TOPICAL DIPHENYLHYDANTOIN IN THE TREATMENT OF NON-MALIGNANT CHRONIC LOWER LIMB ULCERS AT MUHIMBILI NATIONAL HOSPITAL, DAR-ES-SALAAM, TANZANIA.

By

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A dissertation submitted in partial fulfilment of the requirements for the degree of Master of Medicine (Surgery) of the University of Dar-es-salaam.

University of Dar-es-Salaam

July 2002

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CERTIFICATION

The undersigned certifies that he has read and hereby recommend for acceptance by the University of Dar-es-salaam this dissertation entitled, "*Topical Diphenylhydantoin in the treatment of non-malignant chronic lower limb ulcers at Muhimbili National Hospital, Dar-es-salaam, Tanzania*" in partial fulfilment of the requirements for the degree of Master of Medicine (Surgery).

.....

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ACKNOWLEDGEMENT

I express my gratitude to Prof. P.M.R Carneiro, my supervisor, who offered tireless expert guidance and encouragement throughout. Furthermore, he provided a very friendly and productive working environment necessary for this work.

I wish to thank Prof.C.Mkony, the Head of Department of Surgery who frequently reviewed my various drafts during the fowarding processes and gave his constructive criticism.

I also thank Prof.M.R Aziz ; Head of Surgical Firm I and Dr.Yongolo whose review and constructive criticisms encouraged me, their advice contributed enormously to my scientific and academic career.

I thank Prof.L.E.K. Lema and Dr. R.E Kisanga, heads of surgical firms II and III respectively, under whom I had the pleasure of working.

I thank Dr. L.Museru, the Director of Muhimbili Orthopaedic Institute (MOI) for allowing me to use his patients in my study.

I owe my thanks to other members in the department including Prof. J.K. Shija, Prof. M.M.Aboud, Dr. E.N. Sayi , Dr. N.A Mbembati , Dr. R.I Mhina, Dr. Kategile and Lecturers from the basic Sciences for their immense and valuable contribution during my training.

I also extend my thanks to Prof. Matee of the Department of Microbiology and Immunology for giving me the opportunity to utilize their Laboratory and taking care of the ulcer swabs.

I also appreciate the assistance and collaboration I obtained from the nurses of both the surgical and orthopaedic wards who helped me clean the ulcers and apply the topical medication .

I am indebted to Mrs Mpembeni and Mr C. Makwaya of the department of epidemiology and biostatistcs for their valuable assistance in data analysis.

Lastly but with most love, I thank my wife Stella and my children Everestina and Evans who despite missing me provided comfort and moral support. My wife also played a major secretarial role in producing this manuscript; to them I am very grateful.

DEDICATION

To my parents, my father John Mathias and my mother Juliana Malimi who were instrumental in educating me.

ABSTRACT.

Chronic ulcer is a distressing surgical condition causing considerable morbidity. Long-standing ulcers have been implicated to be pre-malignant conditions. Routine use of commonly known antiseptics has been shown to be of limited value though most of these agents work by reducing bacterial wound contamination and super-infection, thereby allowing bodily regeneration mechanisms to take effect. This can be expensive and often associated with prolonged hospital stay. A prospective randomized clinical trial to determine the effectiveness of Diphenylhydantoin powder in the treatment of chronic non-malignant leg ulcers was conducted for a period of 14 months at Muhimbili National Hospital (MNH) on patients admitted to surgical wards. During the study period, August 2000 to September 2001 a total of 102 patients with chronic ulcers were enrolled into the study, of these 50 (49%) constituted the diphenylhydantoin group; while 52 (51%) were treated with a solution of calcium hypochlorite (Eusol) who formed the control group. The effectiveness of the drug in alleviating patients' symptoms and promoting healing was determined clinically and perfoming bacterial culture studies of the ulcer before institution of any therapy and on subsequent days following application of the topical drugs. Parameters for assessment included pain, discharge, formation of healthy granulation tissue, rate of reduction in ulcer surface area and swabs from ulcers for culture and sensitivity was perfomed on three occasions. Data

was analysed by manual and Epi Info 6 (2000) computer programme for statistical testing of comparable variables.

Male patients accounted for 65.7% while females constituted 34.3%. The mean age in the diphenylhydantoin group was 32.5±14.9 years, while in the control group 34.2±14.7 years. Patients were equally distributed in the treatment groups and there was no statistical difference in age and sex ($p>0.05$). Prior to commencement of treatment surgical debridement was carried out if required and then all ulcers were washed with normal saline daily followed by application of a thin film of diphenylhydantoin powder and dressed with dry gauze in the study group, while in the control group dressing with a solution of calcium hypochlorite soaked in a gauze was done. Patients were followed up at weekly intervals and the various parameters to assess ulcer healing were recorded. Significant reduction in pain was noted on the 14th ($p<0.05$) and 21 st day ($p<0.01$), however no significant difference was noted on the 7th and 28th days ($p>0.05$). Both treatment groups showed progressive negative bacterial culture, no significant difference was noted ($p>0.05$) Reduction of ulcer pus discharge on 7th ,14th, and 21st days was noted to be more in the study group as compared to the control. This difference was statistically significant ($p<0.05$). However, no difference was noted on the 28th day. The rate of formation of healthy granulation tissue was noted to be significantly faster in patients treated with diphenylhydantoin powder than the control group on days 14 and 21 ($p<0.001$). However no significant difference was noted

on the 7th and 28th day ($p>0.05$). Significant reduction in ulcer surface area was noted throughout the period of assessment in the diphenylhydantoin group when compared to the control group ($p<0.05$). The healing response in different ulcers was also influenced by the nature of the ulcer and presence of underlying systemic disease.

This study has shown the beneficial effects of diphenylhydantoin powder to achieve faster healing of chronic ulcers. The drug when used topically relieves patient symptoms including pain and discharge, causes faster rate in formation of healthy granulation tissue and reduction in the size of ulcers. No adverse effects were noted during the treatment period. The drug is cheap and easy to use and therefore efforts should be made to make it available throughout our resource scarce country. Health workers at all levels should be educated on how to use the drug on patients with chronic ulcers.

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DEFINITION OF TERMS AND ABBREVIATIONS

M.N.H		Muhimbili National Hospital
M.O.I		Muhimbili Orthopaedic Institute
%SA		Percentage surface area
%RSA	=	$\frac{(A_0-A_i) \times 100}{A_0}$

Whereby:

%RSA	Percentage reduction in surface area
A_0	initial surface area of ulcer
A_i	Surface area on day of assessment
CNSI;	Chronic non specific inflammation (As appears in Table 4)
Unhealthy discharge;	an ulcer with presence of foul smelling, purulent or green discharge
Healthy discharge;	an ulcer with serous discharge
Unhealthy granulation;	presence of pale, blue or patchy granulation with score (1)
Healthy granulation;	presence of complete granulation.
Severe pain	Pain experienced by patient requiring analgesics

1. CHAPTER ONE

1.1 INTRODUCTION

Chronic ulcer is one of the most costly unsolved problems in health care[1, 2]. It is a common condition in any surgical practice and presents one of the surgical management difficulties. Though worldwide the overall magnitude of the problem is unknown, disease conditions leading to chronic ulceration vary from place to place. In the western world, leg ulcer is a leading cause of morbidity among older subjects and the majority of these ulcers are due to venous or arterial diseases[3]. While, in West Africa[4], Buruli ulcer is the common ulcer encountered particularly in Ivory Coast, Benin and Ghana. The incidence of Buruli ulcer in Ghana is 20.7 per 100,000 making it the commonest while, ulcers resulting from leprosy ranks the second with an overall incidence of 9 per 100,000. Ulcers have been known for a long time. About 400 years BC, Hippocrates wrote: "In case of an ulcer it is not expedient to stand, especially if the ulcer be situated on the leg". In the Tropics, tropical ulcers occur in various groups of individuals, the most affected social class are those of lower socio-economic status and in particular those associated with poor hygiene. Individuals included in this class are peasants, pastoralists and hunters in whom minor trauma is very common. Ulcers of other origin, for example vascular or neuropathic, may occur in any social class and develop into chronicity. Reported incidence following community survey in Lothian, North-West London

and Southampton shows an overall prevalence of about 0.2% [5]. The prevalence of leg ulcers in Europe is well documented varying between 0.18%and1% in different countries[6,7,8,9]. There are about 400,000 patients with leg ulcer disease in U.K at any one time, 100,000 have leg ulcers requiring treatment. This costs about £ 600 million/year[10]. In USA it is estimated that approximately 5% of diabetics develop ulcers and this results in about 60,000 amputations per year[1]. The range of medical costs has been estimated at US dollars 24,000 to 36,000 per patient and the overall cost of diabetic ulcers including loss of productivity could be as high as US dollar 20 billion per year. There are about 175,000- 250,000 spinal cord injuries in United States and 7,000 to 10,000 new patients per year. Approximately 25 - 85% develop pressure ulcers and the cost estimates range from US Dollars 14,000 to 25,000 per patient for medical, surgical and nursing care. Bacterial wound infection; venous stasis, animal bites and burn injury are some of the common causes leading to chronic ulcers. Chronic ulcer requires special attention as it frequently causes purulent discharge and creates unconducive environment not only to the patient himself but also to neighbours with a psychological implication. Chronic ulcers especially those due to nutritional deficiency (tropical ulcers), trophic, venous and chronic ulceration of old burn scars have been implicated as pre malignant conditions [11,12,13,14,15]. A non-healing wound in an area of previous trauma may become cancerous termed as Marjolin's ulcer.

These are virulent malignancies that arise from old areas of trauma. The causation may be multifactorial but they appear to arise because the dense scar of the lesion does not allow normal immunological surveillance of the area by the host and therefore the host cannot destroy the malignant transformation occurring within a scar[1,2]. Tropical ulcers almost invariably occur in the leg. Wood in 1968[16] noted that 70% of Epitheliomatous ulcer occurring in the lower limb arises in scars of old healed ulcers or burns. Hutt and Burkitt in Uganda [17] found that most squamous cell cancers occurred in tropical ulcer scars. Hutt and Wright 1968 remarked that these cancers arise mostly in healed tropical ulcers rather than active ulcers[18]. Semambo[19] and Nelson reported a series of 300 chronic tropical ulcers in Uganda and found that 9 had already transformed into malignancy.

The primary goal of management of chronic ulcers healing by secondary intention is to create optimal conditions for healing by contraction and epithelialization or to prepare the wound bed for flaps or skin grafting[1, 2,20,21]. In order to accelerate wound healing several approaches with various antiseptics have been tried. All have been shown to have limited value and therefore the need for continued research. Many reported series have indicated that topical application of diphenylhydantoin powder is beneficial in the management of chronic ulcers [22,23,24,25,26,27,28,29].

A randomised clinical trial to assess the effect of topical diphenylhydantoin on rate of wound healing in chronic ulcers was conducted over a period of 14 months between August 2000 and September 2001 in the department of Surgery, Muhimbili National Hospital, Dar es Salaam, Tanzania.

1. 2 LITERATURE REVIEW.

1.2.1 Historical Perspectives:

A surgical dressing is generally applied to a wound to stem bleeding, absorb exudates, ease pain and provide protection for the newly formed tissue[20]. A variety of substances over the ages have been used for this purpose [30,31,32]. These include cobwebs, dung, leaves, animal fat, honey and other exotic agents. Undoubtedly, many of these materials could be contaminated with microorganisms making them a potential source of infection.

In reviewing the historical aspects of burn management, Artz[33] reported that in 1827 Syme proposed the use of dry cotton wool dressing, applied with firm pressure on burn wounds. The author also noted that Lizfrank in 1835 recommended wet dressings containing sodium and calcium chloride and later in 1858, Passavant promoted the use of saline baths. When Lister demonstrated the importance of cleanliness and good aseptic practice in medicine and surgery in the late 19th century as written in Sabiston Textbook of Surgery[34], the quality of the materials used in wound management also began to improve. In 1880, Joseph Gamgee developed the famous composite dressing pad that still bears his name. He sometimes used it medicated with iodine or phenol. In the early part of this century, gauze impregnated with paraffin were introduced as non-adherent dressings for the treatment of burns and similar types of wounds; medicated

version of these 'tulle gras' dressings were subsequently manufactured, some of which are still in use at the present time. Besides these agents, others include ointment containing cod-liver oil; sulphanilamide, eucalyptus oil, bismuth nitrate and zinc oxide have been thought to promote healing by stimulating formation of granulation tissue. Biological agents using maggots were introduced in the "maggot debridement therapy", in the belief that maggots separate the necrotic tissue from the living tissue making surgical debridement easier[35].

1.2.2 Anatomy and function of the skin.

The skin is the largest organ in the body that is in contact with atmosphere and subjected to frequent trauma. Anatomically, the surface of the body is covered by skin integument together with certain specialized derivatives of the skin[36]. The skin is composed of two layers, the outer or epidermis, and beneath this, the dermis or corium of vascular dense connective tissue. These two layers adhere firmly to each other and form a membrane that varies in thickness from 0.5mm to 4mm on more in different parts of the body. Skin is also classified as thick or thin. Thick skin is found on the palms of the hand and soles of the feet. Thin skin covers the remainder of the body. The primary function of the skin is to serve as a protective barrier against the environment[36]. Loss of the integrity of large portions of the skin as a result of injury or illness may lead to major disability or even death. The skin protects the body from injurious substances and influences; it

provides a barrier to invasion by microorganisms, it helps to regulate the temperature of the body, sweating causes excretion of water and various waste products of metabolism. It is the most extensive sense organ of the body for the reception of tactile, thermal and painful stimuli.

1.2.3 Physiology of wound healing.

Wound healing and wound management form the fundamentals in surgical practice [37,38]. Knowledge of the biology of tissue injury and its repair is very important. Although healing generally proceeds without complication, impaired healing and wound infection are leading causes of morbidity in surgical patients. When the continuity of the skin has been disrupted by abrasion, burn, contusion or laceration, coordinated series of events are set in motion. Traditionally wound healing has been divided into phases. Although this division is artificial it does demarcate the chronological landmarks that characterize the wound healing process[38].

1.2.3.1 Fibroplasia

During the phase of fibroplasia, most important events take place that lead to creation of a scar and hence wound strength[39]. It comprises inflammatory, proliferative and maturation phases.

(i). Inflammatory phase

Inflammation is the initial response to injury comprising vascular and cellular responses[39,40,41].

- ***Vascular response*:**

Tissue damage initiates vasoconstriction, which lasts for 5-10 minutes; this is the initial vascular response to bleeding. Injury activates the coagulation cascade so that platelet adhesion and aggregation lead to formation of a clot. Platelets also release several intracellular substances into the extra cellular space that affects other processes in wound repair[42].

These include:

- Prostaglandins, which affect leucocytes.
- Thromboxane, which produces potent vasoconstriction.
- Chemotactic factors which attract leucocytes
- Biogenic amines, which enhance vascular tone and permeability.
- Fibroblastic proliferation factors
- Collagenase and its inhibitors

Following vasoconstriction, there is a period of active vasodilatation and increased permeability of smaller blood vessels. Some vasoactive substances, such as serotonin and histamine released in the tissues increase the permeability of the microcirculation and cause dilatation of the venules.

Enzymes destroy the vasoconstrictor, noradrenaline, therefore potentiating vasodilatation and increasing the permeability process. Proteolytic enzymes attack the endothelium and activate Kallikrein; this activates the Kinins, which in turn markedly increase vascular permeability thereby increasing the amount of protein and cells within the wound[43,44]. These events continue to increase vascular permeability for approximately 72 hours. At this time the first signs of inflammation can be observed; erythema and warmth from vascular dilatation, oedema from increase in cells and plasma and pain from the tension produced by the oedema.

- ***Cellular response.***

The cellular response begins soon after the vascular changes are observed[45]. Within a few hours of injury, leucocytes stimulated by chemotactic factors, migrate by diapedesis across the walls of vessels into the injured area. If there is no infection or foreign body, the number of leucocytes decreases rapidly in the wound. Conversely, if bacteria or foreign materials are present the inflammatory process continues. Leucocytes release their hydrolytic enzymes to assist in the break down of bacteria and debris. When the inflammatory phase is prolonged by invasive bacteria and devitalised tissue, the physiological response may be in excess of that caused by the initial injury.

Mononuclear, phagocytosing cells and transformed macrophages appear in the wound after 1- 2 days and they release a number of chemotactic and growth factors that activate and stimulate the formation of fibroblasts and blood vessels. Within 3-4 days of injury, macrophages become the dominant cell type. The duration and intensity of the inflammatory reaction determines the amount of scar tissue formed. Only a small amount of collagen is deposited across an inflamed wound and, since it is collagen that ultimately provides strength, there can be no real gain in strength until the inflammatory phase ends and collagen deposition begins[46]. It is only when successful closure occurs, the next stage can begin.

(ii). Substrate or lag phase

In the first 3 - 5 days following injury, there is little gain in tensile strength of the healing wound[38]; this has been termed the 'lag phase'. During this time, the wound debris is disposed off and the fibroblasts and endothelial cells in the capillaries migrate. It has been suggested that this period of active preparation would be more appropriately termed the 'substrate phase'. This phase includes the period of acute inflammation whereby fibroblasts appear at the edges of the wound within the first 24 hours of injury and peak at 3-5 days.

The mucopolysaccharide ground substance is believed to provide an environment for the subsequent deposition of collagen, and may even be involved in the later maturation of the collagen fibres during the maturation phase[41,46]. Whatever their eventual function, there is no evidence that the glycoproteins, mucopolysacharide or other elements of the ground substances make any contribution to the tensile strength of the wound during the substrate phase. Indeed, the principle contributor to the strength of the healing wound in the first 24 hours is the fibrin clot[42].

(iii). Proliferative phase

The proliferative phase of wound repair extends from day 5 until the third week following injury. During this period there is proliferation of epithelial and connective tissue[39,47]. It is marked by collagen deposition, which increases steadily before it plateaus. Fibroblasts migrate into the wound and proliferate, becoming the predominant cell type by the fifth day. They begin the deposition of the extra cellular matrix including collagen. At this point the wound begins to gain strength. After 5 weeks the number of fibroblasts decreases[48].

(iv). Maturation or remodelling phase.

This is characterized by increasing strength without an increase in collagen content[47,49,50]. This is the predominant phase after 3 weeks, it continues until the wound heals completely. A dynamic equilibrium exists between collagen breakdown and synthesis. Strength is gained with increasing cross-links and alignment of collagen fibres along lines of tension. The wound is an up-regulated process until remodelling at this point, acute and chronic inflammatory cells diminish gradually, angiogenesis ceases, and fibroplasia ends[1,51]. During this phase, which may last a year or longer, the scar flattens, the redness fades and pruritis disappears. Fibroblasts themselves appear to be responsible for the resorption and production of new collagen; the zone of activity in which this chemical process takes place is localized to an area approximately 0.75mm on either side of the wound edge. If tension is placed on the wound, the random collagen fibres appear to line up parallel to the forces of tension. Within 3 months the scar becomes flatter, soften and lighter in colour. The collagen becomes thicker and denser, and the blood vessels constrict and disappear.

1.2.3.2 Epithelialization

The process whereby keratinocytes migrate and divide to resurface partial thickness loss of skin or mucosa or migration of epithelial cells from the wound edges across the wound[38,52]. Migration begins within 12 hours and an intact monolayer may be formed between well-approximated tissues within 24 hours. The movement of epithelial cells across an injured area is predictable and relentless, continuing as long as the wound is denuded of epithelial cells. The fundamental process initiating this division and migration has not been elucidated; but it continues until the epithelial cell comes into contact with another epithelial cell and is known as contact inhibition[38]. This process takes place in partial thickness skin graft donor sites, abrasions, blisters and superficial thickness dermal burns.

1.2.3.3 Wound contraction (or intussusceptive growth).

This is one of the most powerful mechanical forces in the body. Controversy still exists regarding the exact biological mechanism of the process. Wound contraction enables large wounds close with minimal scarring; it is a form of tissue remodelling and may represent a vestige of a function now lost. Since a wound must be closed before the next stage of healing process starts, the body

attempts to accomplish this by epithelialization and contraction[46,52,53].

Epithelial cell migration helps to cover the open wound. Wound shrinkage at the edges also contributes to the process of wound closure. Contraction may be inhibited by stress on the wound.

In the early 1970's researchers[1] noted the presence of fibroblast-like cells in contracting wounds, which had smooth muscle components in the cytoplasm and named them myofibroblast. It has been demonstrated that the rate of wound contraction depends on the number of myofibroblasts within the wound[1,2]. Other studies[3,8,54] have suggested that myofibroblasts are distributed throughout the wound and not only on its edges. Thus, the entire granulation tissue serves as a contractile organ in which the myofibroblasts contract the wound; this is followed by collagen deposition and cross-linking to maintain the degree of contraction.

1.2.3.4 Wound strength

The wound gains very little strength during the initial several days of the inflammatory phase. As the wound enters the proliferative phase, the strength of the wound will parallel the rapid rise in collagen content for about 3 weeks. At this time the collagen content and the rate of increasing wound strength plateau and would theoretically be ideal for the removal of all

sutures were it not for the adverse cosmetic results. Even at 3 weeks, the wound only has 40% of the strength of undivided tissues and far less strength than strong suture material. At 6 to 8 weeks, a wound will achieve 70% of its strength; wound remodelling will increase wound strength for at least the next two years though ultimate strength will never equal that of uninjured tissue[38,39].

1.2.3.5 Types of wound healing

The recognized types of wound healing[1,39,40] include.

(i) Primary intention.

Most clean surgical wounds and traumatic injuries are managed by primary closure. The tissues are approximated using sutures, staples or tapes. Repair occurs through the phases of wound healing namely inflammatory, epithelialization, fibroplasia and remodelling. With time, synthesis, deposition and cross-linking of collagen provide the tissue strength and integrity. The healed wound contains minimal quantities of granulation tissue, usually only a thin scar remains, which may be virtually undetectable when fully mature[46].

(ii) Secondary intention (spontaneous closure)

In wounds that have sustained a significant degree of tissue loss as a result of trauma, it may sometimes be impossible to bring the edges of the wound together or contraindicated if there is a

serious risk of infection with subsequent likelihood of wound dehiscence. In these situations it may be necessary to leave the wound open to granulate and then achieve closure spontaneously or by delayed primary closure (see below). During the healing process, the inflammatory phase takes place; the wound becomes progressively filled with granulation tissue composed of collagen and proteoglycans[47,52]. The production of granulation tissue continues until the base of the original cavity is almost level with the surrounding skin. At this stage the epithelium around the wound becomes active and begins to grow over the granulation tissue thereby restoring the integrity of the epidermis.

Occasionally the production of granulation tissue continues even after the wound cavity has been filled leading to the formation of hypergranulation tissue, or 'proud flesh'. This is sometimes associated with the use of occlusive dressings and can be removed by the application of caustic agent such as a silver nitrate pencil or short-term local application of a suitable corticosteroid cream /ointment.

A further equally important part of the healing process is contraction, a mechanism by which the margins of the wound are drawn towards the centre. This, however, produces an area of scar tissue. Contraction may take place in all healing wounds

but it is significant in large wounds that are left to heal by granulation and epithelialization. Contraction takes place at a rate of approximately 0.6 - 0.7mm/day and is independent of the size of the wound[20].

(iii) Delayed primary closure (healing by third intention).

In this type of healing approximation of tissue by sutures, staples or tapes is delayed for several days following surgery or trauma. Delay in wound closure is to prevent infection due to significant bacterial contamination, foreign bodies, extensive tissue trauma, or poor blood supply to the area. In these circumstances the wound is left open for about three to four days before closure is effected. During the period that the wound remains open, moist sterile saline dressings should be changed at least twice a day to optimise the wound closure. In this type of wound that is being prepared for delayed primary closure, angiogenesis[40,51] proceeds to provide enhanced blood supply and needed oxygen. Leucocytes are attracted to destroy and remove bacteria and the phases of healing progress to improve the wound environment for soft tissue closure. Wound tensile strength following delayed primary closure with time becomes the same as that of a primary closed wound.

(iv) Grafting and flap formation.

A skin graft is a portion of skin (composed of dermis and epidermis) that is removed from one anatomical site and placed on a wound elsewhere on the body[55,56]. If successful, grafting ensures that the wound will heal rapidly thus reducing the chances of infection, scarring and the time spent in hospital.

The disadvantage of this technique is that the patient has to endure with an additional wound and it is often reported that the pain associated with the donor site is worse than that caused by the original injury. The most commonly used are partial thickness grafts, which are usually 300-375 μm thick. These are removed from a suitable donor site such as the thigh or buttock, using a special knife (Humby) or dermatome, which can be adjusted to ensure that the harvested skin is of the required thickness. As the cells of epidermal tissue are also found in the base of sebaceous glands and hair follicles, the donor site heals rapidly usually within 10 - 14 days.

Full thickness grafts: These are needed in a more specialist application such as reconstructive surgery. They may contain hair, fat and sebaceous glands[57]. They have the advantage that they are cosmetically more acceptable and less likely to cause contracture compared to split thickness grafts. They are also used when a neurovascular bundle or cortical bone must be

covered with tissue. They have the disadvantage that in some cases, the donor site will itself require the application of a partial thickness graft if the wound is too large to be closed by suture. The success of a graft depends upon a number of factors, the most important of which is the presence of a good vascular bed to supply the metabolic needs of the transplanted tissue. Stress on the graft itself, infection, the formation of seromas and haemetomas are major causes of graft failure[1, 2].

(v) Skin flaps:

Skin flaps differ from grafts in that the relocated tissue is not completely separated from the body. In this technique a portion of skin and subcutaneous tissue is raised on three sides and rotated, or transposed to cover an adjacent area of skin loss. In this way the entire flap continues to receive blood supply from its original vasculature until it becomes established at the transposed site. The maximum size of the flap depends upon the anatomical site from which it is raised, the nature and distribution of the major blood vessels. In a variation of this technique, a flap of tissue (musculo-cutaneous) is relocated completely with its attached blood supply and this is known as free flap transfer[20, 38].

1.2.3.6 Factors affecting the wound healing process.

Though healing of wounds occur through phases, a number of local, systemic and growth factors affect the rate of healing which may accelerate or retard the healing process[1,2,3,20,40,41,46].

(i) Local factors:

- ***Blood supply:***

Blood supply is highly required to the wound, to supply with nutrients and oxygen, which assist in laying down ground substances. In areas where there is poor blood supply healing is usually retarded .

- ***Obesity:***

Problems of obesity are often associated with underlying illness such as poor vascularized fatty tissue in diabetics who are prone to infection and hence prolong wound healing.

- ***Wound infection:***

50% of all postoperative wound complications result from infection[20,39,46]. The high incidence of wound infection is associated with prolonged postoperative stay, breaks in surgical technique, complicated (burst abdomen) wound closure procedures. Presence of seroma, foreign body and haematoma tends to increase risk of wound infection.

It is obvious that wound infection prolongs the inflammatory phase, and hence delay wound healing.

- ***Tension:***

Tension in the wound may result from poor primary wound closure, inadequate haemostasis or poor technique in insertion of drainage. Tension in the wound may lead to wound hypoperfusion, hypoxia as well as direct mechanical disruption that impair healing.

(ii) Systemic factors:

Several systemic factors are known to affect wound healing process which include:

- ***Nutrition:***

In an otherwise healthy individual there is no evidence that the addition of vitamin supplements or other particular nutritious food improves wound healing[38]. Protein deficiency is believed to retard vascularization and lymphatic formation, lower resistance to infection and inhibit several phases of wound healing. In such an environment there is a delay in both fibroplasia and the development of wound tensile strength. The amino acid, D_L–methionine, appears to be necessary for the fibroblastic synthesis of mucopolysaccharides and collagen. However, although the administration of methionine appears to restore healing

process to normal rate, the reversal of protein starvation requires time. Trauma abruptly alters the metabolic status quo.

The posttraumatic state is marked by:

- Increased heat production
- Gluconeogenesis
- Negative nitrogen, potassium, sulphur and phosphorus balance
- Early hyperglycaemia
- A modification in carbohydrate utilization

In addition, serum concentrations of free fatty acids are elevated with,

- Ketosis, sodium chloride and water retention
- Potential depletion of ascorbic acid, thiamine, riboflavin, nicotinamide and vitamin A and possibly, deficiencies of trace metals such as zinc, copper and Iron.

- ***Vitamin C***:

Vitamin C is an essential cofactor for collagen synthesis. Man cannot synthesize vitamin C and can normally store sufficient amounts for 4–5 months only. Ascorbic acid is an active reducing agent in the production of superoxide radicals, and an important intermediary in respiration and

in the synthesis of antibacterial substances by leucocytes. In addition, vitamin C or rather its ester equivalent ascorbate is required for the hydroxylation of proline and lysine. In vitamin C deficiency collagen lysis outstrips synthesis causing:

- Weakening of collagen
- Increased capillary permeability, fragility, rupture and haemorrhage.

- ***Vitamin A:***

Accelerates healing of skin lesions and wounds even when applied locally. Deficiencies causes retardation of the following: epithelialization, wound closure, collagen synthesis and cross-linkage of new collagen. Vitamin E appears to enhance the absorption, storage and utilization of vitamin A. The liver has large stores of vitamin A and supplements are not required in routine surgical procedures. When postoperative nutritional problems are associated with pre-existing diseases such as malignancy, advanced age with diabetes, gastrointestinal disorders (such as Crohn's, or cirrhosis) severe malnutrition will ensue. These patients exhibit tendency to extreme weakness, great susceptibility to anaesthetic shock, impaired function of the liver and gastrointestinal tract, retarded wound healing, serious

infection associated with prolonged hospitalisation and high mortality rate.

- ***Oxygen:***

Oxygen is essential for normal wound healing [54,58,59] whereby large amount is consumed during the process. The capillary injuries occurring in wounds reduce the availability of oxygen. Thus the oxygen requirement for repair is greater when the local circulation is least able to satisfy the need. The minimal PO_2 necessary for healing is not known, though measurements have established that fibroblasts replication was potentiated at PO_2 of 30 – 40 mmHg, however collagen synthesis required higher PO_2 values. Studies indicate that the rate of epithelial cell proliferation in wound is directly proportional to the PaO2 at the wound site[20,58]. Oxygen increases the efficiency of collagen formation. It has been suggested that lack of oxygen may result in the formation of inadequately hydroxylated collagen and weakening of the tensile strength in the

Wounds [54, 58].

- ***Microenvironment host resistance and infection:***

The defence mechanisms of the body are usually sufficient to control infection and promote wound healing[37]. They cannot however function efficiently in an environment heavily

contaminated with debris, necrotic tissue, haematoma, and virulent or abundant bacteria. In these conditions bacteria may compete effectively with the phagocytes, fibroblasts, endothelial cells and epithelium, leading to infection and delayed wound healing. Patients who are susceptible to infection because of impaired host defences include the aged with debilitating illnesses, diabetics, cancer patients, patients who have sustained major trauma or undergone major surgery, those with Human Immunodeficiency virus (HIV) infection and patients having chronic renal disease with uraemia[37,38,39,41,46].

- ***Chemotherapy:***

Chemotherapy adversely affects early stages of wound healing and should be delayed for at least one week after surgery.

- ***Steroids:***

The use of steroids is known to impair every phase of wound healing. The inflammatory phase is shortened and the production of cytokines, kinins and other vasoctive factors are impaired.

- ***Radiation:***

Radiation therapy by dose related has been shown to impair healing in both acute and chronic stages.

(iii). Role of growth factors in wound healing.

- Growth factors are polypeptides or glycoproteins that are synthesized by cells[1,60,61]. They provide all the communication for cell-cell interactions and have important pharmacological roles in many biological processes and disease conditions of the body. They play a role in mitogenesis, chemotaxis, extracellular matrix production, cell differentiation and influence the production of cytokines by other cells. They are also important in the regulation of fibrogenesis, healing of chronic wounds and skin grafts, vascularization and perhaps even in the control of tumour growth or malignant cell replication[61,62]. Cytokines are ideally wound hormones and may take various forms:
- Endocrine substances which are secreted by one cell and then circulate in the blood stream to reach distant target cells (somatomedins and insulin like growth factors)
- Paracrine which is produced by one cell and affect an adjacent target cell (TGF_{β} PDGF)
- Autocrine, which are factors secreted by one cell and then act on receptors of the same cell.

- Intracrine are factors produced by a cell and remain active in the same cell.

Cytokines may either be competence factors, which get the cell into G_1–phase such as PDGF or possibly progression factors such as IGF–1, which act to promote the cell through the proliferation cycle. In addition to cell division, cytokines stimulate cells to migrate to the wound site and produce specific components needed for matrix repair. These include proteins, enzymes, proteoglycans and attachment glycoproteins. Cytokines have been named according to either the original cell of isolation or from their mechanism of action. There are various types of cytokines that affect wound healing:

(a). Epidermal growth factor (EGF)

It is derived from platelets, saliva, urine, milk and plasma. It is mitogenic in all epithelial cells of the cornea, lung, breast, gastrointestinal tract and keratinocytes in the skin. It stimulates fibroblast proliferation hence increasing hyaluronic acid in extracellular matrix[62,63], and the formation of granulation tissue; it is also chemotactic for endothelial cells.

(b). Platelets derived growth factor (PDGF)

The active dimmer is found in high concentration in platelets, macrophages, endothelial and smooth muscle cells. It is chemotactic and mitogenic for fibroblasts, smooth muscle cells, monocytes and neutrophils. It may be important in

pathologic proliferative disorders such as malignancies, atherosclerosis, cirrhosis, pulmonary fibrosis and rheumatoid arthritis.

(c). Transforming growth factor beta (TGF_β)

It is derived from platelets, polymophonuclear cells, T-lymphocytes, macrophages, and many other tissues and cells. It is strongly chemotactic for monocytes and fibroblasts. It increases collagen and fibronectin production by fibroblasts and inhibits their breakdown so as to increase extracellular matrix. It is thought to be very important in the down regulation of mitogenesis and may be largely involved in neoplasia.

(d). Transforming growth factor alpha ($TGF\alpha$)

It is derived from activated macrophages, platelets, keratinocytes and many other cells. It is mitogenic for all epithelial cells in the cornea, lung, breast, GI tract and keratinocytes in skin. $TGF\alpha$ stimulates epithelial growth and migration as well as angiogenesis[62,64]. It increases hyaluronic acid in the extracellular matrix and hence formation of granulation tissue.

(e). Fibroblast growth factor (FGF)

It is derived from brain, pituitary, macrophages and many other tissues and cells. It exists in several forms including

acid and base; all forms bind strongly to heparin. It is chemotactic and mitogenic for endothelial cells, epithelial cells and fibroblasts. Its major role may be angiogenesis. It stimulates matrix deposition and plays a role in wound contraction[65].

(f). Interleukins (Il –1)

This is produced by macrophages, lymphocytes and many other tissues and cells. It stimulates fibroblast proliferation, and is chemotactic for neutrophils.

(g). Tumour necrosis factor (TNF)

It is derived from macrophages, mast cells and T–lymphocytes and stimulates fibroblast proliferation.

(h). Keratinocyte growth factor (KGF)

It is derived from fibroblasts and stimulates epithelial cell proliferation.

(i). Insulin like growth factor-1 (IGF –1)

It is derived from liver, plasma and fibroblasts. It stimulates synthesis of sulphated proteoglycans, collagen and fibroblast proliferation.

(j). Human growth hormone (hu-GH)

Derived from pituitary and thus circulate in plasma. It is anabolic and stimulates insulin growth factor-1 (IGF–1)

(k). Interferon (IFN)

Derived from Lymphocytes and fibroblasts. It inhibits fibroblast proliferation and collagen synthesis.

Careful orchestration of growth factors is responsible for normal healing at a near maximal rate. Alterations in the production and responsiveness to these potent compounds will likely contribute to a large number of proliferative disorders such as excessive scarring and neoplasm, as well as impaired wound healing. It has been shown that absence of viable receptor complex in cytokines involved in wound healing e.g. TGF contributes to wound chronicity[1],[60, 66].

Cytokines and their release during phases of wound healing[1]

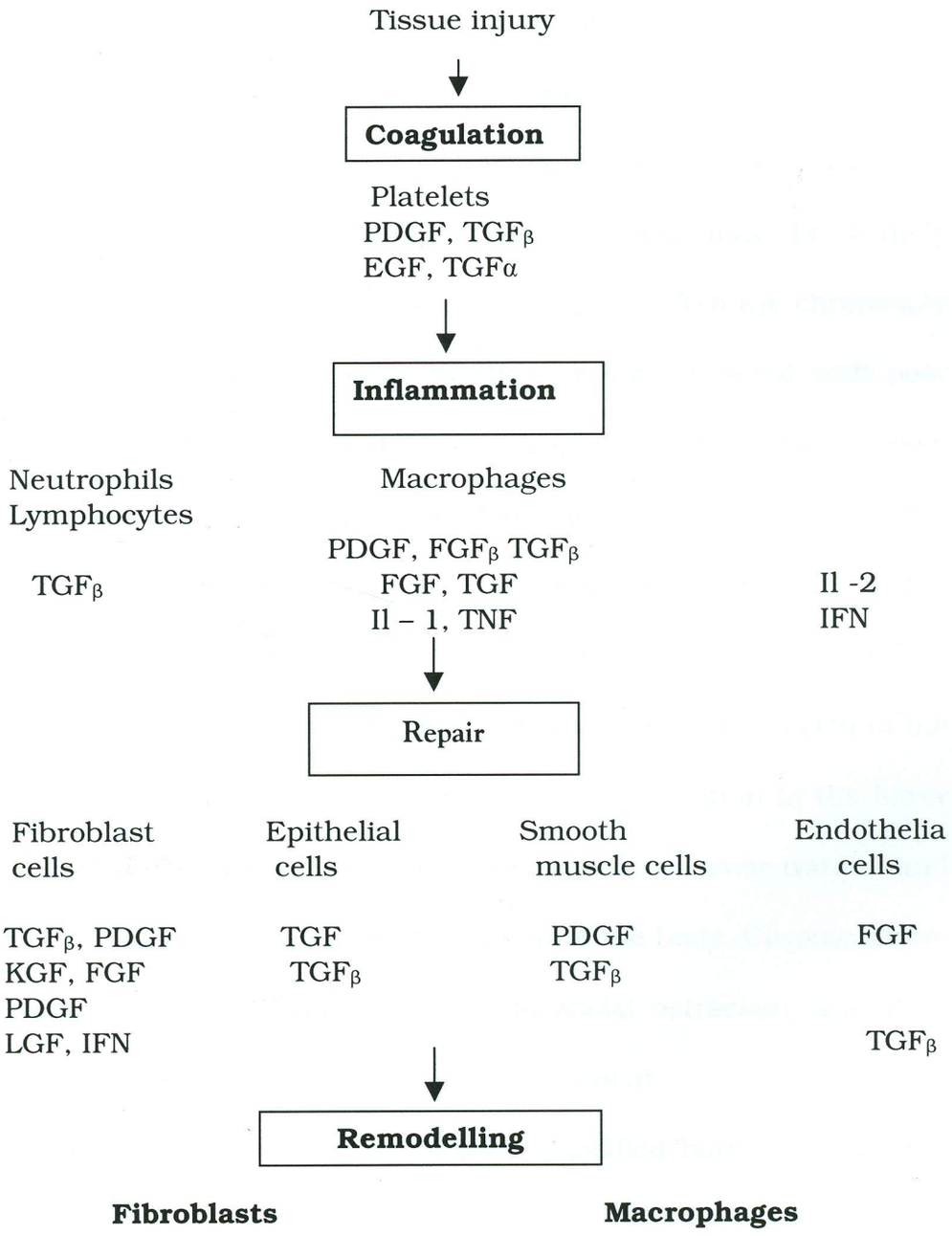


Fig 1.

1.2.4 Pathology of chronic ulcers

An ulcer is a break in the continuity of an epithelial surface of an organ usually with an inflammed and granulation base[67,68]. All chronic ulcers are characterized by presence of cells of chronic inflammation namely macrophages, plasma cells and lymphocytes, they also have processes of fibrosis and angiogenesis[2,69]. Ulcers may be acutely inflammed with surrounding areas of cellulitis but often are chronically inflammed with adherent sloughs. Weak ulcers are covered with poor quality granulation tissue (pale and atrophic) and exhibit delayed epithelial healing. Clinically the period of transformation to chronicity varies, but it is regarded as early as two (2) weeks onwards[69]. Untreated chronic cutaneous ulcers are in contact with the atmosphere and therefore predisposed to bacterial contamination. Any skin region of the body may be affected but the commonest site of location is the lower limb[8,9]. This is because of frequent predisposition to minor trauma and presence of relative ischaemia in that region of the body. Chronic ulcers are distressing, demoralizing may lead to social ostracism, and their chronicity provides challenges to surgical treatment.

Chronic non-neoplastic ulcers have been classified both clinically and pathologically[2, 3,67,68].

1.2.4.1 Clinical classification.

Based on clinical classification[67] an ulcer may be;

(i). **Spreading**; where the surrounding skin of the ulcer is inflamed and the floor is covered with slough without any evidence of granulation.

(ii). **Healing**; when there is granulation tissue in the floor of the ulcer, the surrounding skin is not inflammed and the edges shows bluish outline of the growing epithelium. There may also be slight serous discharge.

(iii). **Callous**; when there is pale granulation tissue on the floor of the ulcer, there is considerable induration at the base, edge and surrounding skin and this type of ulcer shows no tendency towards healing.

1.2.4.2 Pathological classification;

Pathological classification[2, 3,67,68] is based on the aetiological agents and these include:

(i) Non- specific ulcers: -

(a). Traumatic: Extensive ulcers resulting from trauma following mechanical, physical, electrical, X-ray burn, or chemicals e.g. from the application of caustics. These ulcers usually heal quickly and do not become chronic. They may however result into chronicity if they are supervened by infection, the wound area is ischaemic or if they occur in patients who have been on

long-term systemic steroid therapy, where because of dermal atrophy, even minor trauma may result in damage to full thickness of the skin.

(b). Ischaemic arterial ulcers

Ischaemic (vascular) ulcer is usually initiated by minor trauma on an ischaemic limb. The anterior tibial region above the ankle is the commonest site for these types of ulcers. There is a history of trauma or constant pressure by some object with symptoms of intermittent claudication or rest pain and previous problems with healing of skin. These ulcers are seen in arteriosclerosis, Buerger's or Raynaud's diseases. They may be associated with other conditions[68] such as rheumatiod arthritis, especially with Felty's syndrome and in patients with Erythema induratum (Bazin's ulcer). The skin is atrophic and the ulcer is shallow with poor quality granulation tissue with absent or impaired epithelial healing. The whole area has poor blood supply; the limb feels cold, hairless with poor dermal nails and frequently associated with chronic paronychia. Superinfection may occur causing cutaneous warmth and hyperemia and one has to exclude ischaemia.

(c). Gravitational ulcers (venous, varicose, stasis ulcers)

These are commonly seen in surgical practice. They are chronic and tend to recur after healing and cause considerable morbidity. The typical location is on the medial side of the leg above the malleolus but they are also found on the lateral aspect of the ankle[70]. Occasionally extensive ulcers may result appearing as circumferential lesions around the lower leg. They have a typical appearance and are usually associated with signs of chronic venous insufficiency. Dermatitis is often present in the surrounding skin with brown discoloration, thickening of the skin and oedema of the limb. The ulcer may contain an area covered with slough, or flesh red granulation tissue, which is surrounded by a thin pink zone. It may extend deeply to involve the bone. Pain is felt around the edge of the ulcer and often occurs during the repeated healing / breakdown cycles from the base of the ulcer as it involves nerve endings. The pathophysiology of venous ulcer is not fully understood. The condition is more commonly seen in patients with extensive varicose veins and in patients in whom there is a past history of deep venous thrombosis[71]. This may cause perforator vein incompetence between the deep and superficial veins and may occur in isolation or in conjunction with saphenofemoral incompetence. During activity the contraction

of calf muscles pumps the blood in a cephalad direction due to the valves in the deep venous system. The failure of this system results in the transmission of high-pressure venous blood to the superficial capillary venous system and may lead to extravasation of plasma and blood cells. There are several theories that try to explain the pathogenesis of venous ulceration[70,71,72]. One theory postulates that the leakage of soluble fibrinogen is deposited as fibrin in perivascular spaces. This acts as a barrier to diffusion of metabolites and restricts vessel contraction and dilatation. A more recent theory suggests that white cells, which are trapped in this system, may have abnormal function and cause tissue damage[72]. In time the subcutaneous tissue becomes firm with fibrous leather–like feel called liposclerosis. Oedematous legs in patients with cardiac failure do not commonly develop ulcers and so the central venous pressure cannot be the sole cause for this condition[72,73]. In elderly population, about 30% of patients with venous ulcer have an ischaemic element. Marjolin's ulcer may complicate venous ulcer.

(d). Pressure sores (Decubitus ulcers)

These occur over the sacral region, the back of the heel, elbow and back. They may be painless or painful at some time during their occurrences or healing. Many of the afflicted

patients are paraplegic or unconscious and unable to communicate any discomfort. Impaired mobility, old age and neurological disease such as disseminated sclerosis, often accompanied by incontinence of urine and faeces due to sphincter disturbances make pressure sores one of the commonest complication of the chronic invalid or the critically ill[5,58].

Pathogenesis: A localized constant pressure over a contact area causes ischaemia with eventual necrosis, first of the underlying subcutaneous fat and subsequently of the skin, which is sensitive to pain usually at the edge of the ischaemic area where cell death is incomplete[58,59].

(e) Ulcer artefacts;

These are self – inflicted injuries by patients resulting into ulcers.

(f). Others

Other types of ulcers include those associated with underlying diseases such as gout, anaemia, avitaminosis and rheumatoid arthritis. In gout the skin over the gout deposit (tophi) ulcerates and the chalky contents exude.

(ii) Specific ulcers:

In this group there is a well-defined cause of the ulcer, which need specific investigation, and the treatment is based on the causative agent. They include:

(a). Meleney's ulcer:

These are seen in postoperative wounds either after the operation of a perforated viscus or after drainage of empyema thoracis; it is also found rarely on the dorsum of the hand. It is due to the symbiotic action[67] of microaerophilic nonhaemolytic streptococcus and haemolytic staphylococcus aureus. Clinically it is an undermined ulcer with a lot of granulation tissue on the floor. A deep purple zone, which in turn is surrounded by an outer zone of erythema, surrounds it, it is painful and causes toxaemia, deterioration of general condition occurs if untreated. The ulcer may extend to involve underlying tendon and bone. The destructive process is accentuated by the presence of an increased metabolic rate, such as pyrexia from postoperative chest infection.

(b). Ulcers associated with malnutrition;

These are tropical ulcers, which occur in legs and feet of people in tropical countries usually infected by Vincent's organism (Bacteroides fusiformis) following small abrasions.

(c). Neuropathic ulcers

These arise from repeated trauma to an insensitive area of the skin in conditions like diabetic neuropathy, disseminated sclerosis or in leprosy.

- ***Diabetic foot ulcer***: is seen in diabetics whereby there is usually a degree of ischaemia from arteriosclerosis. The latter is a small vessel disease, which interferes with peripheral circulation, and there is also damage to peripheral nerves[74,75,76]. Neuropathy and atherosclerosis make the limb insensitive and a slight injury to the glucose-laden tissue may cause chronic infection and subsequent ulceration[77].

(d). Bazin's ulcer (Erythrocyanosis frigida)

This is seen in fatty adolescent girls particularly in their calves as purplish nodules followed by development of indolent ulcers.

(e). Martorell's or hypertensive ulcers;

These are found in patients suffering from hypertensive heart disease, it affects the legs where patches of skin necrosis first develop; it is generally not associated with atherosclerosis[5].

(f). Tuberculous ulcer

This mostly results from bursting of caseous lymph nodes. This type of ulcer may also develop when a cold abscess due to bone or joint tuberculosis breaks out onto the surface. The ulcer is generally slightly painful. Such ulcer is usually seen in the neck, axilla, and groin. The most characteristic feature of this ulcer is its edge, which is thin, reddish blue and undermined. There is pale granulation tissue with scanty serosanguinous discharge on the floor and induration at the base, which indicates that the ulcer is a chronic one.

(g). Lupus vulgaris :

This is a form of cutaneous tuberculosis, occurs commonly in the face and hand and the usual victims are children and young adults. It starts very superficially as a single, or multiple cutaneous nodules, which gradually turn into multiple superficial ulceration of the skin. These ulcers remain active at the periphery and spread outwards whereas in the centre they gradually heal. Due to its destructive nature at the periphery it is called 'lupus' which means wolf. Squamous cell carcinoma may result from the scar of lupus vulgaris to form Marjolin's ulcer.

(h) Buruli ulcer

This is an ulcer due to mycobacterium ulcerans, an extracellular mycobacterium. The disease starts as a painless though sometimes itchy, swelling in the skin. From these marble-like nodules concealed destruction spreads for days or weeks. A toxin secreted by a proliferating microbe ravages the subcutaneous fat layer, destroying so much tissue that the skin above finally dies and sloughs away. A lesion may spread over the full chest or back or an entire limb. Occasionally the infection chews its way to end even through a bone[78,79]. There is no proven drug treatment for Buruli ulcer so physicians can only limit the bacterium's spread by surgically excising the dead tissue and covering the wound with skin grafts. Over the past century cases of Buruli ulcer have appeared in twenty-five countries around the world and in Africa the western part of the continent being more affected[4,78,79,80].

(i). Syphilitic ulcers

These are ulcers due to treponema pallidum infection and occur during the different stages of the disease resulting into hard chancre, mucous patches, or Gummatous ulcer, which occurs in the late stage.

(j). Leprosy ulcer

These ulcers arise due to infection by mycobacterium leprae. The bacteria destroys the nervous system making the skin and tissue insensitive and hence predisposition to trauma.

1.2.5 Wound care and dressing agents

Most chronic ulcers of various causes will heal by secondary intention provided the underlying causative factors are managed. Occasionally the biological healing process is complex and may lead to delay in wound healing. Advances in wound management show a progression from conventional to modern techniques, and there is continued search for better agents. All these are aimed at accelerating wound healing and therefore shorten the duration of hospital stay thereby reducing costs and help these patients adapt to socially acceptable life. The principles underlying treatment of chronic ulcer is based on clear determination of the exact aetiology (diagnosis) and its management, in addition to adequate drainage and desloughing of the base of the ulcer. In trophic ulcers due to leprosy, curettage, dorsal incision, metatarsectomy and posterior tibia decompression have been the usual practice. For over two centuries, the standard wound-cleansing agent has been the use of hypochlorite solution. Recently it has been found to be an irritant and toxic to tissues and impairs formation of healthy granulation tissues[1,2]. This disadvantage revolutionised the use of 0.9% sodium chloride solution (normal saline),

which has been found to be safe to tissues. However, the mechanism of both hypochlorite and normal saline in promoting wound healing has remained the same in that they increase hydration of slough and necrotic tissue, which is then removed by autolysis[20]. The use of either of these agents has been combined with prior surgical debridement, bed rest, and daily dressings with or without antibiotics. The routine use of antibiotics in chronic wounds has been controversial; in general they are unwarranted in granulating wounds since healthy granulation tissue form a very effective natural barrier against bacterial invasion and is virtually impermeable to both topical and systemic antibiotics. The indication of antibiotic therapy in management of ulcers is limited to patients with infected ulcers with cellulitis and ulcers of specific bacterial origin such as syphilitic or tuberculous.

Recently new surgical dressings and wound management materials have been introduced[20,81,82]. Some of these dressings react chemically with the exudate or change their physical state to form a gel or viscous semisolid covering the surface of the wound. They are called interactive materials and produce changes in the microenvironment on the wound surface that can have a dramatic effect upon the healing process. They tend to become more wound specific depending on the state of the ulcer. No single dressing is suitable for the management of all types of ulcers and usually different types of dressings are ideally

suited for the treatment of a single ulcer during the various stages of the healing process[82,83].

1.2.5.1 Material selection in ulcer management.

The selection of the most appropriate product in any given situation can be extremely difficult. In order that an appropriate dressing material is selected for a particular ulcer, a classification system has been adopted; this is based upon the physical appearance and nature of the ulcer. Using this classification and knowledge of the properties of the individual dressing, it is possible to identify those products that are likely to be best suited for the management of a given ulcer at any particular stage in the healing process, and since healing is a dynamic process, therefore the properties that are required in a dressing may change with the healing progress. The classification represents different types of ulcer and shows the various stages through which an ulcer may undergo during healing[82,83]. Moreover the presence of infection may influence the choice of wound dressings. For the selection of the latter, ulcers may be classified as follows;

(i). Black and necrotic, these are covered with a hard dry black necrotic layer.

(ii). Yellow with slough, which are covered (or filled) with a soft yellow slough.

(iii). Granulating, which are clean with tissue loss

(iv). Epithelializing, these are clean and superficial

The principle stages in dressing selection are;

Stage1: Cleansing and removal of debris;

Given favourable conditions, tissue that has been anoxic and non-viable for example as a result of sustained application of high levels of pressure will often separate spontaneously from the healthy layer beneath. This occurs as a result of autolysis and is presumably due to macrophage activity and the action of proteolytic enzymes. However if such an area is exposed to an atmosphere for extended periods, the epidermis, which is unable to control the loss of moisture vapour, becomes dehydrated and hard with a black-leathery appearance. As this process continues, it becomes progressively more difficult for autolysis to take place and the separation of the dead tissue or slough may be delayed indefinitely. It follows therefore, that any product that reverses this process of dehydration will assist in the removal of necrotic tissue. Depending on the severity of the condition, the necrotic layer may be relatively superficial or extremely deep, often a large cavity develops beneath the surface of the black eschar, which is filled with dead or partially liquefied tissue; in some cases these can extend right down to the bone. Rehydration of this type of wound can be achieved effectively by the use of an occlusive dressing, which not only rehydrates it but also retains the moisture that would otherwise be lost as vapour. Granuflex, hydrocolloid or hydrogels may be used for this purpose.

Stage 2. Granulation and vascularization.

In the granulation stage of healing, it is generally agreed that a dressing is required that provides a moist environment, good thermal insulation and a reasonable fluid-handling capability. It has been suggested that an acidic environment and low oxygen tension may accelerate the formation of granulation tissue[20,21]. In practical terms, the management of ulcers with significant tissue loss depends upon a number of factors including the size, site, shape and origin of the lesion. Ulcers in this stage may respond well to the use of a silastic foam stent. In heavily wounds with a significant degree of tissue loss including leg ulcers and some infected surgical or traumatic wounds, these frequently respond well to treatment with an alginate dressing such as sorbsan.

Stage 3. Epithelialization.

Once a defect has become filled with granulation tissue, epithelialization takes place. In this final stage of the healing process, a dressing is required that provides a moist environment and will not adhere to the surface of the wound. An acid pH may be an advantage; it has also been demonstrated that in the management of clean, low exudate wounds, the oxygen permeability of the dressing can influence the rate of epithelial cell growth. Many different materials are available for use in this situation[20,21,81,82,83,84]. These include, semi permeable film, and hydrocolloid sheets, which are used in clean low exudate epithelializing

ulcers, whereas alginate island dressings, hydrophilic and polyurethane foam are used in clean, with medium to high exudate epithelializing wounds.

1.2.5.2 Classification of Modern wound dressing agents

There are a number of modern wound dressing agents[82,83,84,85] that have been classified depending on their mode of action as follows:

(i). Films:

These are semi occlusive (semi permeable) polyurethane or copolyester dressings. Vapour–permeable films and membranes allow the passage of water vapour and oxygen but not of water or microorganisms. They are highly conformable, convenient to use, provide moist healing environment and some are translucent enabling constant observation of the wound. However water loss, may occur at a slower rate than that generated by exudates, so that fluid accumulates under the dressing which can lead to tissue maceration and to wrinkling at the adhesive contact site (with risk of bacterial entry). They are indicated in acute partial or full thickness wounds with minimal exudates and in nondraining primarily closed wounds. They are most commonly used as secondary dressings over gels. Examples include Opsite, Bioclusive, Tegaderm and Blisten film.

(ii). Hydrocolloids:

These contain hydrophilic colloidal particles (quan, karaya, gelatic, carboxymethyl cellulose) in an adhesive mass usually polyisobutylene. They are usually presented as an absorbent layer on a vapour–permeable film or foam. Owing to their occlusive nature of their backing they are not considered suitable for treatment of clinically infected or very heavily exuding wounds, but are suitable for softening eschars or for promoting granulation[86,87]. They debride soft necrotic tissue by autolysis. They are indicated in acute or chronic partial or full thickness wounds. Examples of hydrocolloid dressings include; Cutinova, Duoderm, Comfell and Intrasile.

ii). Hydrogels

These contain 80-99% water, cross-linked polymer such as polyethyleneoxide, polyvinyl pyrollidone or acrylamide. They are most commonly supplied as amorphous, cohesive materials that can take up the shape of a wound. They are indicated in acute or chronic partial thickness wound with minimal exudate. These dressings are generally used to provide liquid to dry slough wounds and facilitate autolytic debridement but they may also have ability to absorb limited amount of exudates[1,84,85]. Examples are Vigilon, Elastogel and Geliperm.

(iv). Foams

These are either hydrophilic or hydrophobic dressings. They are non-occlusive, usually polyurethane or gel film coated with high absorbance. They are indicated in acute or chronic, partial thickness wounds that are highly secreting and requires mechanical debridement. They debride by autolysis, have high absorbency rates and are water vapour permeable. They include,Cutinova plus, Lyofoam,and Alleryn.

(v). Impregnates

These are fine mesh gauze impregnated with moisturizing, antibacterial or bactericidal compounds, which are non-adherent. They are indicated in acute or chronic partial thickness wounds with minimal to moderate exudates. They do not adhere to the wound, promote re-epithelialisation, and require secondary dressing. They include Aquaphor gauze, Adeptic, Scarlet red and Biobrane.

(vi). Absorptive powders and pastes.

These consist of starch, copolymers or colloidal, hydrophilic particles, which can absorb up to 100 times their weight.

They are indicated in chronic full thickness wounds having large amounts of exudates. They have high absorbency and debride necrotic and fibrous material from wounds. They include: Granulat, Debrisan and Duoderm granules.

(vii). Alginate dressings

These are non-woven composite fibres of calcium alginate, cellulose like polysaccharide[88,89]. The gelling characteristics vary according to the product used, some only form gel to a limited extent on a partially gelled sheet that can be lifted off and others form an amorphous gel that can be rinsed off with water or physiological saline. They are highly absorbent and therefore suitable for moderate or heavily exuding wounds but not for eschars or dry wounds. They include sorbsan and kaltostat.

Despite these various new agents for wound dressing are now available in the market, most are expensive and may not be available in developing countries like Tanzania.

1.2.5.3 Edinburgh University Solution of Lime (Eusol) as a standard antiseptic used in treatment for chronic ulcer.

Eusol is an antibacterial agent, which has long been used in the treatment of infected wounds in various countries including Tanzania. It is a calcium hypochlorite solution and used as a wound-cleansing agent.

It contains 0.25% W/V of available chlorine buffered with boric acid to a pH of 7.5–8.5. It is comparable to Dakin's solution in that it has to be

freshly prepared and is only stable for about two weeks when stored in a tightly closed bottle[20].

Hypochlorite solutions have been known for over two hundred years[1,20]. Eau de jevelle, a solution of potassium hypochlorite was first used as a bleaching agent in 1782 and a solution of sodium hypochlorite (known as Eau de Labarraque) was used as a disinfectant as early as 1820. Semmelweis[19] later used similar solutions with great success in preventing the spread of puerperal sepsis. Chemically, hypochlorite solutions are highly reactive combining rapidly with amine groups on proteins to bring about chlorination, oxidation and hydrolysis of nitrogenous materials[1,20]. It is this property of interaction with proteins that give hypochlorites their antimicrobial activity hence used as disinfectants, lavatory cleaners and water purification agents. Hypochlorites even at very low concentration have marked inhibitory effects on enzyme systems by reacting with all forms of proteins. They are rapidly inactivated by presence of pus, serum and other organic matter. A solution of sodium hypochlorite is very alkaline (pH11) and consequently irritant to skin. Dakin recognized this problem and described a buffered hypochlorite solution in 1915, which still bears his name.

The biological activity and stability of sodium hypochlorite solution is pH dependent. When stored correctly, a solution of sodium hypochlorite at pH 11 is stable for several months but at lower pH values the stability

is dramatically reduced. Unfortunately undissociated hypochlorous acid, the chemically active form reaches a maximum concentration around pH 5.5 at which point the solution is at its most unstable state and under these conditions decomposition is very rapid. For these reasons solutions of hypochlorite used in wound management are buffered to a point somewhere between these values in order to reduce their irritant effect as well as achieve a compromise between biological activity and chemical stability.

Milton solution (Richardson-Vicks) [20] is a commercially available solution containing 1% sodium hypochlorite and 16.5% sodium chloride; it is widely used as a 1 in 80 dilution to sterilize babies' feeding utensils. For wound management application a 1 in 4 dilution is generally used; this contains 0.25% W/V available chlorine with a pH of 10.5–11.2. Despite the difference in pH of these two solutions, these dilutions of Milton solution is often supplied as Eusol equivalent. Other preparations include chlorasm and chloramines BP[20].

Dakin's solution was used extensively during the First World War as an antiseptic and wound-cleansing agent [90], and had a dramatic effect in reducing wound sepsis. Hypochlorite is believed to have a property of assisting in the quick dissolution of necrotic tissue by rapidly combining with protein to form a soluble product thereby dissolving the necrotic tissue, pus and plasma clots. However, further experimental work demonstrated the failure of necrotic tissue to be dissolved when

hypochlorite solution is applied necessitating the initial surgical debridement of a slough if present[90]. Barton and Barton [91] suggested that the use of hypochlorites together with other antiseptics or antibiotics used topically can cause release of endotoxins and other toxic substances by bacteria present in the wound which can then lead to acute renal failure. Recently it has been shown that dressing with hydrocolloid and hydrogels can enhance wound debridement[92,93,94,95]. It is thought that this occurs by increasing hydration of the slough and necrotic tissue, which is then removed by autolysis, a process which takes place at the interface between the slough and the healthy tissue beneath. It is highly likely that this simple mechanism is also responsible for the debriding action of a hypochlorite solution rather than a direct chemical reaction upon the slough itself. If this is the case it is probable that plain saline soaks would be almost as effective as a cleansing agent for chronic ulcers. In addition saline does not cause any irritation to the wound or to the surrounding skin.

In one prospective study it was shown that pressure sores treated with hypochlorite solution healed more slowly than those treated with hydrocolloid dressing[96]. There has been considerable interest on the effect of hypochlorite and other antiseptic solutions on wound healing. In the Rabbit ear chamber preparation, Eusol and chloramines have been shown to permanently disrupt capillary circulation in granulated tissue and thus cause a significant delay in wound healing[97]. It has

also been shown that hypochlorite-treated wounds have a significant increase in DNA levels owing to the presence of elevated number of polymorphonuclear leucocytes indicating an increased inflammatory response as compared to saline treated wounds. In a more recent and detailed study, Kozol et al [98] has shown that hypochlorites cause marked morphological changes in fibroblasts, endothelial cells and in neutrophils. It has also been shown that hypochlorites impair chemotactic function, this implies that hypochlorites impair the natural defence mechanism of the body and therefore wound healing. The role of antiseptic and topical agents in wound management is currently controversial. Leaper and Simpson [99] concluded that prolonged use of antiseptic may delay wound healing and in particular hypochlorites could lengthen inpatient stay and delay return to normal life. The study suggested that a sterile solution of 0.9% sodium chloride is all that is required. But despite this hypochlorites are still widely marketed, and used as standard treatment for wounds and ulcers in many hospital centres including Muhimbili National Hospital.

1.2.5.4 Role of phenytoin in treatment of chronic ulcers

Phenytoin (diphenylhydantoin) a hydantoin derivative is a pharmacological agent that has been employed in the treatment of many conditions including epilepsy, cardiac arrhythmias; muscle disorders trigeminal and related neuralgias and other types of pain since 1938[100]. Recently there has been increasing evidence supporting earlier

observation that phenytoin has a distinct beneficial role in the biology of wound healing[23-29,101,102,103,104]. It accelerates the formation and maturation of collagen fibres, stimulate fibroblast cell proliferation and inhibit collagenase activity. The ability to reduce pain also suggests that it should be useful in treatment of chronic ulcers[105-111]. This information stimulated an investigation into possible beneficial effects of phenytoin in periodontal disease. In 1958 Shapiro[24] found that oral phenytoin pre-treatment improved pain and accelerated healing of surgically created gingivitis in patients with periodontal disease. This proved to be one of the initial well-controlled studies that confirmed accelerated wound healing with epithelial proliferation following phenytoin therapy. This was both substantiated and extended to topical application of the drug, which also caused decreased inflammatory response and enhanced collagen deposition in gingival tissue[112,113,114]. Examining effects of phenytoin on the general process of wound healing, laboratory investigations have demonstrated dramatic increase in wound tensile strength in primarily closed skin wounds[115] as well as accelerated healing of corneal wounds and mandibular fractures in rabbits[116,117,118,119]. Other laboratory studies have revealed phenytoin stimulated fibroblast growth in tissue cultures, inhibition of collagenase and collagen peptidase activity[120]. The use of phenytoin in patients with recessive epidermolysis bullosa has been shown to provide clinical improvement by increasing collagen content and decreasing degradation

of collagen, hence constituting regulation of tissue[120]. Experiments in rats with use of phenytoin has been shown to decrease dermal water, fat and catabolic effects of cortisol in skin by reduction of hexosamine nitrogen and collagen fraction[121,122]. Other studies have shown that phenytoin has inhibitory action on extracellular degradating enzymes by inhibiting β-gluconidase, lactate dehydrogenases and release of lysosomal enzymes, hence preventing degradation of proteoglycans[123,124,125]. Recently phenytoin has been shown to inhibit the release of some chemical mediators of inflammation by inhibiting the generation of 6-Ketoprostaglandin F_{1a} and thromboxane B_2 that are potent vasoconstrictors. It is effected in a dose depedent manner perhaps by binding to a glucocorticoid receptor site[126]. Rwanyuma[127] while comparing phenytoin with silverex in the treatment of small to moderate acute burn injury in paediatric patients; found significant relief in pain (P=0.01), faster reduction in surface area and bacterial wound contamination. In 1965, Simpson et al [128] reported a careful clinical study showing marked improvement in the rate of healing in chronic cutaneous leg ulcers (venous stasis, decubitus, diabetic, trophic ulcers of leprosy as well as partial thickness burn injury) when phenytoin was administered to 30 patients. This study used matched-pair, double blind and cross over techniques. The subjects were paired according to the severity of their ulcers and then randomized to groups that received oral phenytoin or a placebo capsule. The ulcer area was

determined by planimetry, ulcers were also rated clinically at the start of the study at 8 weeks, and at 13 weeks. There was marked improvement seen in the ulcers of the phenytoin treated group. Significant alleviation of patients' symptoms and acceleration in connective tissue repair was demonstrated. Statistical analysis of the ulcer areas showed benefit from Phenytoin compared to placebo ($P<0.05$); the placebo treated patients in fact had their ulcers worsening. A smaller oral dose of phenytoin (200mg/day) was adequate for healing compared to larger doses (400-600 mg/day). Two studies at the University of Guadalajara in Mexico [129,130] have demonstrated significant improvement in pain and healing of both chronic cutaneous ulcers and acute burns with topical phenytoin application. It was shown that topical application of phenytoin was effective compared to using it orally only in terms of accelerating wound healing and shortening hospitalisation period. However combined topical and oral medication had further improved reduction in duration of stay in hospital though this was not statistically significant when compared to topical application alone. Oluwatosin et al[131] compared topical honey with phenytoin in the treatment of leg ulcers and found significant reduction in the size of the ulcers in the phenytoin treated patients. The study concluded that topical phenytoin was superior to honey. No adverse reactions such as dermatitis, chemical toxicity, or hypersensitivity reaction using phenytoin have been noted in the published studies[22-29,101-109,112,113,114]. The absorption of phenytoin when applied topically on the ulcer is minimal[93], Amadosoul in 1989, reported serum concentration levels of less than 2ng/ml, when it was applied at 50mg per

2. CHAPTER TWO

2.0 STUDY RATIONALE AND STATEMENT OF THE PROBLEM

Patients with chronic ulcers are frequently seen at Muhimbili National Hospital Surgical Units. They are either referred from neighbouring districts, peripheral hospitals or adjacent regions and some ulcers have already developed malignant transformation. Common types of ulcers seen are chronic infected traumatic ulcers, tropical ulcer, diabetic ulcer, ulcers as a result of chronic non-specific inflammation, necrotizing fascitis of the scrotum (Fournier's gangrene), venous ulcer and infected burn injury. These patients usually need admission and daily dressing with some routine antiseptic with the intent of giving an appreciable time to allow the wound to granulate and thereafter discharge the patient when the wound has healed spontaneously or been skin grafted. At Muhimbili National Hospital (M.N.H) the topical antibacterial agents, which are routinely used, include Eusol, Dettol, silverex cream, hydrogen peroxide and honey. Bed rest and a high protein diet are encouraged in the treatment of chronic ulcers.

All these procedures aim at reducing bacterial wound contamination and super infection thereby allowing repair or regeneration mechanisms to take place. This is often associated with prolonged hospitalisation, which is very expensive and causes a great deal of distress not only to the patient but also to relatives who take care of the patient.

The use of topical phenytoin with its property of inducing fibrogenesis [28,109] increasing collagen synthesis, inhibition of proteolytic and tissue degrading enzymes thereby promoting wound healing will help shorten healing time and alleviate patient symptoms, allow short duration of stay in hospital, and reduce cost of treatment. Given the encouraging healing properties, the Author felt it appropriate to carry out a study on its use on ulcers in this party of the world as no study has been done hitherto. This in turn will hopefully provide an alternative improved approach in the treatment of patients with chronic ulcers at Muhimbili National Hospital.

3. CHAPTER THREE

3.0 OBJECTIVES

3.1. Broad Objective

To determine the effectiveness of phenytoin applied topically in the treatment of non-malignant chronic lower limb ulcers.

3.2. Specific Objectives

1. To determine the duration that is required with phenytoin treatment to make the ulcer sterile/discharge free and compare with those treated with Eusol.
2. To determine the rate of ulcer healing in phenytoin treated patients and compare to those treated with Eusol.
3. To determine the analgesic effect of phenytoin in the treatment of chronic ulcers.
4. To determine the type of chronic ulcers that respond better to topical phenytoin.

4. CHAPTER FOUR

4.0 PATIENTS AND METHODS

4.1. Study population and design

This study was a simple randomised clinical trial that recruited all eligible patients admitted to surgical wards with non-malignant chronic lower limb ulcers who had fulfilled the inclusion criteria. Patients were randomised using a table of random digits 0 to 9. Every patient who had presented with a chronic ulcer had their detailed history regarding mode of onset, duration of illness, pain, discharge and any other associated illness recorded. This was followed by physical examination to determine ulcer location, size, and characteristic of the floor, discharge and type of granulation tissue. A clinical diagnosis was then made after this initial assessment to determine the nature of the ulcer.

4.2. Subject selection

In addition to clinical assessment the inclusion and exclusion criteria were as follows

4.2.1 Inclusion Criteria

(i) A patient with chronic ulcer without features of malignancy as determined by physical examination and/or histopathology findings.

(ii) Patients with diabetic ulcers, post-traumatic ulcer, postoperative wounds/ulcer, and tropical ulcer were the targets of this study.

(iii) Patients of both sexes and all age groups from and above twelve (12) years.

(iv) Patients with ulcer surface area of up to 300cm^2 as determined by the product of the greatest vertical and transverse diameters. The cut off point (300cm^2) was taken because the average rate of cicatrisation varies with the size of the ulcer[132].

4.2.2. Exclusion Criteria

(i) All patients with malignant ulcer as determined by clinical assessment and confirmed by histopathology findings.

(ii) Patients with ulcer initially thought to be non-malignant clinically but proved histopathologically to be malignant during the course of treatment, as it was not healing. This was in particular to patients who did not show healing response during the second and third week of treatment in the diphenylhydantoin and control groups respectively, whereby a biopsy was mandatory.

(iii) Patients presenting with gross cellulitis, deep slough, ischemic gangrene due to major blood vessel involvement.

(iv) All patients with chronic ulcers who are on oral phenytoin.

(v) All patients with chronic ulcers who are known to have adverse reaction to phenytoin; also all patients who would develop an adverse reaction in the course of treatment, the treatment was to be stopped and a report made later.

(vi) Pregnant women.

(vii) Patients on Immunosuppressive or steroid therapy.

4.3. Sample size and sampling technique

It is estimated that diphenylhydantoin powder when applied as a thin film over the surface of non-malignant chronic ulcers will be effective with a good healing response in about 80% of patients'[22]. In comparison, using an antiseptic or normal saline which work by the same mechanism of increasing hydration of slough and necrotic tissue which is then removed by autolysis, these have been found to be effective in only about 50% of patients who have had good healing response[20].

The sample size was calculated from a general formula derived from the EPI Info 6(2000) computer programme:

$$N = \frac{(U + V)^2 [P_1 (100-P_1) + P_2 (100 - P_2)]}{(P_1 - P_2)^2}$$

Whereby:

N, Is the desired sample size

P_1, The proportion of patients treated with diphenylhydantoin powder, with a good response that is 80%.

P_2, The proportion of patients treated with a buffered solution of calcium hypochlorite with a good response that is 50%.

U, The power of the study, that is the probability of finding a significant result estimated at 80% and hence u =1.28.

V, The percentage point of the normal distribution corresponding to the two sided significant level that is the error 5% and hence v = 1.96.

Substituting these values in the above formula a minimum sample size of forty-eight (48) patients was required per arm of treatment. Hence a total of ninety-six (96) patients was the minimum number of patients required in this study. 102 patients were enrolled of which 50(49%) were treated with diphenylhydantoin and 52(51%) were in the control group and treated with Eusol.

4.4. Data Collection

4.4.1 Patient demography.

Patients' demography characteristics were collected using a questionnaire. All patients' details regarding his or her ulcer was obtained by clinical evaluation and entered. Patient's age, sex and address (district) were also recorded; the duration of the ulcer in weeks was also noted. The site of the ulcer on the limb, whether located proximal third, mid third, distal lateral, distal front or distal medial of the foot or other sites (heel, plantar or toes) was also recorded. The

margin, edge and floor of the ulcers were assessed to exclude possibility of malignancy and if suspicious a biopsy was done.

4.4.2 Parameters for assessment.

The ulcers were inspected regularly to assess parameters of healing and these were recorded at weekly intervals. They included:

4.4.2.1 Nature of discharge.

The nature of discharge was characterized into the following;

(i) Unhealthy discharge: when there was presence of foul smelling, purulent or greenish discharge and this was scored 1 point.

(ii) Healthy discharge: when there was a serous discharge, which scored 2 points.

(iii) No discharge: which scored 3 points.

4.4.2.2 Ulcer and pain.

The presence of pain was recorded and grouped into:

(i) Severe, if the patient had intolerable pain requiring analgesics and given a score of 1 point.

(ii) Bearable (mild to moderate), if the pain was present but patient could tolerate and was given score of 2 points.

(iii) No pain; and given a score of 3 points.

4.4.2.3 Ulcer and Granulation tissue.

The Characteristic of granulation tissue present on the floor of the ulcer was also recorded. It was classified as follows:

(i) Unhealthy granulation, when there was pale, blue, or patchy granulation.

(ii) Healthy granulation, when there was a 'red' granulation tissue suitable for skin grafting.

4.4.2.4 Size of the ulcer.

The size of the ulcer was determined by calculating its surface area, which in turn was obtained by measuring the greatest vertical and transverse diameter and finding the product. The measurements were obtained by using a tape measure and the result recorded in cm^2 to the nearest one decimal point. Subsequent measurements were done at weekly intervals for four weeks and the percentage reduction in surface area

$(A_0-A_i)/A_0 \times 100\%$ cm^2 recorded.

4.4.2.5 Culture and sensitivity.

Swabs from the ulcers were taken for aerobic bacterial culture and sensitivity prior to commencement of treatment (day 0) and subsequently on days 7 and 14. When the culture yielded no bacterial growth, no further swabs was taken from the ulcer.

Techniques for culture:

Pus swabs were collected from the ulcer floor and sent to the microbiology laboratory where they were cultured on Mac Conkey and blood agar plates. Both plates were then incubated at 37°C overnight. The next day the plates were inspected for any evidence of bacterial growth. If present, Gram stain and sensitivity testing using discs impregnated with antibiotics was done. The common antibiotics tested for were Gentamycin, Clavulinic-Amoxycillin, Ampicillin, Chloramphenicol, Erythromycin and Cloxacillin. The culture report showing causative organism and sensitivity pattern was recorded.

4.4.2.5 Treatment and Follow up.

Patients who had fulfilled the inclusion criteria underwent whenever required surgical debridement and cleaning of the ulcer with normal saline before the application of a topical medicament.

(i) Study patients: After cleaning the ulcer with normal saline, a thin uniform film of diphenylhydantoin powder was sprinkled over the floor of the ulcer and dressed with dry gauzes

(ii) Control patients: The ulcer was similarly cleaned with normal saline and dressed with calcium hypochlorite solution (Eusol)

- The dressings were done on a daily basis and pus swabs were taken prior to dressing on days 0, 7 and 14. Follow up assessment of the

healing process was done on a weekly basis on days 7, 14, 21 and 28 and recorded in the questionnaire (see appendix). Patients were followed up until either the ulcer had healed by contraction/scar formation or were ready for skin grafting by the 28th day of treatment.

4.5 Data analysis

Data was extracted from the questionnaire, frequency distribution tables constructed and analysis was done; statistical testing of data for comparison purpose between the two groups was done using a computer with the help of the EPI Info 6(2000) computer programme. Statistical significance was assessed using Chi-Squared test for categorical variables, student-t test and analysis of variance for means. The statistical significance level of P-value less than 5% ($p<0.05$) was used for comparison.

4.6 Ethical consideration.

The study was conducted after being granted permission by the department of surgery and obtaining clearance by the college research and publication committee as well as the hospital ethical committee. Relevant information was given to the patient and an informed consent obtained; parent or guardian gave consent in case of children.

4.7 Limitations of the study.

Though the study was successful done it was limited in some aspects;

- Due to lack of facilities it was difficult to measure serum level concentrations of the absorbed drug.
- Anaerobic organisms could not be cultured because of lack of facilities.
- Despite of the fact that a large number of patients have shown early formation of health granulation tissue suitable for skin grafting, these patients could not undergo skin grafting at appropriate time mainly because of lack of theatre time.

• CHAPTER FIVE.

5.1 RESULTS

The study was done over a period of 14 months, between August 2000 and September 2001. A total of 102 patients with chronic non–malignant ulcers were enrolled in the study. The study group consisted of 50 patients who were treated with topical application of diphenylhydantoin powder while 52 patients were treated with Eusol and formed the control group.

Table 1: Sex distribution

	Treatment					
Sex	**Diphenylhydantoin**		**Eusol**		**Total**	
	N	**%**	**N**	**%**	**N**	**%**
Male	30	(44.8)	37	(55.2)	67	(65.7)
Female	20	(52.1)	15	(42.9)	35	(34.3)
Total	**50**	**(49.0)**	**52**	**(51.0)**	**102**	**(100.0)**

$\chi^2 = 1.41$, df 1, P>0.05

There were 67 (65.7%) male and 35 (34.3%) female patients. There was no significant difference in sex distribution between the two treatment groups (Table 1)

Table 2: Age Distribution.

Age group (Years)	Diphenylhydantoin N	Control N	Total N	(%)
12-16	3	8	11	(10.8)
17-21	5	5	10	(9.8)
22-26	9	3	12	(11.8)
27-31	11	9	20	(19.6)
32-36	6	5	11	(10.8)
37-41	4	6	10	(9.8)
42-46	4	1	5	(4.9)
47-51	3	6	9	(8.8)
52-56	5	9	14	(13.7)
TOTAL	**50**	**52**	**102**	**(100.0)**

The mean age in the diphenylhydantoin group was 32.5 (± 14.9) years with a range of 12 to 55 years while in the control group the mean age was 34.2(± 14.7) years with a range of 12 to 56 years. A relatively higher proportion (19.6%) of patients were in the age group of 27-31 years. There was no significant difference in the age distribution between the groups. $\chi^2 = 2.27$, df 1, $p>0.05$ (Table 2)

Table 3: Distribution according to Aetiology of Ulcer

Aetiology of Ulcer	Diphenylhydantoin	Control	Total	
	N	N	N	%
Trauma	16	12	28	(27.5)
CNSI	10	12	22	(21.6)
Burn	7	9	16	(15.7)
Diabetic	5	8	13	(12.7)
Tropical	7	6	13	(12.7)
Animal bite	3	2	5	(4.9)
Venous	2	3	5	(4.9)
Total	**50(49%)**	**52(51%)**	**102**	**(100.0)**

**CNSI, Chronic Non-Specific Inflammation.

The aetiology of the ulcers varied, majority of the patients had ulcer following trauma (27.5%). Other circumstances which led to chronic ulcers were animal bites, infected burn wounds, diabetes mellitus, chronic non-specific inflammations (C.N.S.I), tropical and venous ulcer. (Table 3)

Table 4. Distribution according to site of ulcer

Site of ulcer	N	%
Dorsum of foot	26	(25.5)
Distal lateral	20	(19.6)
Mid third	17	(16.7)
Distal frontal	15	(14.7)
Others (heel, plantar, toes)	7	(6.9)
Proximal third	5	(4.9)
Total	**102**	**(100.0)**

The site of the ulcers on the leg was multiple, the majority being on the dorsal aspect of the foot (25.5%). Other sites on the leg were proximal third, mid third, distal lateral; distal medial, distal front. The remaining were from the plantar aspect, heel and toes. (Table 4)

Table 5: Duration of ulcer

Duration (Months)	Diphenylhydantoin	Control	Total	
	N	N	N	%
<2	29	28	57	(56.0)
3-4	9	11	20	(19.6)
5-6	2	6	8	(7.8)
7-8	4	0	4	(3.9)
9-10	1	2	3	(2.9)
11-12	0	0	0	(0.0)
>12	5	5	10	(9.8)
Total	**50**	**52**	**102**	**(100.0)**

The duration of the ulcer ranged from 3 weeks to 39 months and 3 weeks to 32 months in the diphenylhydantoin and control groups respectively. In both groups the median duration of ulcers was 1 month. There was no statistical difference in duration of ulcer between the groups T=0.26, p>0.05. (Table 5)

Table 6: Distribution according to severity of pain.

Day of assessment	Treatment group	Severity of pain			Remarks
		Severe	Bearable	No Pain	
Initial day (0)	Diphenylhydantoin	27	23	0	p > 0.05
	Control	24	28	0	
Day 7	Diphenylhydantoin	4	45	1	p < 0.05
	Control	11	40	1	
Day 14	Diphenylhydantoin	7	28	15	p > 0.05
	Control	6	35	11	
Day 21	Diphenylhydantoin	5	10	35	p > 0.05
	Control	1	31	20	
Day 28	Diphenylhydantoin	0	8	39	p > 0.05
	Control	0	14	37	

The severity of pain in the treatment groups was graded into severe, bearable (mild to moderate) and no pain. The distribution of severity of pain in the two groups is shown in Table 6. Patients presented with various intensity of pain however there was no significant difference between the treatment groups on admission ($p>0.05$). On day 7 of treatment there was a relatively higher proportion of patients with bearable pain in the diphenylhydantoin group as compared to those treated with Eusol, this difference was statistically significant ($p<0.05$). By day 14, three patients in the diphenylhydantoin group who had shown improvement in pain developed severe pain. However, the majority of patients in both treatment groups showed progressive alleviation of pain symptoms and there was no statistical difference

between the groups on 14th, 21st, and 28th days ($p>0.05$). By the 28th day, 3 patients in the diphenylhydantoin group and one in the control group were already discharged as the ulcers had either healed spontaneously or skin grafted and therefore do not appear in the table.

Table 7: Follow-up assessment of pain during treatment

Day of assessment	Presence of pain	Diphenylhydantoin	Control	Remarks
Initial Day (0)	Yes	27	24	χ^2=0.0, p>0.05
	No	0	0	
Day 7	Yes	27	24	χ^2=0.0, p>0.05
	No	0	0	
Day 14	Yes	21	23	χ^2=0.04,p<0.05
	No	6	1	
Day 21	Yes	10	18	χ^2=7.4, p<0.01
	No	17	6	
Day 28	Yes	7	8	χ^2=0.47,p>0.05
	No	20	16	

Patients who had initially presented with severe pain in the diphenylhydantoin and control groups (54% and 46.2% respectively) were further followed up to assess progress of pain intensity. Significant reduction in the severity of pain was noted in patients who were treated with diphenylhydantoin powder on the 14th day ($p<0.05$). This difference was even higher on the 21st day of treatment ($p<0.01$). No significant difference was noted on the 28th day (Table 7).

Table 8: Rate of clearance of discharge.

Period of treatment	Treatment	Nature of Discharge						Remarks
		Unhealthy		Healthy		No Discharge		
		N	%	N	%	N	%	
Initial	Diphenylhydantoin	49	(98.0)	1	(2.0)	0	(0.0)	χ^2 =0.0
day(0)	Control	51	(98.1)	1	(2.0)	0	(0.0)	p>0.05
Day (7)	Diphenylhydantoin	37	(74.0)	13	(26.0)	0	(0.0)	χ^2=6.15,
	Control	48	(92.3)	4	(7.7)	0	(0.0)	p<0.05
Day (14)	Diphenylhydantoin	12	(24.0)	35	(7.0)	3	(6.0)	χ^2=4.6,
	Control	23	(44.2)	29	(55.8)	0	(0.0)	p<0.05
Day (21)	Diphenylhydantoin	3	(6.0)	28	(56.0)	19	(38.0)	χ^2=0.23,
	Control	5	(9.6)	32	(61.5)	15	(28.9)	p>0.05
Day (28)	Diphenylhydantoin	2	(4.3)	8	(17.0)	37	(78.7)	χ^2=3.00,
	Control	3	(5.9)	16	(31.4)	32	(62.7)	p>0.05

Both treatment groups showed an improvement in clearance of discharge; however, a higher proportion of clearance was noted on days 7 and 14 in the diphenylhydantoin group as compared to the control group, this difference was statistically significant ($p<0.05$). By the 21st and 28th day, though there were also a higher proportion of patients with clearance of discharge in the diphenylhydantoin group as compared to the control group this difference was not statistically significant ($p>0.05$). By the 28th day, 3 patients in the

diphenylhydantoin group and one patient in the control group had been discharged as the ulcers had either healed spontaneously or skin grafted (Table 8).

Table 9: Bacteria Isolated on Initial Culture.

Organisms	Diphenylhydantoin	Control	Total	
			N	(%)
Pseudomonas	33	23	56	(54.9)
S.aureus	3	8	11	(10.8)
E. Coli	2	8	10	(9. 8)
Pseud+S.aureus	6	3	9	(8. 8)
Klebsiella	1	6	7	(6. 9)
Proteus	3	1	4	(3 .5)
S.aureus +E.coli	0	2	2	(2. 0)
Pseud+Klebsiella	1	0	1	(1. 0)
S.aureus+Klebsi	0	1	1	(1. 0)
No Bact.growth	1	0	1	(1. 0)
Total	**50(49%)**	**52(51%)**	**102**	**(100.0)**

For the exception of one ulcer in the diphenylhydantoin group, all pus swabs taken for culture and sensitivity at start of study showed positive bacterial growth (Table 9). The commonest bacterium isolated was Pseudomonas species 54.9%. Staphylococus aureus was isolated in 10.8% other bacteria included E-coli, Klebsiella and Proteus species. Double infection was seen in 13 patients, these were mainly Pseudomonas with Staphylococus aureus (9); Staphylococcus with E.coli (2), Pseudomonas with Klebsiella (1); Staphylococus with Klebsiella (1).

Table 10: Pattern of antibiotic sensitivity.

Organism	Drug sensitivity
Pseudomonas	Gentamycin, Kanamycin
Stephylococus aureus	Cloxacillin, Erythromycin
E – Coli	Gentamycin, Augmentin + Chloramphenicol
Klebsiella	Ciprofloxacin, Augumentin, Chloramphenicol
Proteus	Gentamycin, Chloramphenicol.

The organisms isolated were sensitive to antibiotics generally available, however sensitivity tests were also limited to the common available antibiotic sensitivity testing discs. (Table10). A combination of Gentamycin and Augmentin (Clavulinic acid + Amoxycillin) would cover most bacteria that infect chronic ulcers seen in our environment.

Table 11: Bacterial infection clearance rate.

Day pus swab taken	Negative bacterial culture	Diphenylhydantoin N	(%)	Control N	(%)	Remarks
Initial Day (0)	Yes	1	(2.0)	0	(0.0)	$\chi^2 = 1.05$,
	No	49	(98.0)	52	(10.0)	p>0.05
Day (7)	Yes	15	(30.6)	14	(27.0)	$\chi^2 = 0.17$,
	No	34	(69.4)	38	(73.0)	p>0.05
Day (14)	Yes	15	(44.0)	15	(39.5)	$\chi^2 = 0.09$,
	No	9	(56.0)	23	(60.5)	p>0.05

** It should be noted that once a swab has revealed a negative bacterial culture, no further swabs were taken from the ulcer.

Clearance of bacterial growth was seen in both treatment groups on follow up with a relatively higher proportion of negative bacterial cultures in diphenylhydantoin group compared to the control group 30.6% and 27% on the 7th day, 44% and 39.5% on 14th day respectively (Table 11.) However these differences were not statistically significant (p >0.05).

Table 12: Healthy granulation tissue formation rate.

Period of treatment	Presence of healthy granulation	Diphenylhydantoin	Control	Remarks
Initial Day (0)	Yes	0	0	χ^2=0.00,
	No	50	52	p>0.05
Day 7	Yes	3	0	χ^2 = 3.21,
	No	47	52	p>0.05
Day 14	Yes	31	5	χ^2 = 30.63,
	No	19	47	p<0.001
Day 21	Yes	44	25	χ^2 = 18.56,
	No	6	27	p<0.001
Day 28	Yes	45	43	χ^2 = 3.5,
	No	2	8	p>0.05

None of the patients included in the study had healthy granulation tissue at commencement of treatment. By the 7th day, 3 patients in the diphenylhydantoin group had developed healthy granulation and none in the control group (Table 12). There was relatively higher proportion of patients whose ulcers had healthy granulation tissue by the 14th and 21st days of treatment in the diphenylhydantoin group compared to the control group and this difference was highly statistically significant (p< 0.001). However, by the 28th day of treatment the difference was not statistically significant between the two treatment groups (p>0.05). Spontaneous closure of the ulcer had occurred in 3 patients in the diphenylhydantoin group while one patient had skin grafting in the

control group by the 28th day. They were discharged and therefore do not appear in the table.

Table 13: Decrease in ulcer mean surface area.

Assessment day	Diphenylhydantoin	Control	Remarks
Day 7	20.9 ± 13.5	14.9 ± 17.4	T= 1.954, p<0.05
Day14	40.3 ± 21.0	29.2 ± 17.4	T = 2.877, p<0.01
Day 21	54.6 ± 22.3	43.1 ± 17.3	T = 2.896, p<0.01
Day 28	66.5 ± 22.0	58.7 ± 18.1	T = 2.000,p< 0.05

The mean ulcer area in the diphenylhydantoin and control groups was 119.8±89.6 and 79.6±71.7 respectively on admission. The surface area in the diphenylhydantoin group ranged from 14–300cm^2 and in the control group it was 12–300 cm^2. While undergoing treatment there was a relative high rate in reduction in the surface area of the ulcers in the diphenylhydantoin group as compared to the control group, these differences were statistically significant (Table 13)

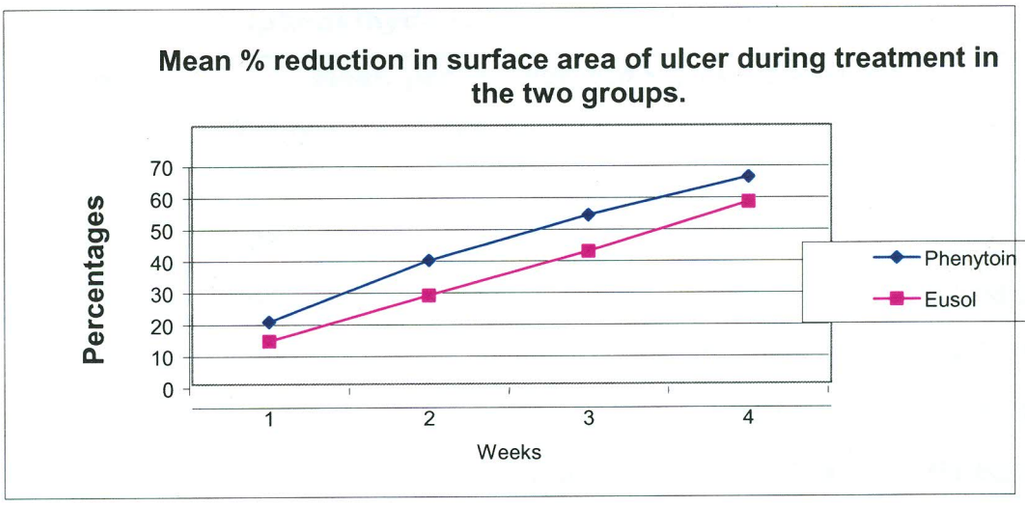


Fig 2.

In both treatment groups there was progressive healing by contraction, however it was faster in the diphenylhydantoin group compared to the control group (Fig 2).

Table 14: Mean percentage reduction in surface area of the diphenylhydantoin treated ulcers.

Nature of ulcer	Mean percentage reduction of surface area			
	7 day	14 day	21 day	28 day
Animal bite	31.0± 9.2	55.1± 16.7	61.4± 20.5	69.7± 15.5
Trauma	20.6± 5.0	43.5± 25.4	57.6± 7.0	71.6± 18.4
Tropical	23.5± 18.8	38.6± 24.0	55.9± 20.8	67.1±19.7
Burn	22.1± 12.3	38.5±17.7	52.9± 14.0	68.1± 15.7
CNSI	19.9 ± 7.8	37.0± 13.2	52.6± 17.9	61.6± 27.9
Diabetic	17.6± 15.8	37.6± 28.9	47.1± 31.4	54.8± 33.3
Venous	16.4± 16.1	23.1± 25.3	36.8± 27.7	55.4± 21.8

**CNSI, Chronic Non-Specific Inflammation.

All the different types of ulcers treated with diphenylhydantoin showed progressive healing as seen by their progressive reduction in surface areas during treatment (Table 14).

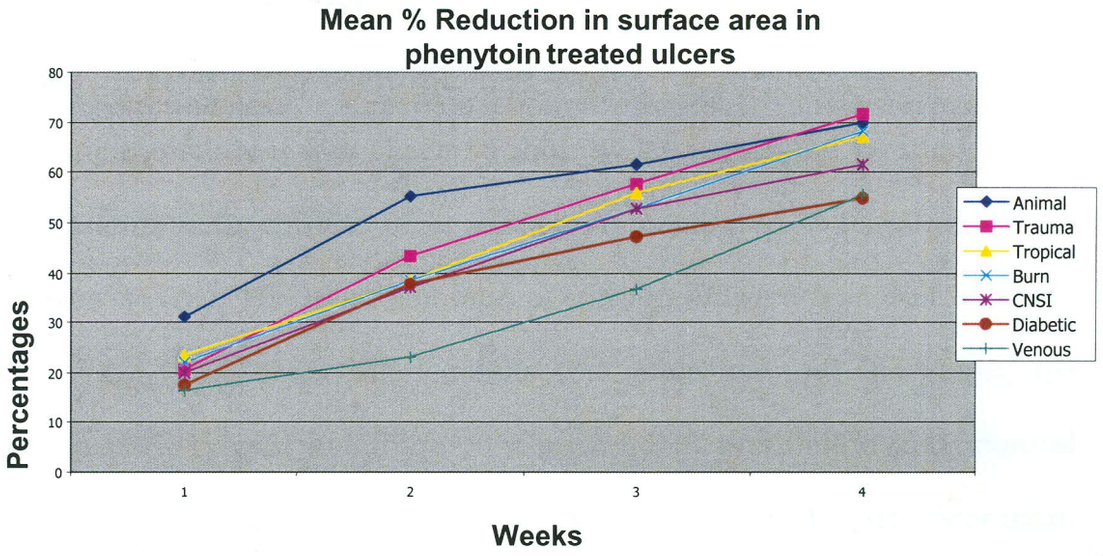


Fig. 3.

The mean percentage reduction in surface area was relatively higher in ulcers due to animal bites, followed by those due to trauma, while venous and diabetic ulcers responded less favourably. (Fig 3)

5.2. DISCUSSION

5.2.1. Variation in occurrence of ulcer.

During the study it was observed that, males accounted for the majority of patients with chronic ulcer disease as compared to females, (65.7% versus 34.3%). The most common age group affected was that of 27-31 years old. This observation entails that male sex and the young age group (27-31years) are frequently involved in production and manual work therefore predisposes to trauma and hence chronic ulceration. Majority of the ulcers were located in the dorsal aspect of the foot (25.5%). The common occurrence of ulcers in the lower limb is due to the fact that it is frequently subjected to trauma and the limb often does not get enough rest for the wound to heal. This means that ulcers in the lower limbs are difficult to treat and therefore presents one of the surgical management difficulties.

5.2.2 Nature of ulcers

A chronic ulcer may arise due to various aetiological agents; the present study revealed subjects developed chronic ulcers following animal bite, diabetes mellitus; venous insufficiency; trauma; burn injury and chronic non-specific inflammation including tropical ulcers. The transformation from an acute inflammation to chronic ulcer is indicated by presence of cells of chronic inflammation such as macrophages, plasma cells, and lymphocytes and in addition there are processes of

fibrosis and angiogenesis. Clinically this period varies but it may be as early as 2 weeks[69]. However, in this study, patients who presented with non-healing wounds or ulcers of 3weeks duration or more were regarded to be having chronic ulcers.

5.2.3 Status of chronic ulcers

5.2.3.1. Chronic ulcer and pain

Due to the chronic nature of ulcers patients may or may not have pain. In this study presence of severe ulcer pain requiring analgesics was found in 27 (54%) patients in the diphenylhydantoin group while, in the control group there were 24 (46.2%) patients. Pain is usually pronounced in an acute on chronic inflammatory condition due to elaboration of chemical mediators of inflammation. The physical response to pain is also affected by individual personality and anxiety. It is a significant symptom in most patients as it causes a lot of discomfort and distress. In this study, both treatment groups showed progressive pain alleviation. There was no significant difference between the two groups in severity of pain when assessed on days 7and 28. There was, however, a significant relief in pain noted on days 14 and 21 of assessment, whereby a high proportion of patients in the diphenylhydantoin group were pain free compared to the control group ($p<0.05$). Previous studies have reported on the analgesic effects of diphenylhydantoin[24,129].

The local analgesic property of diphenylhydantoin is thought to be mediated by its selective depression of repetitive neuronal activity and synaptic transimission[23]. Another study attributed phenytoin's analgesic effect due to its combined action of membrane stabilizing property including modulation of ion influx and selective depression of repetitive neuronal activity acting together[103].

5.2.3.2 Clearance of discharge.

Prolonged ulceration particularly with unpleasant odour and discharge may not only demoralize the patient but also lead to social ostracism. During the study, ulcers with unhealthy discharge, which were characterized by presence of purulent, green or bloody discharge were seen in both treatment groups. Progressive clearance of discharge occurred by the 7th and 14th days in both groups though there was a relatively higher proportion of ulcers having the discharge cleared when on diphenylhydantoin treatment compared to those on Eusol and this difference was statistically significant ($p<0.05$). By the 21st and 28th days of treatment, both groups had shown complete reduction in ulcer discharge but the difference was not statistically significant ($p>0.05$). Previous studies have noted similar findings whereby there was significant clearance of ulcer discharge in patients treated with topical diphenylhydantoin. Modaghegh.S et al[105] also noted a decrease in exudation and oedema.

5.2.3.3 Chronic ulcers and bacterial contamination.

Bacterial infection is a common complication of chronic ulcers, some of these are nosocomial. In this study, bacteria were isolated in all except one ulcer. In 13 patients double infection was cultured from the ulcers. Most of the organisms isolated were gram-negative bacteria; the only gram-positive bacterium was staphylococcus aureus. Bacteria are generally present when there is a discharge but occasionally are not isolated from culture studies, as was seen in one case. In this study clearance of bacterial growth was seen in both treatment groups with a relatively higher proportion of negative cultures in the diphenylhydantoin group compared to the control group. No significant difference was noted between the two groups ($p>0.05$). However other studies have shown significant reduction in bacterial wound infection by diphenylhydantoin[26,28,105,129].

The mechanism of reduction in bacterial contamination by diphenylhydantoin is thought to be mediated through a change in pH and improvement in local circulation[103].

5.2.4 Treatment and healing

5.2.4.1 Rate of formation of healthy granulation

The importance of rapid wound healing is emphasized, the hallmark of surgery is to achieve complete wound healing as soon as

possible and this will enable a short hospital stay, reduce cost, minimise burden and stress to both patients and hospital staff. Healing of ulcer should either be complete closure or provide a base with healthy granulation tissue for skin grafting or secondary surgical wound closure. Since its discovery in 1908[133], diphenylhydantoin was noted to promote wound healing by stimulation of fibroblastic activity that lead to maturation of granulation tissue and formation of collagen fibres. In this study it was observed that formation of healthy granulation tissue was faster in the diphenylhydantoin treated patients compared to those treated with Eusol. In the first week there were three patients whose ulcers had healthy granulation tissue in the diphenylhydantoin group while none in the control group. During the second week there were more patients with healthy granulation tissue in the diphenylhydantoin group 31(62%) patients compared to 5(9.6%) patients in the control group, the difference being highly statistically significant ($p<0.001$). This significant statistical difference was also noted by the third week of treatment. By the end of fourth week, three patients managed with diphenylhydantoin powder had their ulcers completely healed, while one patient in the control group had been skin grafted. By the end of fourth week the majority of patients in both groups had demonstrated formation of healthy granulation tissue but there was no statistical significant difference ($p>0.05$). It was also observed that the mean

duration of formation of healthy granulation tissue was shorter in the diphenylhydantoin group compared to the control group (14.7 ± 5.3 versus 21.5 ± 4.7 days respectively). This finding has also been reported in other studies [26,105].

5.2.4.2 Rate of wound contraction

In the first week of treatment the mean percentage reduction in ulcer surface area was 20.9 ± 13.5 % in the diphenylhydantoin group while in the control group it was 14.9 ± 17.4%. This difference was statistically significant ($p<0.05$). On subsequent days of assessment namely 14th, 21st and 28th, the rate of ulcer healing was even more faster in patients managed by diphenylhydantoin compared to the control group and this difference was highly statistically significant ($p<0.01$) as demonstrated in figure 1. The effect of diphenylhydantoin in promoting wound healing observed in this study is consistent with the findings of other series [24,107,109,114,116,117]. There are several mechanisms by which diphenylhydantoin promotes wound healing. It has been shown to inhibit the release of chemical mediators of inflammation by inhibiting the generation of 6-Ketoprostaglandin F_{1a} and Thromboxane B_2 which are potent dose dependant vasoconstrictors and act by binding to a glucocorticoid receptor site[126]. Diphenylhydantoin also increases fibroblastic proliferation, inhibits release of lysosomal and cytoplasmic enzymes and decrease catabolic effect of cortisol[111,123,124,125].

It further inhibits the degrading enzymes such collagenase and peptidases hence increasing collagen content and new blood vessel formation leading to increase in tensile wound strength[104,108,109,110-117] and therefore promote healing.

5.2.5 Healing response with diphenylhydantoin in chronic ulcers of various aetiology.

When comparing healing of ulcers of different aetiology treated with diphenylhydantoin, those due to animal bites and trauma responded better than those due to venous insufficiency and diabetes. The poor response in diabetic patients can be explained by the fact that these patients have defective immunological defence mechanisms associated with impaired phagocytosis and neuropathy that frequently predisposes them to mechanical trauma and infection[134]. Diabetics also have deranged metabolism being more in catabolic state rather than anabolic and therefore laying down of ground substances and fibroangiogenesis are also impaired[134]. On the other hand, venous ulcer results from incompetent and defective valves in the veins, leading to increase pooling of blood, increased interstitial fluid transfer across the capillary membrane and lymphoedema[135,136]. There is also increased connective tissue in the subcutaneous tissue or sometimes haemosiderin may be deposited leading to scarring secondary to excess protein in interstitial tissue thereby predisposing to trauma, infection and phlebitis. Despite using various topical antibacterial agents,

the poor integrity of the surrounding skin, lymphoedema and frequent occurrence of thrombophlebites can explain the slow healing rate of venous ulcers. The good healing response observed in ulcers following animal bites and trauma, may be explained by the fact that, in these two types ulcers there is good preservation and integrity of the surrounding tissue yet no underlying systemic diseases.

5.3 Conclusion

Chronic ulcer remains a challenging clinical problem in surgical practice. It can cause considerable disability and very distressing as intractable ulcers are usually associated with unpleasant odour, discharge and pain. This may demoralize the patient leading to social ostracism. Furthermore, prolonged ulceration or the resulting scar may predispose to malignant transformation[19,22]. Hitherto surgical approaches in the management of chronic ulcer in our environment has not shown encouraging results and the need to continue researching for better methods is advocated. Chronic ulcers resulting from different causes, causing considerable morbidity are frequently seen at Muhimbili National hospital in the general surgical units. Research should aim at improving care, alleviate patient's symptoms and shorten duration of hospital stay. This prospective controlled clinical trial has demonstrated the effectiveness of diphenylhydantoin powder when compared with a solution of calcium hypochlorite (Eusol), which is a commonly used antibacterial agent for infected wounds and ulcers at MNH. Diphenylhydantoin also alleviated pain by the 14th day of treatment; caused earlier clearance of discharge by the 7th and 14th days. There were also a higher proportion of patients with no bacterial growth. Diphenylhydantoin is effective in promoting healing of ulcers as it caused faster rate in formation of healthy granulation tissue and wound contraction sometimes causing complete ulcer closure or

encouraged early healthy granulation tissue formation for skin grafting and then discharge from hospital. By minimizing morbidity and distress these patients are able to enjoy a more socially acceptable life.

As seen in other series, diphenylhydantoin is cheap[137], 100g of the powder cost about 1 US Dollar and patient can use 50mg per square centimetre surface area of ulcer[132]. In contrast to Eusol, which is expensive, 100mls costs 0.5 US Dollar and patient can use at least 25mls per dressing.

5.4 **Recommendations**:

- The beneficial effect demonstrated by diphenylhydantoin in the treatment of chronic ulcers and its ease of application suggests that health personnel working in peripheral dispensaries, health centres and district hospitals could be instructed on how to use and apply the drug on chronic ulcers thereby minimising referrals to a large hospital like MNH. This will reduce costs, congestion and overstaying at referral hospitals and patients could be closer to their families.
- More detailed study will probably be needed to evaluate the optimal dose required including checking serum levels of diphenylhydantoin to avoid exceeding therapeutic levels. Its mechanism of antibacterial activity could also be further studied.

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