RESEARCH ARTICLE

Effect of Topical Insulin on Diabetic Foot Ulcers

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ABSTRACT

Diabetic foot ulcers developing in a patient with long standing diabetes is a cause of morbidity and amputation in many patients. Our understanding of non healing of these ulcers at cellular and molecular levels needs to expand, to enable us to develop efficacious and cost effective therapeutic regimens

Methods: The effect of topical insulin on diabetic foot ulcers was studied over a period of 2 years in a single surgical unit.

Conclusion: Topical Insulin was found to be beneficial in chronic wound healing and it's role on neutrophil function and neo angiogenesis evaluated by measurement of MIP-2 and VEGF levels.

Keywords: Diabetic foot ulcers, Topical insulin, VEGF, MIP-2

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INTRODUCTION

It is estimated that 463 million adults with an age range of 20–70 years have diabetes, of which 25% will acquire a non-healing Diabetic Foot ulcer (DFU) in their lifetime.[1] Chronic ulcers are behind most of amputations, and it is estimated that one leg gets amputated because of diabetes every 30 seconds.[2] Diabetic foot is profoundly susceptible to a multitude of insults secondary to impaired vascular and neurological state. Manifestations range from asymptomatic, nail deformities, callus formation, skin lesions, foot ulcers and involvement of the bone.

Our understanding of the non-healing of DFU at cellular and molecular levels needs to expand into developing novel and low-cost therapy so that amputations may be prevented.[2] Neutrophils are the cells behind the inflammatory response besides macrophages. They clean exogenous pathogens through phagocytosis and release enzymes and reactive oxygen species (ROS) for killing bacteria and/or other intruders.[3] At the same time, prolonged neutrophil infiltration may lead to possible impaired wound healing. It explains that although they perform as traumatic scavengers and assist wound healing, they also affect wound healing negatively, specifically with excess and/or hyper-functional neutrophils. Hence, suppressing neutrophil infiltration might result in enhanced wound healing, especially in asepsis.[4] Macrophages secrete a protein having a molecular mass of approximately 6000 Da and an affinity for heparin. This protein (macrophage inflammatory protein 2 (MIP-2)) is a potent chemotactic agent for human polymorphonuclear leukocytes.[5]

In wounds, VEGF is produced by neutrophils, platelets, and macrophages. Acting through tyrosine kinase receptors expressed on endothelial cells, its core function in wounds is to induce and maintain wound vasculature.[6] Diminished VEGF production and reduced angiogenesis are considered to help in repairing the impaired tissue in diabetic patients.[7]

Standard therapeutic modalities for DFU include debridement, antibiotics, pressure offloading, dressing regimens, hyperbaric oxygen, and topical growth factors. At the same time, even among recent available therapies, more than half of chronic wounds remain intractable.[8,9]

New treatment strategies in wound healing such as bioengineered dressings and cellular applications, aim to replace senescent resident cells and re-establish the normal cycle.[10,11] However, they are costly and even cumbersome to carry out daily.

Wound healing is influenced by several agents, such as insulin-like growth factor (IGF) and human acidic fibroblast growth factor (rh-aFGF).[12] *In-vivo* studies express that IGF can stimulate endothelial cells' and fibroblasts' proliferation and differentiation and promote granulation tissue regeneration, helping wound healing. Since Bunting discovered insulin in 1921, various outcomes beyond blood glucose regulation

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Stage	Grade			
	O	I	II	III
A (no infection or ischemia)	Pre or Post ulcerative lesion completely epithelialized	Superficial wound not involving tendon, capsule on bone	Wound penetrating to tendon or capsule	Wound penetrating to bone or joint
B	Infection	Infection	Infection	Infection
C	Ischemia	Ischemia	Ischemia	Ischemia
D	Infection & ischemia	Infection & ischemia	Infection & ischemia	Infection & ischemia

have been recorded. Systemic insulin treatment encourages increasing the expression of neutrophil adhesion molecules and various cell surface receptors to reinforce the cellular functions of migration, phagocytosis, and bactericidal actions.[13] Many pre-clinical and clinical studies have presented the positive outcomes of insulin on wound healing, but a suitable method for routine clinical use of topically applied insulin has not been documented.[14-16]

Application of topical insulin for the management of chronic diabetic foot ulcers can therefore be an inexpensive method of accelerating wound healing time, thereby decreasing morbidity and stress on the socio-economic health of the patient and decreasing the ever-increasing demand on the limited health resources present in our country

The current study is a prospective observational study that aims to analyze the effect of topical insulin on diabetic foot ulcer healing, evaluate its effect on neutrophil function via MIP-2 and angiogenesis via VEGF by immunohistochemistry.

Patients and Methods

This was a prospective observational study carried out in a single surgical unit of Department of General Surgery, Institute of Medical Sciences, Banaras Hindu University in collaboration with Department of Endocrine and Metabolism, and Department of Pathology between August 2017 and July 2019. Patients presenting with diabetic foot ulcer for > 6 weeks were enrolled in the study, while those with concomitant malignancy, severe peripheral vascular disease with impalpable pulses and pregnant women were excluded from the study design.

A detailed history and examination on the wound were done for all the patients. The wound was graded using University of Texas Diabetic wound classification.[1]

University of Texas Diabetic Wound Classification Relevant hematological tests (CBC, Blood sugar – Fasting and Post Prandial, HbA1c, RFT, LFT) were carried out to assess the patient's general condition and nutritional status.

Anemia and hypoproteinemia, if present, were treated. Glycemic control was maintained. Dead and necrotic tissue was debrided under local anesthesia. Systemic antibiotics were given in both groups based on culture and sensitivity.

The area of the wound was obtained as the product of the two largest perpendicular diameters measured using a ruler (mm), which were marked using sterilized translucent graph paper. Pre-procedural biopsy as well as tissue culture was sent for all the patients. The solution containing 10 units H Insulin (R) in 10 mL normal saline was sprayed on each 10 cm^2 wound with a syringe and covered with sterile cotton gauze. The limb was positioned to prevent solution run-off from the wound.

The patient was admitted or kept in daycare to evaluate the side effects of topical insulin. Blood glucose levels were measured with a glucometer 10 minutes before and 1-hour after therapy. Adverse events were assessed and recorded, including headache, sweating, palpitation and vertigo (due to hypoglycemia).

The patients were followed up to 6 weeks from the date of the first procedure with a series of photographs, along with tissue MIP-2 and VEGF expressions via immunohistochemistry at baseline and 6 weeks. Based on the intensity of color and percentage of cells attained scores, the summation of which resulted in the total score.

For VEGF, intensity scores were assigned numeric values: 0 (no staining), 1 (weak staining), 2 (moderate staining) or 3 (strong staining). Proportion scores of 0, 1, 2 and 3 indicate 0%, 1–25%, 26–50% and >50% positively stained cells. Total score of ≥ 3 was taken as positive. Evaluation of MIP-2 scoring was done as per College of American Pathologists Guideline 2013 Recommendation. Intensity and proportion scores were similar as above and a total score of ≥ 3 was taken as positive. Comparative analysis was done between baseline and post-therapy VEGF and MIP-2 expressions.

Statistical Analysis

Statistical analysis was performed using SPSS 20.0 software Windows version (Inc., Chicago, USA). For paired samples, paired student's T test was used. For categorical variables, chi square test was used. A p-value less than 0.05 was considered statistically significant.

Observation

Over a period of 2 years (between August 2017 and July 2019), a total of 48 patients with DFU were enrolled in the study. All patients underwent a thorough history, examination, and pre-procedural biopsy to rule out malignancy. Topical insulin (10 units regular insulin in 10 mL saline/ 10cm^2) was applied on the wound up to 6 weeks. Clinical parameters, photographs, tissue VEGF and MIP-2 expression via immunohistochemistry were assessed at baseline and end of 6 weeks.

Most of the patients (89.4%) were above 40 years, with a mean age of 56.67 ± 13.36 years. There was a male preponderance ratio of 1.4:1. The mean BMI was 23.49 ± 3.836 kg/m^2, showing that chronic diabetic foot ulcers were not confined to patients with higher BMI.

Most of the patients had long-standing diabetes, mean duration being 8.81 ± 7.111 years however 12.5% of patients had diabetes

Table 1: Baseline parameters

		Frequency (n=48) (%)
Symptoms	Neuropathy	40(83.3)
	Pain	36(75)
	Discharge from wound	40(83.3)
Recurrence of ulcer	Present	18(37.5)
	Absent	30(62.5)
Laterality	Unilateral	47(97.9)
	Bilateral	01(2.1)
Site	Dorsum of foot	23(47.9)
	Planter aspect of foot	07(14.6)
	Over heel	02(4.2)
	Combined	16(33.3)
Wound area (cm^2)	< 20	10(20.8)
	20–50	26(54.2)
	> 50	12(25)
Wound Grade	1	12(25)
	2	21(43.8)
	3	15(31.2)
Wound Culture	Positive	34(70.8)
	Negative	14(29.2)

Table 2: Baseline Investigations

		Frequency (n=48)(%)
Hb (gm/dL)	< 7	6(12.5)
	7–10	25(52.1)
	> 10	17(35.4)
HbA1c	< 6.5	3(6.2)
	6.5–8	26(54.2)
	8–10	13(27.1)
	>10	6(12.5)

Table 3: Comparison of VEGF expression before and after insulin application

	Baseline		*Post therapy*	
	Frequency (n=40)	Percentage (%)	Frequency (n=40)	Percentage (%)
Positive	19	47.5	31	77.5
Negative	21	52.5	9	22.5

Table 4: Comparison of MIP 2 expression before and after insulin application

	Baseline		*Post therapy*	
	Frequency (n=40)	Percentage (%)	Frequency (n=40)	Percentage (%)
Positive	21	52.5	15	37.5
Negative	19	47.5	25	62.5

for just less than a year. 72.9% of patients had ulcers persisting for 6 weeks to 6 months.

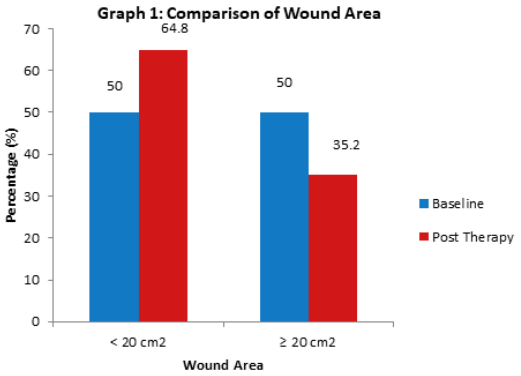
Discharge from wounds and neuropathy were the most common presenting symptoms (83.3%) in our study, while pain was present in 75 and 37.5% of patients with recurrent ulcers. Both limbs were involved only in 1 patient (2.1%), while the right foot was the most commonly affected side (54.16%). Chronic diabetic foot ulcers could be observed all over the foot, with maximum occurrence on dorsum of foot (47.9%). Most of the patients (54.2%) had wound surface area of > 20 to 50 cm^2 and 43.8% had Grade 2 wound. The culture was positive in 70.8% of the wounds, the infection being polymicrobial in nature. The organisms most commonly isolated were *Staphylococcus, Pseudomonas and Escherichia coli* (Table 1).

64.6% of patients were anemic and the mean Hb of the study group was 9.375 ± 1.9634 gm/dL, whereas 54.2% had HbA1c levels in the range of 6.5–8 with a mean value being 8.296 ± 1.8854 (Table 2).

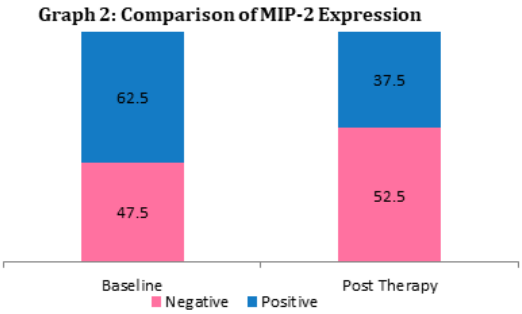
The graph 1 shows a gradual decrease in the wound surface area following topical insulin therapy, although not significant.

In our study, out of 48 patients, slides from 8 patients were rejected due to improper sectioning or inadequate tissue on IHC. So comparison could be made only for 40 patients. As seen above VEGF expression increased significantly following insulin therapy from 47.5 to 77.5% (p= 0.005). Expression of VEGF was mostly cytoplasmic but nuclear expression was also seen in two cases (Table 3).

Table 4 shows that MIP-2 expression decreased following topical insulin application from 52.5 to 37.5% (Graph 2). However, the difference was not statistically significant (p=



Graph 1: Comparison of wound area



Graph 2: Comparison of MIP-2 expression

Table 5: Correlation between baseline VEGF and MIP-2 expressions with wound area

	Wound Area	*Percentage (%)*		*Correlation*
		Positive	*Negative*	
VEGF	< 20 cm^2	57.9	33.3	$\chi2$=2.431 p=0.119
	≥ 20 cm^2	42.1	66.7	
MIP-2	< 20 cm^2	66.7	21.1	$\chi2$=8.386 p=0.004
	≥ 20 cm^2	33.3	78.9	

Table 6: Correlation between baseline VEGF and MIP-2 expressions with wound grade

	Wound Grade	*Percentage (%)*		*Correlation*
		Positive	*Negative*	
VEGF	1	10.6	28.6	$\chi2$=2.035 p=0.362
	2	52.6	42.8	
	3	36.8	28.6	
MIP-2	1	19.1	21.0	$\chi2$=0.030 p=0.985
	2	47.6	47.4	
	3	33.3	31.6	

0.177). MIP-2 expression was mostly cytoplasmic but the nuclear expression was also present in some cases.

Table 5 shows that baseline tissue MIP-2 expression was significantly associated with wounds having area < 20 cm^2 (p=0.004), while there was no significant correlation between expression of VEGF and wound area at baseline.

According to Table 6, no significant correlation was found between baseline expressions of VEGF and MIP-2 and wound grade as determined by University of Texas classification.

DISCUSSION

Diabetic foot ulcers are difficult to treat and a major cause of morbidity among diabetic patients, thus posing a challenge to the treating physician. The wound healing process consists of a well-orchestrated chain of distinct but interlinked events like coagulation, inflammation, migration, proliferation, regeneration and remodeling.

The phase of inflammation begins after the hemostasis and the first cell type recruited in the acute wound is neurophil. They peak at around day 2, and then the macrophages come into play from day 2 to day 5. Apart from remaining pathogems from wound they secrete cytokines and growth factors like TGF-B, and VEGF that promote angiogenesis and attract fibrobl asks, which lay extracellular matrix.[17]

This process is somehow deranged in DFU. Insulin, a growth factor, is believed to stimulate angiogenesis, collagen formation, and extracellular matrix formation. Insulin-like growth factor, which has a high sequence of similarity to the hormone insulin has *in-vivo* studies shown to stimulate keratinocytes and fibroblasts.[18]

In our study, 10 units of H Insulin® was dissolved in 10 mL N. saline and applied on the ulcer. Weekly wound measurements revealed a decrease in the wound area from 40.17 ± 39.6 cm^2 to 27.26 ± 26.48 cm^2.

Various biomarkers have been assessed from time to time to assess the healing process in DFU. Inflammatory biomarkers like ESR, CRP, procalcitonin have been clinical trial subjects (NCT04025853). Tissue biomarkers like c-myc and phosphorylated glucocorticoid receptor (NCT04591691) are being used to assess wound healing. We have assessed VEGF and MIP-2 as biomarkers that give information regarding the neoangiogenesis and macrophage dysfunction in the diabetic non-healing wound. VEGF expression significantly increased after topical insulin application, from 47.5 to 77.5% patients with a p-value of 0.005. VEGF is one of the most potent angiogenesis-stimulating growth factors. It acts in a paracrine manner on both endothelial cells and dermal microvessels.[19]

A randomized, double-blind, placebo-controlled study on the use of local insulin-zinc injection at skin donor site reported that wounds healed faster with insulin injection at the local site (Zhang XJ 2007).[19]

Topical insulin decreased the neutrophil infiltration in wounds as evidenced by decreased expression of MIP-2, a marker of wound neutrophil infiltration indicating rapid resolution of the inflammatory phase.

CONCLUSION

Topical application of insulin achieved greater wound area reduction than other standard-of-care dressings. It helps improve VEGF levels and diminish MIP-2 levels, stalling the prolonged inflammatory phase. Insulin is easily available, economical, and safe for diabetic foot ulcers.

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