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# THE EFFICACY AND SAFETY OF LOCAL INSULIN INFILTRATION VS NORMAL SALINE DRESSINGS IN WOUND HEALING IN DIABETIC FOOT ULCERS

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## 1. INTRODUCTION

Diabetes Mellitus is known to man to be one of the oldest diseases. In 2015, an estimated 415 million people had diabetes worldwide, with type 2 DM making up for about 90% of the cases. This represents 8.3% of the adult population, with equal rates in both women and men. And the trend suggests that it is on the rise.[1] Diabetic foot ulcers are one of the major complications of diabetes mellitus. The vascular changes and neuropathy all contribute to changes in to the lower extremity called the diabetic foot.[2] Wound healing involves cell adhesion, migration, proliferation, differentiation and apoptosis at cellular and molecular levels.[3] Abnormalities in the factors of wound healing contribute to defective wound healing in diabetic ulcers including decreased growth factor production, angiogenic response, macrophage function, collagen accumulation, epidermal barrier function and keratinocyte and fibroblast migration and proliferation. In diabetes, ulcers fail to re-epithelize properly making them prone to infection. The inter relationship of all these factors results in gangrene and ultimately amputation. [4] Nowadays there are various types of dressings available and these serve a pivotal part in the healing of footulcers. [5] Topical dressings include one of the major modalities of care for diabetic foot ulcers. Different types of moist dressings and topical agents are used today. [6] Conventional dressings respond poorly, thereby increasing hospital stay or total duration of treatment and decreasing the quality of life. Several therapeutic modalities are available to effect wound healing such as skin grafts, hydrocolloid dressings etc. but some may not be economically suitable for the patient. [7]. Topical Insulin promote wound healing by activating serine-threonine kinase (AKT) and extracellular signal regulated protein kinase (ERK) pathway. Insulin stimulates the growth and development of different cell types and affects proliferation, migration, and secretion by keratinocytes, endothelial cells, and fibroblasts. [8] There have been numerous animal studies which have successfully proven the efficacy of insulin application locally on wounds. Few human studies have been conducted where insulin was sprayed topically over the wound surface. This doesn't standardise the amount of insulin actually remaining in contact with the wound. To overcome this, it was decided to infiltrate the wound locally in our study. The purpose of this prospective, randomized study is to investigate the efficacy and safety of local insulin infiltration in management of patients with diabetic foot ulcers.

## 2. REVIEW OF LITERATURE

The population of Diabetics is predicted to rise to 109 million cases out of a complete estimated population of 1.5 billion in India by 2035.The prevalence of an impaired glucose tolerance test (IGT) within the Indian population is around 8.7% in urban areas and 7.9% in rural areas. 35% of this population eventually develop type 2 diabetes mellitus. Hence India is facing a real crisis. [9] Type 2 diabetes is characterized by hyperglycemia in the presence of hyperinsulinemia due to peripheral insulin resistance. Diabetes is related to various complications associated with microvascular, macrovascular, and metabolic etiologies. They include cerebrovascular, cardio- vascular, and peripheral arterial disease; retinopathy; neuropathy; and nephropathy. Diabetic foot is one of the long-term complications of diabetes. Lifetime risk for foot ulcers in diabetes is 15%. Diabetes continues to be one of the most common underlying cause of non-traumatic lower extremity amputations. [10]

Mean age at diagnosis of diabetic foot and mean age for major amputations was significantly lower in Indian population as compared to Western literature. This could be the sole reason to explain favorable results seen in Indian populations especially with reference to survival at 2 years after major amputation, contralateral limb amputation rate and above knee to below knee amputation rates. Older patients reported in Western literature were more likely to have advanced atherosclerotic disease involving heart, cerebral circulation, peripheral circulation and renal circulation, thus adversely affecting mortality and contralateral limb amputation rate. Above knee amputation was more common in Western population as compared to Indian population and above knee to below knee amputation ratio was 1:2 vs. 1:17 in Western vs. Indian series.

Majority of Indian patients had an infection as a dominant feature in non-neuro ischemic foot. In such cases local debridement, control of infection and diabetes improved the rate of limb salvage. If the infection was fulminant, minor or at the most below knee amputation was enough to stop the advancing infective process. However, the Western patients were older and neuro ischemic limbs were common with advanced atherosclerosis and multi-system involvement hence making above knee amputation perhaps the right choice to reduce the overall mortality. [11]

In a population-based meta-analysis, the cost of treating foot ulcer or any patient undergoing amputations was significantly higher in the USA or Sweden when compared to India. The difference in cost of treatment between these countries is obviously due to marked economic disparity in two populations. Although cost of private treatment in India is less, majority of the patients bear the entire cost of the treatment as they are not medically insured. Hence it is imperative to find the most cost effective and yet a safe method of dressing. [12] Caroline C Naves in her study titled “The Diabetic Foot: A Historical Overview and Gaps in Current Treatment” had mentioned three principles of treatment of ulceration of the foot which included sharp debridement, off-loading pressure, and education about foot care and footwear. [13] Patrick Laing in his study “The development and complications of diabetic foot ulcers” showed that Neuropathy and ischemia, the two common complications of diabetes mellitus, are the primary underlying risk factors for the development of foot ulcers and thereafter the complications. [14]

### 2.1 About Local Insulin Infiltration

The biological effects of local insulin application have been suggested to be associated with several molecular mechanisms:

Research consistently highlights the importance of the insulin receptor, a transmembrane molecule, activated by insulin, IGF-I and IGF-II. It belongs to a large class of tyrosine kinase receptors found in all cell types, including keratinocytes and fibroblasts [15]. Liu, et al. [16] showed that topical application of insulin to wounds stimulates keratinocyte migration. This migra¬tory enhancement involves the PI3K-Akt pathway, and identifies Rac1, a small GTPase, as a molecule activated downstream of PI3K-Akt [17]. Activation leads to translocation of Rac1 to the plasma membrane, followed by activation of Rac1 substrate, the integrin α3 and the extracellular matrix molecule laminin332 [18]. The effects of insulin on keratinocyte migration led

to propositions that insulin-accelerated wound healing involves in¬creased expression of the integrin α3β1 in keratinocytes as well as an increase in the levels of LN332. The latter molecule is a matrix protein secreted by migrating keratinocytes at the leading edge [19], where it mediates kera¬tinocyte polarity and cell migration [20]. After a traumatic skin event, quiescent epidermal keratinocytes are activated and ex¬press α6β4 and α3β1 integrins, which control their migration on LN332 and facilitate the development of the basal membrane. So, insulin stimulates keratinocyte integrin α3 expression and LN332 deposition, and that suppression of these proteins in vitro or in vivo inhibits insulin-induced keratinocyte migration and wound healing, strongly suggesting a critical role for these molecules in the action of insulin to stimulate healing. Howev¬er, the relationship between LN332 and cell migration remains controversial. Some studies have implicated LN332 in inhibiting cell migration [21], whereas others support a role for LN332 in promoting keratinocyte migration [22].

Expression of the insulin receptor, IRS-1, IRS-2, ERK and Akt are increased in the tissue of wounds compared to intact skin, suggesting that the insulin signalling pathway may have a critical role in this process. Akt, in particular, can phosphorylate proteins that regulate cell survival, lipid and glycogen synthesis [23,24]. Recently, data demonstrate that Akt activation is an important step for VEGF release in skin wounds, through a post-transcrip¬tional mechanism in keratinocytes [25,25], and is necessary for vascular maturation and angiogenesis during cutaneous wound healing [26]. These various pathways are inhibited in the injured skin of diabetic rats and correlate with a delay in the time re¬quired for complete wound healing. Lima et al. [27] showed that insulin signalling pathways are promoted in the injured skin of normal rats, whereas these pathways are attenuated in diabetic animals due to insulin deficiency. However, when injured skin of diabetic rats is treated with a topical insulin cream, an acceler¬ation of wound healing occurs, together with a recovery in the proteins of the insulin signalling cascade [28]. Therefore, expres¬sion of proteins involved in the early phase of insulin exposure, namely, IRS-1,2 and Akt, are increased in healing tissue when compared to healthy skin.

Insulin stimulation of ERK involves the tyrosine phosphory¬lation of IRS proteins, which in turn interact with the adapter protein, Grb2 (growth factor receptor-bound protein-2), recruit¬ing SOS exchange protein to the plasma membrane for activation of Ras (one member of a large family of small molecular weight GTP-binding proteins) [74].Once activated, Ras acts as a molec¬ular switch, stimulating a serine kinase cascades through the stepwise activation of Raf, MEK (protein kinase that activates MAP kinases) and ERK. Activated ERK can translocate into the nucleus, where it catalyses the phosphorylation of transcription factors, stimulating a transcriptional program that leads to cel¬lular proliferation or differentiation [29]. The protein levels of ERK are increased in injured skin, suggesting that the ERK sig-nalling pathway can also play a direct role in the regulation of cellular growth and differentiation. It is important to emphasise that ERK activation is necessary for keratinocyte pro-migratory signalling pathways [30,31]. It is now well established that an increase in the migration of endothelial progenitor cells from bone marrow to wounded skin accelerates wound healing [32]. The regulation of this process is complex and involves activation of eNOS in the bone marrow by VEGF that is produced in the site of injury, enhancing the mobilisation of endothelial progen¬itor cells, which are recruited to the cutaneous wound site by an increase in tissue levels of SDF-1α [33].

Other proposed mechanisms of action for insulin in wound healing exist, such as the enhancement of expression of neutro¬phil adhesion molecules to reinforce the cellular functions of migration, phagocytosis and bactericidal actions [34]. Although neutrophils act as traumatic scavengers and help wound healing in some ways, neutrophils also have a negative impact on wound healing, particularly when excess or hyper-functional neutrophils accumulate in the wound environment. Suppression of neutrophil infiltration could promote wound healing. Chen, et al. [35] provided observations regarding the number and func¬tion of neutrophils with or without low-dose topical insulin and a preliminarily investigation of the underlying mechanisms. It was shown that topical insulin application decreases neutrophil infiltration by inhibiting MIP-

2 expression and advanced neutro¬phil resolution. So, insulin regulates the inflammatory response during wound healing and findings support local and systemic administration of insulin could be an effective treatment for skin wound incisions [36].

## 3. AIM AND OBJECTIVES

**Aim:** To study the efficacy and safety of local insulin infiltration vs normal saline dressings in wound healing in diabetic foot ulcers

**Objectives:**

(i) Primary objective: to study the efficacy of local insulin infiltration vs normal saline dressings in wound healing in diabetic foot ulcers

(ii)Secondary objective: to assess the safety of local insulin infiltration

## 4. MATERIALS AND METHODS

(i) Study Setting: This study was carried out in Sagar Hospital, a tertiary referral healthcare in Jayanagar, Bangalore.

(ii) Study Duration: This study was carried out for period of 1 and 1/2 years from December 2018 to May 2020.

(iii) Study Design: prospective, randomized control study

### *4.1* ***Inclusion Criteria***

1.Age group above 18 years.
2.Diabetic patients with a foot ulcer of Wagner's grade I and II.
3.Written and informed consent.
4.Type I or type II diabetics

### *4.2* ***Exclusion Criteria***

1.X-Rays showing features of osteomyelitis. (Wagner's Grade III and above)
2.Presence of Peripheral Vascular Disease
3.Presence of Ischemic Heart Disease
4.Any known immunological disorders, connective tissue disorders or malignancies.

### *4.3 Study Population*

Patients with diabetes mellitus (inpatients or outpatients) with a foot ulcer (Wagner Grade 1 and 2) and willing to take part in the study were included.

### *4.4 Method*

A detailed history was taken for all patients presenting with a diabetic foot ulcer (inpatient as well as outpatient department) including mode of onset of ulcer, duration and progress of ulcer. Also, information about their current status of Diabetes in terms of duration, associated comorbidities if any, history of smoking and medication history in terms of use of OHA (Oral Hypoglycemic Agents) or Insulin and other drug history was taken. The patients were put through the following set of investigations that included:

(i) Complete blood count (CBC)

(ii) Random blood glucose (RBS)

(iii) Blood urea nitrogen (BUN)

(iv) Creatinine

(v) HbA1c

(vi) Liver Function Test

(vii) X-ray of the involved foot

(viii) Doppler Study to rule out PVD

(ix) Coagulation study

(x) Wound Swab / Pus for culture and antibiotic sensitivity

Patients who were satisfying the inclusion and exclusion criteria and consented for taking part in the study underwent initial wound management in terms of debridement (cleaning of wound, removal of necrotic debris, frank slough, pus and any foreign body). Surgical debridement of dirty wounds (either in outpatient basis or operation theatre) was done and once the wound was rid of all dead, necrotic material and pus, the wound was considered ready and actual intervention as per the study was initiated. The time required for preparing the ulcers, that is the time from the day of first visit till initiation of study was considered as wound preparation time and was not considered in final calculation of time to wound healing. If a patient was anemic, appropriate correction measures were taken. Other factors that may directly or indirectly affect the study such as low levels of albumin and azotemia were corrected as far as possible prior to initiation of study and aim was made to keep them reasonably under control during the entire course of the study. All patients were treated empirically with Cap. Amoxicillin-Clavulanic acid 625mg after taking a wound swab and sent for culture and sensitivity and later antibiotics were changed according to pus culture and sensitivity reports.

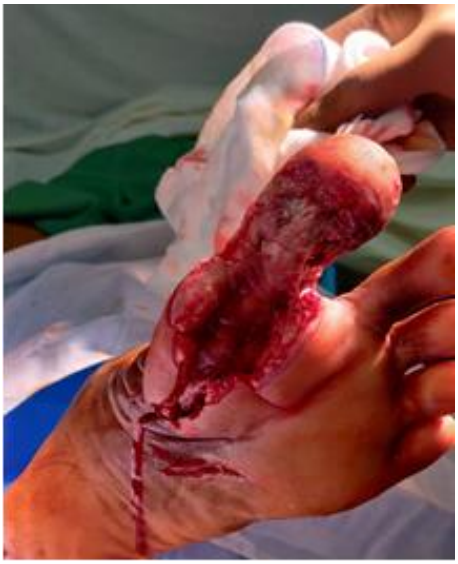


**Fig. 33: Diabetic Foot Ulcer**

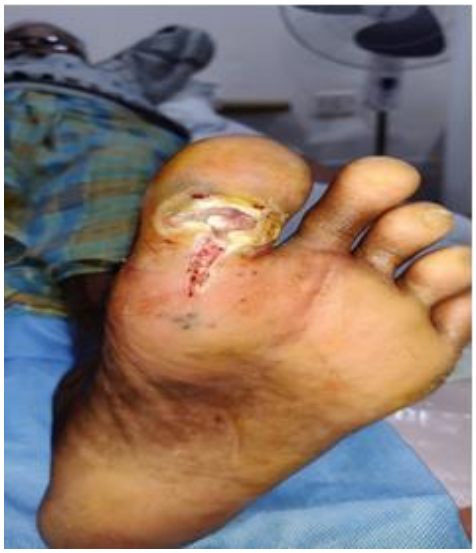


**Fig. 34: Extensive wound debridement done**

Thereafter all the participants were randomly allotted into two groups (Insulin and Normal Saline) by using the Random Number Generator ( www.randomizer.org).

Wounds area was measured using a tracing paper placed on the wound to mark the wound borders. The two largest perpendicular diameters were measured using a ruler in cm. To calculate the wound area, these two diameters were multiplied to obtain area of ulcer in cm2.

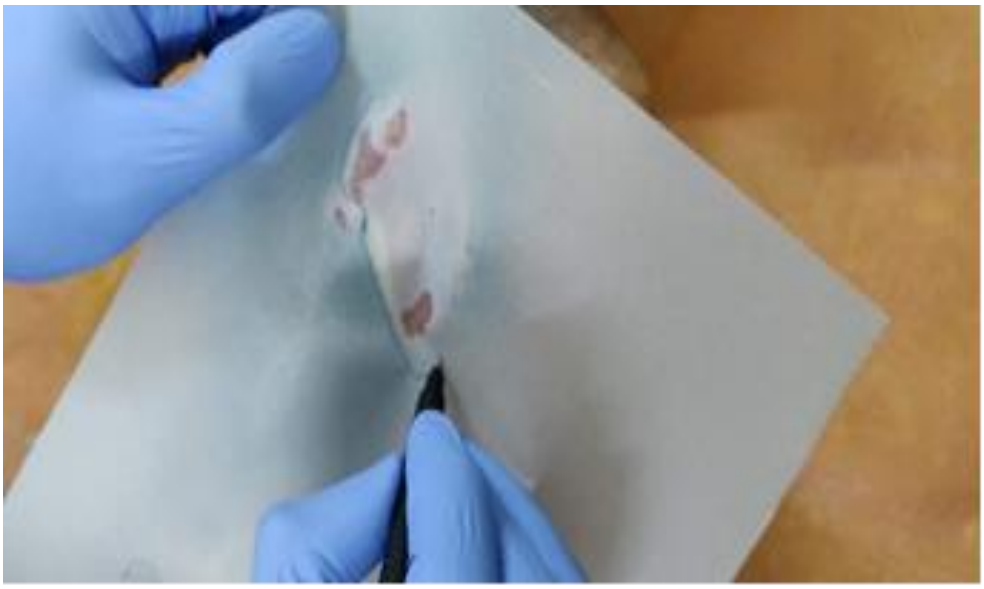


**Fig. 35: Wound border being traced with a tracing paper**

In case of Insulin Group, the ulcers were cleaned 1st with betadine and then thoroughly washed off with normal saline and left to dry. Following which the wound was infiltrated with 10 units (0.25 ml) of human soluble insulin (Actrapid® - 40IU/ml) mixed in 1 ml normal saline (0.9%) for each 10 cm2 of wound. The solution was prepared after determining area of the wound and dose calculated accordingly following which it was injected into the ulcer with an insulin syringe and then covered with sterile cotton gauzes. For wound areas less than 10 cm2, a standard dose of 5 units (0.1ml) of insulin Actrapid® was mixed with 1 ml of normal saline and infiltrated into the wound. A standard 5-point infiltration technique was followed for all patients according to the schematic shown below.

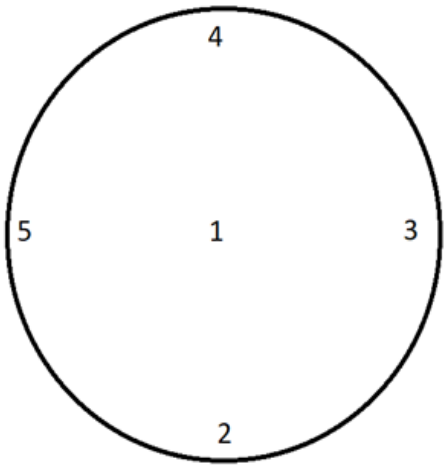



**Fig. 36: Schematic diagram for infiltration of insulin into the wound**

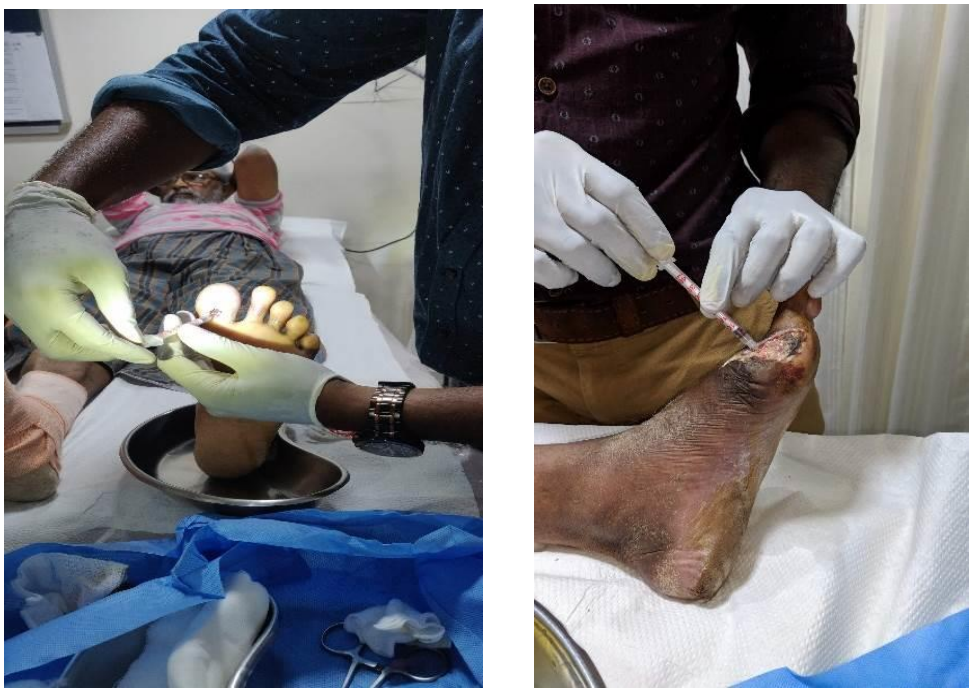


**Fig. 37: Insulin infiltration into the wound using insulin syringe**

In Normal Saline group, the ulcers were first cleaned with betadine followed by normal saline without insulin and covered with a normal saline soaked sterile gauzes.

**Fig. 38: Wound being cleaned with betadine and normal saline**

Alternate day dressings were done for both study groups. The recordings were carried out at the end of each week of therapy for a period of 4 weeks or till ulcer had healed completely, whichever was earlier.

**Fig. 39: Normal saline soaked gauze being applied on wound**

For measuring safety profile of injecting insulin into the wound, the capillary blood glucose was measured immediately before administration of insulin and then repeated 2 hours after administration. Documented hypoglycemia or more than 25% drop of peripheral blood glucose from pre administration levels was taken as significant. During the time post administration, the patient was observed for any signs of hypoglycemia and if present, all arrangements were kept to manage it accordingly. Patients were also explained about symptoms of hypoglycemia and was told to call the investigator immediately if any of the symptoms appeared after 2 hours. In case of in patients, the ward sisters on duty were also explained to look out for symptoms of hypoglycemia and inform regarding the same.

• **SAMPLE SIZE:** The prevalence of diabetic foot ulcer was estimated to be 15% of all diabetic patients. [1,2]

The sample size (n) was estimated to be 50. This was calculated using the following formula

$$n = \frac{z^2 pq}{d^2}$$

Wherein z (at 95% confidence levels) = 1.96
p (estimated prevalence of diabetic foot ulcer) = 0.15
q (1-p) = 0.85
d (Precision taken) = 10% = 0.1

• Using this formula, the sample size came up to 50:

• **STATISTICAL ANALYSIS:** The data collected was entered in Microsoft Excel and analyzed using SPSS software 25. The primary end point was complete healing of the ulcer. Analysis was performed on the intent-to-treat sample. The categorical demographic data like sex, age categories, history of smoking and whether on OHA or insulin were expressed as proportions. Continuous data like age, duration of diabetes, duration of foot ulcer was measured as mean and standard deviation. The comparison of effectiveness between the cases and controls based on area and reduction of area at different time intervals were measured using an independent t test. The effect of levels of albumin, hemoglobin, HbA1C, total count, duration of diabetes and duration of ulcer between the cases and controls were assessed using an independent t test. A p value of less than 0.05 was considered as statistically significant.

• **ETHICAL CONSIDERATIONS:** Ethical and Scientific Committee clearance was taken before initiation of the study. Various other insulin studies had already showed that spraying of insulin over the wound was safe. However, since this study involved injecting of the insulin into the wound, for safety considerations, the patients were observed for hypoglycemic episodes after administration of insulin until 2 hours of administration. They were explained all the symptoms of hypoglycemia and told to contact the investigator if the symptoms did appear. All the patients enrolled for the study had provided written consent which explained the reason for the study, duration of the study and the possible complications they might face. All patients were provided the contact number of the investigator in case of any queries or difficulties.

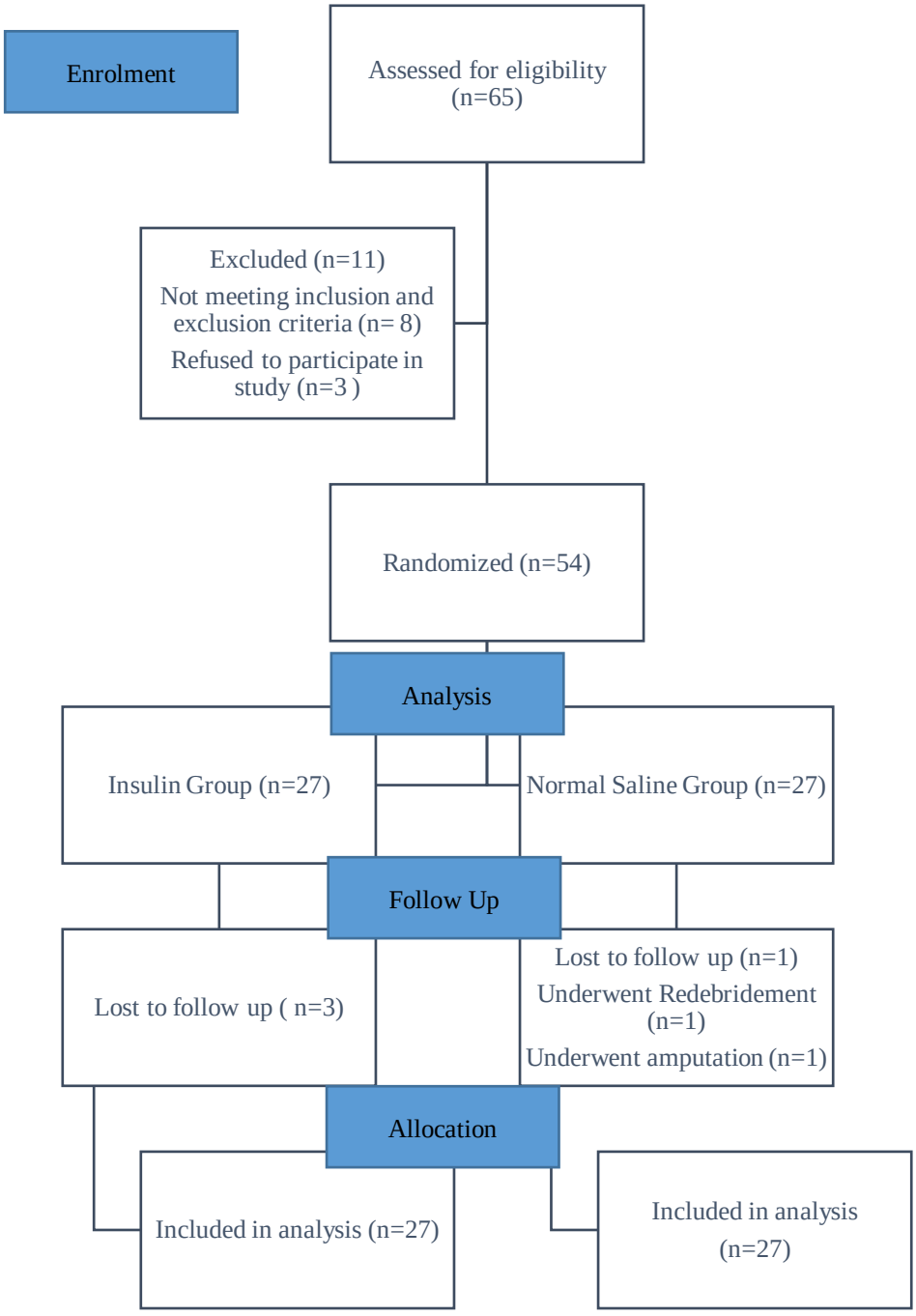



**Fig. 40: CONSORT Diagram**

## 5. RESULTS

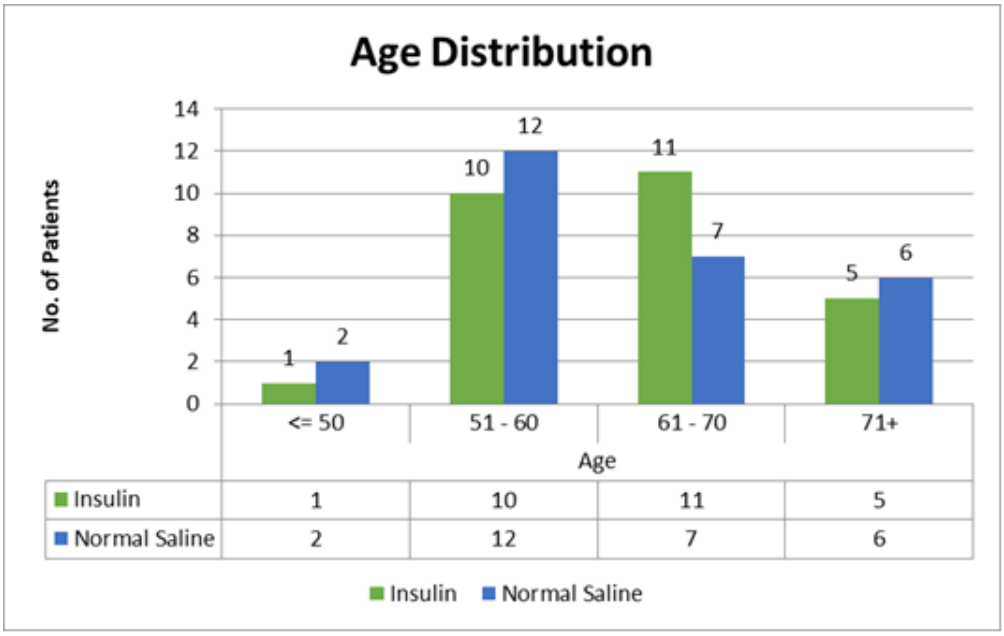
This was a prospective randomized controlled clinical study carried out in Sagar Hospital, a tertiary referral healthcare centre in Jayanagar, Bangalore This study was carried out for a period of 1 and 1/2 years from December 2018 to May 2020.

A total of 65 patients were assessed for eligibility in the trial, out of which 8 patients were excluded as they failed to meet the inclusion and exclusion criteria. 3 of the patients were diagnosed to have underlying osteomyelitis on X ray and 5 patients were diagnosed with underlying peripheral vascular disease on Doppler Studies. 3 patients refused to participate in the study. 54 patients were finally randomized for the trial into two groups with 27 patients in insulin group and 27 patients in normal saline group.

### 5.1 Demographic Assessment

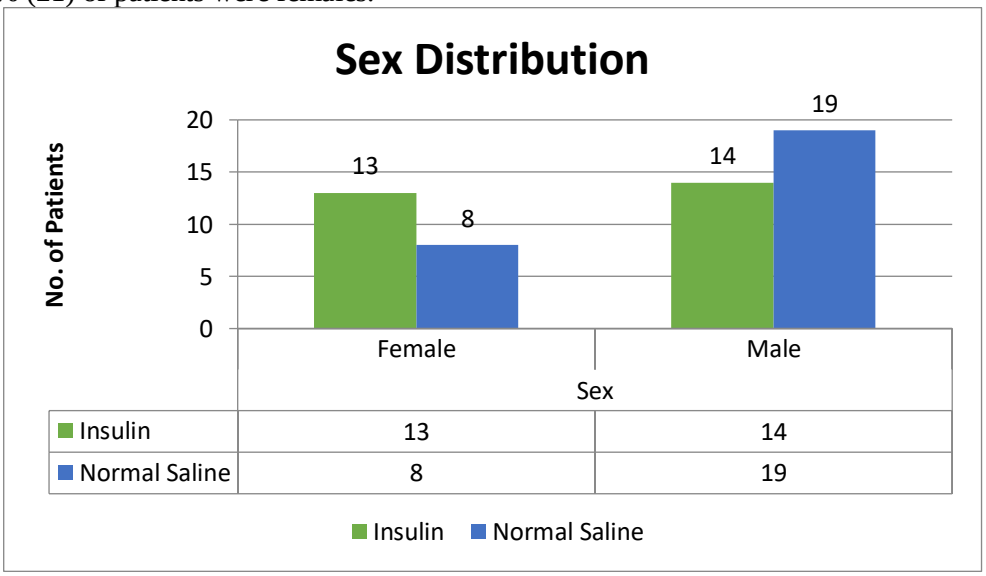
The following graph shows the age wise distribution of patients in our study group.

The age distribution in our study group varied from 47 years to 80 years. 74% of the study population was within age group 51-70 years. The mean age of the study population was 63.040 ± 8.877 for insulin group and 62.590 ±8.697 for normal saline group.



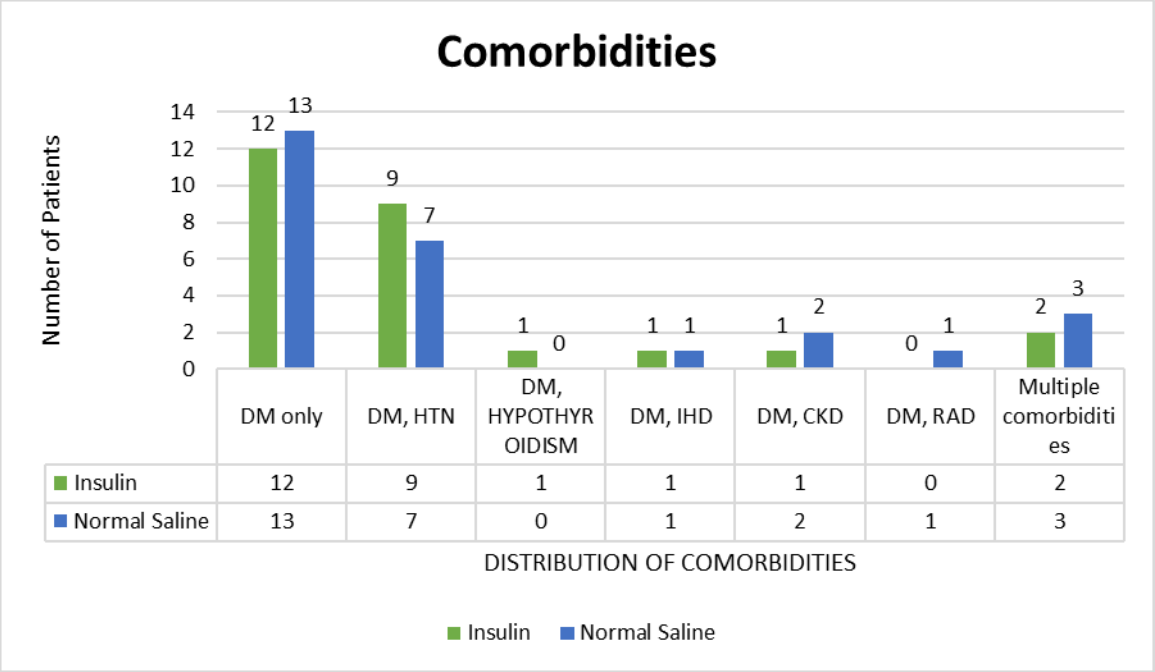

**Graph 1: Age wise distribution of patients**

The graph below shows the gender wise distribution of patients in our study group. 61% (33) of patients were males and 39% (21) of patients were females.



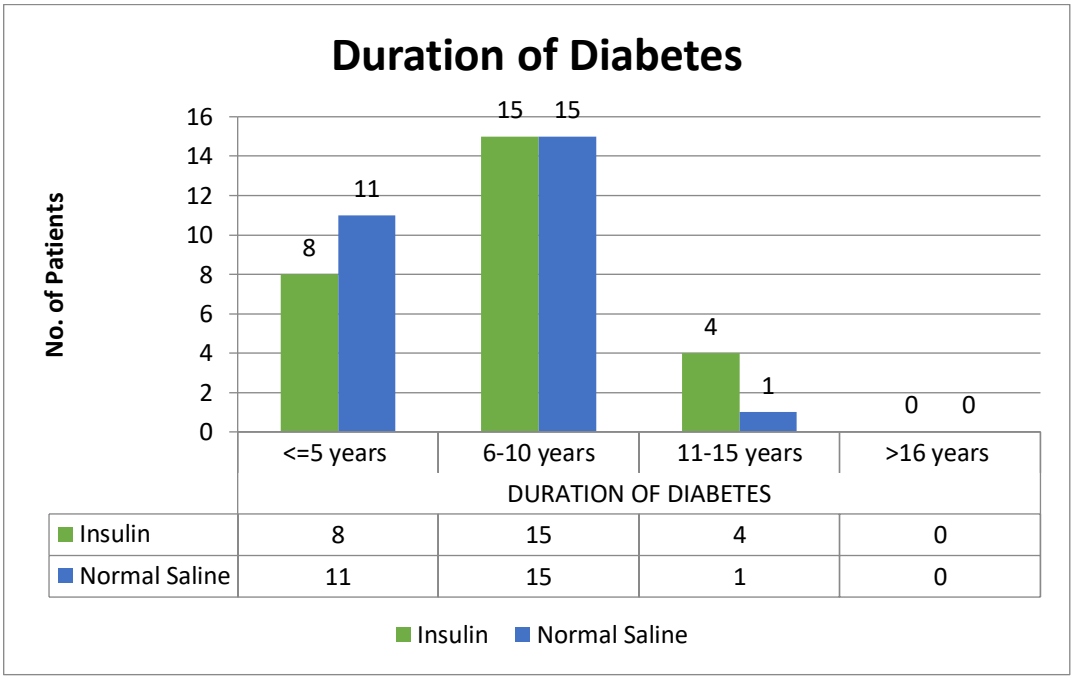

**Graph 2: Gender wise distribution of patients**

The graph below shows the distribution of co-morbidities among the patients in our study group.



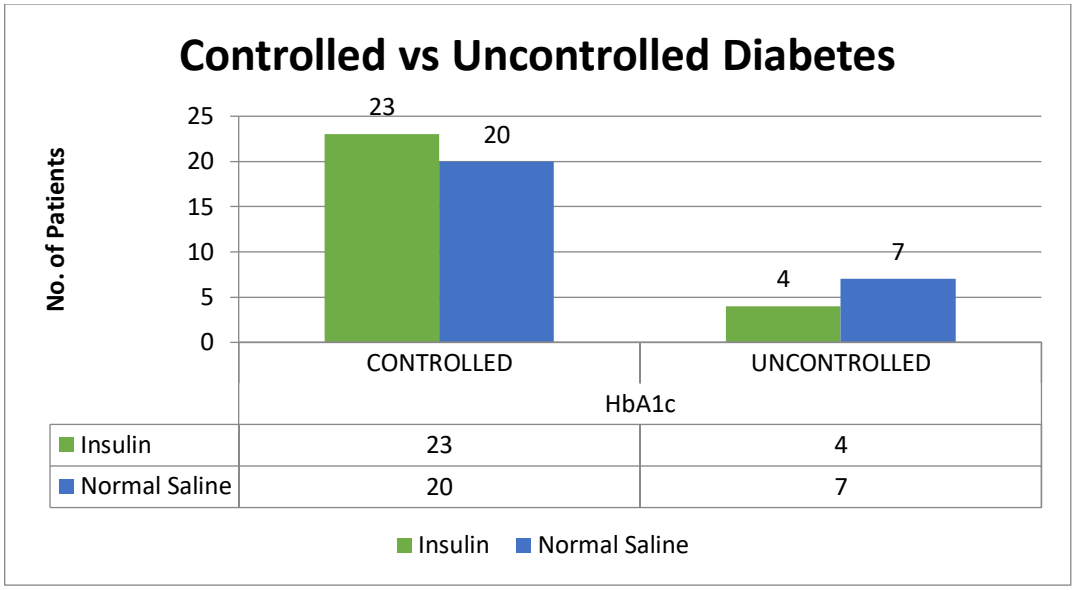

**Graph 3: Co-morbidity wise distribution of patients**

In terms of duration of Diabetes Mellitus in our study population, 4 patients in the insulin group and 1 patient in the normal saline group had a duration of Diabetes more than 10 years.



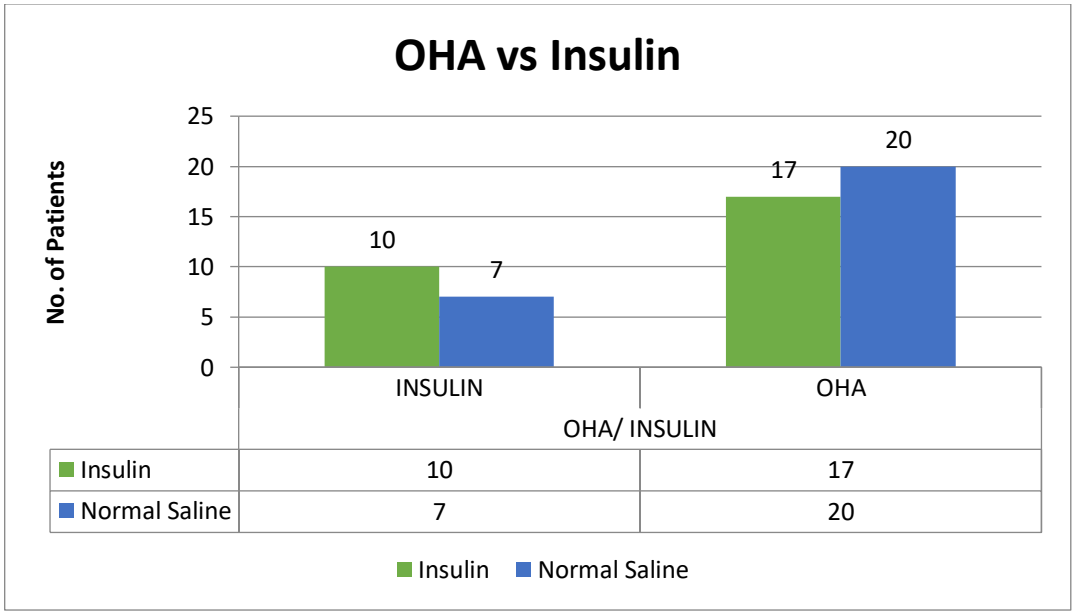

**Graph 4: Duration of Diabetes Mellitus**

In terms of whether the patient's Diabetes was controlled or uncontrolled by levels of HbA1c, 79.6% patients had controlled diabetes. (HbA1c ≤ 7.5)



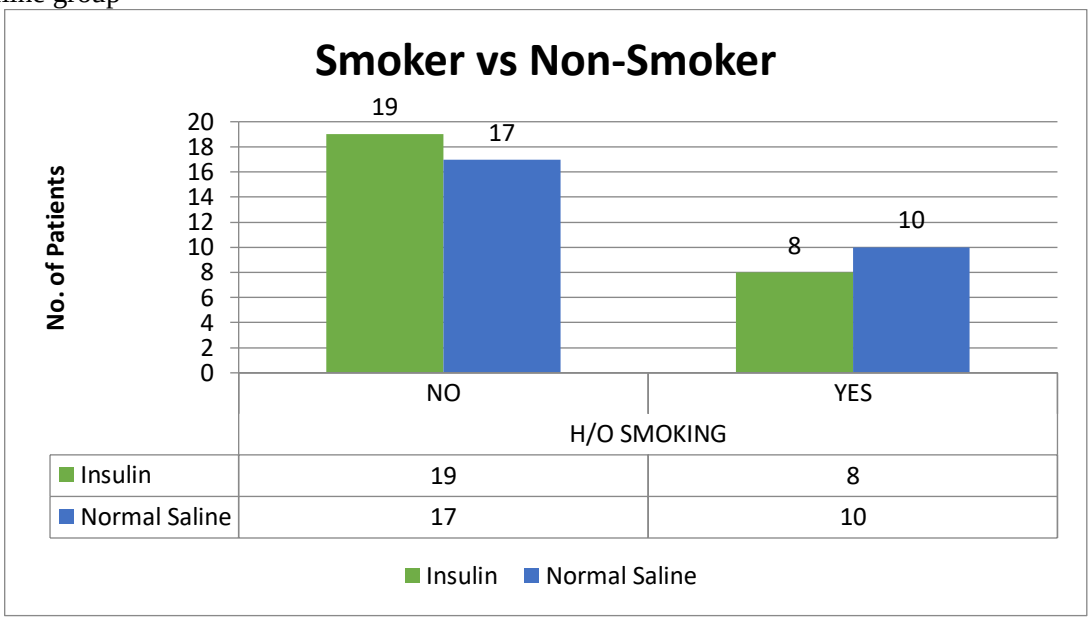

**Graph 5: Controlled vs Uncontrolled Diabetes**

68.5% of patients were on oral hypoglycemic agents (OHA) for diabetes control while 31.5% patients were on insulin.



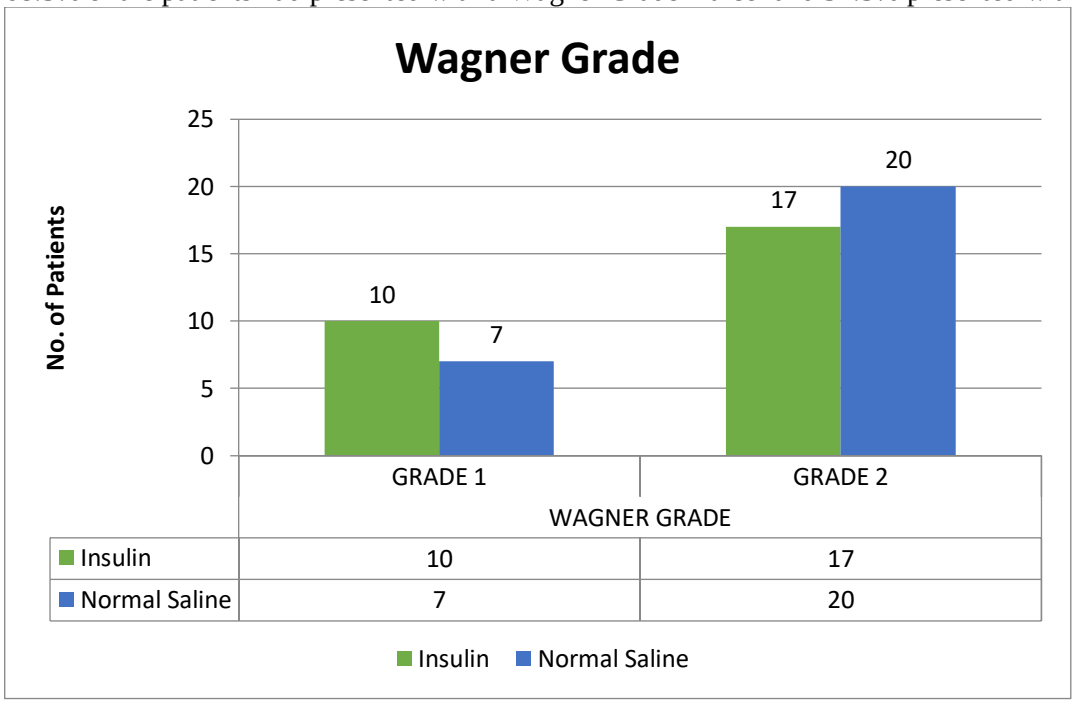

**Graph 6: OHA vs Insulin**

34% of the study population were smokers out of which 45% belonged to the insulin group and 55% belonged to normal saline group



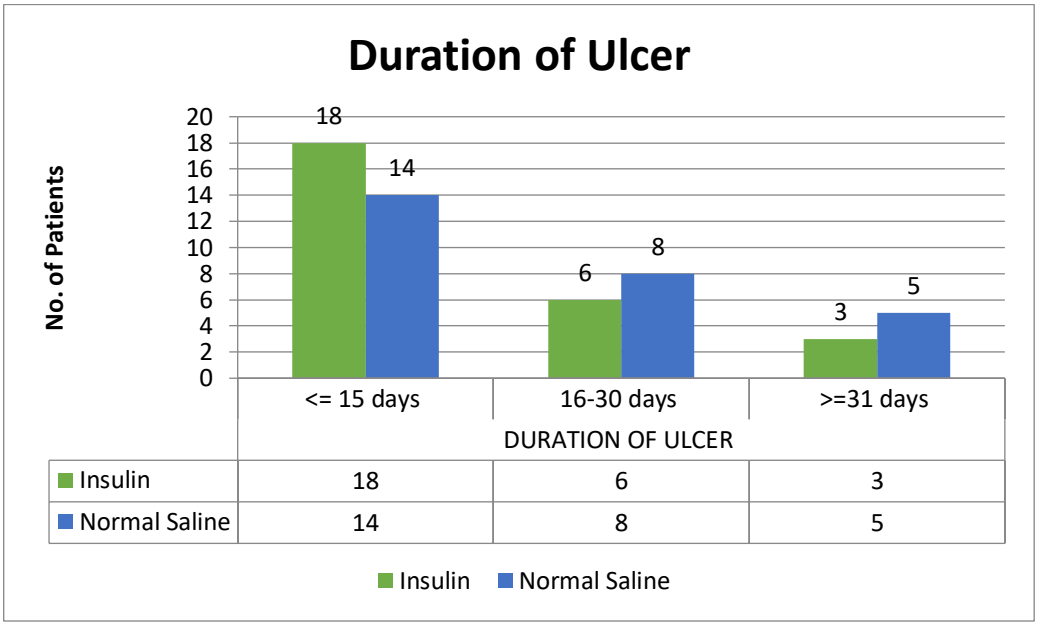

**Graph 7: Smoker vs Non-Smoker**

- 68.5% of the patients had presented with a Wagner Grade 2 ulcer and 31.5% presented with a Grade 1 ulcer.



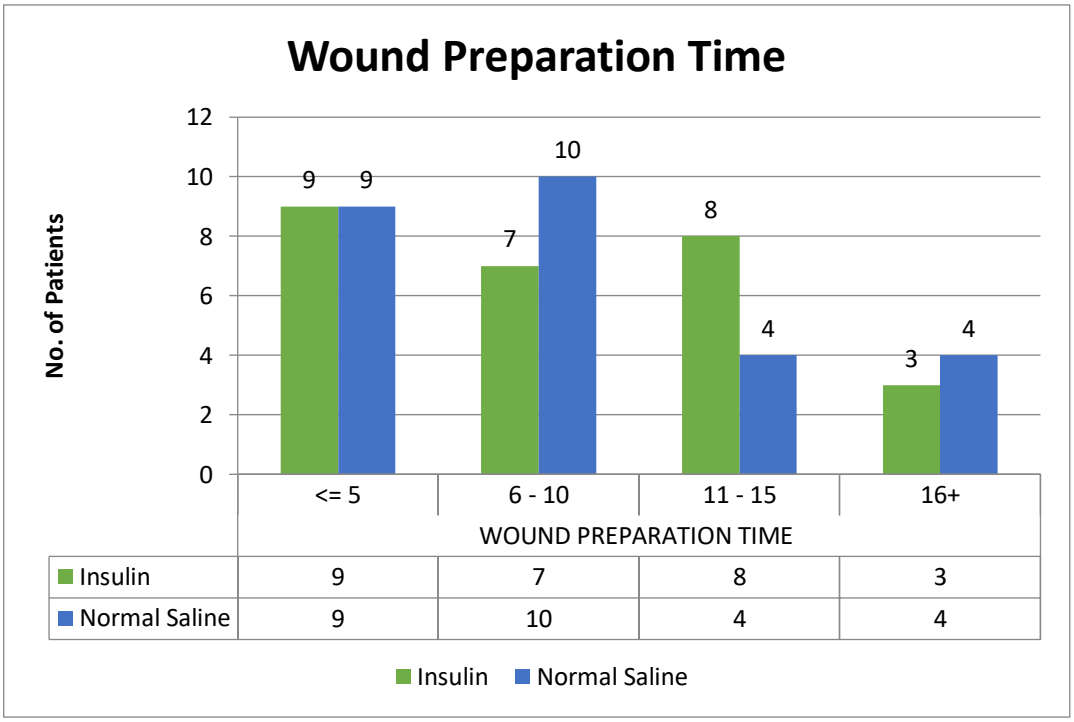

**Graph 8: Number of patients with Grade 1 and Grade 2 ulcer**

In terms of duration of ulcer, 59.3% of the study population had an ulcer for 2 weeks and 14.8% had an ulcer that had not healed for more than 1 month.




**Graph 9: Duration of Ulcer**

87% of the study population had a wound preparation time that lasted up to 15 days. Only 7 patients required a prolonged wound preparation time. The main reason for a prolonged wound preparation time for these 7 patients was because of various factors that included persistent pus discharge despite debridement that warranted re debridement.




**Graph 10: Wound Preparation Time**

When we compare the two groups of patients in terms of baseline characteristics, we can see that the two groups are comparable.

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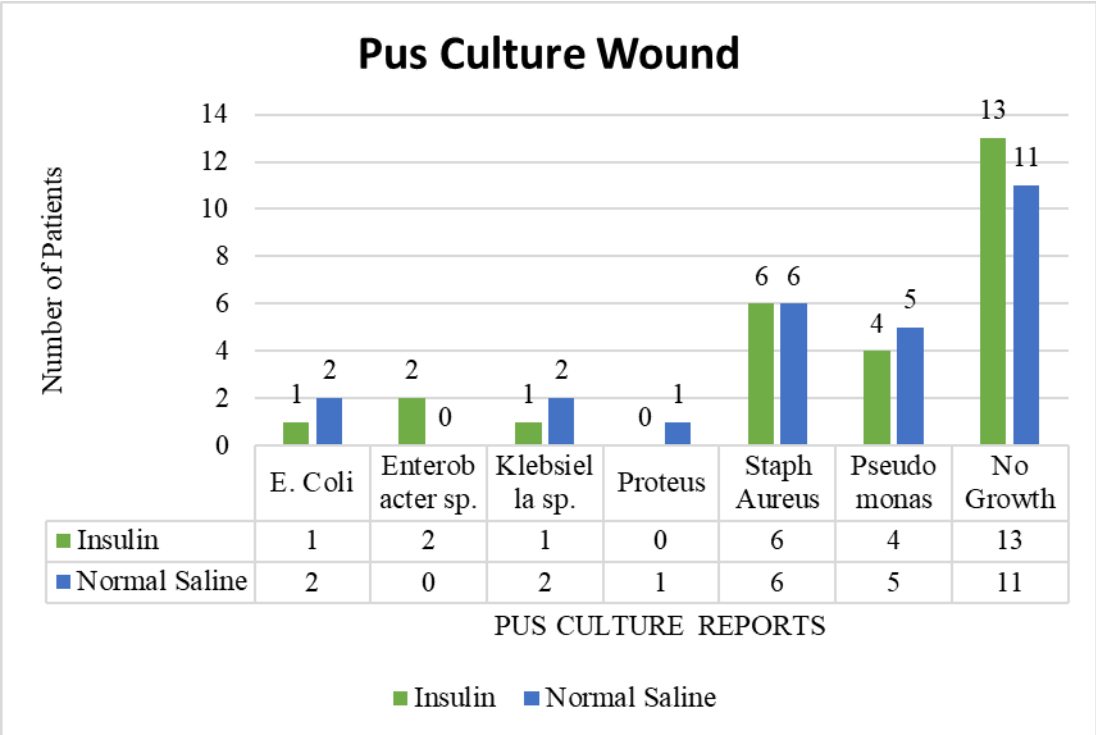
**Table 4: Comparison of Baseline Characteristics between groups**

| | Insulin Group | Normal Saline Group | P Value |
|---|---|---|---|
| Age (Mean) | 49-80 (63.04) | 48-79 (62.6) | P= 0.853 |
| Sex Ratio (M: F) | 14:13 | 19:8 | P= 0.163 |
| Duration of Diabetes (Mean) | 7.48 years | 6.77 years | P= 0.321 |
| Patients with uncontrolled Diabetes | 4 | 7 | P= 0.311 |
| OHA: Insulin | 17:10 | 20:7 | P= 0.379 |
| Smokers | 8 | 10 | P= 0.564 |
| On Anticoagulant/Antiplatelets | 5 | 2 | P= 0.224 |
| Patients with Wagner 2 ulcer | 17 | 20 | P= 0.379 |
| Wound preparation time taking more than 2 weeks | 3 | 4 | P= 0.571 |
| Duration of ulcer more than 1 month | 3 | 5 | P=0.693 |
| Hemoglobin (Mean) g/dL | 10.31 | 13.67 | P=0.328 |
| Albumin (Mean) g/dL | 3.685 | 3.570 | P=0.497 |
| BUN (Mean) mg/dL | 13.780 | 15.520 | P=0.355 |

When we look into the microbiological culture spectrum across the groups, we see that most of the cultures reported no growth. Most common organism isolated in cultures was Staph Aureus followed by Pseudomonas, E. coli and Klebsiella.

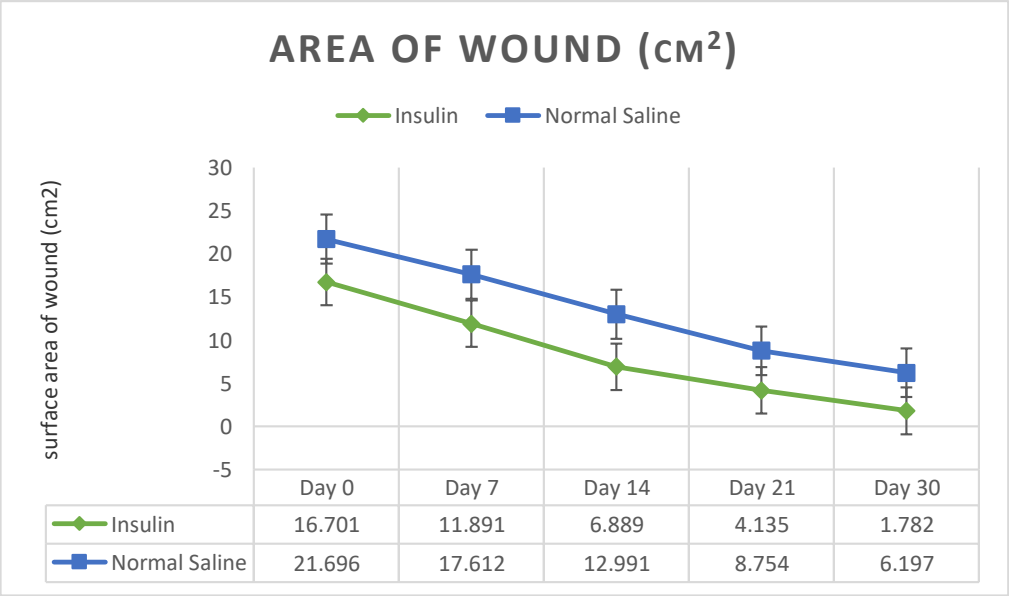



**Graph 11: Pus Culture from Wound**

- We compared the mean surface area between the two groups at day 0, 7,14, 21 and 30. We saw that the difference in the mean surface area between the insulin group and the normal saline group at day 0 and day 7 was comparable with a p value of 0.178 and 0.091 respectively. However, the mean surface area between the two groups were statistically significant at day 14, 21 and 30 respectively.

**Table 5: Comparison of mean surface area of ulcer**

| | **Group** | **Number of patients** | **Mean (cm2)** | **Std. Deviation (cm2)** | **Significance** |
|---|---|---|---|---|---|
| SURFACE AREA DAY 0 | Insulin | 27 | 16.701 | 10.567 | P=0.178 |
| | Normal Saline | 27 | 21.696 | 15.778 | |
| SURFACE AREA DAY 7 | Insulin | 25 | 11.891 | 8.101 | P=0.091 |
| | Normal Saline | 27 | 17.612 | 14.637 | |
| SURFACE AREA DAY 14 | Insulin | 24 | 6.889 | 6.024 | **P=0.031*** |
| | Normal Saline | 25 | 12.991 | 12.030 | |
| SURFACE AREA DAY 21 | Insulin | 23 | 4.135 | 4.030 | **P=0.040*** |
| | Normal Saline | 24 | 8.754 | 9.710 | |
| SURFACE AREA DAY 30 | Insulin | 19 | 1.782 | 1.761 | **P=0.015*** |
| | Normal Saline | 22 | 6.197 | 7.380 | |

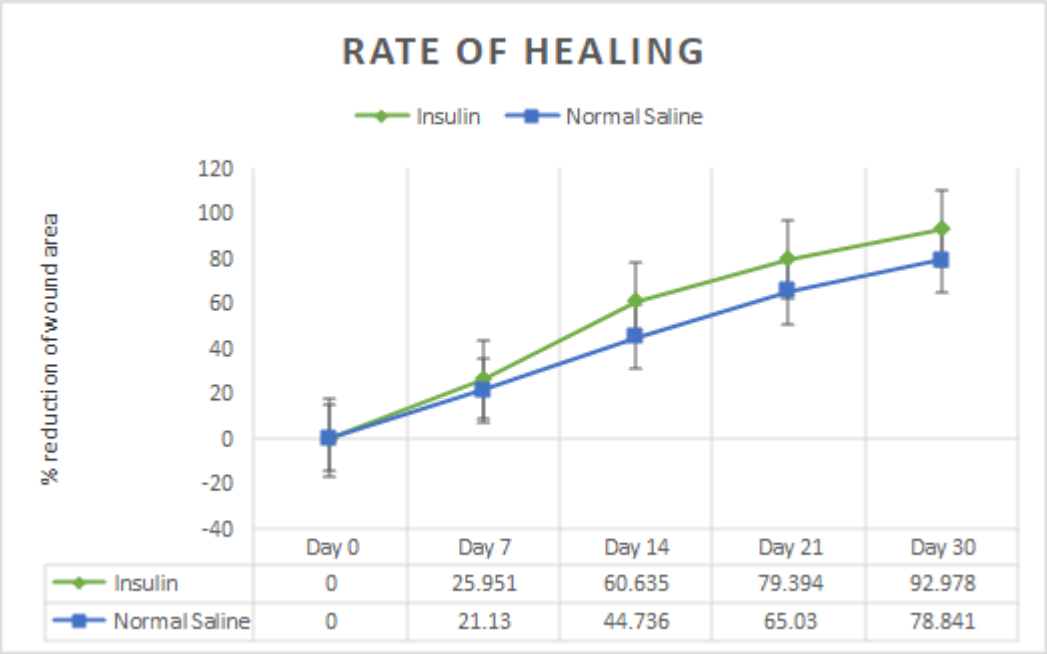



**Graph 12: Reduction in ulcer surface area over 30 days in each group.**

We calculated the rate of healing of ulcer between the two study groups given by the percentage reduction in surface area of the ulcer as per the formula [(Size of ulcer at Day0 – Size of ulcer at Dayx)/ Size of ulcer at Day0]x100. On comparison between the two groups, we saw that the rate of healing was not significantly different between the two groups till day 7, however the difference was significant on day 14, 21 and 30.

**Table 6. Comparison of rate of healing between the study groups**

| | **Group** | **Number of patients** | **Mean(cm2)** | **Std. Deviation (cm2)** | **Significance** |
|---|---|---|---|---|---|
| % reduction in surface area at day 7 | Insulin | 25 | 25.951 | 27.056 | P=0.467 |
| | Normal Saline | 27 | 21.130 | 20.143 | |
| % reduction in surface area at day 14 | Insulin | 24 | 60.635 | 15.064 | **P=0.004*** |
| | Normal Saline | 25 | 44.736 | 20.894 | |
| % reduction in surface area at day 21 | Insulin | 24 | 79.394 | 11.947 | **P=0.002*** |
| | Normal Saline | 24 | 65.030 | 17.821 | |
| % reduction in surface area at day 30 | Insulin | 23 | 92.978 | 6.332 | **P=0.001*** |
| | Normal Saline | 24 | 78.841 | 15.544 | |




**Graph 13. Rate of wound healing**

- While analyzing the effect of duration of diabetes on the rate of wound healing, we saw that patients with a duration of diabetes < 5 years had better rate of wound healing as compared to patients with longer duration of diabetes.

**Table 7: Effect of duration of diabetes on the rate of wound healing**

| **Group Statistics** | | | | | | |
|---|---|---|---|---|---|---|
| **Group** | | **DURATION OF DIABETES** | **N** | **Mean** | **Std. Deviation** | **T-test (significance)** |
| Insulin | Rate of wound healing at day 30 | <= 5 years | 7 | 98.075652 | 2.1270281 | **P=0.007*** |
| | | 6+ years | 16 | 90.747415 | 6.2897991 | |
| Normal Saline | Rate of wound healing at day 30 | <= 5 years | 11 | 83.227037 | 12.0681581 | P=0.211 |
| | | 6+ years | 13 | 75.129413 | 17.5834105 | |

- Age of the patient didn't have any effect in rate of wound healing in our study population.

**Table 8: Age distribution and rate of wound healing**

| **Group Statistics** | | | | | | |
|---|---|---|---|---|---|---|
| Group | | Age | N | Mean | Std. Deviation | T test (significance) |
| Insulin | Rate of wound healing at day 30 | <=60 years | 10 | 93.992625 | 6.6608960 | P=0.513 |
| | | 61+ years | 13 | 92.197073 | 6.2219318 | |
| Normal Saline | Rate of wound healing at day 30 | <=60 years | 12 | 78.574151 | 15.7918373 | P=0.935 |
| | | 61+ years | 12 | 79.107496 | 15.9895054 | |

- The gender distribution in our study population did not directly affect the rate of wound healing in our study population.

**Table 9: Gender Distribution and rate of wound healing**

| **Group Statistics** | | | | | | |
|---|---|---|---|---|---|---|
| Group | | Sex | N | Mean | Std. Deviation | T test (significance) |
| Insulin | Rate of wound healing at day 30 | Male | 12 | 92.676457 | 6.3810694 | P=0.818 |
| | | Female | 11 | 93.306429 | 6.5719366 | |
| Normal Saline | Rate of wound healing at day 30 | Male | 16 | 76.240518 | 16.8940555 | P=0.215 |
| | | Female | 8 | 84.041435 | 11.6589898 | |

- Although history of smoking is a recognized risk factor in rate of wound healing, it didn't directly impact rate of healing in our study population.

**Table 10: History of smoking and rate of wound healing**

| **Group Statistics** | | | | | | |
|---|---|---|---|---|---|---|
| Group | | H/O SMOKING | N | Mean | Std. Deviation | T test (significance) |
| Insulin | Rate of wound healing at day 30 | YES | 7 | 92.752802 | 6.3613468 | P=0.913 |
| | | NO | 16 | 93.076162 | 6.5256934 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Normal Saline | Rate of wound healing at day 30 | YES | 10 | 73.153332 | 15.2716281 | P=0.133 |
| | | NO | 14 | 82.903318 | 14.9452437 | |

- The level of albumin did not affect the rate of wound healing in our study population.

**Table 11: Albumin levels and rate of wound healing**

| **Group Statistics** | | | | | | |
|---|---|---|---|---|---|---|
| Group | | ALBUMIN (g/dL) | N | Mean | Std. Deviation | T test (significance) |
| Insulin | Rate of wound healing at day 30 | <= 3.50 | 10 | 93.438615 | 5.7823888 | P=0.767NS |
| | | 3.50+ | 13 | 92.623235 | 6.9366039 | |
| Normal Saline | Rate of wound healing at day 30 | <= 3.50 | 14 | 80.962601 | 11.4297229 | P=0.486NS |
| | | 3.50+ | 10 | 75.870335 | 20.2968270 | |

- When we tried to identify if duration of ulcer had any influence in rate of healing, we found it was not clinically significant in our study population.

**Table 12: Duration of ulcer and rate of wound healing**

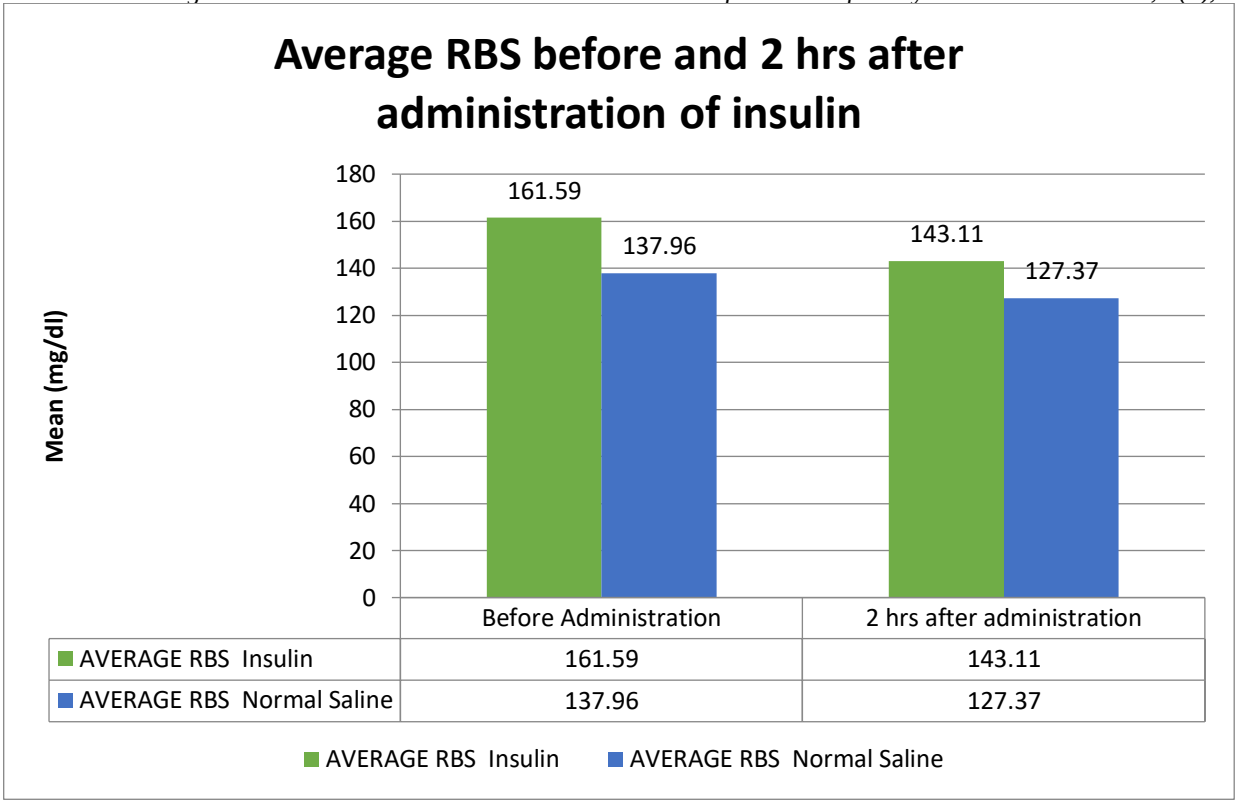
| | | | | | | |
|---|---|---|---|---|---|---|
| Group | | DURATION OF ULCER (Days) | N | Mean | Std. Deviation | T test (significance) |
| Insulin | Rate of wound healing at day 30 | <= 29 | 19 | 93.256996 | 6.6296346 | P=0.656 |
| | | 30+ | 4 | 91.651319 | 5.2416099 | |
| Normal Saline | Rate of wound healing at day 30 | <= 29 | 15 | 77.485252 | 16.8417900 | P=0.593 |
| | | 30+ | 9 | 81.100110 | 13.7507808 | |

- When we tried to see if the levels of hemoglobin ha any direct effect on the rate of healing, we saw that it did not influence rate of healing in our study population.

**Table 13: Levels of Haemoglobin and rate of wound healing**

| **Group Statistics** | | | | | | |
|---|---|---|---|---|---|---|
| Group | | HAEMOGLOBIN (g/dl) | N | Mean | Std. Deviation | T-test (significance) |
| Insulin | Rate of wound healing at day 30 | <= 10.00 | 9 | 93.316868 | 4.3149518 | P=0.824 |
| | | 10.01+ | 14 | 92.759742 | 7.5009170 | |
| Normal Saline | Rate of wound healing at day 30 | <= 10.00 | 10 | 80.184853 | 12.4298420 | P=0.713 |
| | | 10.01+ | 14 | 77.880803 | 17.8363099 | |

In context of safety of insulin being injected directly into the wound, it was decided at the beginning of the study that any episode of documented hypoglycemia after administration of insulin into the wound or >25% reduction in levels of peripheral blood glucose levels from pre administration levels was considered as significant. In this regard, no documented episodes of hypoglycemia were recorded in any patient. When we compared the average reduction in levels of peripheral blood glucose, none of the patients had ≥ 25% drop of levels of peripheral blood glucose in insulin group and was comparable to normal saline group.




**Graph 14: Average RBS before and 2 hrs after administration of insulin**

## 6. DISCUSSION

Diabetic Foot ulcers in patients with diabetes is a relatively common entity and may end up frequently into lower limb amputations unless a quick, pre-determined, rational and multidisciplinary approach to the management is taken.

Diabetic foot ulcers in patients is resource consuming and can particularly be of concern in a country like India where majority of the population are poor and illiterate and hence due to lack of information about the nature of the disease, presents late and when fulminant infection had usually set in and the only way out is by either an extensive wound debridement or an amputation. Foot infection and subsequent amputation of a lower extremity are the most common cause of hospitalization among diabetic patients. Various methods of treatment are available for patients with diabetic ulcer with its own pros and cons. However, many of them are costly and not a feasible option for majority of the Indian population. In this regard, various indigenous methods of dressings had been tried and tested including honey, foams, hydrocolloid gels or iodine. What has been probably less explored is the use of insulin in the wound dressings. Insulin is cheap and is a good alternative to other costly dressings.

Insulin-like growth factor (IGF), which has a high sequence of similarity to the hormone insulin, has been shown through in vivo studies to stimulate the proliferation, migration, and extracellular matrix excretion by keratinocytes, endothelial cells, fibroblasts, and it even promotes the reformation of granulation tissue. It has been proven previously in numerous animal studies that insulin does improve rate of wound healing. However, its application in human studies are limited. The few human studies that have explored its potential had either attempted to spray the insulin over the wound which does not effectively confirm how much of the insulin actually remained at the ulcer site and how much of it had dripped off or evaporated or they had fixed the dose of insulin that would be applied to the wound irrespective of surface area of wound, which meant that despite variations in size of the ulcer, the amount of insulin being administered would effectively remain the same.

In our study we attempted to address this issue by pre determining a variable dose of insulin as per the surface area of the wound and directly injecting it locally into the ulcer floor so that none of it is wasted and remains in the wound.

Ours was a prospective randomised controlled study that had involved 54 patients after excluding the patients who did not meet the inclusion or exclusion criteria or were not willing to consent for the intervention.

The exclusion criteria were determined based on the fact that late presentations of diabetic foot usually have an underlying osteomyelitis or is usually associated with peripheral vascular disease and in most cases have a poor outcome that ultimately result in amputations.

The 54 patients were randomly allotted to the two groups based on the sequence that was decided before the study was initiated using the online randomisation application with 27 patients in the insulin group and 27 in the normal saline group. 3 patients in insulin group and 1 patient from the normal saline group were lost to follow up after 1 week of starting intervention. 1 patient in the normal saline group underwent re debridement after initiation of intervention at day 10 and 1 patient went for ray amputation of the great toe at day 17. However as per the intent-to-treat assessment, they were included in final statistical analysis.

In our study population, majority were within the age group of 51-70 years and males comprised 61%. Most of the patients had a history of diabetes between 6- 10 years and 79.6% of them had good to average glycaemic control. About 1/3 of the patients were on insulin therapy for glycaemic control. Baseline physiological characteristics were comparable between the two groups.

The average wound surface area in the study groups were 16.7 cm2 in case of insulin group and 21.7 cm2 in case of normal saline group at day 0 and 1.8 cm2 and 6.2 cm2 respectively at day 30 which was statistically significant. ($p< 0.05$) The average reduction of wound surface area was approximately 14.9 cm2 in case of insulin group and 15.5 cm2 in normal saline group. When we look at the percentage reduction of wound surface area which indirectly implies rate of wound healing, we saw a significant increase in rate of wound healing in case of insulin group (92.98 cm2 at day 30) compared to normal saline group (78.841 cm2 at day 30) ($p< 0.001$). Similar results were stated by Swaminathan in his study that showed that topical insulin was efficacious for restoring normal epithelialization in foot ulcers. The average size of the ulcer in his study was 4.1 cm2 in insulin group, and 3.9 cm2 in saline group and he had demonstrated statistically significant difference ($P < 0.05$) in the improvement of ulcer size after treatment. [37]

Praveen et al. showed the complete healing time achieved in insulin versus saline group was 30.63 ± 6.5 days and 60.47 ± 23.31 days, respectively, with significant p value <0.0001. Similar results were demonstrated in our study. [38]

In our study, none of the patients in the insulin group had developed any episode of hypoglycaemia after intervention and the drop of levels of peripheral blood glucose was comparable to that of normal saline group. Similar findings were observed in a study conducted by Stephan et al. In their study they had demonstrated that administration of insulin into the wound was found to be safe and effective for pressure ulcer management even at a dose of 1 U/cm2. None of the study participants in the insulin group had developed hypoglycaemia, and blood glucose levels before and after insulin application did not change significantly ($P>0.05$). [39].

CONCLUSION:

Our study has demonstrated that insulin infiltration into the wound for dressing is a cost-effective method for diabetic foot ulcer management when compared to normal saline. During the 4 weeks of study, statistically significant differences in reduction in wound area were observed between insulin and normal saline gauze dressings. Our study also demonstrated that insulin infiltration into the wound is safe without any complication of hypoglycaemia. However, as we had only observed for superficial ulcers and ulcers of short duration, the long-term effect of local insulin infiltration on neuropathic ulcer healing and other chronic wounds remains to be examined.

Another limitation of the study was that we had not taken the depth of the wound while calculating rate of healing due to technical difficulties involved in measurement of depth of ulcer.

## 7. AUTHOR(S) CONTRIBUTION

The authors agreed to have no connections or engagements with any group or body that provides financial and non-financial assistance for the topics and resources covered in the article.

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**10. PLAGIARISM POLICY**

The authors declare that any kind of violation of plagiarism, copyright, and ethical matters will be handled by all authors. Journalists and editors are not liable for the aforesaid matters.

**11. CONFLICT OF INTEREST**

The authors declared that no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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