



# A comparative study showing effect of local insulin injection on wound vascularization seen in color Doppler imaging in patients with diabetic foot ulcer disease before and after therapy

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## ABSTRACT

**Background:** Diabetic foot ulcer disease is one of the most common complications of diabetes, especially in developing nations like India. Management and successful treatment of diabetic ulcers have been under rigorous research and interventions for the past few decades now. This study is an attempt to analyze the effects of a relatively newer treatment modality for diabetic foot ulcers. **Aims and Objectives:** The primary objective of this study was to determine results after treating patients of diabetic foot ulcers with local insulin injection therapy and compare with the placebo group after 1 week of treatment. **Materials and Methods:** In this study, 50 patients with diabetic foot ulcer disease were enrolled, of which 25 patients were randomly selected for the intervention group and the rest were kept in a control group. In the intervention group, patients were treated by injecting insulin preparation at the ulcer site whereas in control group, normal saline was used. Wound assessment was done by measuring the ulcer size and by determining the ankle peak systolic velocity (APSV) using color Doppler technique before and after the treatment in both intervention and control groups. **Results:** Mean ulcer size before the treatment (7.19 cm<sup>2</sup>) was higher than post-treatment size (3.48 cm<sup>2</sup>) and the difference was statistically highly significant (P=0.00) while there was no significant difference in ulcer size post-treatment by insulin or non-insulin (normal saline) group (P=0.274). Mean APSV before the treatment (30.75 cm/s) was lower than post-treatment velocity (43.50 cm/s) and the difference was statistically highly significant (P=0.00) while there was no significant difference in APSV post-treatment by insulin or non-insulin (normal saline) group (P=0.061). **Conclusion:** It meant that insulin and normal saline were equally effective in reducing ulcer size and improving APSV without being superior to one another.

**Key words:** Diabetic foot ulcer; Local insulin therapy; Diabetic foot; Wound treatment

## INTRODUCTION

Diabetes has become a major threat due to variations in demography, culture, and ageing factors. It poses a huge economic burden and is a primary causative factor for blindness, lipoprotein abnormalities, or mitochondrial

dysfunction causing cardiovascular diseases, renal failure, and amputation in individuals. The World Health Organization (WHO) has reported 24 million cases of diabetic neuropathy, 5 million cases of retinopathy, and 6 million cases of amputation due to diabetes.<sup>1</sup> International diabetes federation<sup>2</sup> gave a prevalence of approximately 387

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million people globally who suffer from diabetes which is estimated to rise to 592 million by 2035. The prevalence of diabetes in India in 2000 was 31.7 million in 2013, the prevalence has risen to 65.1 million and is expected to reach 79.4 million by 2030. However, the current estimates revealed that there are at least 150 million people who are living with diabetes worldwide of which 2/3<sup>rd</sup> of them belong to developing countries. In patients with diabetic foot ulcers, numerous factors such as increased blood glucose (locally and systemically), inefficient wound angiogenesis, and fibrous tissue deposition lead to the slow healing of local ulcers and delayed appearance of wound granulation tissue. This can be overcome by that local treatment with insulin may improve wound healing in diabetes, as suggested by clinical and animal experiments. A previous animal study demonstrated that insulin could reduce inflammation and increase collagen deposition, thus inducing accelerated burn wound healing. Furthermore, when insulin is injected diffusely into the wound, it can accelerate wound re-epithelialization. This re-epithelialization and accelerated healing may be caused by insulin promoting protein synthesis and it points toward potential use of insulin in the process of wound healing or non-healing ulcers. Controlling the role of insulin is primarily dedicated to controlling the systemic blood sugar levels. However, newer research throws light of the role of insulin in restoring the function and structure of the vasculature and improving angiogenesis which are currently the key problems to be solved for wound healing in patients. The aim of the present study was to investigate the effect of local insulin injection on wound vascularization seen in color Doppler imaging in patients with diabetic foot ulcer disease before and after therapy.

### Aims and objectives

The aim of this study is to assess the effects of local insulin injection on ulcer healing in terms of changes seen in ulcer size and APSV and compare it with the placebo group. To evaluate the effect of smoking on ulcer healing.

## MATERIALS AND METHODS

**Grouping:** The 50 patients enrolled in this study were randomly allocated to either the insulin group (n=25) or the control group (n=25).

### Inclusion criteria

The following criteria were included in the study:

1. All diabetic patient having foot ulcer disease
2. Yellow wound without much granulation tissue
3. Age >20 years.

### Exclusion criteria

The following criteria were excluded from the study:

1. Other causes of foot ulcer such as venous ulcer, vasculitis, amyloidosis, and neuropathy
2. Wagner's Grade 3 or more.

In the insulin group, one-half of the calculated dose of recombinant (regular) human insulin was diluted with physiological saline to a total volume of 1 ml and was then injected diffusely into the base of the diabetic foot ulcer. The remaining half dose of insulin was subcutaneously injected into the abdominal wall. The insulin injection was performed twice a day. In the control group, half of the calculated dose of human insulin was subcutaneously injected into the abdominal wall and 1 ml normal saline was injected into the base of the diabetic foot ulcer. The injection was performed twice a day. Both groups received injections for 7 consecutive days.

### Wound treatment

The eschar and necrotic tissue attached to the wound was removed. As a support for late repair, the tendons and nerves without obvious liquefactive necrosis were reserved, and soft tissues were retained to the greatest extent possible. To avoid the effect of the drug on the local blood glucose level, all the wounds were cleaned with physiological saline.

### Insulin dosage

All patients in the intervention group were treated with 1 IU/cm<sup>2</sup> of regular human insulin, half of which was injected into the wound and remaining half was used for systemic administration.

### Ethical clearance

The study was ethically approved by the Ethics Committee Registration No. ECR/922/inst/UP/2017 issued under Rule 122DD/of the Drugs and Cosmetics Rule 1945.

### Data analysis

Data were recorded on Microsoft Excel and analyzed using SPSS software 16 version. Quantitative data were presented as means ± standard deviations and qualitative data were presented as frequency. The paired *t* test was used to compare normally distributed continuous variables between groups and P<0.05 was considered as significant.

## RESULTS

The primary objective of this study was to determine results after treating patients of diabetic foot ulcers with local insulin injection therapy and compare with the placebo group.

The mean age of study samples was 57.84 years and it comprised of 37 (74%) males and 13 (26%) females (Figure 1 and Table 1).

Among 50 participants, 23 (46%) subjects had positive history of smoking while remaining 27 (54%) had no history of smoking. In the study, it was found that the mean ulcer size and ankle peak systolic velocity (APSV) pre-treatment was approximately similar among smokers and non-smokers, similarly in post-treatment, also they were approximately similar without significant difference between them ( $P=0.922$ ,  $0.914$  and  $0.482$ ,  $0.274$ , respectively) (Table 2).

It was found that mean duration of illness was 7.05 years with maximum being 25 years and minimum 6 months (Table 3).

When correlation between duration and APSV was studied, it was found that there was significant negative correlation between duration of diabetes with pre-treatment ankle peak systolic pressure (Pearson correlation= $0.672$   $P=0.00$ ), which means as duration of diabetes increased APSV decreased. Similarly, a weak positive correlation of duration of diabetes with ulcer size was found but it was statistically not significant ( $P=0.717$ ) (Figure 2).

Except for five non-responders, the rest 45 participants responded to the treatment significantly with or without use of insulin. The mean ulcer size of  $7.0996\text{ cm}^2$  in the intervention group reduced to  $2.9112\text{ cm}^2$  and a mean ulcer size of  $7.2816\text{ cm}^2$  reduced to  $4.0568\text{ cm}^2$  in the control group (Table 4).

Table 1: Age (years) statistics	
n	50
Mean	57.84
SD error of mean	1.442
SD	10.195
Range	40
Minimum	40
Maximum	80

Table 2: Group statistics for smoking						
Variable	Smoking	n	Mean	SD	Std. Error Mean	P-value
Pre-treatment ulcer size ( $\text{cm}^2$ )	Yes	23	7.2539	4.2039	0.8766	0.922
	No	27	7.1367	4.1592	0.8004	
Post-treatment ulcer size ( $\text{cm}^2$ )	Yes	23	3.8600	3.7546	0.7829	0.487
	No	27	3.1637	3.2828	0.6318	
Pre-treatment APSV (cm/s)	Yes	23	30.6500	7.7790	1.6220	0.914
	No	27	30.8900	7.5410	1.4510	
Post-treatment APSV (cm/s)	Yes	23	41.8300	10.9410	2.2810	0.274
	No	27	44.9300	8.8530	1.7040	

Similarly, the mean APSV of  $31.52\text{ cm/s}$  in the intervention group increased to  $46.0\text{ cm/s}$  and a mean APSV of  $30.04\text{ cm/s}$  increased to  $41\text{ cm/s}$  in the control group (Table 5).

## DISCUSSION

When results of both the groups were compared, it was concluded that there was no significant difference in ulcer size (post-treatment) in insulin or non-insulin (normal saline) group ( $P=0.274$ ). It means insulin and normal saline were equally effective in reduction of ulcer size without being superior to one another. Furthermore, there was no significant difference in APSV (post-treatment) in insulin or non-insulin (normal saline) group ( $P=0.061$ ). It means that insulin and normal saline were equally effective in improving peak systolic velocity without being superior to one another.

It was found that there was no correlation of smoking with ulcer size and APSV and it did not affect the treatment outcome. Although out of 50 patients, in only five patients, the wound was found to be non-healing and did not respond to the treatment. Moreover, among those five non-responders, four of them had current or past history of smoking. On application of Chi-square test, there was no association of smoking with ulcer healing as stated earlier (Chi-square with Yates correction –  $1.288$ ,  $P=0.256$ ).

A systemic review and meta-analysis performed by Sridharan and Sivaramakrishnan<sup>3</sup> which was carried out as a systematic compilation of existing literature regarding the

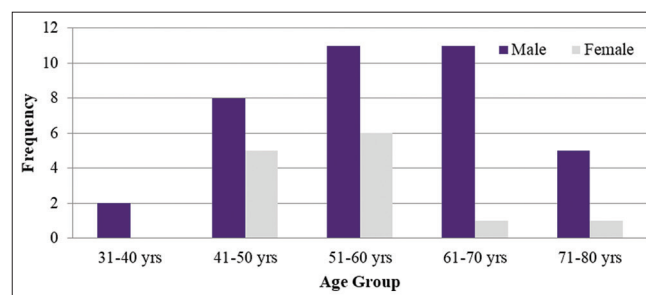
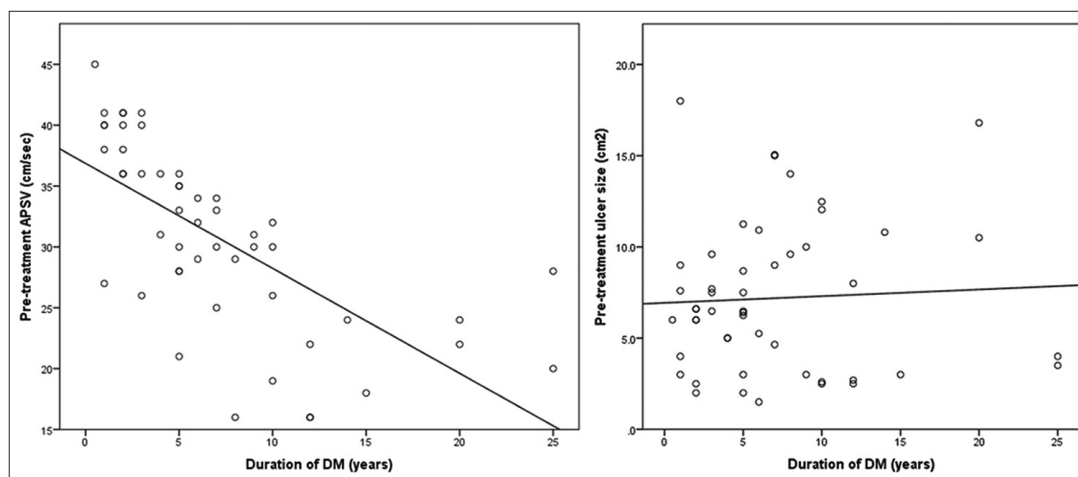


Figure 1: Age-gender-wise distribution



**Figure 2:** Correlation of duration of diabetes mellitus with pre-treatment ankle peak systolic velocity and ulcer size

**Table 3: Duration of DM (years) statistics**

n	50
Mean	7.05
Std. Error of Mean	0.84
SD	5.91
Range	24.50
Minimum	0.50
Maximum	25.00

**Table 4: Descriptive statistics**

Ulcer size (cm <sup>2</sup> )	Group	Mean	SD	n
Pre-treatment	Insulin	7.0996	3.9291	25
	NS	7.2816	4.4150	25
	Total	7.1906	4.1373	50
Post-treatment	Insulin	2.9112	3.4036	25
	NS	4.0568	3.5468	25
	Total	3.4840	3.4886	50

**Table 5: Descriptive statistics**

APSV (cm/s)	Group	Mean	SD	n
Pre-treatment	Insulin	31.52	7.747	25
	NS	30.04	7.480	25
	Total	30.78	7.574	50
Post-treatment	Insulin	46.00	9.170	25
	NS	41.00	10.124	25
	Total	43.50	9.888	50

efficacy and safety of topical administration of insulin for healing wounds. They obtained seven eligible studies and the quantitative synthesis yielded a significant result for topical insulin only in the wound area reduction. The study did not observe any significance either favoring or refuting the use of topical insulin in the following outcomes: wound healing rate, percent growth of granulation tissue, and microvessel density.

Stephen et al.,<sup>4</sup> performed a randomized and controlled trial to assess the effect of topical insulin versus normal

saline in pressure ulcer healing. They found that their study suggested that topical insulin significantly increases the rate of pressure ulcer healing compared with normal saline.

In another study by Wang et al.,<sup>5</sup> local application of low-dose insulin, high dose insulin, and normal saline were compared, in improving wound healing after deep burn surgery. They found that insulin was superior to normal saline in wound healing.

The results of this study were found to deviate from the findings of similar studies done in the past. This could be attributable to the multifactorial and complex nature of wound healing process. Treating a diabetic foot ulcer requires adequate debridement, pressure offloading, moist wound care, controlling infection, and revascularization of an ischemic limb.<sup>6</sup>

The presence of necrotic tissue over the wound affects the wound healing. In this study, although ulcers of Wagner's Grade 3 or more were excluded but several patients with Grade 2 ulcers required serial debridement which was done surgically.

Another significant factor in determining wound healing is wound infections. Infection in a diabetic foot ulcer may vary from aerobic or anaerobic bacteria and fungal to even poly-microbial in nature. Since wound cultures and sensitivity patterns of infections were not included in the study and all patients were kept under some form of oral antibiotic therapy, the response to treatment could have varied significantly.

Another important factor that influences wound healing process is adequate limb perfusion. In this study, APSV was used to determine limb perfusion. In a prospective



study conducted by Bishara *et al.*,<sup>7</sup> found that at a cutoff value of 35 cm/s, it was found that the APSV showed a sensitivity of 92.9% (95% confidence interval [CI] 82–97%), a specificity of 90.6% (95% CI 76–96%), a positive predictive value of 92.9%, and a negative predictive value of 90.6% in predicting non-healing of diabetic foot lesions. In our study, we found that 32 out of 50 participants had a pre-treatment APSV of below 35 cm/s which included all five non-responders as well.

Hence, it can be safely deduced from the results of the study that use of local insulin therapy or not is not the only factor that contributes to wound healing process in a diabetic foot ulcer. Rather a combined approach including surgical wound debridement, treating infections, and proper care of wound enables an adequate response in terms of wound healing.

#### Limitations of the study

The most significant limitation of this study was a relatively smaller sample size due to effect of ongoing pandemic of Covid-19. Due to the unavailability of thermography and immunohistochemistry techniques, the wound assessment could only be done clinically and on colour Doppler studies. The ongoing pandemic posed a challenge on regular follow up of the enrolled patients.

#### CONCLUSION

Following can be concluded from the results of the study –

1. The mean age of study sample was 57.84 years. Maximum patients (17) belonged to sixth decade of life followed by 13 falling in fifth decade.
2. 74% were male and 26% were female participants.
3. Among 50 participants 23 (46%) had positive history of smoking. 4 out of 5 non-responders had habit of smoking in past. There was no association of smoking with ulcer healing on application of Chi square testing. This could be due to smaller sample size of the study.
4. Mean duration of diabetes mellitus in all patients was 7.05 years. As duration of illness increased the APSV decreased but no correlation was found between duration of DM with ulcer size.
5. Except for 2 patients in the insulin group and 3 in the normal saline group all patients responded to treatment significantly. And the outcome in both groups did not differ significantly.
6. Hence both insulin and normal saline were found to be

equally effective in ulcer healing in terms of reduction in ulcer size and increment in APSV.

7. The doses of insulin (1 IU/cm<sup>2</sup>) used in the study was found to be safe as no patient suffered from post injection hypoglycemia.
8. For determination of the effective dose of insulin, analysis with a larger sample size is recommended.

The outcomes and results of the study clearly defines that both insulin and normal saline were equally effective in terms of ulcer healing. It is recommended that in future studies a larger sample size be taken and variables like wound culture patterns and antibiotic sensitivity be considered as well.

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**Authors' Contributions:**

**MS-** Concept and design of study; **SS-** Methodology and review of literature; **SJS-** Interpretation of results; **SU-** Review of literature, data collection, statistical analysis, drafting manuscript, and corrections.

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