EFFICACY OF HONEY DRESSING VERSUS MECHANICAL DEBRIDEMENT IN HEALING OF ULCERS WITH BIOFILMS A COMPARATIVE STUDY

By

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In

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Under the guidance of

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DR.SURYAPRAKASH REDDY

ABSTRACT

Chronic wounds are the significant health problems globally. The treatment and management of chronic wounds is challenging to the health care providers. Microbial Bioburden in wounds are the important factors responsible for the chronicity of wounds. The effective management of bacterial bioburden is an essential element of wound care. Bacteria can exist in at least two different phenotypic growth forms: the first being single, fast-growing cells i.e. the planktonic form; the second as aggregated communities of slow-growing cells in a biofilm form[1].

Management of biofilm in chronic wounds is rapidly becoming a primary objective of wound care. However management of biofilm is an undeniably complex task. Beyond the basic steps of initial prevention (use of anti-biofilm agent), removal (debridement, de-sloughing) and prevention of reformation (use of antimicrobial agents), patient, environmental and clinical parameters that must be considered.

Honey on the wound bed not only draws material out of the wound, but also prevents biofilm formation and cross-contamination. It provides a barrier effect on an open wound preventing further infection from external contamination.

Method of Collection of Data:

All patients admitted in the department of general surgery in Shri B.M Patil Medical collage during the study period of October 2015 to June 2017 with ulcers were initially subjected to the identification of biofilm in ulcers. Ulcers with biofilm were included in the study. A total of 90 patients were taken up for study and divided randomly into Honey and Debridement group with 45 in each. Ulcers were treated with dressing soaked with honey in honey group and debridement with povidone

iodine dressings in debridement group. Once in 5days ulcers were evaluated for the presence of biofilm and assess the healing process and once the ulcers were healed completely or the culture was sterile or negative for biofilm were underwent definitive surgery. Statistical analysis was done by Fisher's exact test and Chi square test.

RESULTS:

In our study total of 90 patients were included. Most of the patients were in the age group of 61-75yrs in honey group and 45-60yrs in debridement group with mean age of 49.8±19.0yrs and 53.4±17.5yrs in honey and debridement groups respectively with male predominance in both groups 82.2%. Most of the ulcers were chronic 60% in honey and 68.9% in debridement group. S.aureus was the common organism isolated in this study. Mean time for formation of healthy granulation tissue was 14.7±5.4 in honey group whereas 17.9±7.5 in debridement group which was significant (p=0.025). All patients were discharged after the definitive management without any complications, 40% patients in honey group and 57.8% patients in debridement group were discharged after 30days with mean hospital stay was 34.1±15.7days & 36.0±15.8days in honey and debridement groups respectively.

CONCLUSSION:

Honey dressing is more effective when compared to the mechanical debridement with povidone iodine dressing in achieving complete healing, reducing the hospital stay and increasing the comfort (i.e repeated debridement under local or spinal anesthesia, cost and pain) to the subjects with chronic wounds.

KEY WORDS: Honey, Biofilm, Granulation tissue, Split thickness skin graft, healing process.

LIST OF ABBREVIATIONS USED

SL.NO	-	SERIAL NUMBER
N	-	NAME
A	-	AGE
S	-	SEX
M	-	MALE
F	-	FEMALE
IP.NO	-	IN PATIENT NUMBER
DOA	-	DATE OF ADMISSION
DOS	-	DATE OF SURGERY
DOD	-	DATE OF DISCHARGE
STSG	-	SPLIT THICKNESS SKIN GRAFTING
SS	-	SECONDARY SUTURING
SEC INT	-	SECONDARY INTENTION
BKA	-	BELOW KNEE AMPUTATION
GROUP H	-	GROUP HONEY
GROUP D	-	GROUP DEBRIDEMENT

MRSA	-	METHICILLIN RESISTENT STAPHYLOCOCCUS AUREUS
EC	-	ESCHERICHIA COLI
EF	-	ENTEROCOCCUS FECALIS
KO	-	KLEBSIELLA OXYTOCA
KP	-	KLEBSIELLA PNEUMONIAE
SA	-	STAPHYLOCOCCUS AUREUS
YRS	-	YEARS

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INTRODUCTION

Chronic wounds are the significant health problems globally. Chronic wounds fail to progress through the expected healing process in a timely manner. Health care costs related to the management of chronic wounds still forms a major burden. The treatment and management of chronic wounds is challenging to the health care providers. Microbial Bioburden in wounds is one of the important factor responsible for the chronicity of wounds. The effective management of bacterial bioburden is an essential element of wound care. Wound infection results from complex interaction between an individual's immune system, condition of the wound and number and virulence of bacteria present. Bacteria can exist in at least two different phenotypic growth forms: the first being single, fast-growing cells i.e. the planktonic form; the second as aggregated communities of slow-growing cells in a biofilm form[1].

Management of biofilm in chronic wounds is rapidly becoming a primary objective of wound care. However management of biofilm is an undeniably complex task. Management of ulcer should include basic steps of initial prevention (use of anti-biofilm agent), removal (debridement, de-sloughing) and prevention of reformation (use of antimicrobial agents). In addition patient, environmental and clinical parameters must be considered.

Honey has been used to treat the wounds since many years as it contains antibacterial activity, osmotic effect, de-sloughing activity etc. in this antibiotic era, no studies have shown the development of resistance to honey. There are studies done

in vivo show the eradication of biofilm by the honey. Earlier studies conducted in our institution showed existence of biofilm in 60% of ulcers. Biofilm in these ulcers could be eradicated with the topical application of honey. Hence this study was taken up mainly to compare the efficacy of honey in eradicate the biofilm as a topical agent in wound healing in ulcers with biofilms with the conventional methods as these biofilms are the major factor in preventing the healing of wounds.

AIMS AND OBJECTIVES

- To detect biofilm in wounds
- To compare the efficacy of honey dressing versus mechanical debridement in healing of ulcers with biofilm.

REVIEW OF LITERATURE

Chronic wounds are the significant health problems globally. Chronic wounds failed to progress through the expected healing process in a timely manner. Health care costs related to the management of chronic wounds will forms a major burden. The treatment and management of chronic wounds is challenging to the health care providers. Traditionally basic wound care consists of surgical debridement, irrigation of wounds manually and regular dressings along with systemic or topical antibiotic therapy. The prevalence and incidence of chronic wounds and their associated complications continue to escalate in spite of tremendous progress in science of wound healing[1].

PROCESS OF WOUND HEALING[2]

It is a process by which the body repairs the damaged tissue which involves a series of stages in an organized way and follow four stages including haemostatic, inflammatory, proliferative and remodelling phases.

- HAEMOSTATIC PHASE:

During the immediate reaction of the tissue to injury haemostasis and inflammation occur. This phase represents an attempt to limit the damage by stopping the bleeding sealing the surface of the wound and removing any necrotic tissue, foreign debris or bacteria present. This phase is characterized by increased vascular permeability, migration of cells into the wound by chemotaxis, secretion of cytokines and growth factors into the wound and activation of migrating cells.

- INFLAMATORY PHASE:

During the acute tissue injury, blood vessel damage results in the initial intense vasoconstriction of arterioles and capillaries followed by vasodilatation and increased vascular permeability. Erythrocytes and platelets adhere to the damaged capillary endothelium, resulting in plugging of capillaries and leading cessation of haemorrhage. Platelet aggregation results in the triggering of intracellular signal transduction pathway and results in the release of biologically active proteins like platelet-derived growth factor (PDGF), transforming growth factor-beta, insulin-like growth factor type-1, fibronectin, fibrinogen etc. mast cells adherent to the endothelial surface release the histamines and serotonin, resulting in increased permeability of endothelial cells and causing leakage of plasma from the intravascular space to extracellular compartment.

Macrophage is the one cell that is truly crucial to wound healing in that it serves the release of cytokines and stimulate many of the subsequent process of wound healing. It appears at the same time that neutrophils disappear. They induce apoptosis of PMNs. Chemotaxis of migrating blood monocytes occur within 24 to 48 hrs. These macrophages secrete numerous cytokines and growth factors, a pro-inflammatory cytokine is an acute phase response cytokine.

T lymphocytes appear in significant number in the wound on 5^{th} day with peak occurring on 7^{th} day. Lymphocytes exert most of their effects on fibroblasts by producing stimulatory cytokines such as IL-2 and fibroblasts activating factor and inhibitory cytokines such as TGF-β, TNF-α and IFN-ϒ.

- PROLIFERATIVE PHASE:

As the acute response of haemostasis and inflammation begin to resolve, the scaffolding is laid for the repair of wound through angiogenesis, fibroplasia and epithelialization. This stage is characterized by the formation of granulation tissue which consists of a capillary bed, fibroblasts, macrophages and a loose arrangement of collagen, fibronectin and hyaluronic acid.

- Angiogenesis:-

Angiogenesis is the process of new blood vessel formation and is necessary to support a healing wound environment. After injury, activated endothelial cells degrade the basement membrane of post capillary venules, thereby allowing the migration of cells through this gap. Division of these migrating endothelial cells results in tubules or lumen formation. Eventually, deposition of the basement membrane occurs and results in capillary maturation. Angiogenesis is mediated by vascular cell surface adhesion molecule-1 (VCAM-1), fibroblast growth factor (FGF), PDGF, and TGF- β.

Angiogenesis appears to be stimulated and manipulated by a variety of cytokines predominantly produced by macrophages and platelets. VEGF, a member of PDGF family of growth factors, has potent angiogenic activity. It is produced in large amounts by keratinocytes, macrophages, endothelial cells, platelets and fibroblasts during wound healing. Cell disruption and hypoxia, hallmarks of tissue injury, appear to be strong initial inducer of potent angiogenic factors at the wound site.

- Fibroplasia:-

Fibroblasts are specialised cells that differentiate from resting mesenchymal cells in connective tissue; they do not arrive in the wound clot by diapedesis from circulating cells. After injury, the normally quiescent and sparse fibroblasts are chemo attracted to the inflammatory site, where they divide and produce the components of the ECM. After stimulation by macrophage and platelet derived cytokines and growth factors, fibroblast which is normally arrested in G0 phase, undergoes replication and proliferation.

The primary function of fibroblasts is to synthesis collagen, which they begin to produce during the cellular phase of inflammation. The time required for undifferentiated mesenchymal cells to differentiate into highly specialised fibroblasts accounts for the delay between injury and the appearance of collagen in a healing wound. This period, generally 3 to 5 days, depending on type of tissue injured, is called the lag phase of wound healing. Fibroblasts begin to migrate in response to chemotactic substances such as growth factors (PDGF, TGF-β), C5 fragments, thrombin, TNF-α, eicosanoids, elastin fragments, leukotriene B4, and fragments of collagen and fibronectin.

The rate of collagen synthesis declines after 4weeks and eventually balances the rate of collagen destruction by collagenase (MMP-1). At this point, the wound enters the phase of collagen maturation. The maturation phase continues for months or even years.

- EPITHELIALIZATION:-

Re-epithelialization of wounds begins within hours after injury. Initially, the wound is rapidly sealed by clot formation and then by epithelial (epidermal) cell migration across the defect. Keratinocytes located at the basal layer of the residual epidermis or in the depths of epithelium-lined dermal appendages migrate to resurface the wound. Epithelialization involves a sequence of changes in wound keratinocytes-detachment, migration, proliferation, differentiation, and stratification. If the basement membrane zone is intact, epithelialization proceeds more rapidly.

If the basement membrane zone is not intact, it will be repaired first. The absence of neighbouring cells at the wound margin may be a signal for the migration and proliferation of epidermal cells. Local release of EGF, TGF-α, and KGF and increased expression of their receptors may also stimulate these processes.

- Extracellular Matrix:-

The ECM exists as a scaffold to stabilise the physical structure of tissues, but it also plays an active and complex role by regulating the behaviour of the cells that contact it. The wound matrix accumulates and changes in composition as healing progresses, balanced between new deposition and degradation. The provisional matrix is scaffold for cellular migration and composed of fibrin, fibrinogen, and fibronectin. GAGs and proteoglycans are synthesised next and support further matrix deposition and remodelling. Collagens, which are predominant scar proteins, are the end result. Attachment proteins, such as fibrin and fibronectin, provide linkage to ECM through binding to cell surface integrin receptors. Stimulation of fibroblasts by growth factors induces upregulated expression of integrin receptors, thereby facilitating cell matrix interactions.

- Collagen structure:-

Collagens are found in all multicellular animals and are secreted by variety of cell types. They are a major component of skin and bone and constitute 25% of the total protein mass in mammals. There are at least 20 types of collagen, the main constituents of connective tissue being types 1, 2, 3, 5, and 11. Type-1 is the principal collagen of skin and bone and is the most common. In adults, the skin is approximately 80% type-1 and 20% type-3.

- Collagen synthesis:-

Vitamin c deficiency is characterised by the gradual loss of pre-existing normal collagen, which leads to fragile blood vessels and loose teeth. A numb of factors can affect collagen synthesis. Vitamin-C (ascorbic acid), TGF-β, INF-1, and INF-2 increase collagen synthesis. IFN-ϒ decreases type-1 procollagen m RNA synthesis and glucocorticoids inhibit procollagen gene transcription, thereby leading to decreased collagen synthesis.

- MATURATION PHASE:-

Wound contraction occurs by centripetal movement of the whole thickness of the surrounding skin and reduces the amount of disorganised scar. Wound contracture, in contrast, is physical constriction or limitation of function and is a result of the process of wound contracture. Wound contracture appears to take place as a result of a complex interaction of the extracellular materials and fibroblasts, which is not completely understood.

- FACTORS THAT INHIBIT WOUND HEALING:-

1. Infection
2. Ischaemia
    - Circulation
    - Respiration
    - Local tension
3. Uncontrolled Diabetes mellitus
4. Ionizing radiation
5. Advanced age
6. Malnutrition:- Hypoalbunemia and Hypoproteinaemia.
7. Vitamin deficiencies
    - Vitamin –C
    - Vitamin -A
8. Mineral deficiencies
    - Zinc
    - Iron
9. Exogenous drugs
    - Doxorubicin (Adriamycin)
    - Glucocorticosteroids.

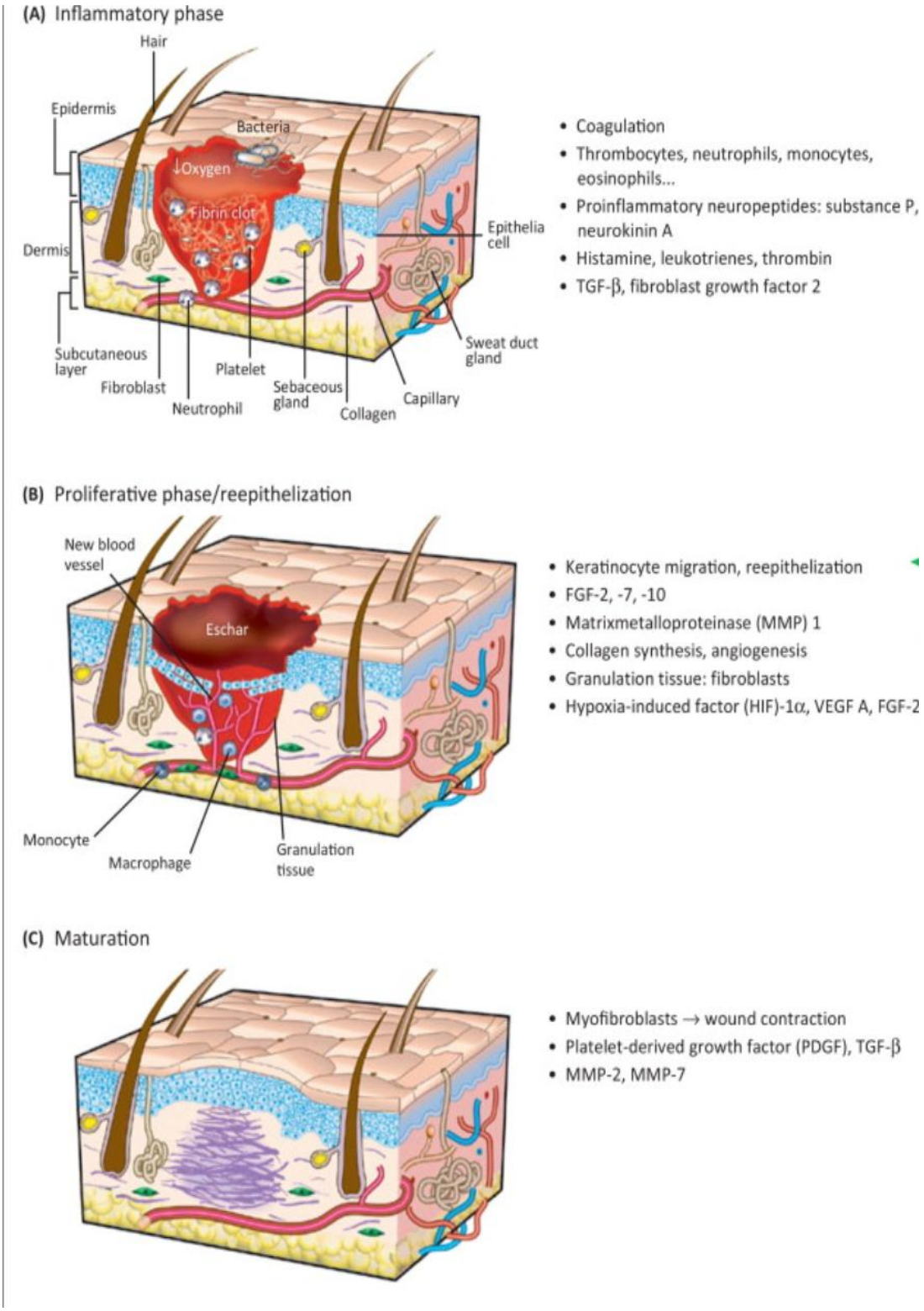


Figure 1: diagrammatic representation of stages of healing.

CHRONIC WOUND:

A chronic wound is a wound that is arrested in the inflammatory phase of wound healing and cannot progress further[2]. The presence of necrotic tissue, foreign material, and/or bacteria impedes the wound's ability to heal by producing or stimulating the production of pro-inflammatory cytokines, elevated matrix metalloproteases, and excessive neutrophils.

In this process, the building blocks (chemotactants, growth factors, mitogens, and so on) necessary for normal wound healing are either rendered inert or destroyed. This hostile environment also allows bacteria to proliferate and further colonize the wound by constructing protected colonies known as biofilm. Over 90% of chronic wounds contain bacteria living within biofilm construct. Chronic wounds cause a significant burden to healthcare systems as well as morbidity and mortality to mankind.

MICROBIAL BIOBURDEN:

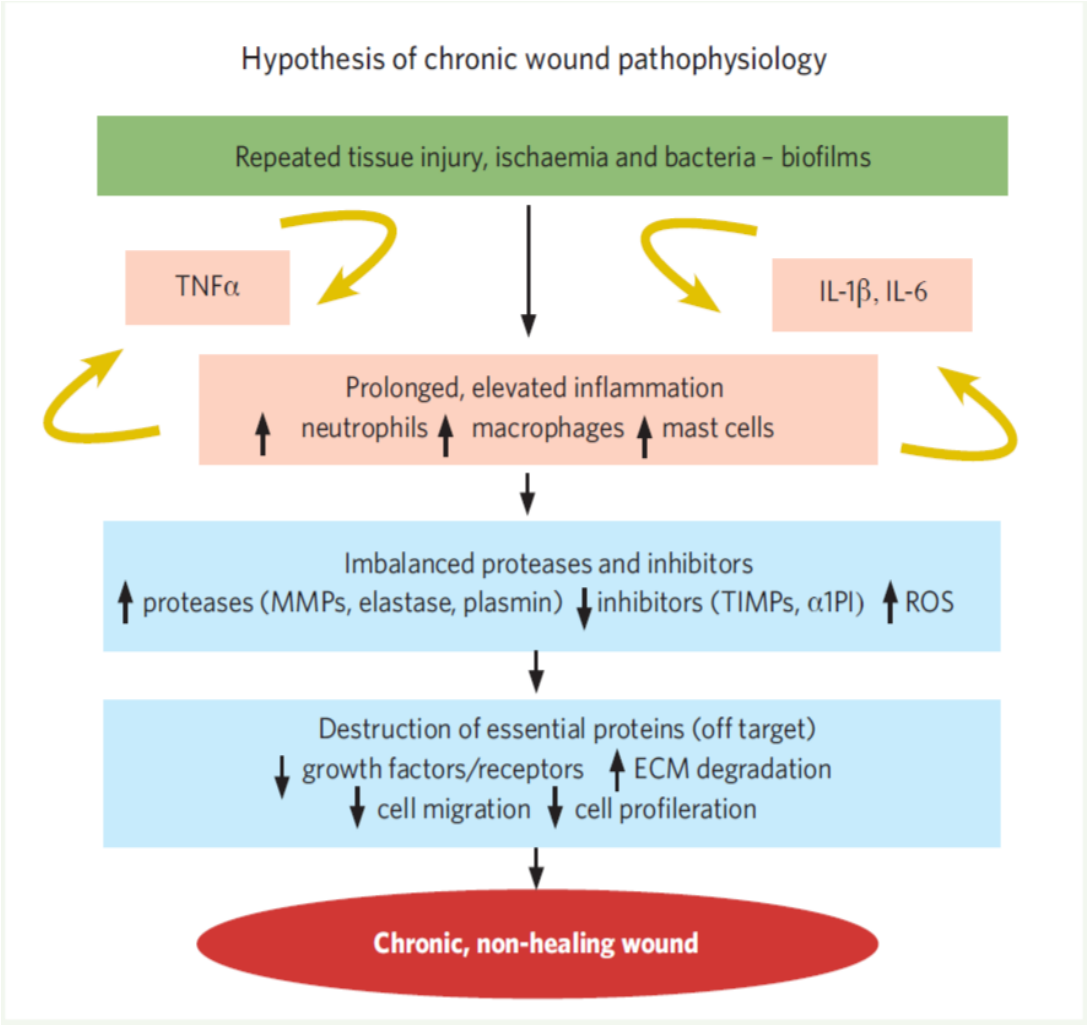
Microbial Bioburden in wounds are the important factors responsible for the chronicity of wounds. The effective management of bacterial bioburden is an essential element of wound care. Wound infection results from the complex interaction between an individual's immune system, the condition of the wound and the number and virulence of bacteria present. Although there are no clinical studies on the impact of specific microorganisms on the healing process, clinicians agree that infection causes serious delays in healing as a result of the expression of bacterial virulence factors[3].

These factors are believed to damage the wound bed in a variety of ways[3].

- Microorganisms consume nutrients and oxygen required for wound repair
- Protease virulence determinants (e.g. elastase) damage the extracellular matrix
- White cell function is impaired by the release of short chain fatty acids produced by anaerobes. Endotoxins stimulate production of interleukins: Tumor-necrosing factor and matrix metalloproteinases (MMPs).
- Free oxygen radical production increases and Imbalances occur between MMPs and tissue inhibitors of metalloproteinases (TIMPs).
- Fibroblast production is decreased or delayed, collagen disorganised and scar strength decreased.
- Additional consequences for the patient may include increased pain and discomfort, inconvenience, and life threatening illness.

  Adverse consequences for the healthcare system may be extended hospital stay, heightened risk of litigation and increased treatment costs incurred by extra antibiotic and dressing usage, as well as extra staff costs.

Hypothesis of chronic wound pathophysiology and biofilms[3]



Most chronic wounds have become chronic due to maltreatment, and they undoubtedly have substantial amounts of bacterial biofilm, but when many chronic wounds receive correct treatment such as compression in case of venous ulcer and/or off-loading in diabetic foot ulcers, they start to heal even without adding antibiotics or antiseptics. Bacterial biofilm play a pivotal role in the development and maintenance of chronic wounds. The detection and localisation of biofilms in chronic wounds provide useful clinical information, in particular in assessing and directing the effectiveness of debridement. Development of biofilms in acute wounds leads to

chronic inflammation characterised by elevated levels of pro-inflammatory cytokines that leads to increased numbers of neutrophils, macrophages and mast cells that secrete proteases and ROS that become chronically elevated and accidently (off-target) destroy proteins that are essential for healing, leading to a chronic, non-healing wound[4].

BIOFILM:

Biofilms were probably first recognised by Anthony Leeuwenhoek who noticed microbial attachment to his own tooth. Later on it was forgotten for nearly 2 centuries.

In 2000, several mechanisms were proposed to explain the phenomenon of resistance within biofilms, including delayed penetration of antimicrobial into the biofilm extracellular matrix, slowing of growth rate of organisms within the biofilm, or other physiological changes brought about by interaction of the organisms with a surface[5].

"Biofilms are complex microbial communities containing bacteria and fungi. The microorganisms synthesise and secrete a protective matrix that attaches the biofilm firmly to a living or non-living surface. Biofilms are dynamic heterogeneous communities that are continuously changing. They may consist of a single bacterial or fungal species, or more commonly, may be polymicrobial i.e contain multiple diverse species[6]".

Biofilms are also found in chronic wounds and are suspected to delay in the healing. "Electron microscopy of biopsies from chronic wounds found that 60% of the specimens contained biofilm structures in comparison with only 6% of biopsies from

acute wounds. Since biofilms are reported to be a major factor contributing to multiple chronic inflammatory diseases, it is likely that almost all chronic wounds have biofilm communities on at least part of the wound bed[7]".

In 2007, in a clinical study on chronic wounds specimens were obtained from 77 subjects, 50 chronic wound specimens were evaluated by microscopy. 30 were characterized as containing biofilm (60%) and 8 acute wound specimens had biofilm (6%) [8].

In 2007, a study on biofilm-based wound management in subjects with critical limb ischemia, it was observed that , Biofilm Based Wound Control strategies significantly improved healing frequency in comparison with previously published study. These findings demonstrate that effectively managing the biofilm in chronic wounds is important component of consistently transforming "non-healable" wounds into healable wounds[9].

Bacteria can exist in at least two different phenotypic growth forms, first being single, fast-growing cells i.e. the planktonic form; the second as aggregated communities of slow-growing cells in a biofilm form.

FORMATION OF BIOFILMS

Stage one: Reversible surface attachment:-

The initial attachment of these free floating planktonic microorganisms which are responsible for the formation of the biofilms is reversible. Under natural circumstances these free floating organisms tend to attach to the surfaces either living or dead and forms the biofilms[10, 11].

Stage two: Permanent surface attachment:-

To promote the survival of these microorganisms they multiply, differentiate and firmly attached to the surface and change the gene expression patterns. This is usually the result of a type of bacterial communication known as quorum sensing[12].

Stage three: Slimy protective matrix/biofilm:-

Once firmly attached, the bacteria start secreting a matrix known as extracellular polymeric substance (EPS). This is a protective matrix or 'slime'. Initially Small bacterial colonies form biofilm. The exact composition of EPS varies according to the microorganisms present, but generally consists of polysaccharides, proteins, glycolipids and bacterial DNA. Bacterial DNA released by living or dead bacteria is thought to provide an important structural component for biofilm EPS matrix. Various secreted proteins and enzymes help the biofilm to become firmly attached to the wound bed. Fully mature biofilms continuously shed planktonic bacteria, micro-colonies and fragments of biofilm, which can disperse and attach to other parts of the wound bed or to other wounds, forming new biofilm colonies[13].

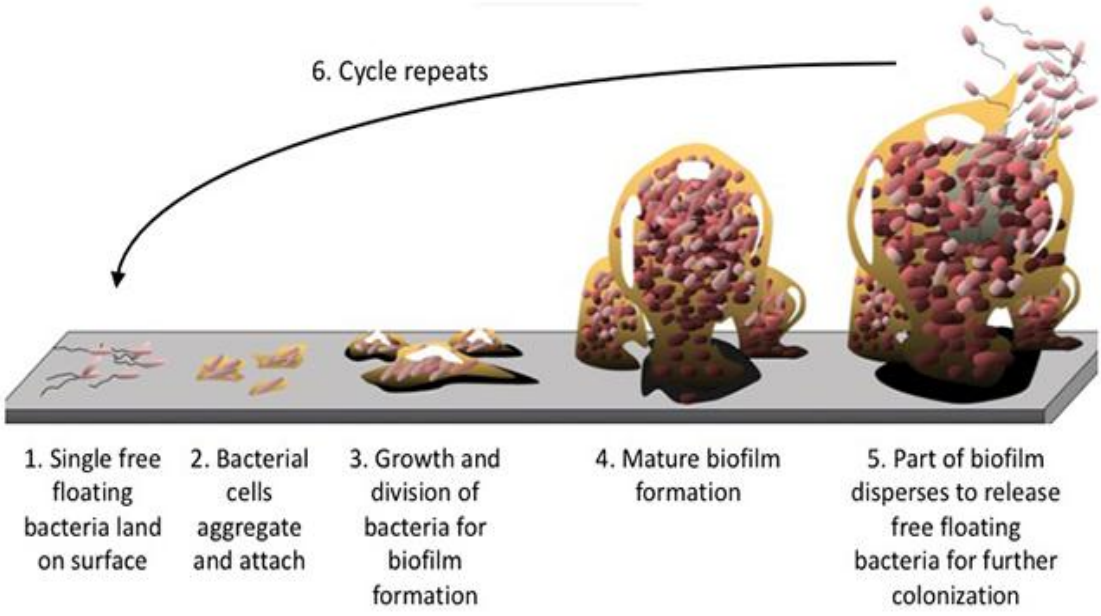


Figure 2: Schematic representation of polymicrobial biofilm formation

INHIBITION OF WOUND HEALING BY BIOFILM

The exact mechanisms by which biofilm impairs the healing processes of wounds remain ambiguous. Current data suggest the wound is kept in a vicious inflammatory state preventing normal wound healing cycles from occurring. The pathways behind this are not clear, but several systemic and local factors contribute to the occurrence and maintenance of a chronic wound.

At the systemic level, physiological factors include diabetes mellitus, venous insufficiency, malnutrition, malignancy, oedema, repetitive trauma to the tissue and impaired host response. The majority of chronic wounds will heal if the predisposing factors are treated properly; for example, oedema reduction in venous leg ulcers, off-loading in diabetic foot ulcers and pressure ulcers, along with moist wound healing principles.

At local level bacteria colonise all chronic wounds; the most commonly reported are Staphyloccocus aureus and Pseudomonas aeruginosa — two renowned biofilm formers. Study done by Ennis et al in 2000 stated that chronic wounds were 'stunned in the inflammatory phase of healing'. In normal wound healing trajectories this phase would be proceeded by a proliferative phase, where the function of PMN are gradually overtaken by macrophages, and fibroblasts begin to rebuild the tissue[14].

Study done by Gjodsbolk[15] et al in 2006 93.5% of chronic leg ulcers contained S. aureus and 52.2 % harboured P. aeruginosa, but only the ulcers with P. aeruginosa were characterised by larger wound sizes and slower healing rates. This could be explained by the ability of P. aeruginosa to eliminate polymorphonuclear leucocytes (PMN) by secreting rhamnolipid[16]. This glycolipid is controlled though the quorum sensing system and is probably one of the main mechanisms behind the lack of eradication of P. aeruginosa in chronic wounds.

The biofilms interfere with normal wound healing, apparently by 'locking' the wound bed into a chronic inflammatory state that leads to elevated levels of proteases (matrix metalloprotease and neutrophil elastase) and reactive oxygen species (ROS) that damage proteins and molecules that are essential for healing. A large percentage of bacteria in biofilm communities are metabolically dormant, which generates tolerance to antibiotics. Highly chemically reactive disinfectant molecules frequently react with the components of the biofilm Exo-polymeric matrix, depleting their concentration and impeding their penetration deep into the biofilm matrix.

In 2010 Kim et al stated that several proteins revealed through proteomic analysis had putative links to delayed wound healing. These included α-haemolysin, alcohol dehydrogenase, fructose-bisphosphate aldolase, lactate dehydrogenase and

epidermal cell differentiation inhibitor. While acute infections tend to produce the classic signs and symptoms of wound infection, such as inflammation, pain, heat, redness and swelling, microbes growing as biofilm produce a distinctly different pattern, often recognised as chronic infection[17].

In 2015, Marano et al identified that migration and proliferation of human epidermal keratinocytes were decreased by derivatives from biofilms of P. aeruginosa and S. aureus. Employing proteomic analysis allowed Marano et al to map S. aureus activity to a protein, while P. aeruginosa activity was more likely due to a small molecule[18].

The members of the biofilm community possess different genotypic and phenotypic traits, resulting in a structure that is heterogenous, dynamic and recalcitrant to antimicrobials and the immune response. Antibiotics fail to eradicate biofilms due to poor penetration, metabolic inhibition, protected quiescent bacteria (persisters) and other mechanisms. In vitro investigations have shown that bacteria in mixed-species biofilm communities can act synergistically in ways not observed in planktonic bacteria[19, 20].

Incorporating biofilms into the model for microbial infection and wound chronicity may better explain the biochemistry and cellular biology of the chronic wound environment. For example, chronically elevated pro-inflammatory cytokines (tumour necrosis factor-alpha, interleukin-1, alpha and gamma interferons), increased matrix metalo proteases levels (MMP-2, 8 and 9) and increased elastase can be explained by the possible effects of a biofilm on the host's innate immune system. Biofilms may also influence fibroblast senescence, keratinocyte impairment and the failure of endothelial cells to initiate angiogenesis[21].

Significant alterations occur during biofilm maturation. For example, during the development of a monoculture biofilm, more than 50% of the protein expressed by the bacteria can differ several-fold, depending on the biofilm's stage of development[22]. This enhanced expression of proteins is thought to aid biofilm resistance to antimicrobials and the host's immune response. The biofilm's strengths are found in its heterogenicity (different protein expression), interspecies cooperation and intercellular matrix structure[23, 24].

The most metabolically active cells in the biofilm are located near the non-attached surface where they grow, reproduce, slough and behave similarly to planktonic cells. These metabolically active cells are the most vulnerable to the effects of antibiotics, antiseptics and host defences. Bacteria that are more deeply embedded in the biofilm's extracellular matrix are sheltered from external perturbations, less metabolically active and more resistant to an array of antimicrobial therapies[25, 26].

Systemic treatment strategies are required for infected chronic wounds, whereas in non-infected wounds where the presence of biofilm is impeding healing, strategies can be adopted to break up the biofilm. Alternately, attempts can be made to prevent initial biofilm formation in patients or wounds judged to be at high risk[27].

In 2014, a study conducted on biofilms in vitro wound biofilm model has been developed, in which "biofilms of clinically relevant bacteria (P. aeruginosa and S. aureus) are established on sterile cellulose discs, incubated in Simulated Wound Fluid (SWF) and allowed to mature. Biofilms were then transferred to fresh SWF and challenged with a test dressing (in this instance a Silver Non-adherent Alginate dressing [SNA]*) or a control dressing† for a defined contact exposure time. Biofilms

are also visualized by Scanning Electron Microscopy (SEM). SEM of modelled wound biofilms revealed micro colonies and putative biofilm Exo polysaccharide, indicative of biofilm formation. SEM of modelled wound biofilms revealed complex bacterial biofilm microstructures indicating that stable, mature biofilms were generated within 24 hours. Both antimicrobial dressings showed anti-biofilm activity against Pseudomonas in this in vitro model; however, the efficacy of both dressings was affected by the composition of media used in the assay. Performing the assay in a complex nutritional environment rendered the honey-containing dressing inactive against 72 hours old Pseudomonas biofilms. The silver containing dressing also showed a decrease in activity under these more clinically relevant conditions, particularly when the level of serum was increased; however, it still led to a reduction in biofilm total count within 24 hours. These results are interesting and should be considered when evaluating data generated using in vitro biofilm models[28]".

Detection and localisation of biofilms in chronic wounds provide useful clinical information that helps in assessing the healing of wounds and strategies required to eradicate the biofilm from wounds. Identification of biofilm in clinical practice is also difficult, with few guidelines available to facilitate its recognition.

Keast[29] et al 2014 propose four main features that may increase suspicion of the biofilm presence, as follows:

1. Antibiotic failure

2. Infection of >30 days' duration

3. Friable granulation tissue

4. A gelatinous material easily removed from wound surface that quickly rebuilds.

CLINICAL INDICATORS OF BIOFILM IN CHRONIC WOUNDS[3]	
Excessive moisture/ exudate	Excessive moisture encourages biofilm development
Poor quality granulation tissue	High burden may present as friable granulation tissue
Signs and symptoms of local infection	Secondary signs of infection are more typical of biofilm infection
Antibiotic failure or recurring infection following antibiotic cessation	Antibiotic failure is the hallmark of biofilm infection. The use of antibiotics is still controversial regarding biofilm management. It has been suggested that- without the use of concurrent strategies for biofilm management- efficacy may be as low as 25-30%
Negative wound culture	Routine cultures will only pick up the free floating bacteria not those within the biofilm.
Non-healing in spite of optimal wound management and host support	Biofilm defences include resistance to UV light, biocides, antibiotics and host defences. Biofilm can quickly reconstitute but strategically does not kill its host.
Infection lasting >30days	Infection of <30days duration may also contains biofilm, planktonic infection would not persists >30days.
Responds to steroids	Inflammation is the by-product of biofilm, thus a good response to these treatments suggests presence of biofilm. Decreasing inflammation removes the primary source of nutrition.
Gelatinous material easily removed from the wound surface	Research suggests that biofilm can reform within 24-72 hours.

Targeted therapies could be used to improve healing in cases where microbial biofilm is a causal component of chronic wounds as opposed to non-pathogenic colonisation[30];

1. Early use of systemic antibiotics directed at planktonic bacteria

2. Unique strategies to make microbes more susceptible to antimicrobials for clearance by the host immune system

3. Therapies directed at preventing a prolonged inflammatory component of wound healing.

Cooper[31] et al in 2014 developed novel strategies to prevent and treat biofilm in chronic wounds which confer:

1. Preventative action, interfering with either microbial attachment or processes involved in biofilm maturation or removal, and/or disruption of mature biofilm.
2. Action against existing biofilm, removing or disruption of the biofilm and prevention of reformation.

STRATEGIES FOR PREVENTION AND TREATMENT OF BIOFILM:-

Once the likelihood of biofilm presence is established, an appropriate treatment strategy should be determined, taking into account that there are several stages of biofilm formation. A proactive approach to treatment recognises that there is no one-step solution for treatment of biofilm, but aims to reduce burden and prevent its reconstitution[13].

Wolcott[32] in 2015 states that: ‘Biofilm-based wound care is predicated on using multiple different treatment strategies simultaneously including antibiotics, anti-

biofilm agents, selective antimicrobials and frequent debridement.' Moreover, Hurlow[33] et al 2015caution that while focused activity against the biofilm is paramount, maximising the host response must also be addressed with attention paid to all local and underlying causes of delayed wound healing.

Potential anti-biofilm agents[3]

In practice, physical biofilm disruption in the form of debridement and/or cleansing, followed by use of antimicrobial agents (such as PHMB or silver) to prevent its reformation, is the primary anti-biofilm option available to clinicians at present[34].

However, various potential anti-biofilm agents that interfere with elements of their formation or support and enhance the effect of antimicrobials have been investigated; these are summarised in the following table, categorised by their modes of action. Where such an agent is chosen, this choice should be based on factors including the biocidal capability and length of activity of the active agent, and the capability of the carrier dressing to manage presenting symptoms, such as increased levels of exudate.

Potential anti-biofilm agents[3]		
Mode of action	Examples	Further details
Interference with biofilm surface attachment	Lactoferrin EDTA Xylitol Honey	As part of innate human response mechanism, lactoferrin binds to cell walls causing destabilisation, leakiness and ultimately, cell death[35]. EDTA has been used as a permeating and sensitising agent for biofilm conditions in dentistry and other fields[36]. Xylitol and Honey have also been shown to block the attachment
Interference with quorum sensing, a mechanism of chemical signalling or communication between the cells within biofilm	Farnesol Lberin Aioene Manuka honey	Several agents block or interfere with quorum sensing including these agents. Manuka honey has also been shown to down regulate 3 of the 4 genes responsible for the quorum sensing process[35].
Disruption of extra-cellular polymeric substance(EPS), a protective matrix secreted by and surrounding the biofilm	EDTA	EDTA supports and enhances topical antimicrobials by disrupting the EPS in which microorganisms are encased[36, 37].
False metabolites	Gallium, Xylitol	Low doses of gallium and xylitol have been shown to interfere with biofilm formation[38].
Disruption of existing biofilm	Betain	Current solution favoured in the disruption of biofilm contain surfactants such as betaine, which lower the surface tension of the medium in which they dissolved, allowing dirt and debris to be lifted and suspended in the solution[39, 40].

Preparation of the wound bed, including cleansing and debridement, are important principles of wound management, since wounds must be clean to heal[41]. The concept of TIME (Tissue, Infection/Inflammation, Moisture, edge of wound) is a widely accepted standard of wound management. In the intervening 10 years there have been important developments including, understanding of biofilm presence (and the need for a simple diagnostic), the importance of clinical recognition of infection,

and the value in repetitive and maintenance debridement and cleansing of wounds, which is paramount[42].

Where either slough or necrosis is present in a wound, this non-viable tissue should be removed as it may support the attachment and development of biofilm. The speed of tissue removal should be conducted according to the patient's ability to undergo the procedure, the skill and competence of the practitioner, and the safety of the environment in which the technique is to be performed. A distinction has recently been drawn between removal of slough ('desloughing') and that of necrotic tissue (debridement). In order to ensure effectiveness, it is proposed that neither therapy be conducted as a one-off, with both maintenance debridement and desloughing recommended[43, 44].

Various debridement techniques are available, from surgical (performed in theatre, back to healthy bleeding tissue), and autolytic (use of dressings to facilitate removal of necrotic tissue) through to debridement pads and cloths. The current cleansing solutions favoured to assist in the disruption of biofilm contain surfactants, which lower the surface tension of the medium in which they are dissolved, making it easier to lift off dirt/debris and suspend this in solution, to avoid re-contamination of the wound. Solutions may be added directly to the wound, used as soaks on gauze or used as part of an instillation alongside negative pressure wound therapy[45, 46].

Combination of polyhexanide and betaine, a surfactant, has been identified as effective for autolytic wound debridement. In a randomised controlled trial conducted by Bellingeri[41] et al (June 2010 — December 2013), stated that the solution was found to promote wound bed preparation, reduce inflammatory signs, and accelerate healing of vascular leg ulcers, as well as having a lasting barrier effect. Indeed,

compared with normal saline, the solution was statistically significantly superior ($p<0.001$) in terms of both wound improvement and reduction in inflammatory signs.

Once the wound has been appropriately cleansed and as much non-viable tissue removed as is comfortable for the patient, it is suggested that an antimicrobial product be used to prevent reformation of the biofilm[29].

A number of active antimicrobial agents have been linked to biofilm treatment[3].

- Acetic acid
- Honey[31, 47]
- Iodine
- PHMB
- Silver.

Importantly, these must be used following physical disruption of the biofilm by cleansing and debridement, in order to ensure antimicrobial efficacy.

Honey has been used to treat acute and chronic wound infections since 2500 BC. Honey possesses a number of antimicrobial properties including high sugar content, low pH, and the generation of hydrogen peroxide by the bee-derived enzyme glucose oxidase.

HONEY

"Honey is the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining

with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature[48]".

HONEY COMPOSITION:

Honey is one of the most complex natural foods available today and contains a wide variety of nutrients. Although darker honeys tend to provide higher amounts of minerals than lighter varieties, 100 grams (about 5 tablespoons) of honey contain approximately 304 calories and average 17.1 grams of water, with a range of 12.2 to 22.9 grams[49].

Honey in average contains 82.4 % carbohydrates, based on 100 grams, that includes 38.5 grams of fructose, 31 grams of glucose, 7.2 grams of maltose, and just over 1 gram of sucrose. Fructose and glucose are monosaccharides, or simple sugars, while sucrose is a disaccharide with fructose and glucose linked together. Honey also contains more complex carbohydrates known as oligosaccharides, which are medium-size carbohydrates containing more than three simple sugar subunits, often made up of mono- and disaccharides. These sugars are formed when nectar and honeydew are converted to honey. Oligosaccharides are sometimes referred to as higher sugars, they encourage the growth of "friendly bacteria" in the intestinal tract, which has been found to contribute to good health.

Even though honey contains an average of only 0.5 % protein, amino acids, vitamins, and minerals, it has more nutrients than refined sugars like table sugar and corn syrup. In addition, the body easily assimilates these trace amounts. Vitamins include thiamin, riboflavin, niacin, pantothenic acid, pyridoxine, and ascorbic acid (vitamin C) and minerals include calcium, copper, iron, magnesium, manganese, phosphorus, potassium, chromium, selenium, and zinc[50].

ROLE OF HONEY IN WOUND MANAGEMENT:

Honey has four main properties that helps in eradicating the microorganisms on the wounds

- Osmosis
- High acidity
- Hydrogen peroxide activity
- Variety of phytochemicals that are derived both from the honey bees and the plants they visit.

OSMOSIS EFFECT:

Honey has hyperosmotic property, draws the fluid away from the infected wound. This helps to kill the bacteria because these organisms requires liquid to grow.

Honey works differently from antibiotics, which attack the bacteria's cell wall or inhibit intracellular metabolic pathways. Honey is hygroscopic, meaning it draws moisture out of the environment and thus dehydrates bacteria. This happens because honey is supersaturated solution of sugars. (With 84% of the honey being a mixture of fructose and glucose). The water content in honey is usually only 15–21% by weight. The strong interaction of these sugar molecules with water molecules leaves very few of the water molecules available to support the survival and growth of microorganisms[51,52].

LOW PH (HIGH ACIDITY):-

Honey is very acidic. Its pH is between 3 and 4, which is roughly the same pH as orange or grapefruit juice. Most types of bacteria survive or requires the pH levels between 7.2 and 7.4 and cannot survive at levels below pH 4.0. But if the honey is

diluted (for example, by the release of body fluids from a wound), it may become less acidic, allowing bacteria to grow again.

The osmotic power of honey draws out fluid from the plasma or lymph in the tissues that underlie the wound. This activates the enzyme glucose oxidase, which enables the honey to produce hydrogen peroxide, an important factor in inhibiting bacterial growth and promoting immune activity.

HYDROGEN PEROXIDE ACTIVITY (H_20_2):-

Hydrogen peroxide is another reason why honey can heal. Hydrogen peroxide is made naturally in honey by an enzyme called glucose oxidase, which is added to the plant nectar by the bee. Glucose oxidase is secreted from the hypo-pharyngeal gland of the bee into the nectar to help formulate honey from the nectar.

Full-strength honey has a negligible level of hydrogen peroxide, because this substance is short-lived in the presence of the transition metal ions and ascorbic acid in honey, which cause the hydrogen peroxide to decompose to oxygen and water. In full strength honey, the activity of glucose oxidase is very less and contains hydrogen peroxidase activity when it is diluted which helps in the wound healing.

When it comes to clearing infections, honey supplies low levels of hydrogen peroxide to wounds continuously over time as opposed to a large amount at the moment of treatment. In essence, it becomes a powerful yet effective “slow-release” antiseptic at a level that is antibacterial but does not damage tissue. The mild acidity and low-level hydrogen peroxide release assists both tissue repair and contributes to the antibacterial activity of honey. The antibacterial activity is a major factor in promoting wound healing where infection is present[53].

Dilution with water has not been found to seriously inhibit hydrogen peroxide production in honey, an important factor to consider when treating exuding wounds. “Significant antibacterial activity can be maintained easily when using honey as a wound dressing, even on a heavily exuding wound[54]”.

PHYTOCHEMICALS:

Honey also contains phytochemical factors, which are chemical compounds (such as a carotenoid or phytosterol) that occur naturally in plants. They are found in the nectar that the bees collect. Not only does each plant species supply specific phytochemicals, but the chemical activity can also vary from plant to plant.

In the past few years various researchers have identified an astonishing number of chemicals with antibacterial and antioxidant activity in honey, such as peptides, organic acids, vitamins, and enzymes. One of the most important of these cytokines is tumour necrosis factor, a protein that reduces tissue inflammation, induces the destruction of some tumour cells, and activates white blood cells, which is vital to healing[55].

HONEY ON WOUNDS:

In addition to its direct action on bacteria and other pathogens, honey also promotes healing through its effect on the immune system. Honey at concentrations as low as 1 % has been found to stimulate the proliferation of peripheral blood B-lymphocytes and T-lymphocytes (two kinds of white blood cells that helps defend the body from disease) in cell culture and active phagocytes (cells that ingest bacteria) from blood. These cells activate the body’s natural immune system to fight infection. And at even a 1% concentration, honey can stimulate monocytes in cell culture and

release cytokines including tumour necrosis factor-1, interleukin-1, and interleukin-6[56].

HONEY AND WOUND DEBRIDMENT[56]:

Like other moist wound dressings, honey facilitates the debridement or the removal of dead tissue and foreign matter from the wound bed. It accomplishes through the process of autolysis or by phagocytosis by the digestion of cells by different types of enzymes. Honey produces strong osmotic activity by drawing out lymph fluid from wound tissues. This helps to kill bacteria, because bacteria need liquid to grow.

Honey serves as a protective barrier to the cross infection of wounds. This osmotic action provides a constant supply of proteases (as proteolytic enzymes) that aids in wound debridement also has the ability to wash the surface of the wound bed from below, which aids in the removal of dirt and grit.

"It also helps separate dead tissue from the wound and allows granulation the formation of small connective tissue projections as part of the healing process to occur. In addition, osmosis prevents the new tissue from becoming too soft due to an accumulation of moisture. The osmotic action of honey also removes any risk of skin surrounding a wound becoming macerated [softened] by the moisture accumulating under a dressing. Even when diluted, honey will induce a withdrawal of moisture rather than a hydration of skin. When honey is placed on a wound, osmosis creates a layer of fluid beneath the bandage made up of the honey diluted in plasma or lymph. This makes it impossible for the bandage to adhere to the wound. When the dressing is removed, new-growth tissue isn't torn away[56]".

ANTI INFLAMATORY ACTION:

"Although inflammation is the normal response to injury, prolonged or excessive inflammation can inhibit the healing process. In addition to reducing patient discomfort, reducing inflammation helps reduce the size of the blood vessels within the wound and reduces oedema and exudate, the fluid that exudes from an infected wound. Pressure in tissues caused by oedema can restrict the flow of blood through the capillaries, starving the tissues of oxygen and nutrients that are needed to heal. Both laboratory and clinical studies have proved honey's ability to reduce wound inflammation, especially in skin ulcers and both deep and surface burns. The antioxidant content of honey, which helps get rid of free radical activity, is seen as a factor in this healing effect[57]".

RE-EPITHELIZATION:

"Honey has been proved clinically to stimulate tissue growth in a variety of wounds, including skin ulcers and burns. Honey also can stimulate collagen synthesis, which involves the formation of connective tissue, cartilage, and bone. Honey has also been found to stimulate angiogenesis—the development of new blood vessels—in wounds. This increases oxygen supply to the wound and supplies essential nutrients to the fibroblasts involved in collagen formation. Honey promotes the formation of clean, healthy granulation tissue. This is accomplished in part by supplying glucose to the epithelial cells. These skin-producing cells must build up an internal store of carbohydrate, which provides the energy the cells need to migrate across the surface of a wound. This migration eventually leads to the formation of new skin, known clinically as re-epithelialization"[58].

In 2007, a study was conducted to compare the effect of honey dressing vs an Ethoxy-diaminoacridine plus Nitrofurazone dressing in patients with pressure ulcers. After 5 weeks of treatment , patients who were treated by honey dressing had significantly better PUSH tool (Pressure Ulcer Score for Healing) scores than subjects treated with the ethoxy-diaminoacridine plus nitrofurazone dressing (6.55±2.14 vs 12.62±2.15, P<0..001). By week 5, PUSH tool scores showed that healing among subjects using honey dressing was approximately 4 times the rate of healing in the comparison group. The use of honey is effective and practical[59].

In 2008, a study compared "clean, non-sterile pure honey as a wound dressing to Povidone Iodine (10%) solution as the control, in patients admitted for surgery with Wagner grade-II diabetic foot ulcers. Mean days for healing was recorded and compiled from assessment of wounds by a surgeon blinded to the dressing material. With the use of the honey dressing mean days for healing was 14.4 days with a range of 7-26. The control group took 15.4 days to heal & range was 9-36 days. The p-value was less than 0.005. With a difference in mean days of approximately 1, the p-value makes this data statistically significant and the small difference in days was not due to chance alone but rather to the use of honey as a dressing which allowed for similar healing time between the honey group and control group. While the difference in healing time between the two groups is not drastically different this data is statistically significant and portrays the equality of the two products in healing diabetic foot ulcers[60]".

In 2009, a study conducted on diabetic wounds observed excellent results in treating diabetic wounds with dressing soaked with natural honey. "The disability of diabetic foot patients was minimized by decreasing the rate of leg or foot amputations and thus enhancing the quality and productivity of individual life[61]".

In 2012, surgeons at AIIMS published a paper in the Indian Journal of Surgery, which showed that using honey (procured from beehives on neem trees) healed wounds better and faster than povidone-iodine (betadine), standard ointment and there was significant decrease in the surface area of the wound and pain in the group, where honey was used as wound dressing[62].

In 2014 Jing Lu[63] et al conducted study on biofilm with Manuka honey in vivo stated that honey was able to penetrate through the biofilm matrix and kill the embedded cells in some cases. As has been reported for antibiotics, sub-inhibitory concentrations of honey improved biofilm formation by some S. aureus strains, however, biofilm cell suspensions recovered after honey treatment did not develop resistance towards Manuka type honeys. New Zealand Manuka type honeys, at the concentrations they can be applied in wound dressings are highly active in both preventing S. aureus biofilm formation and in their eradication, and do not result in bacteria becoming resistant[63].

Study conducted by Rose cooper[35] et al in 2011 showed Inhibition of established biofilms of staphylococci and enterococci by Manuka honey was influenced by contact time and concentration. Relatively low concentrations of Manuka honey prevented biofilm formation in MSSA, MRSA and VRE. Planktonic bacteria are more susceptible to Manuka honey than biofilms, but the susceptibility of biofilms of MSSA, MRSA and VRE differed to that of planktonic cultures by a factor of less than ten. Biofilms of MSSA, MRSA and VRE can be prevented and inhibited in vitro with concentrations of Manuka honey that could be used in clinical practice. The efficacy of Manuka honey in inhibiting biofilms in vivo must be tested[35].

MATERIALS AND METHODS

- **SOURCE OF DATA:** This study was carried out in the Department of General Surgery, B.L.D.E.U's Shri B.M Patil Medical College, Hospital and Research centre, Vijayapur.
- **STUDY PERIOD**: One and half years, from October 2015 to june2017.
- **STUDY DESIGN**: Prospective, comparative study of effectiveness of honey dressing versus mechanical debridement in ulcers with biofilm.
- **STUDY SAMPLE**: Total of 90 with 45 in each group.
- **APPROVAL**: Study was approved by the institutional medical ethics committee and written informed consent was obtained from all patients participating in the study.
- **STUDY POPULATION:** Patients came with ulcers during the study period were initially subjected for detection of biofilm in wounds and those who were positive for biofilm are included in the study.
- **INCLUSION CRITERIA:**
    - All the patients with ulcers having the biofilm.
- **EXCLUSION CRITERIA:** patients with ulcers who are
    - Immune compromised
    - HIV positive individuals on ART medication.
    - History of chemotherapy within last 6 months.
    - Radiotherapy to local area of ulcer.

METHOD OF COLLECTION OF DATA

- All eligible patients admitted in the Department of General Surgery in Shri B.M Patil medical college with ulcer during the study period from October 2015 to June 2017 were initially evaluated for the presence of biofilm in ulcers by taking swab cultures from the ulcer. Detection of biofilm will be done by Tube adherence test and Congo red agar test.
- Once the biofilm is detected thorough clinical examination of the ulcer will be done. The study subjects will be randomly divided into two groups, Honey (H) group and Debridement (D) group.
- The honey group was treated with topical application of dressing which were soaked with honey. Dabur honey of 10-30 ml was taken on a sterile gauze piece and diluted with normal saline in ratio of 1:2 and was spread over ulcer bed thoroughly and the ulcer was covered using sterile pads and roller gauze. Consecutive day's regular dressing with honey was done.
- Control group was treated with mechanical debridement and dressed with 10% Povidone Iodine.
- Once in 5days wound assessment was done regarding a) Discharge, b) Foul smell, c) Granulation tissue and d) Size of the ulcer in both the groups.
- The same protocol was followed for consecutive days, ulcer assessment was done using same parameters and culture swab was taken and sent for biofilm detection.
- Ulcers which were free from biofilm or pus culture sensitivity was sterile were taken up for definitive management in both groups.
- Statistical analysis was done by using Fisher exact test and Chi square test and p value <0.05 was considered significant.

CASE 1:

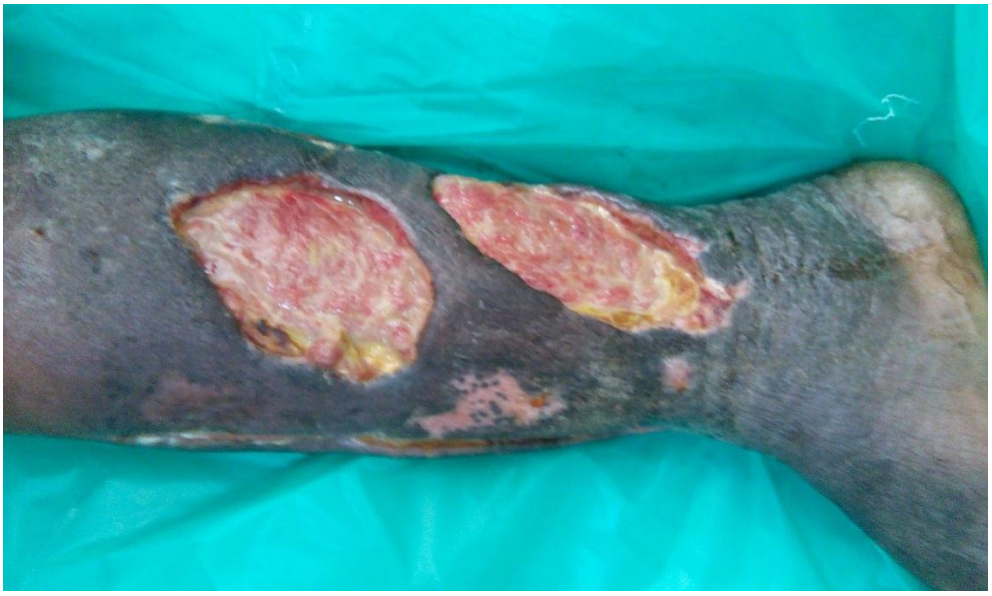


FIGURE 3: chronic non healing ulcer over the right leg with lymphedema.

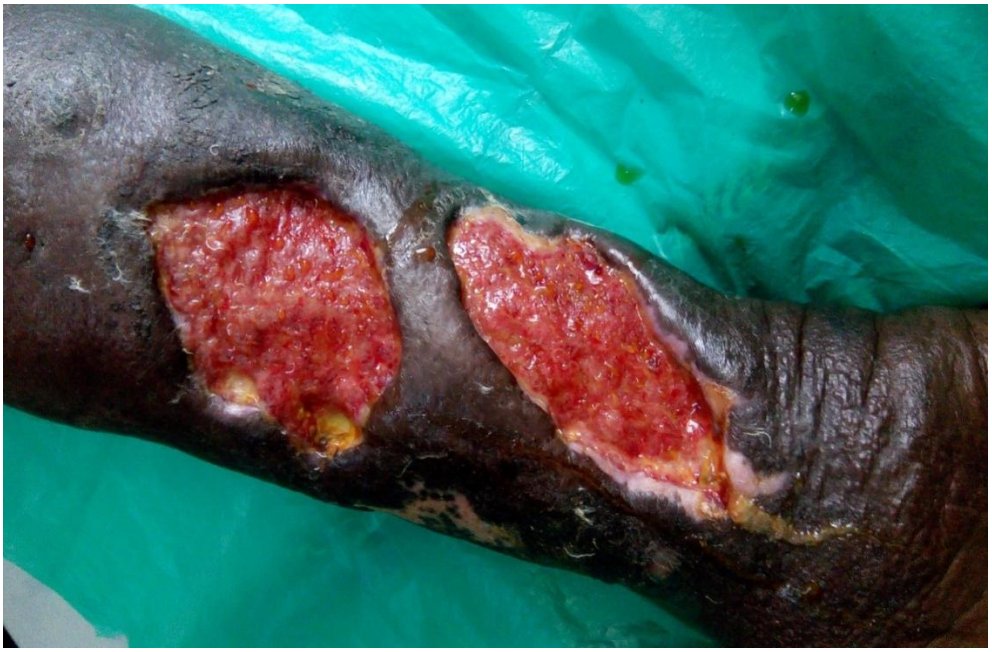


FIGURE 4: 10days after the application of Honey.

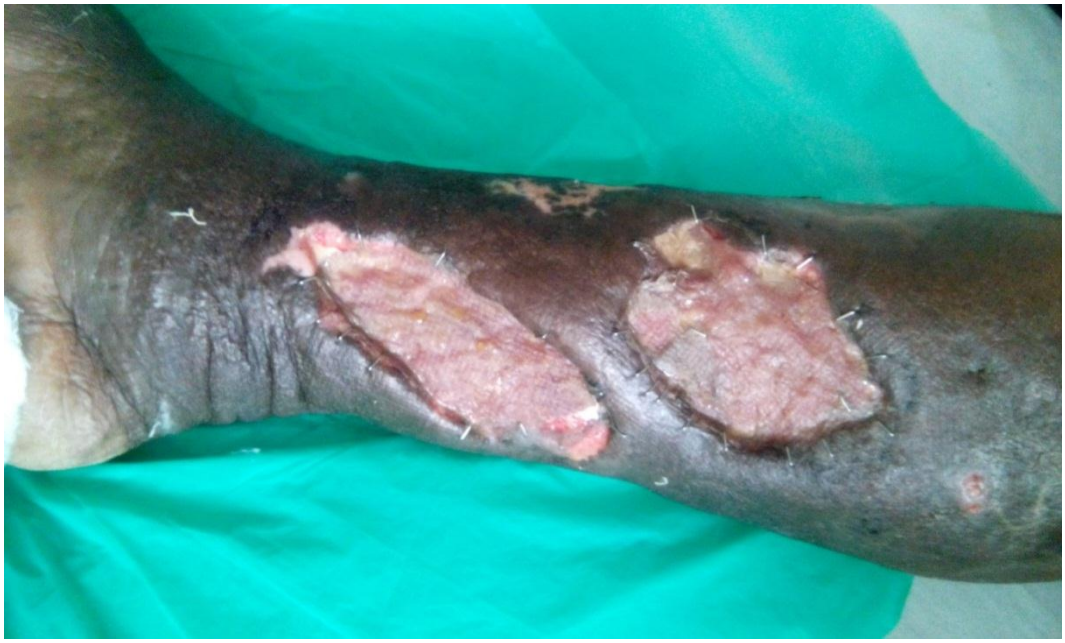


Figure 5: Post STGS Day-3 in Honey group.

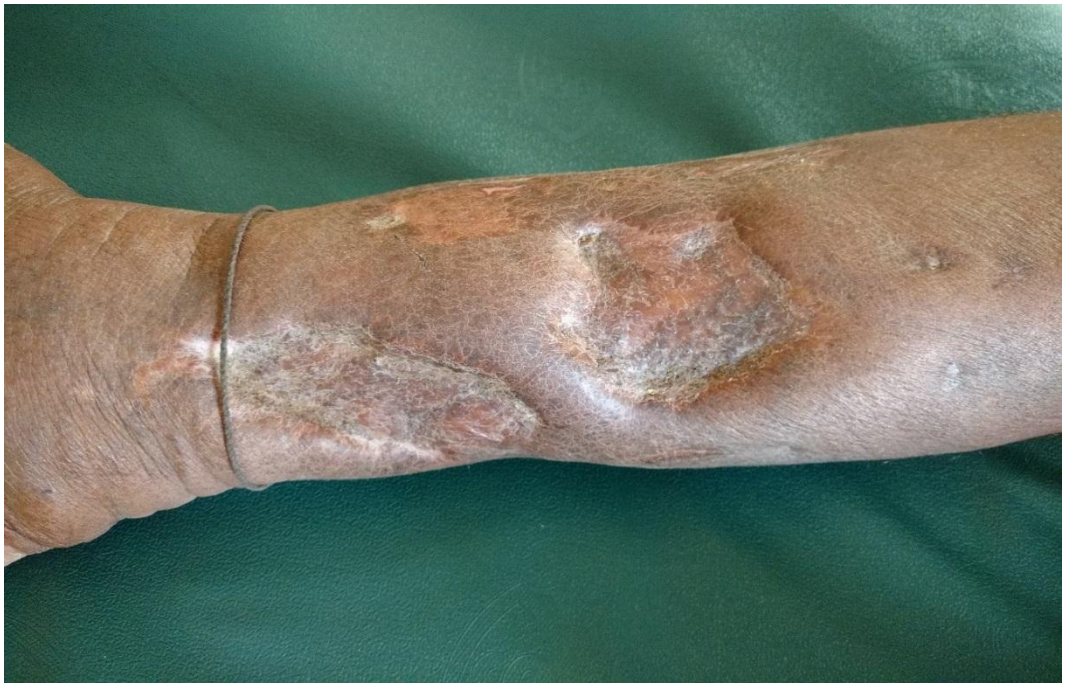


FIGURE 6: After 1month

CASE 2:

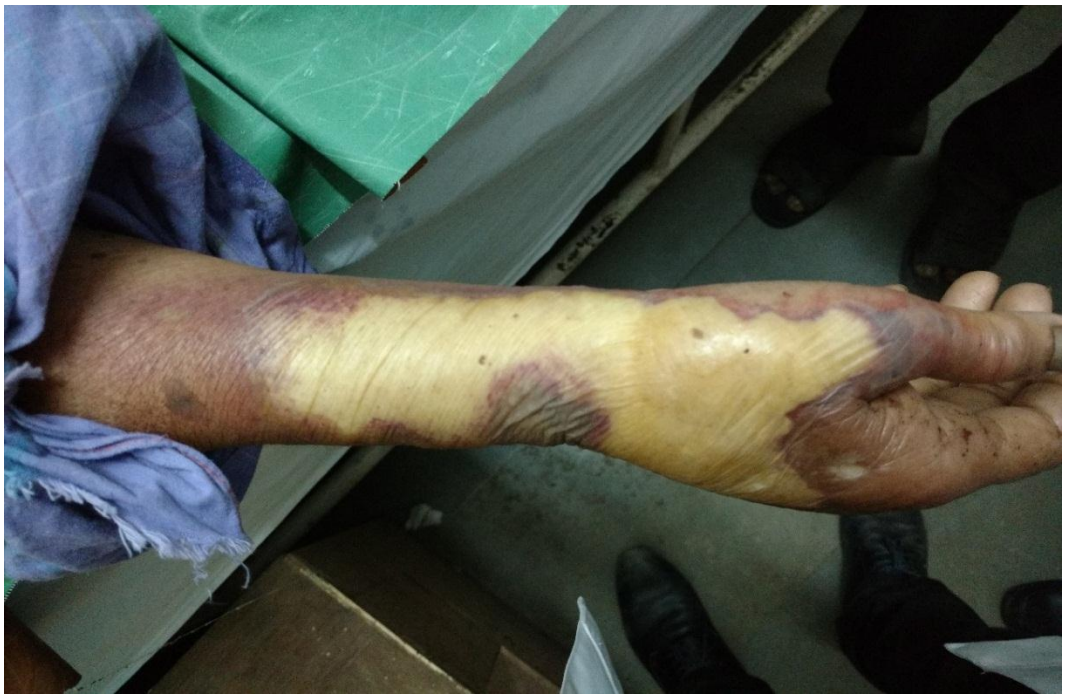


FIGURE 7: NECROTIZING FASCITIS OF RIGHT FOREARM.

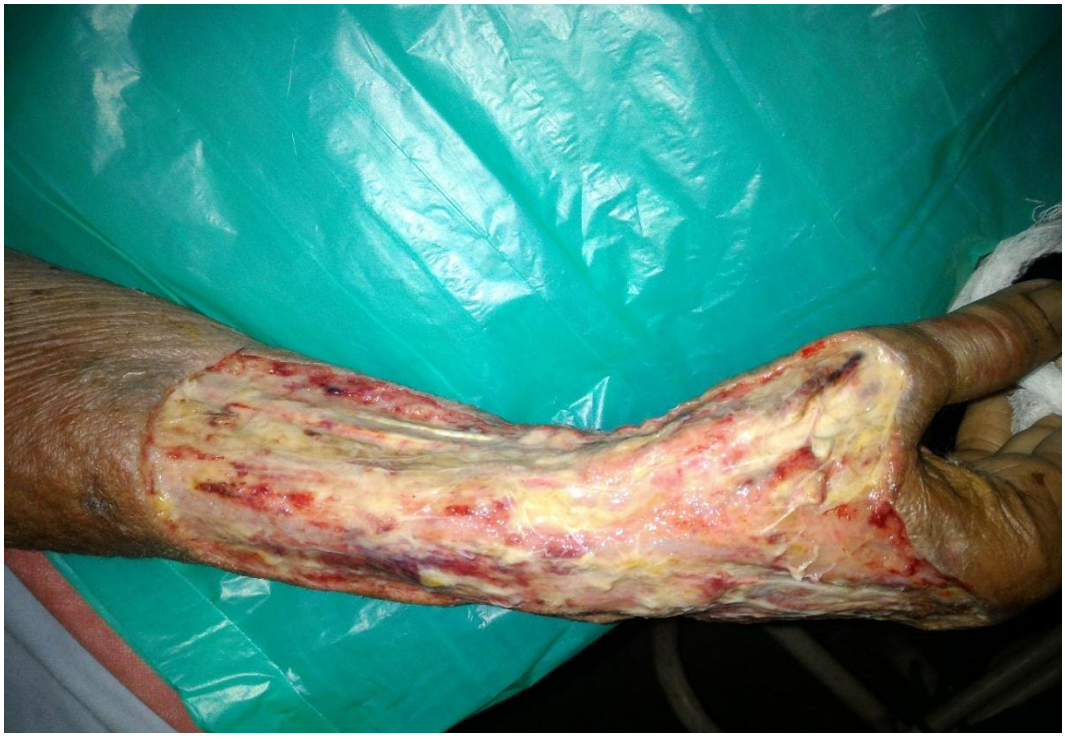


FIGURE 8: ON DAY 5 AFTER APPLICATION OF HONEY.

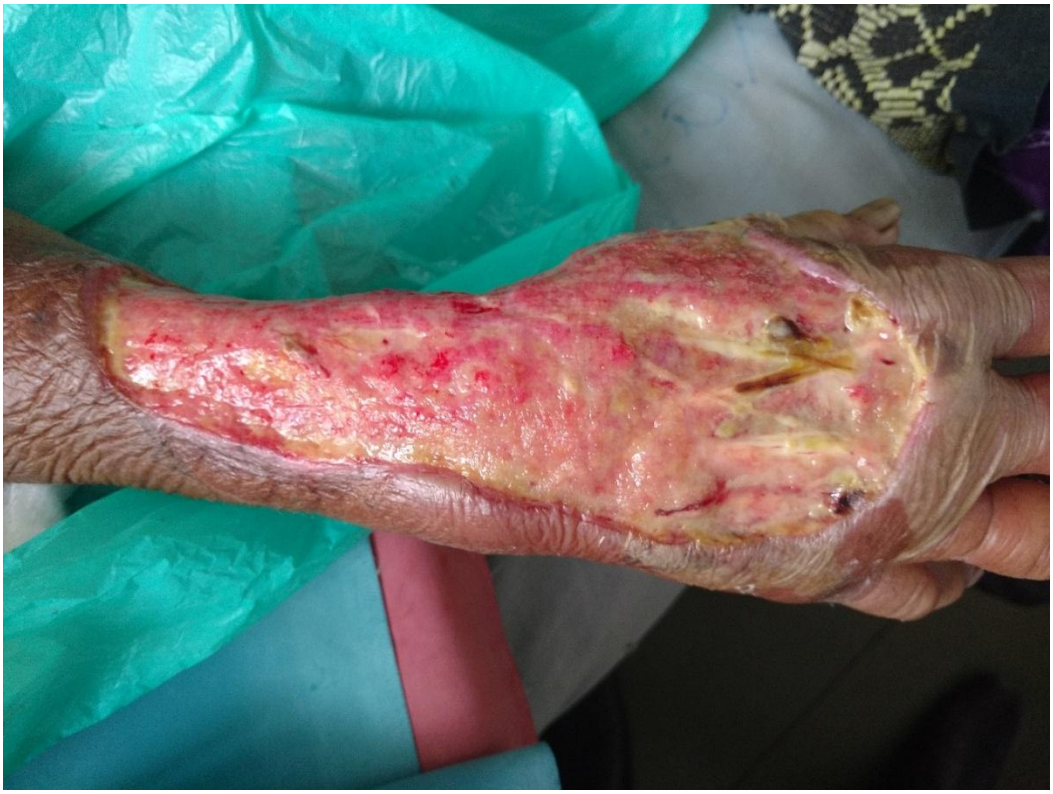


FIGURE 9: ON DAY 10 AFTER APPLICATION OF HONEY

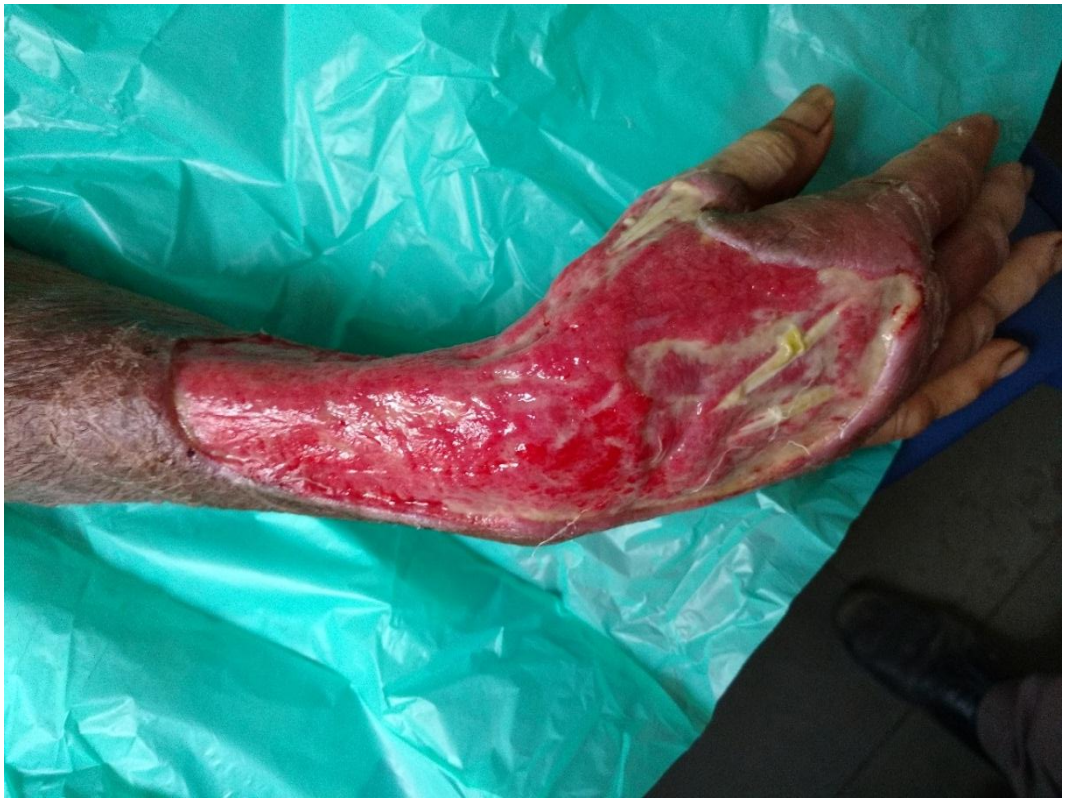


FIGURE 10: ON DAY 15 AFTER APPLICATION OF HONEY.

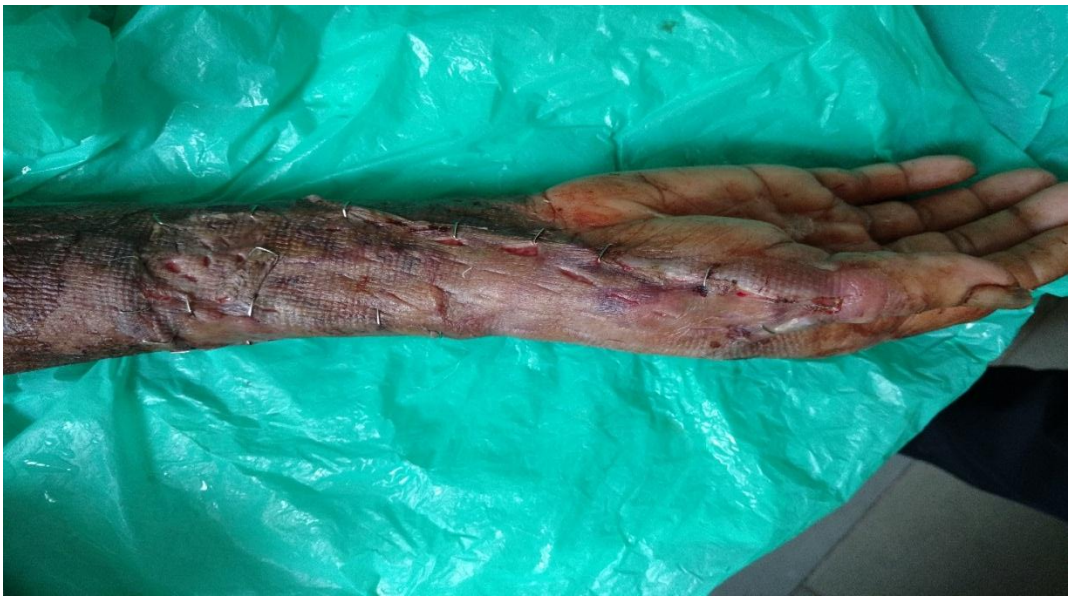


FIGURE 11: POD 5 AFTER SPLIT THICKNESS GRAFTING

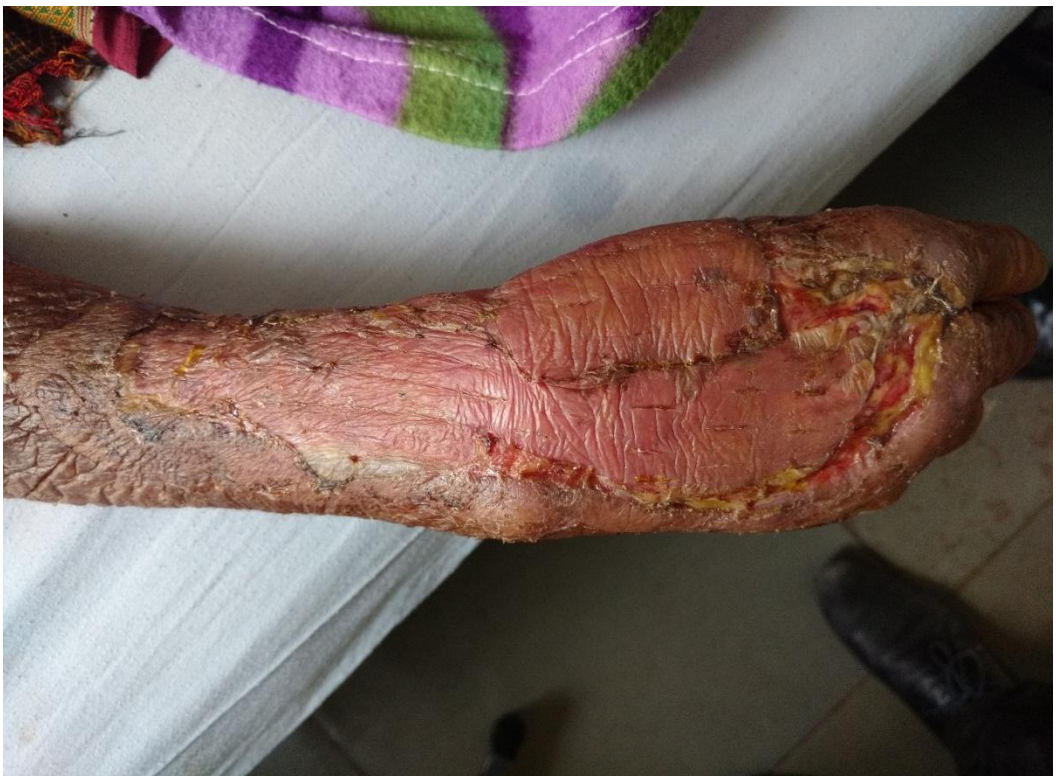


FIGURE 12: POD 10 AFTER SPLIT THICKNESS GRAFTING

CASE 3: ACUTE N.F OVER THE LEFT LEG.

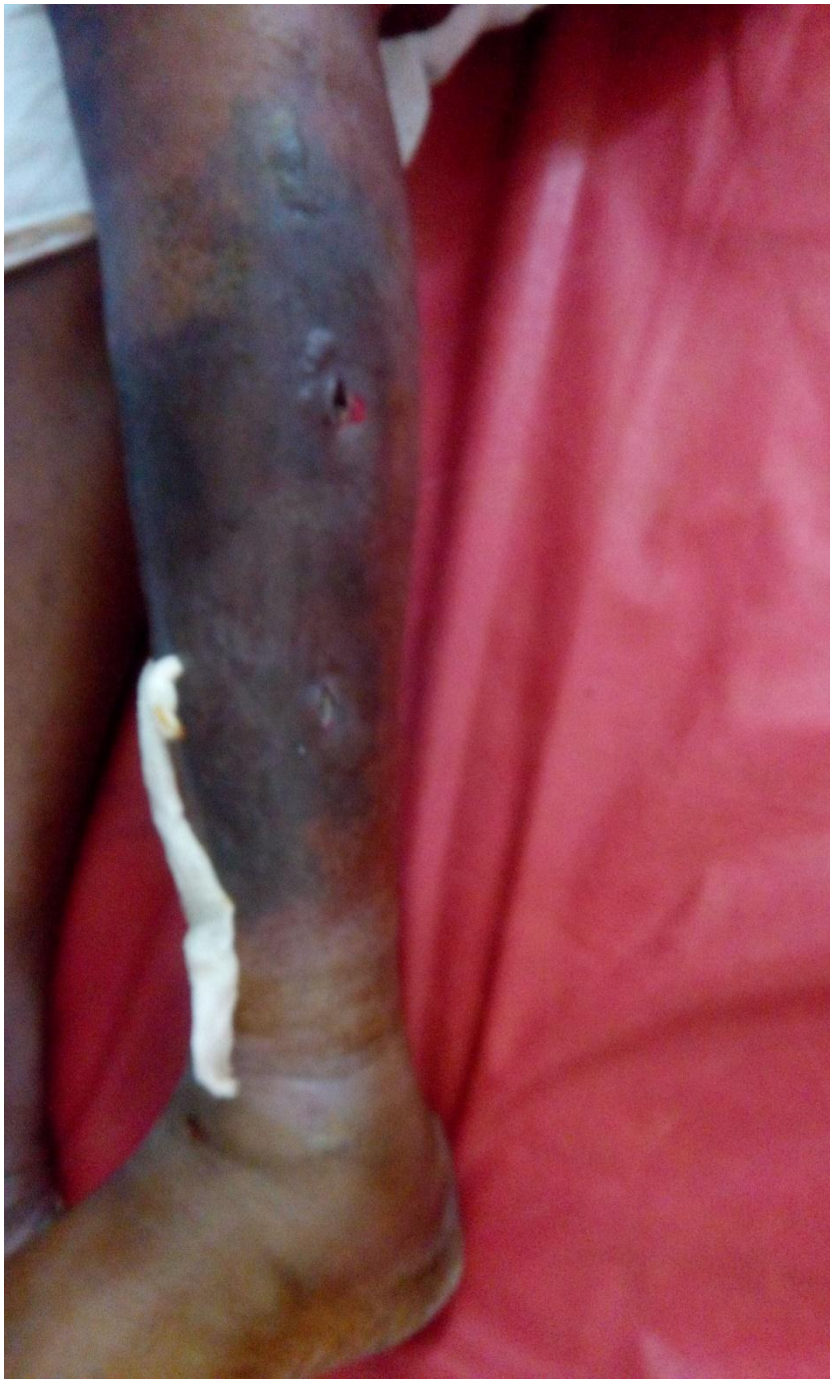


FIGURE 13: ACUTE N.F OVER THE LEFT LEG

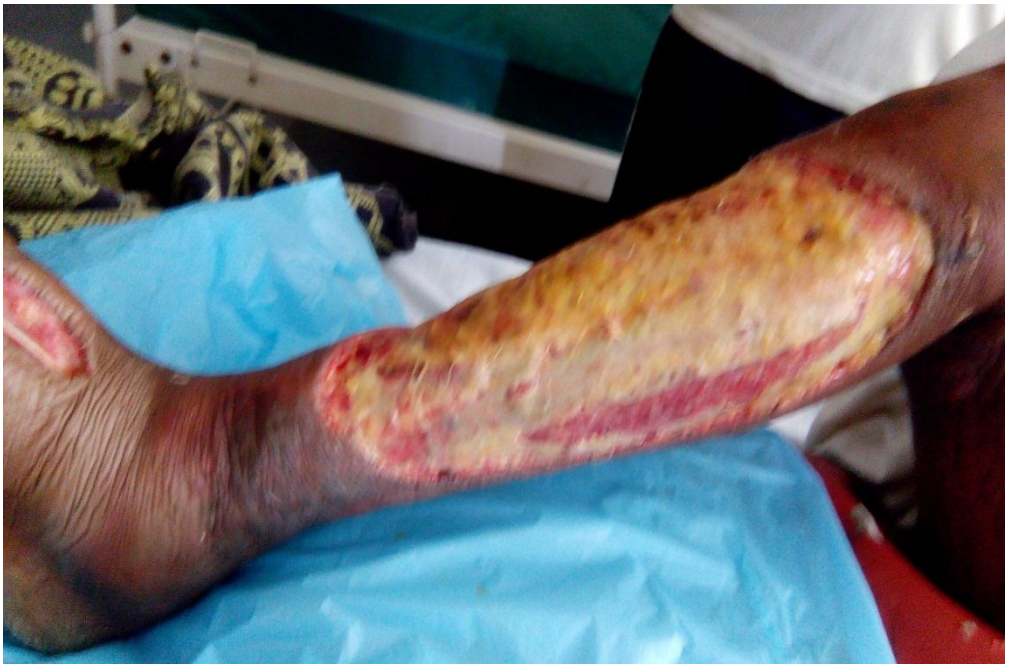


FIGURE 14: DAY 10 AFTER MECHANICAL DEBRIDEMENT

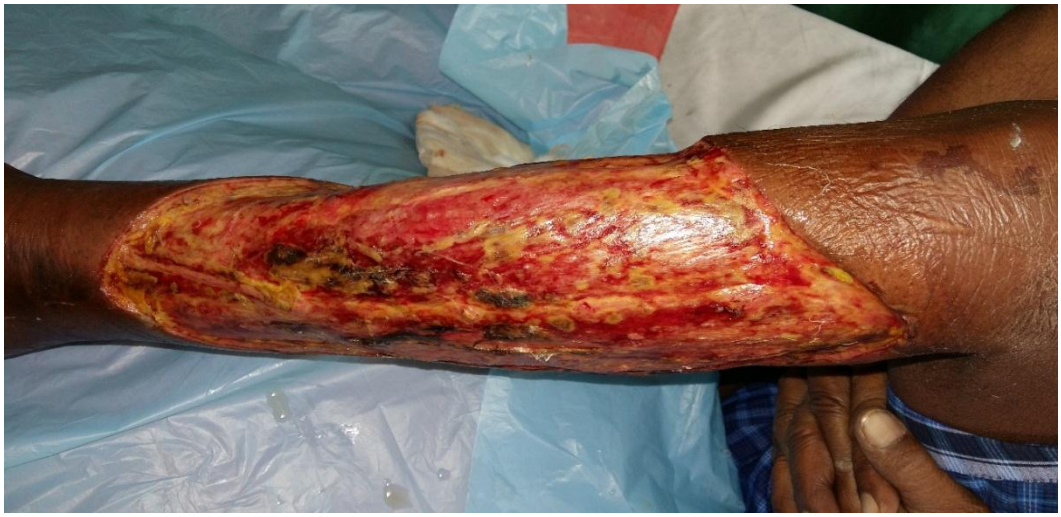


FIGURE 15: DAY 20 AFTER MECHANICAL DEBRIDEMENT.

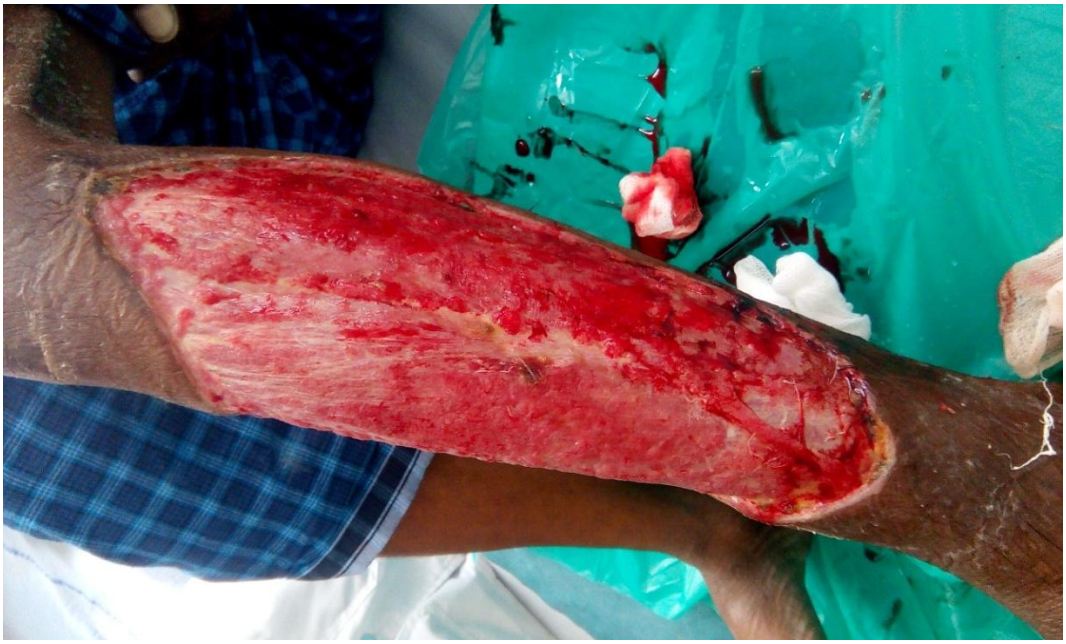


FIGURE 16: DAY 30 AFTER MECHANICAL DEBRIDEMENT.

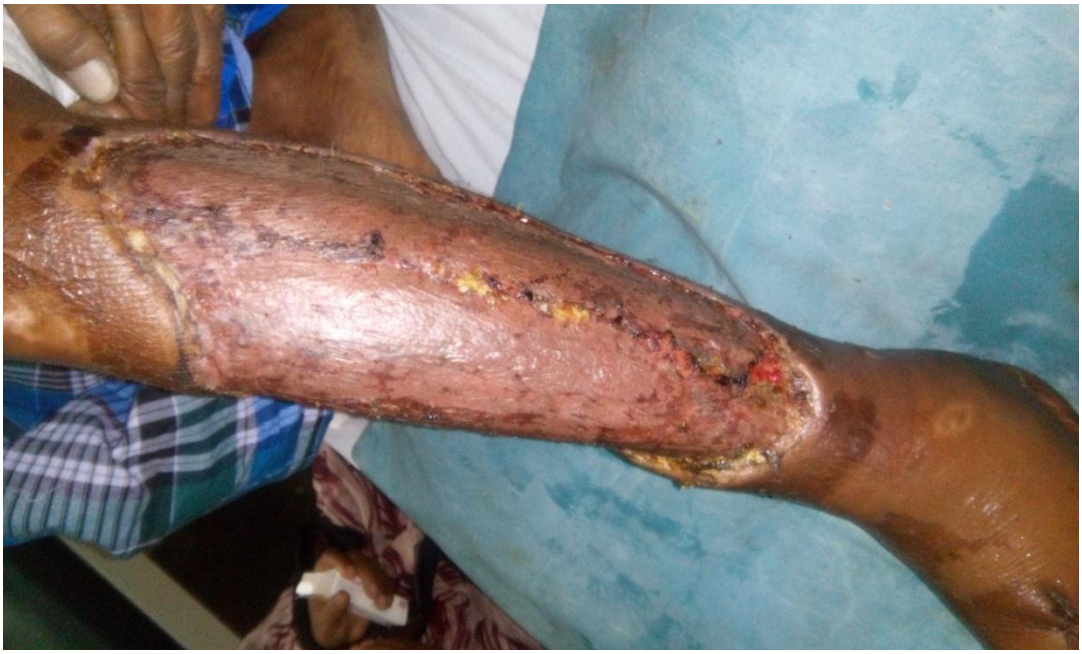


FIGURE 17: POD 10 AFTER SPLIT THICKNESS GRAFTING.

RESULTS

TABLE 1: DISTRIBUTION OF AGE BETWEEN HONEY GROUP (H) AND DEBRIDEMENT GROUP (D)

AGE (YRS)	HONEY GROUP		DEBRIDEMNT GROUP		p value
	N	%	N	%	
≤15	3	6.7	2	4.4	0.405
16-30	7	15.6	2	4.4	
31-45	8	17.8	12	26.7	
46-60	10	22.2	14	31.1	
61-75	16	35.6	13	28.9	
>75	1	2.2	2	4.4	
Total	45	100.0	45	100.0	

CHART 1: DISTRIBUTION OF AGE BETWEEN GROUP H AND GROUP D.

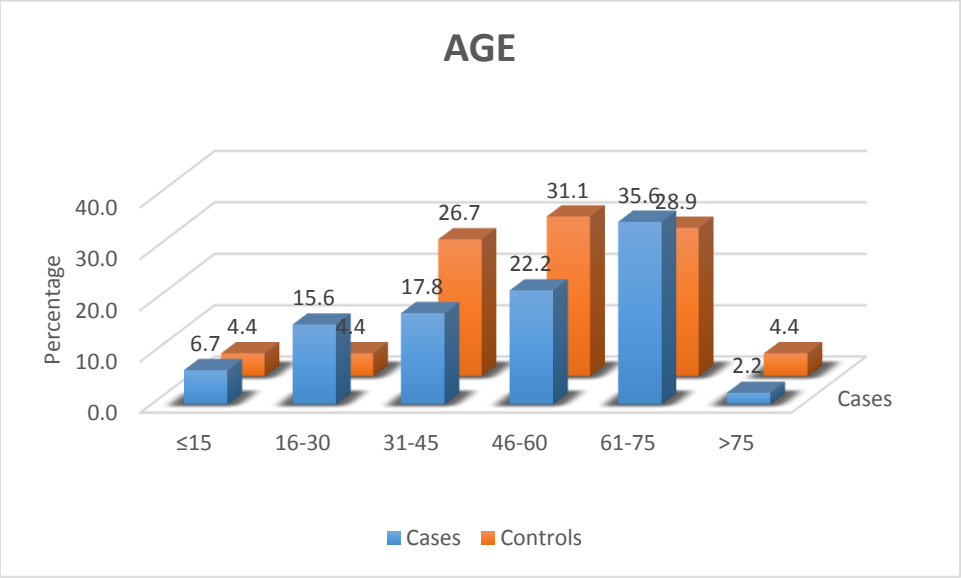


Table 1 and Chart 1 shows the distribution of age between the Group H and Group MD with percentage distribution of age is maximum in between 61-75 years (35.6%) in Honey group and 46-60 years (31.1%) in Group M.

TABLE 2: MEAN AGE BETWEEN HONEY AND DEBRIDEMENT GROUPS

Variable	HONEY		DEBRIDEMENT		p value
	Mean	SD	Mean	SD	
AGE	49.8	19.0	53.4	17.5	0.353

CHART 2: MEAN AGE BETWEEN HONEYAND DEBRIDEMENT GROUPS

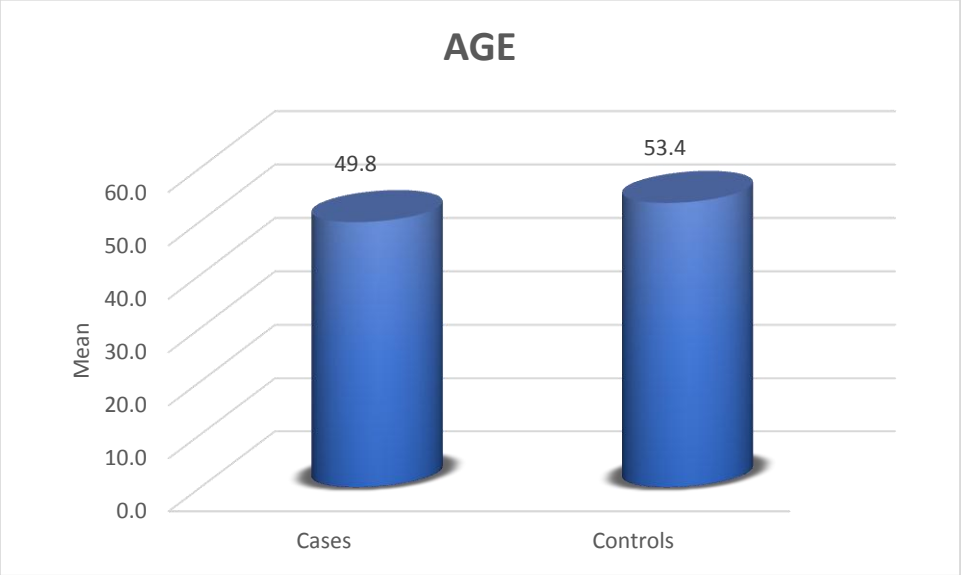


Table 2 and chart 2 shows the mean age between the honey and debridement groups. In this study the mean age 49.8±19 in honey group and 53.4±17.5 in debridement group.

TABLE 3: DISTRIBUTION OF SEX BETWEEN HONEY AND DEBRIDEMENT GROUPS.

SEX	HONEY GROUP		DEBRIDEMENT GROUP		p value
	N	%	N	%	
MALE	37	82.2	37	82.2	-
FEMALE	8	17.8	8	17.8	
Total	45	100.0	45	100.0	

CHART 3: DISTRIBUTION OF SEX BETWEEN HONEY AND DEBRIDEMENT GROUPS

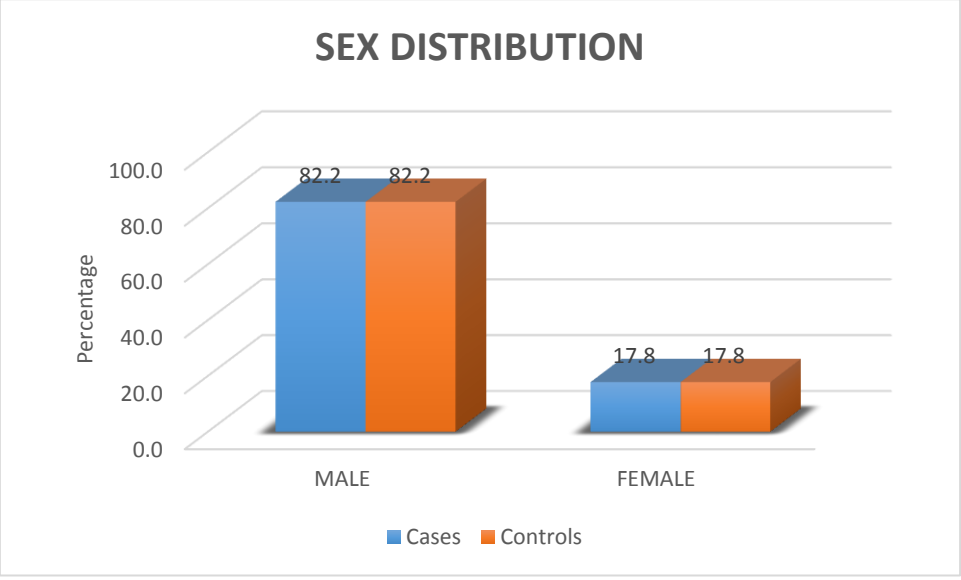


Table 3 and Chart 3 shows the sex distribution among the two groups. In this study in both the groups were male predominant i.e 82.2%.

TABLE 4: DISTRIBUTION OF ULCER BETWEEN HONEY AND DEBRIDEMENT GROUPS

ULCER	HONEY GROUP		DEBRIDEMENT GROUP		p value
	N	%	N	%	
ACUTE	18	40.0	14	31.1	0.509
CHRONIC	27	60.0	31	68.9	
Total	45	100.0	45	100.0	

CHART 4: DISTRIBUTION OF ULCER BETWEEN HONEY AND DEBRIDEMENT GROUPS

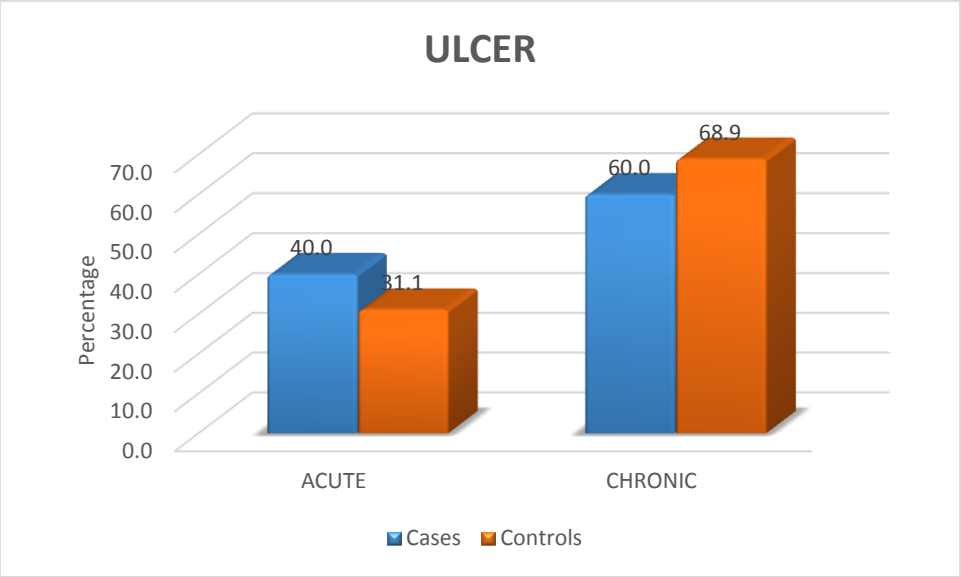


Table 4 and chart 4 shows the distribution of ulcers among the groups. In this study most of the ulcers were chronic 60% and 68.9% in honey and debridement groups respectively.

TABLE 5: DISTRIBUTION OF SITE BETWEEN HONEY AND DEBRIDEMENT GROUPS

SITE	HONEY GROUP		DEBRIDEMENT GROUP		p value
	N	%	N	%	
ANT TRUNK	1	2.2	0	0.0	0.354
BOTH GLUTEAL	0	0.0	1	2.2	
BREAST	0	0.0	1	2.2	
CHST WALL ANT	1	2.2	0	0.0	
FOOT	20	44.4	25	55.6	
FOREARM	1	2.2	2	4.4	
ISCHORECTAL ABSECC	0	0.0	1	2.2	
LEG	12	26.7	11	24.4	
PERIANAL ABCESS	0	0.0	1	2.2	
SACRUM	1	2.2	0	0.0	
SHOULDER	3	6.7	0	0.0	
THIGH	2	4.4	3	6.7	
THUMB	1	2.2	0	0.0	
TOE	2	4.4	0	0.0	
WHOLE BACK	1	2.2	0	0.0	
Total	45	100.0	45	100.0	

CHART 5: DISTRIBUTION OF SITE BETWEEN HONEY AND DEBRIDEMENT GROUPS

Table 5 and Chart 5 shows the distribution of ulcers over the body in both the groups. In this study most of the ulcers were localized to the foot, 44.4% in honey group and 55.6% in debridement group.

TABLE 6: DISTRIBUTION OF COMORBIDITIES BETWEEN HONEY AND DEBRIDEMENT GROUPS

COMORBIDITIES	HONEY GROUP		DEBRIDEMENT GROUP		p value
	N	%	N	%	
HTN	1	2.2	3	6.7	0.289
DM	6	13.3	17	37.8	0.009*
SMOKER	1	2.2	4	8.9	0.137
OTHERS	14	31.1	11	24.4	0.257

Note: *means significant at 5% level of significance (p<0.05)

CHART 6: DISTRIBUTION OF COMORBIDITIES BETWEEN HONEY AND DEBRIDEMENT GROUPS

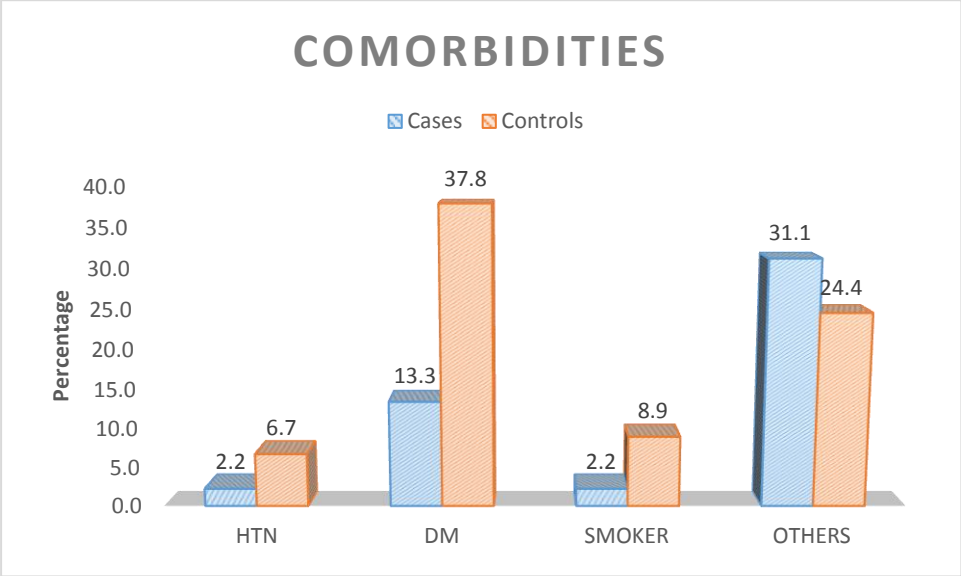


Table 6 and Chart 6 shows the distribution of comorbidities among the groups. In this study 13.3% and 37.8% were diabetic in honey and debridement groups respectively. Others were 31.1% and 24.4% in honey and debridement groups respectively which includes Anaemia, IHD, bedridden due to stroke, CRF and PEM.

TABLE 7: DISTRIBUTION OF ORGANISMS ISOLATED BETWEEN HONEY AND DEBRIDEMENT GROUPS

ORGANISMS ISOLATED	HONEY GROUP		DEBRIDEMENT GROUP		p value
	N	%	N	%	
P.A	16	35.6	22	48.9	0.200
K.P	22	48.9	17	37.8	0.288
ACINOBACTER	3	6.7	0	0.0	0.242
E.COLI	4	8.9	5	11.1	0.725
CITROBACTER KOSETI	7	15.6	4	8.9	0.522
KL. OXYTOCA	4	8.9	1	2.2	0.361
MRSA	6	13.3	3	6.7	0.485
ENTEROCOCS	2	4.4	1	2.2	0.557
STAPHH. AUREUS	24	53.3	21	46.7	0.674
ASPERGILLUS FUMIGATUS	0	0.0	1	2.2	0.494
STREPTOCOCUS	0	0.0	1	2.2	0.494

CHART 7**: DISTRIBUTION OF ORGANISMS ISOLATED BETWEEN HONEY AND DEBRIDEMENT GROUPS**

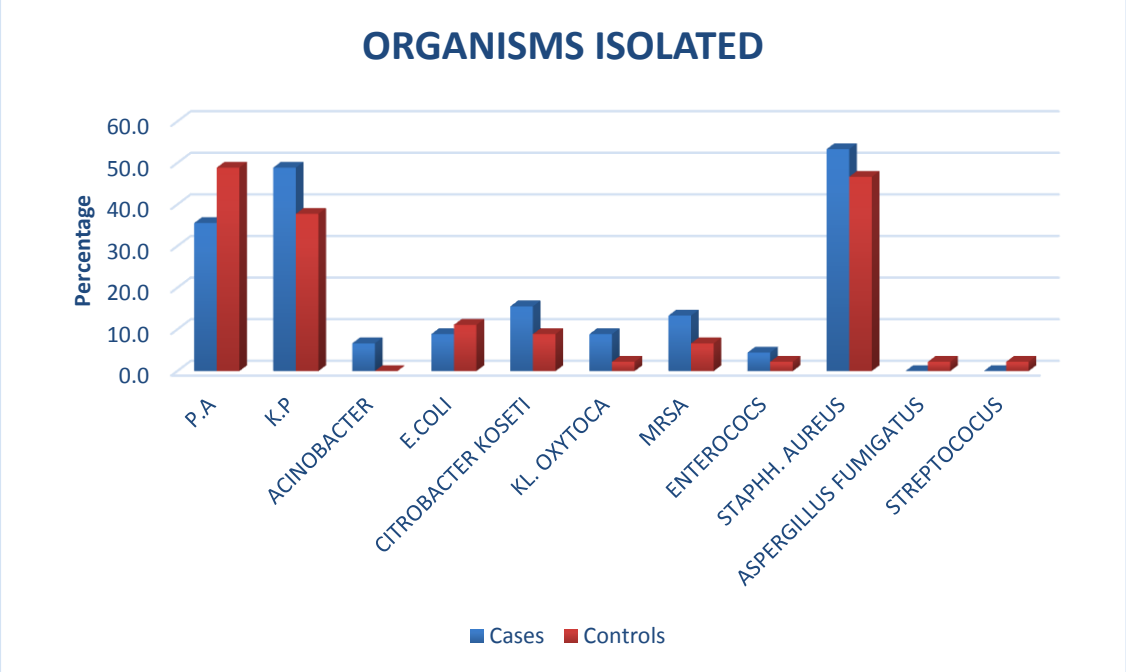


Table 7 and chart 7 shows the organisms isolated between the groups. In this study most common organism isolated was staph aureus 53.3%, K.P 48.9% in honey group, P.A 48.9% and staph aureus 46.7% were isolated in debridement group.

TABLE 8: DISTRIBUTION OF ORGANISMS ISOLATED BETWEEN TYPES OF ULCER AMONG HONEY GROUP.

ORGANISMS ISOLATED	ULCER				p value
	ACUTE		CHRONIC		
	N	%	N	%	
P.A	3	16.7	13	48.1	0.031*
K.P	8	44.4	14	51.9	0.626
ACINOBACTER	0	0.0	3	11.1	0.143
E.COLI	1	5.6	3	11.1	0.521
CITROBACTER KOSETI	1	5.6	6	22.2	0.131
KL. OXYTOCA	0	0.0	4	14.8	0.087
MRSA	4	22.2	2	7.4	0.152
ENTEROCOCS	2	11.1	0	0.0	0.076
STAPHH. AUREUS	9	50.0	15	55.6	0.714
Others	0	0.0	0	0.0	-
Total	18	100.0	27	100.0	

CHART 8**: DISTRIBUTION OF ORGANISMS ISOLATED BETWEEN TYPES OF ULCER AMONG HONEY GROUP.**

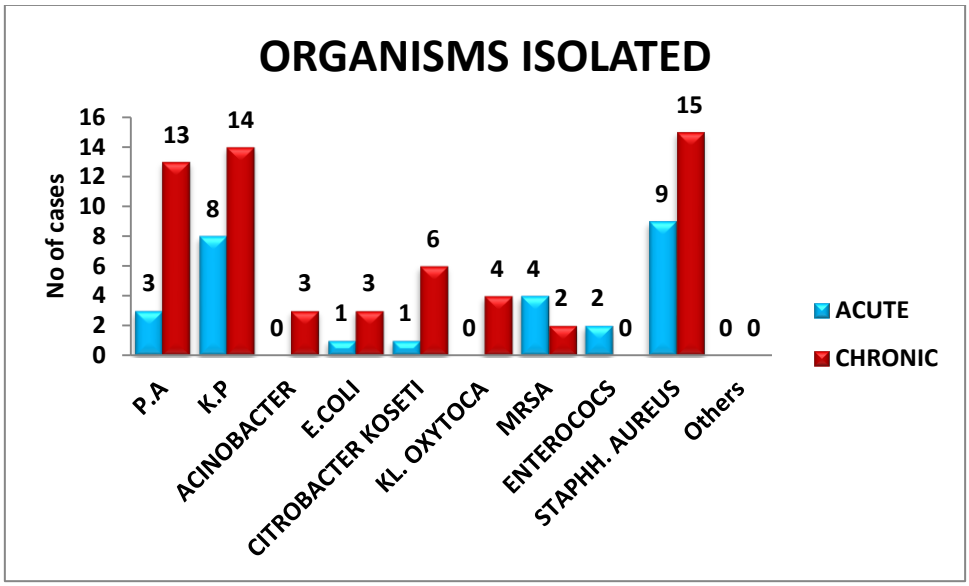


Table 8 and chart 8 shows the distribution of organism isolated between the types of ulcers in the honey group. The commonest organism isolated was staph aureus in both acute and chronic ulcers 50% and 55.8% respectively in honey group.

TABLE 9: DISTRIBUTION OF ORGANISMS ISOLATED BETWEEN TYPES OF ULCER AMONG GROUP D.

ORGANISMS ISOLATED	ULCER				p value
	ACUTE		CHRONIC		
	N	%	N	%	
P.A	9	64.3	13	41.9	0.165
K.P	4	28.6	13	41.9	0.392
ACINOBACTER	14	100.0	31	100.0	-
E.COLI	1	7.1	4	12.9	0.569
CITROBACTER KOSETI	0	0.0	4	12.9	0.159
KL. OXYTOCA	0	0.0	1	3.2	0.497
MRSA	0	0.0	3	9.7	0.228
ENTEROCOCS	1	7.1	0	0.0	0.132
STAPHH. AUREUS	5	35.7	16	51.6	0.322
Others	1	7.1	1	3.2	0.262
Total	14	100.0	31	100.0	

CHART 9: DISTRIBUTION OF ORGANISMS ISOLATED BETWEEN TYPES OF ULCER AMONG GROUP D

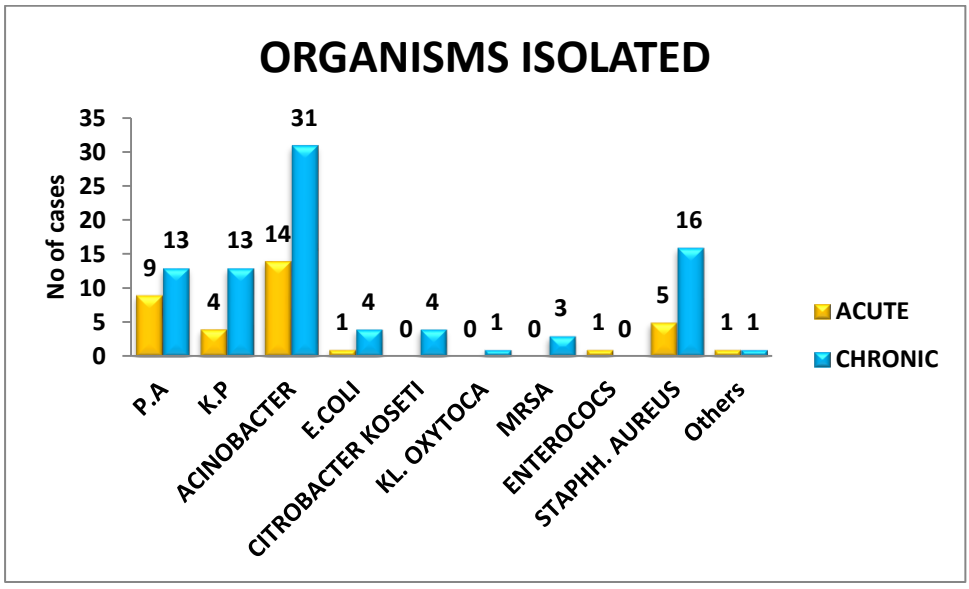


Table 9 and Chart 9 shows the distribution of organisms between the types of ulcers among the debridement group. In this study the most of the ulcers were chronic ulcers and the commonest organism isolated was Acinobacter sps.

TABLE 10: DISTRIBUTION OF GRANULATION TISSUE TIME BETWEEN HONEY AND DEBRIDEMENT GROUPS.

GRANULATION TISSUE TIME (DAYS)	HONEY GROUP		DEBRIDEMENT GROUP		p value
	N	%	N	%	
≤10	12	26.7	12	26.7	0.081
11-15	19	42.2	8	17.8	
16-20	9	20.0	12	26.7	
21-25	4	8.9	7	15.6	
26-30	1	2.2	5	11.1	
>30	0	0.0	1	2.2	

CHART 10: DISTRIBUTION OF GRANULATION TISSUE TIME BETWEEN HONEY AND DEBRIDEMENT GROUPS

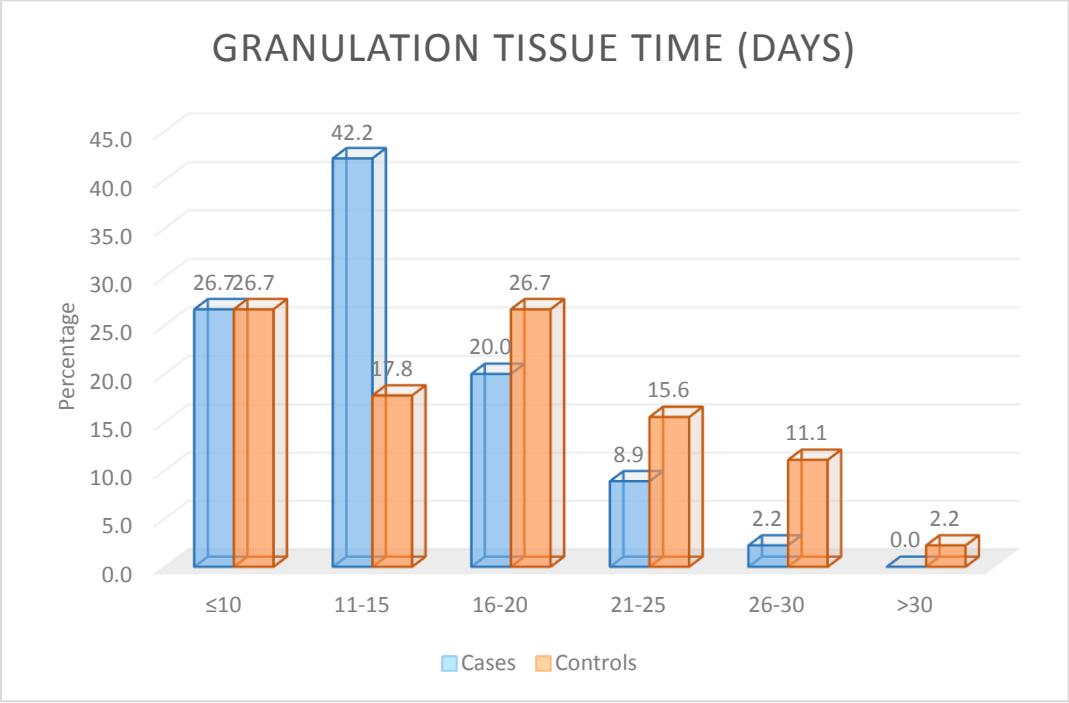


Table 10 and chart 10 shows the appearance of granulation tissue among both groups. In this study, in 42.2% patients' granulation tissue was appeared in less than 2weeks in honey group, where as in debridement group 26.7% patients appeared in 16-20days.

TABLE 11: MEAN GRANULATION TISSUE TIME BETWEEN HONEY AND DEBRIDEMENT GROUPS

Variable	HONEY GROUP		DEBRIDEMENT GROUP		p value
	Mean	SD	Mean	SD	
GRANULATION TISSUE TIME (DAYS)	14.7	5.4	17.9	7.5	0.025*

Note: *means significant at 5% level of significance (p<0.05)

CHART 11: MEAN GRANULATION TISSUE TIME BETWEEN HONEY AND DEBRIDEMENT GROUPS

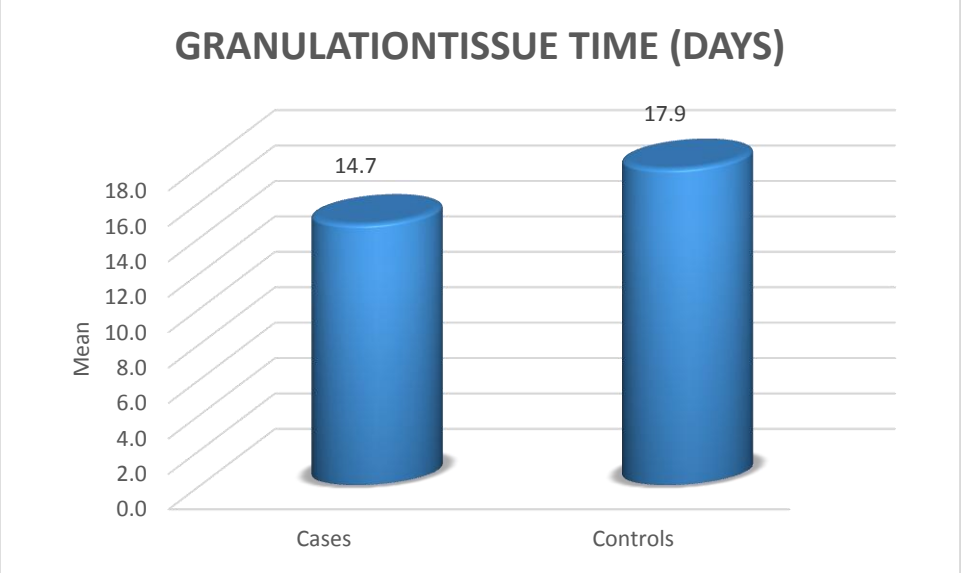


TABLE 11 AND CHART 11 shows the Mean time for the appearance of granulation tissue among the both groups. In this study the mean time was 14.7± 5.4 in honey group and 17.9±7.5 in debridement group which was statistically significant (p=0.025).

TABLE 12: DISTRIBUTION OF SURGERY BETWEEN HONEY AND DEBRIDEMENT GROUPS

SURGERY	HONEY GROUP		DEBRIDEMENT GROUP		p value
	N	%	N	%	
SEC HEALING	3	6.7	8	17.8	0.3
SS	3	6.7	6	13.3	
STGS	33	73.3	28	62.2	
STGS & SS	3	6.7	2	4.4	
OTHERS	3	6.7	1	2.2	
Total	45	100.0	45	100.0	

CHART 12: DISTRIBUTION OF SURGERY BETWEEN HONEY AND DEBRIDEMENT GROUPS

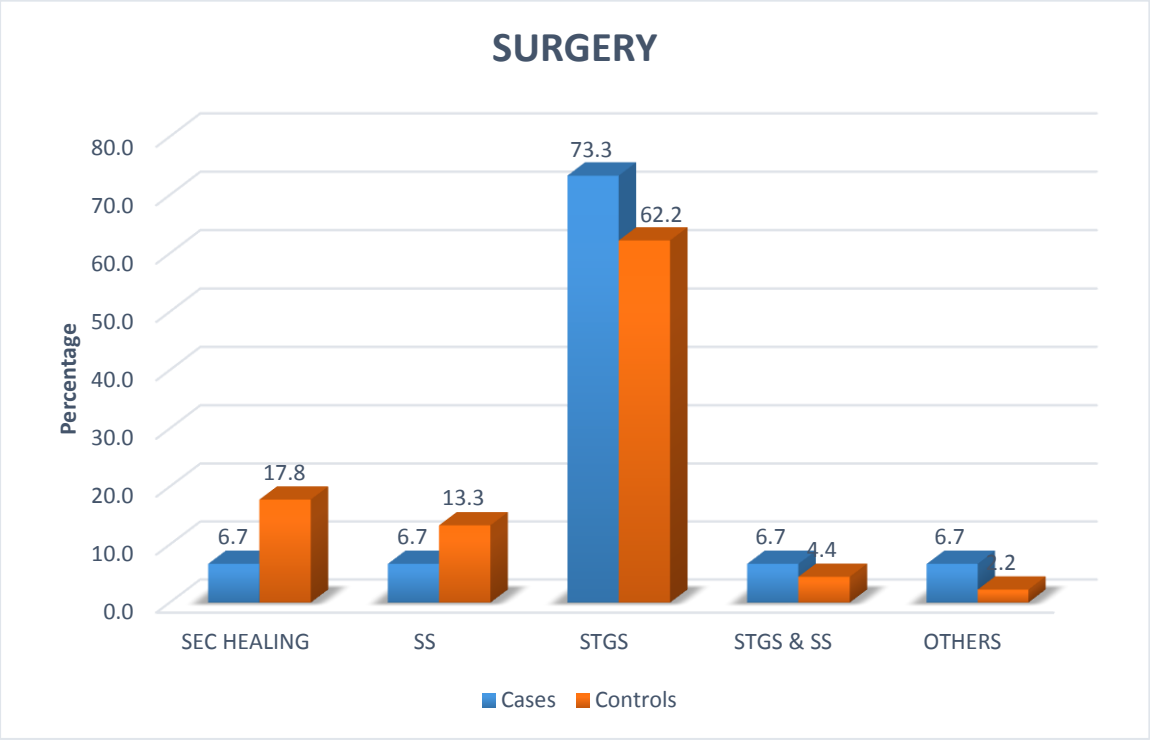


Table 12 and chart 12 shows the percentage of definitive treatment in both the groups. In this study 73.3% and 62.2% underwent split thickness skin grafting in honey and debridement group respectively.

TABLE 13: DISTRIBUTION OF TIME FOR HEALING BETWEEN HONEY AND DEBRIDEMENT GROUPS

TIME FOR HEALING (DAYS)	HONEY GROUP		DEBRIDEMENT GROUP		p value
	N	%	N	%	
≤10	4	8.9	5	11.1	0.022*
11-15	13	28.9	8	17.8	
16-20	10	22.2	4	8.9	
21-25	11	24.4	6	13.3	
26-30	4	8.9	13	28.9	
>30	3	6.7	9	20.0	
Total	45	100.0	45	100.0	

Note: *means significant at 5% level of significance (p<0.05)

CHART 13: DISTRIBUTION OF TIME FOR HEALING BETWEEN HONEY AND DEBRIDEMENT GROUPS

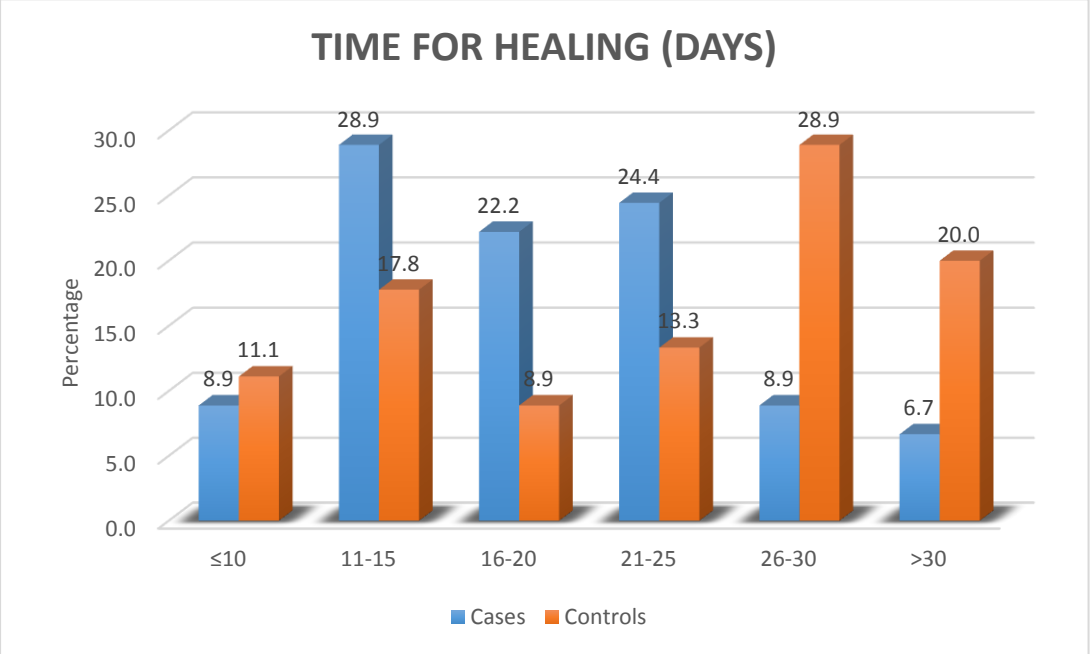


TABLE 13 AND CHART 13 shows the time for healing of wounds with biofilm among the two groups. In this study, 28.9% (13/45) patients treated with honey were healed in the time span of <15days, whereas 28.9% (13/45) patients' treated with debridement were healed in 26-30 days which is significant (p=0.022).

TABLE 14: MEAN TIME FOR HEALING BETWEEN HONEY AND DEBRIDEMENT GROUPS

Variable	HONEY GROUP		DEBRIDEMENT GROUP		p value
	Mean	SD	Mean	SD	
TIME FOR HEALING (DAYS)	21.0	10.5	24.9	10.8	0.084

CHART 14: MEAN TIME FOR HEALING BETWEEN HONEY AND DEBRIDEMENT GROUPS

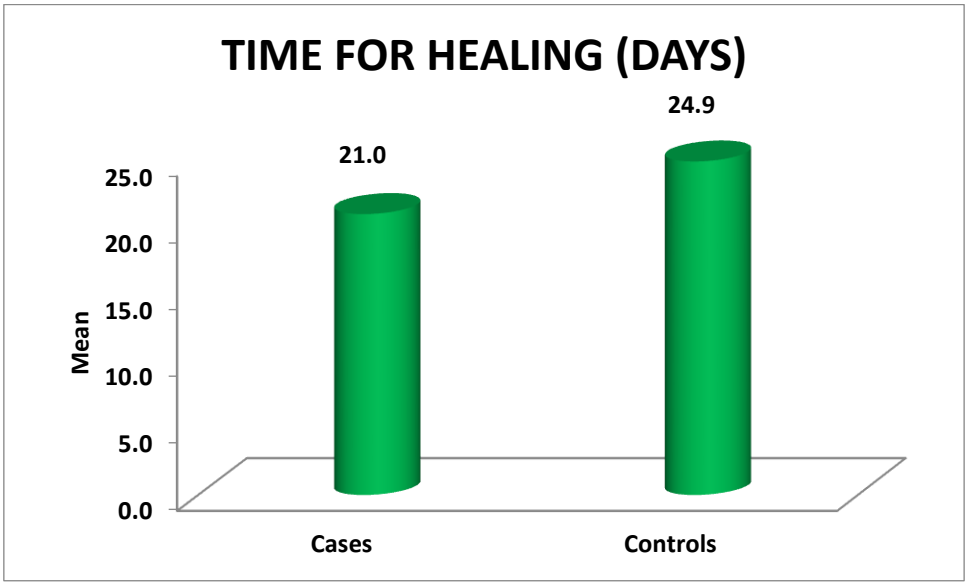


Table 14 and chart 14 shows the mean time healing of wounds among the two groups. In this study the mean time for healing of wounds were 21.0±10.5 in honey group and 24.9±10.8 in debridement group.

TABLE 15: DISTRIBUTION OF HOSPITAL STAY BETWEEN HONEY AND DEBRIDEMENT GROUPS

HOSPITAL STAY (DAYS)	HONEY GROUP		DEBRIDEMENT GROUP		p value
	N	%	N	%	
≤10	0	0.0	1	2.2	0.004*
11-15	1	2.2	6	13.3	
16-20	4	8.9	3	6.7	
21-25	14	31.1	1	2.2	
26-30	8	17.8	8	17.8	
>30	18	40.0	26	57.8	
Total	45	100.0	45	100.0	

Note: *means significant at 5% level of significance (p<0.05)

CHART 15: DISTRIBUTION OF HOSPITAL STAY BETWEEN HONEY AND DEBRIDEMENT GROUPS

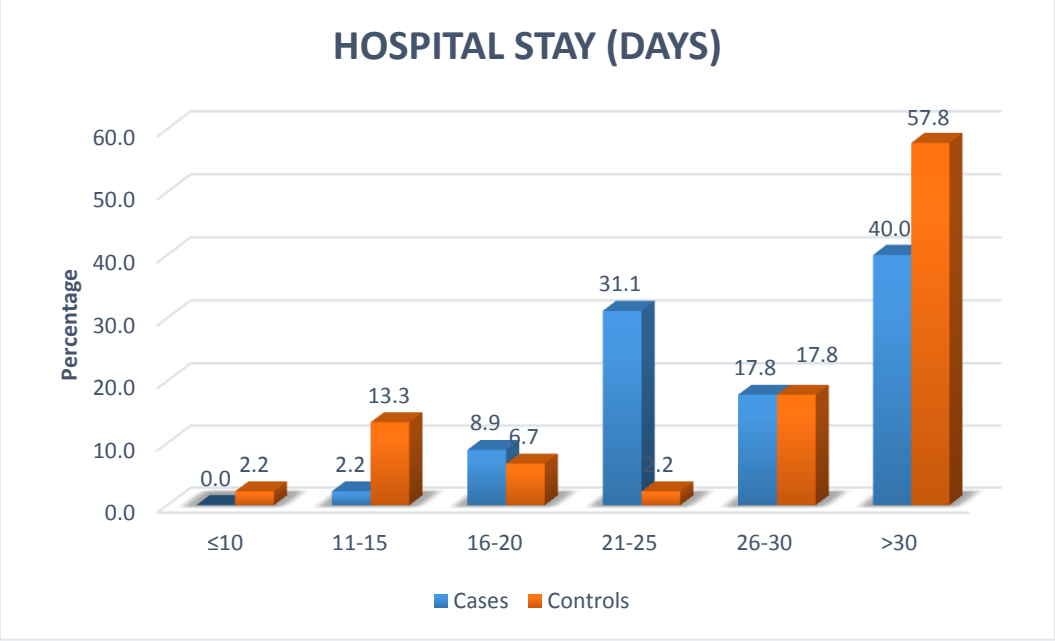


TABLE 15 AND CHART 15 shows the length of hospital stay in days among the two groups. In this study, 40.0% in honey group and 57.8% in debridement group were discharged after 1month which was statistically significant with p value 0.004. 31.1% in honey group were discharged by 3weeks, 17.8% in debridement group were discharged by 4weeks.

TABLE 16: MEAN HOSPITAL STAY BETWEEN HONEY AND DEBRIDEMENT GROUPS

Variable	HONEY GROUP		DEBRIDEMENT GROUP		p value
	Mean	SD	Mean	SD	
HOSPITAL STAY (DAYS)	34.1	15.7	36.0	15.8	0.567

CHART 16: MEAN HOSPITAL STAY BETWEEN HONEY AND DEBRIDEMENT GROUPS

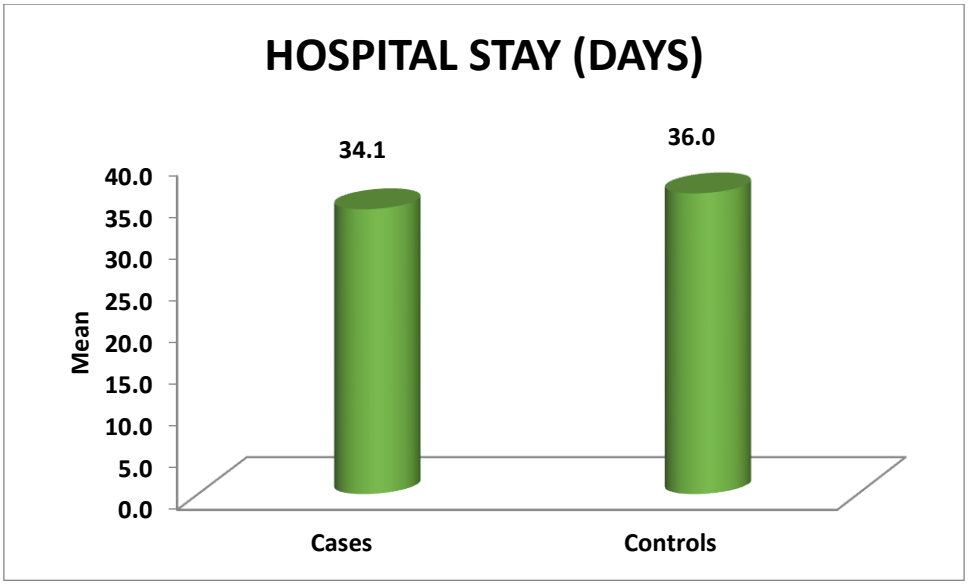


TABLE 16 AND CHART 16 shows the mean duration of hospital stay in days among the both groups. In this study the mean duration of hospital stay was 34.1±15.7 and 36.0±15.8 in honey and debridement groups respectively.

TABLE 17: DISTRIBUTION OF COMPLICATIONS BETWEEN HONEY AND DEBRIDEMENT GROUPS

COMPLICATIONS	HONEY GROUP		DEBRIDEMENT GROUP		p value
	N	%	N	%	
BKA	1	2.2	0	0.0	0.408
GRAFT REJECTION	2	4.4	2	4.4	
POST OP OOZING	2	4.4	0	0.0	
POST OP OOZING & GRAFT REJECTION	1	2.2	0	0.0	
NO	39	86.7	43	95.6	
Total	45	100.0	45	100.0	

CHART 17: DISTRIBUTION OF COMPLICATIONS BETWEEN HONEY AND DEBRIDEMENT GROUPS

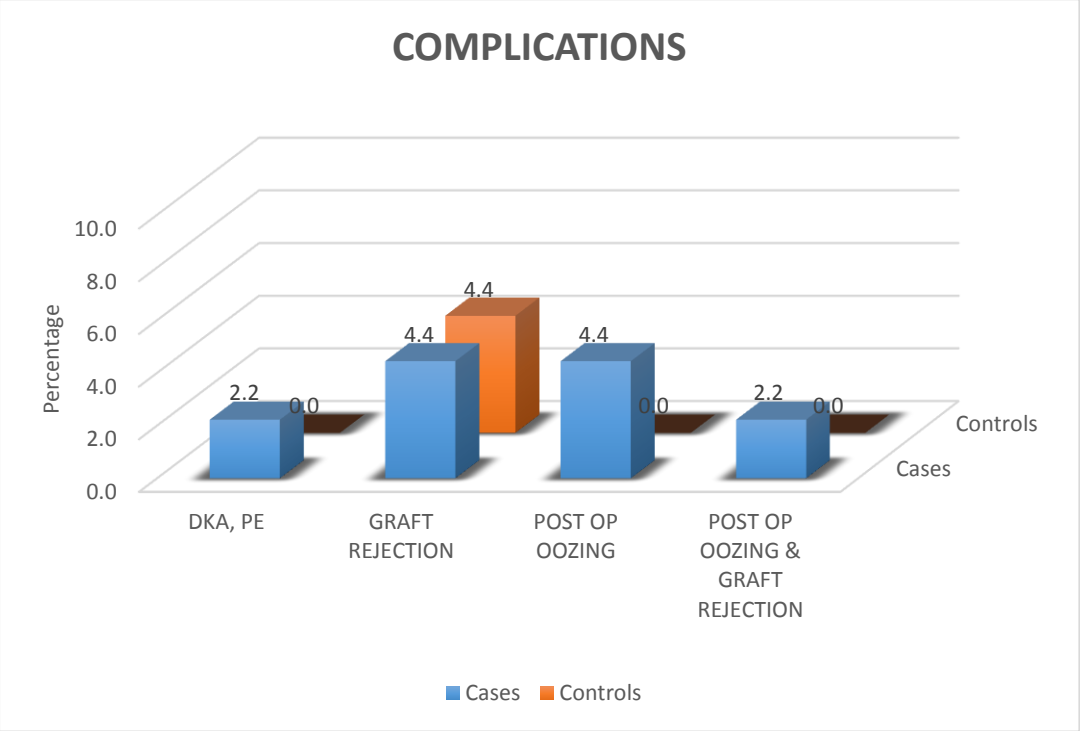


TABLE 17 AND CHART 17 shows the complications among the two groups.

TABLE 18: DISTRIBUTION OF ORGANISMS ISOLATED BETWEEN HONEY AND DEBRIDEMENT GROUPSAMONG DIABETIC PAITIENTS

ORGANISMS ISOLATED	HONEY GROUP		DEBRIDEMENT GROUP		p value
	N	%	N	%	
P.A	2	33.3	9	52.9	0.408
K.P	2	33.3	5	29.4	0.858
ACINOBACTER	6	100.0	17	100.0	-
E.COLI	1	16.7	3	17.6	0.957
CITROBACTER KOSETI	2	33.3	2	11.8	0.231
KL. OXYTOCA	6	100.0	17	100.0	-
MRSA	1	16.7	1	5.9	0.42
ENTEROCOCS	1	16.7	1	5.9	0.42
STAPHH. AUREUS	4	66.7	12	70.6	0.858
Others	0	0.0	1	5.9	0.544

CHART 18: DISTRIBUTION OF ORGANISMS ISOLATED BETWEEN HONEY AND DEBRIDEMENT GROUPSAMONG DIABETIC PAITIENTS

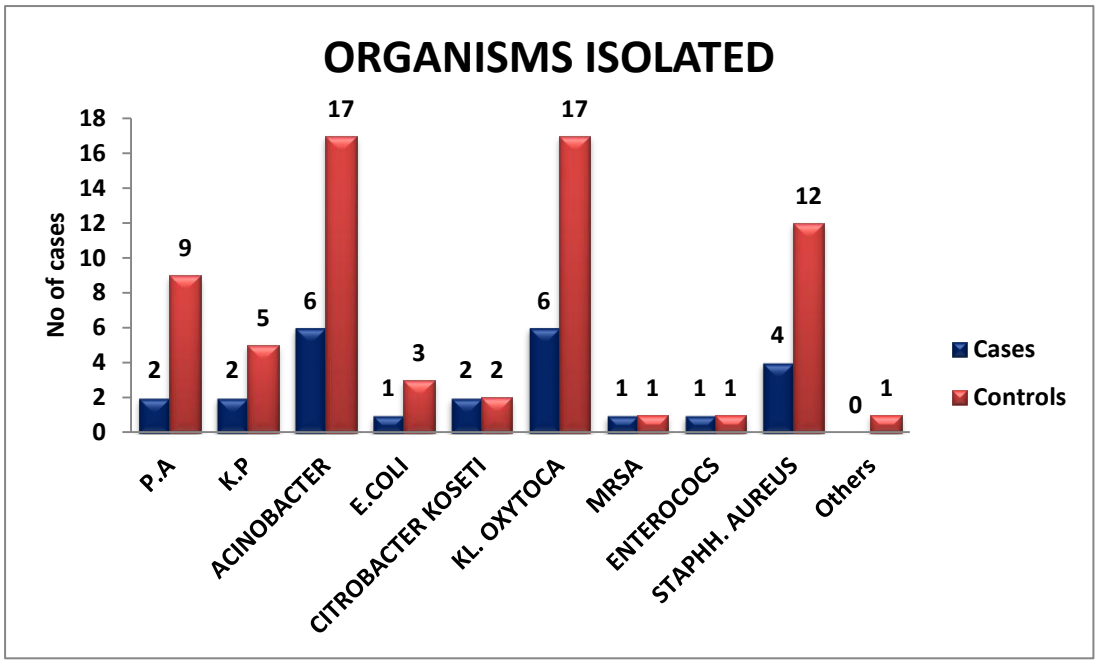


CHART 18 AND TABLE 18 depicts the organism isolated in the diabetic patients in both groups. In this study the commonest organism isolated was Acinobacter sps and Kl. Oxytoca in honey and debridement groups..

TABLE 19: DISTRIBUTION OF TIME FOR HEALING BETWEEN HONEY AND DEBRIDEMENT GROUPS AMONG DIABETIC PAITIENTS

TIME FOR HEALING (DAYS)	HONEY GROUP		DEBRIDEMENT GROUP		p value
	N	%	N	%	
≤10	0	0.0	0	0.0	0.079
11-15	2	33.3	3	17.6	
16-20	0	0.0	2	11.8	
21-25	4	66.7	2	11.8	
26-30	0	0.0	6	35.3	
>30	0	0.0	3	17.6	
Total	6	100.0	17	100.0	

CHART 19: DISTRIBUTION OF TIME FOR HEALING BETWEEN HONEY AND DEBRIDEMENT GROUPS AMONG DIABETIC PAITIENTS

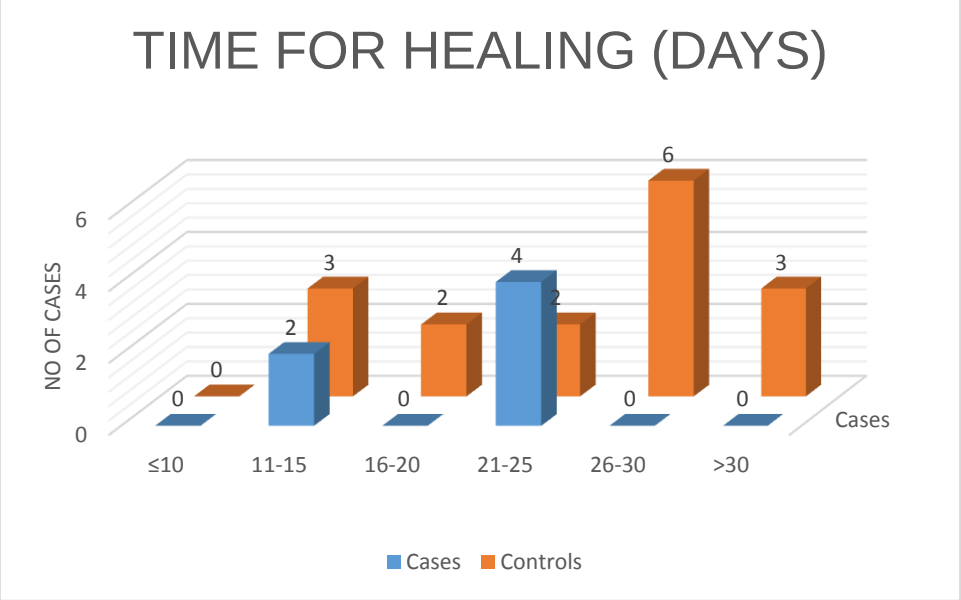


TABLE 19 AND CHART 19 DEPICTS the time for healing of wounds with biofilm among the diabetic patients in both the groups.

TABLE 20: DISTRIBUTION OF GRANULATIONTISSUE TIME BETWEEN HONEY AND DEBRIDEMENT GROUPS AMONG DIABETIC PAITIENTS

GRANULATIONTISSUE TIME (DAYS)	HONEY GROUP		DEBRIDEMENT GROUP		p value
	N	%	N	%	
≤10	0	0.0	4	23.5	0.521
11-15	5	83.3	3	17.6	
16-20	0	0.0	3	17.6	
21-25	1	16.7	3	17.6	
26-30	0	0.0	4	23.5	
>30	0	0.0	0	0.0	
Total	6	100.0	17	100.0	

Chart 20: DISTRIBUTION OF GRANULATIONTISSUE TIME BETWEEN HONEY AND DEBRIDEMENT GROUPS AMONG DIABETIC PAITIENTS

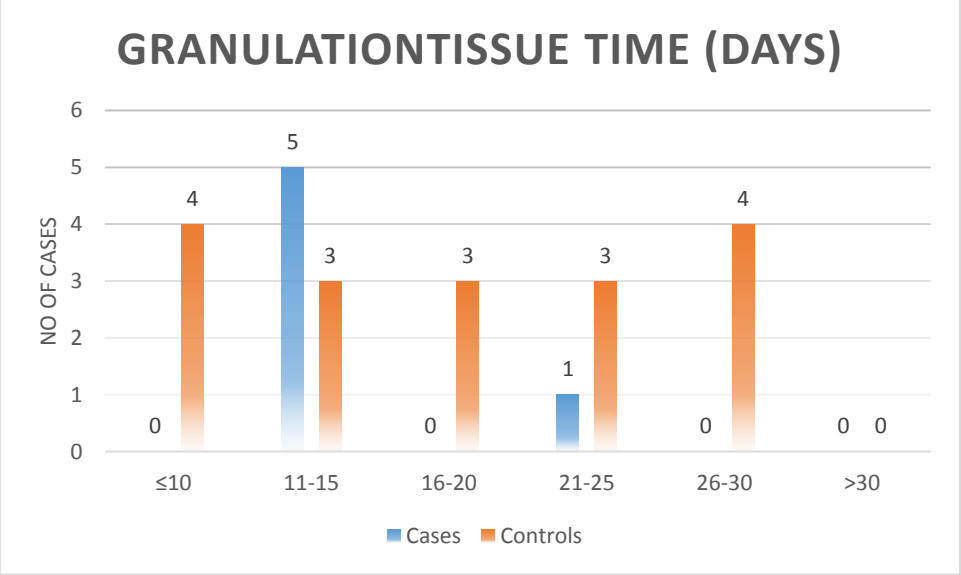


Table 20 and chart 20 shows the time for appearance of healthy granulation tissue in diabetic patients among the two groups. In this study 5/6 patients, the granulation tissue was appeared in 11-15days in honey group and 4/17 patients took 26-30 days for the appearance of granulation tissue in debridement group.

TABLE 21: DISTRIBUTION OF HOSPITAL STAY BETWEEN HONEY AND DEBRIDEMENT GROUPS AMONG DIABETIC PAITIENTS

HOSPITAL STAY (DAYS)	HONEY GROUP		DEBRIDEMENT GROUP		p value
	N	%	N	%	
≤10	0	0.0	0	0.0	0.47
11-15	0	0.0	1	5.9	
16-20	0	0.0	1	5.9	
21-25	1	16.7	0	0.0	
26-30	1	16.7	3	17.6	
>30	4	66.7	12	70.6	
Total	6	100.0	17	100.0	

CHART 21: DISTRIBUTION OF HOSPITAL STAY BETWEEN HONEY AND DEBRIDEMENT GROUPS AMONG DIABETIC PAITIENTS

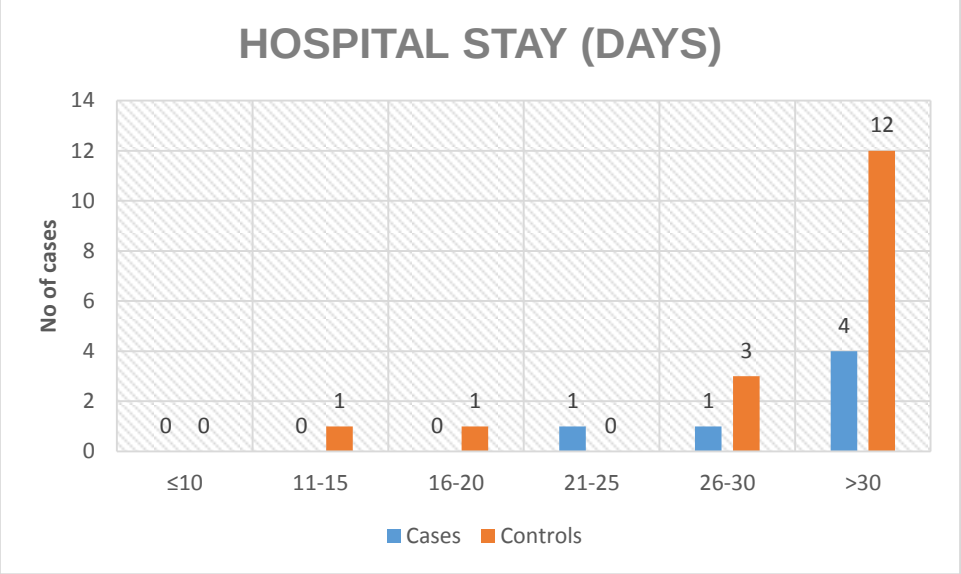


TABLE 21 AND CHART 21 shows the duration of hospital stay among the two groups in diabetic patients. In this study 3/6 patients in honey group were discharged in 26-30 days, 12/17 patients were discharged after 30days in the debridement group.

TABLE 22: DISTRIBUTION OF MEAN PARAMETERSBETWEEN HONEY AND DEBRIDEMENT GROUPS AMONG DIABETIC PAITIENTS

Variable	HONEY GROUP		DEBRIDEMENT GROUP		p value
	Mean	SD	Mean	SD	
GRANULATIONTISSUE TIME (DAYS)	16.5	4.2	19.5	7.3	0.353
TIME FOR HEALING (DAYS)	21.0	5.2	26.6	11.9	0.285
HOSPITAL STAY (DAYS)	38.8	16.6	40.5	15.5	0.829

Table 22 depicts the mean distribution of healing process in the diabetic patients among the two groups.

DISCUSSION

Traditionally basic wound care consists of surgical debridement, irrigation of wounds manually and regular dressings along with systemic or topical antibiotic therapy. The prevalence and incidence of chronic wounds and their associated complications continue to escalate in spite of tremendous progress in science of wound healing. In the context of the continued emergence of antibiotic resistant pathogens, some alternative or "traditional" topical antimicrobials have been reintroduced into modern wound care, one such example being honey. This study mainly compare the efficacy of application of honey topically with the debridement in an ulcers with the biofilm in regarding the healing process.

A total of 90 patients with ulcers having biofilm were randomly divided into honey group and debridement group with 45 patients each.

In this study most of the patients were in the age group of 61-75yrs 16 out of 45 (35.6%) in honey group and 14 out of 45 (46-60) in debridement group (Table 1). The mean age among the two groups was 49.8±19.0 in honey group and 53.4±17.5 in debridement group (Table 2).Most of the patients were male in both groups 37 out of 45 (82.2%) and 8 out of 45 (17.8%) were females (Table 3).

In this study the incidence of biofilm in acute ulcer was 35.55% (18/45, 40.0% in Honey group & 14/45, 31.1% in debridement group, (Table 4) and chronic ulcer was 65.55% (27/45, 60% in honey group & 31/45, 68.9% in debridement group, (Table 4). This is similar to the study done by James GA et al[64], where the incidence of biofilm was 6% in acute ulcers and 60% in chronic ulcers and to study done by SR Swarna et al[65], where the incidence of biofilm was 70% in chronic ulcers and 6% in

acute ulcers and with study done by Anand SR et al[66] where incidence of biofilm was 6.7% in acute ulcer and 93.3% in chronic ulcers.

In this study, organisms isolated were S.aureus (53.3% in Honey & 46.7% in Debridement group), Klebsiella pneumoniae (48.9%, 37.8%), Pseudomonas aeruginosa (35.6%, 48.9%), Citrobacter species (15.6% in honey group) and E.coli (11.1% in debridement group) (Table 7). In a study done by SR. Swarna etal[65] with a sample size of 62, showed S.aureus (29.26%), E.coli (19.51%), P. aeruginosa (19.51%), K.pneumoniae (4.87%), Proteus species (4.87%), Acinetobacter species (4.87%), Citrobacter species (2.43%) (Table 7).

In this study the most common organism isolated was S.aureus 50% (53.3% in honey group &46.7% in debridement group) followed by Klebsiella pneumonia 43.3% (48.9% in honey group & 37.8% in debridement group) which is consistent with a study done by Thomsen TR et al[67] in 2010, where the most common organism isolated was staphylococcus aureus and Study done by Anand SR et al[66] 2014, the commonest organism isolated was S.aureus and P.auruginosa.

In this study, most common organism isolated from the chronic ulcer was S.aureus 55.6% & 51.6% in both honey and control group respectively followed by Klebsiella pneumonia 44.4% & 51.9% in honey and control group (Table 7).

In this study, 42.2% in honey and 26.7% in debridement group had healthy granulation tissue within 11-15days and 16-20days respectively. The mean time for appearance of healthy granulation tissue was 14.7±5.4days in honey group and 17.9±7.5days in debridement group which was statistically significant (p=0.025) (Table 10&11) which is similar to a study done by Anand SR et al[66] 2014, the mean duration of granulation tissue by topical application of honey was 18.1±5.5days and

another study done by Subramanyam M,[68] showed Honey dressing significantly stimulated the rate of burn wound healing as demonstrated by formation of granulation tissue and reduction in wound size especially after 21 and 28 days after burn, whereas in another study by H. Maghsoudi[69] et al, showed Clinical evidence of granulation tissue formation and epithelialization of raw areas were observed in comparative study between 42 patients in honey group and 36 patients in Mafenide acetate group by day 7. In honey-treated patients, all the wounds healed by day 21 (100%) compared to 42 patients (84%) ($p< 0.001$) in the Mafenide acetate treated group.

In this study, ulcers in the honey group were healed within 3weeks (50.1%) when compared to the debridement group healed within 30days (28.9%) which is statistically significant with p value 0.022 (Table 13), with mean duration of healing was 21.0±10.5 days in honey group and 24.9±10.8 days in debridement group. Where as in a study done by Sonia G[62] et al 2015, showed 31% of subjects in the honey dressing group achieved complete healing of chronic wounds at the sixth week which was compared to the wounds treated with povidone solution showed none of the wounds attained complete healing within 6weeks. Another study done by Medhi[70] et al, conducted a meta-analysis to evaluate the efficacy of honey in observational studies as well as in clinical trials in the treatment of wounds, showed complete healing within 4-12 weeks in clinical trials and within 2-9weeks in observational studies.

In this study most of the patients underwent definitive procedure split thickness skin grafting within 3weeks in honey group and within 30days in debridement group.

The mean duration of hospital stay in days in this study was 34.1±15.7 days in honey group and 36.0±15.8days in debridement group. 31.1% in honey group were discharged in less than <25days when compared to debridement group 57.8% discharged more than >30days which was significant with p value 0.004. (Table 16). In a study done by Anand SR[66] et al mean duration of hospital stay was 26.4±3.1days, whereas in a study H.Maghsoudi[69] et al in 2011, Comparison between topical honey and Mafenide acetate in treatment of burn wounds the mean hospital stay in the honey-treated group was 22 ± 1.2 days versus 32.3 ± 2 days in the Mafenide acetate group ($p < 0.005$, significant).

In this study, among diabetic patients in both the groups the mean time for the healthy granulation tissue was 16.5±4.2days and 19.5±7.3days, the mean time for healing of ulcers was 21.0±5.2days and 26.6±11.9days, the mean hospital stay was 38.8±16.6days and 40.5±15.5days in honey and control group respectively (Table 22).

CONCLUSSION

- All patients with acute or chronic wounds with biofilm were effectively managed with the topical application of honey when compared to the mechanical debridement with povidone iodine dressings with significant appearance of healthy granulation tissue, mean duration of healing of wounds and the hospital stay in the patients treated with topical honey.
- Honey dressing is more effective when compared to the mechanical debridement with povidone iodine dressing in achieving complete healing, reducing the hospital stay and increasing the comfort (i.e repeated debridement under local or spinal anesthesia and cost and pain will be more in subjects with debridement) to the subjects with chronic wounds.
- However, additional successful clinical evidence is required with validated laboratory findings to establish honey as one of the most effective alternative topical medicines for treating chronic wounds.
- There were no side effects or reactions found in subjects treated with honey except the pain which was due to low P^H of honey.

SUMMARY

- This study was done in Shri B.M Patil medical college in the department of general surgery during the study period of October 2015 to June 2017, to compare the efficacy of topical application of honey dressings with the debridement in an ulcers with the biofilm. Total of 90pateints with ulcers having biofilm were included in the study and randomly divided into Honey group and Debridement group with 45 patients in each group. Regular dressings were done with topical application of honey in the honey group and debridement with application of povidone solution dressing was done in debridement group depending on the soakage of wound. Definitive treatment was done once the culture sensitivity was sterile and all the patients were discharged once the wound heels completely.
- In our study, we found
  - The mean age was 49.8±19.0yrs in honey group and 53.4±17.5yrs in debridement group with highest number of patients were in the age group of 61-75yrs in honey group and 45-60yrs in debridement group with male predominance in both the groups (82.2%).
  - Most of the ulcers were chronic ulcers in both groups 60% in honey group and 68.9% in debridement group.
  - Organisms isolated were S.aureus (53.3% in Honey & 46.7% in Debridement group), Klebsiella pneumoniae (48.9%, 37.8%), Pseudomonas aeruginosa (35.6%, 48.9%), Citrobacter species (15.6% in honey group) and E.coli (11.1% in debridement group).

- The mean duration of appearance of healthy granulation tissue was 14.7±5.4days in honey group and 17.9±7.5days in debridement group with significant p value=0.025.
- The most of the patients underwent split skin grafting once the wound is healthy and the mean time for healing of wounds with biofilm was 21.0±10.5days in honey group and 24.9±10.8days in debridement group.
- Most of the patients in the honey group was discharged in less than 25days where as in debridement group discharged after more than 30days with significant p value of 0.004 with Mean duration of hospital stay was 34.1±15.7days and 36.0±15.8days in honey and debridement groups respectively.

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ANNEXURES

ETHICAL CLEARANCE CERTIFICATE

B.L.D.E.UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR – 586103
INSTITUTIONAL ETHICAL COMMITTEE

NO/58/2015
20/11/15

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 17-11-2015 *at* 03 pm *scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has accorded Ethical Clearance.*

Title "Efficacy of Honey dressing versus Mechanical debridement in healing of ulcers with biofilms a Comparative study"

Name of P.G. Student : Dr. A. Suryaprakash Reddy
Dept of Surgery.

Name of Guide/Co-investigator : Dr Tejaswini Vallabha
Prof & HOD.

DR.TEJASWINI VALLABHA
CHAIRMAN

Following documents were placed before E.C. for Scrutinization:
1)Copy of Synopsis/Research Project
2)Copy of informed consent form.
3)Any other relevant documents.

CHAIRMAN
Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,BIJAPUR-586103.

INFORMED CONSENT FORM:

TITLE OF THE PROJECT	Efficacy of honey dressing vs mechanical debridement in healing of ulcers with biofilms. A comparative study
PG GUIDE	**DR. TEJASWINI VALLABHA$_{M.S}$** M.S. General Surgery PROFESSOR & HOD Department of Surgery
PRINCIPAL INVESTIGATOR	**DR. A. SURYAPRAKASH REDDY**

PURPOSE OF RESEARCH:

I have been informed that this study will help in comparing the efficacy of honey in eradication of biofilm with mechanical debridement in ulcers with biofilms. I have also been given a free choice of participation in this study. This study will help in proper management of ulcers with biofilms.

PROCEDURE:

The procedure which will be followed as explained by Dr. Suryaprakash Reddy is as follows

- The ulcer will be inspected for a transparent membrane and pus. It will be excised or pus will be collected in a sterile container and transported to the microbiology department and processed immediately. If the specimen is not available then the dead tissue will be collected with sterile swab and processed immediately.
- After the detection of biofilm the ulcer dressing will be done either with honey soaked sterile gauze or by mechanical debridement and ulcer will be packed with sterile pads.

RISK AND DISCOMFORTS:

I understand that I may experience some pain and discomforts during the examination or during my treatment. This is mainly the result of my condition and the procedures of this study are not expected to exaggerate these feelings which are associated with the usual course of treatment.

BENEFITS:

I understand that my participation in the study will help to study efficacy of honey dressing versus mechanical debridement in ulcer healing with biofilms in patients treated in the hospital.

CONFIDENTIALITY:

I understand that the medical information produced by this study will become a part of hospital records and will be subject to the confidentiality. Information of sensitive personal nature will not be part of the medical record, but will be stored in the investigations research file.

If the data are used for publication in the medical literature or for teaching purpose, no name will be used and other identifiers such as photographs will be used only with special written permission. I understand that I may see the photograph before giving the permission.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study to Dr. A. Suryaprakash Reddy in the Department of General Surgery who will be available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of the study, which might influence my continued participation. A copy of this consent form will be given to me to keep for careful reading.

REFUSAL FOR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice. I also understand that Dr. A. Suryaprakash Reddy may terminate my participation in the study after he has explained the reasons for doing so.

INJURY STATEMENT:

I understand that in the unlikely event of injury to me resulting directly from my participation in this study, if such injury were reported promptly, the appropriate treatment would be available to me. But, no further compensation would be provided by the hospital. I understand that by my agreements to participate in this study and not waiving any of my legal rights.

I have explained to _____the purpose of the research, the procedures required and the possible risks to the best of my ability.

_____ _____

Dr. A. SURYAPRAKASH REDDY Date

(Investigator)

WITNESS SIGNATURE

1)

DATE

2)

DATE

STUDY SUBJECT CONSENT STATEMENT:

I confirm that Dr. Suryaprakash Reddy has explained to me the purpose of research, the study procedure, that I will undergo and the possible discomforts as well as benefits that I may experience in my own language. I have been explained all the above in detail in my own language and I understand the same. Therefore I agree to give consent to participate as a subject in this research project.

_____ _____

(Participant) Date

_____ _____

(Witness to signature) Date

PROFORMA FOR CASE TAKING

SL NO: IP NO: H/M

Name: UNIT:-

Age/Sex: DOA:-

Religion: DOS:-

Occupation: DOD:-

Address:

Mobile No:

Chief complaints**:**

History of presenting complaints**:**

Past history:

Comorbidities:

PERSONAL HISTORY:

Diet: Appetite: Bowel/Bladder:

Sleep: Digestion: Habits:

GENERAL PHYSICAL EXAMINATION:

Built: Well/Moderate/Poor

Nourishment: Well/Moderate/Poor

Pallor/Icterus/Cyanosis/clubbing/pedal oedema/ lymphadenopathy

BP: PR: RR:

Temperature: SPo_2:

SYSTEMIC EXAMINATION:

Per Abdomen:

Respiratory System:

Cardio Vascular System:

Central Nervous System:

Local examination of wound:

LABORATORY TESTS

Haemoglobin% : BT: CT:

Total Count : N L E B M

Platelets :

Blood Urea :

Serum Creatinine :

HIV : HB_SAg:

Electro Cardiogram:

Urine routine :

X- RAY of effected part:

Pus for GramStain: Pus for C/S:

Presence of Biofilm: Yes/No

FINAL DIAGNOSIS :

TYPE OF DRESSING: HONEY/DEBRIDEMENT

OBSERVATION OF THE WOUND WITH BIOFILM:

VARIABLES	DAY 5	DAY 10	DAY 15	DAY 20	DAY 25	DAY 30
FOUL SMELL						
DISCHARGE						
GRANULATION TISSUE						
SIZE OF THE ULCER						

Interventions done for the wound:

- Skin grafting:
- Delayed primary closure:
- Secondary suturing:
- Time for healing of wounds with biofilm:

INFERENCE:

REMARKS: