvement in the area of ulcer at 3rd week

PV3- Percentage of improvement in the volume of ulcer at 3rd week

A4- Area of the ulcer at 4th week

V4- Volume of the ulcer at 4th week

PA4- Percentage of improvement in the area of ulcer at 4^{th} week

PV4- Percentage of improvement in the volume of ulcer at 4^{th} week

A5- Area of the ulcer at 5^{th} week

V5- Volume of the ulcer at 5^{th} week

PA5- Percentage of improvement in the area of ulcer at 5^{th} week

PV5- Percentage of improvement in the volume of ulcer at 5^{th} week

A6- Area of the ulcer at 6^{th} week

V6- Volume of the ulcer at 6^{th} week

PA6- Percentage of improvement in the area of ulcer at 6^{th} week

PV6- Percentage of improvement in the volume of ulcer at 6^{th} week