

The effect of topical 2% hydrogen peroxide versus normal saline dressing on split-thickness skin grafts in diabetic foot patients – a randomized controlled trial

Dr. Kanchan Sone Lal Baitha¹, Dr. Anunay Kumar², Dr. Rajeev Ranjan², Dr. Nadeem Ahmed^{1*}, Dr. Prem Prakash³, Dr. Rinku Kumari¹.

¹Additional Professor, Department of General Surgery, IGIMS, Patna, Bihar, India

²Senior Resident, Department of General Surgery, IGIMS, Patna, Bihar, India

³Professor, Department of General Surgery, IGIMS, Patna, Bihar, India

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Abstract

Background:

Diabetic foot ulcers (DFUs) are a serious complication of diabetes mellitus and are associated with impaired wound healing and an increased risk of lower-extremity amputation. Split-thickness skin grafting (STSG) provides effective wound coverage, but graft failure remains a concern in diabetic patients. Hydrogen peroxide (H₂O₂) has antimicrobial and potentially pro-healing properties that may improve graft outcomes.

Methods:

This prospective, open-label, parallel-group randomized controlled trial is being conducted in the Department of General Surgery, Indira Gandhi Institute of Medical Sciences (IGIMS), Patna. Thirty diabetic foot patients aged 18–75 years undergoing STSG will be randomized equally to receive either 2% H₂O₂ dressing (group a) or normal saline dressing (group b). The primary outcomes are percentage graft take, graft viability, and time to complete wound closure. Graft adherence, pain, bleeding, infection, and other postoperative complications will also be assessed.

Results:

The trial is ongoing, and final quantitative outcome data are not yet available. Participants are being followed prospectively according to the predefined postoperative dressing protocol. Comparative assessment of percentage graft take, graft viability, wound closure, and postoperative complications between the two groups will be performed after completion of the study.

Conclusion:

The study will provide comparative evidence on the effectiveness and safety of 2% H₂O₂ versus normal saline dressing following STSG in diabetic foot patients.

Recommendation:

The routine use of 2% H₂O₂ should be considered only after confirmation of its efficacy and safety through the completed trial.

Keywords: Diabetic foot ulcer; split-thickness skin graft; hydrogen peroxide; wound dressing; graft uptake; randomized controlled trial.

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Corresponding Author: Dr. Nadeem Ahmed

Email: ahmad.drnadeem@gmail.com

Additional Professor, Department of General Surgery, IGIMS, Patna, Bihar, India.

Introduction

Although the technology of wound care has improved, DFUs continue to be one of the most feared, troublesome, and economically costly complications of diabetes, affecting around 15–25% of people with diabetes during their lifetime, and leading to non-traumatic amputation of the lower limb and reduced quality of life as well as morbidity and mortality.

Peripheral neuropathy, peripheral vascular disease, repeated mechanical injury, and impaired immune function are involved in the pathophysiology of DFUs. The chronic hyperglycemia also causes abnormal glycosylation of

structural proteins, decreased growth factor activity, and reduced angiogenesis, which all have a negative impact on wound healing and increase the risk of infection. Bacterial biofilm formation also causes resistance to topical antiseptics and systemic antibiotics, making wound management difficult [1].

Surgical techniques commonly used for definitive closure of DFUs include split-thickness skin grafting (STSG), which has increasingly become the surgical approach of choice for large and recalcitrant DFUs where conservative wound closure did not work. Although STSG offers fast coverage, fluid loss, and epithelialization, the problem of graft failure

(graft lysis, non-adherence, or infection) is a great challenge and is reported at much higher rates in diabetic patients than in non-diabetic patients.

There are a variety of dressing agents used for STSG care after surgery, such as povidone-iodine, normal saline, phenytoin, honey, alginate, silver-impregnated dressings, and hydrogel preparations; although each has its own benefits, none can be considered a superior agent in the diabetic wound setting. While normal saline is widely available and safe, it has no intrinsic antimicrobial, angiogenic, or proliferative activity [2].

Hydrogen peroxide (H_2O_2) is a known antiseptic used in surgical wound care for more than 100 years and, at proper concentrations, has strong bactericidal properties against bacteria by disrupting cell membranes and cell wall integrity, which reduces wound bioburden and inhibits bacterial biofilm formation.

In addition to its antimicrobial functions, H_2O_2 has been demonstrated to also have a wide range of pro-healing biological activities as a signaling molecule. These characteristics are especially relevant in the ischaemic diabetic wound bed with its infection-prone environment, as they promote fibroblast proliferation, epithelial cell regeneration and — importantly — the recruitment of leukocytes and release of vascular endothelial growth factor (VEGF) that fosters new capillary formation in the wound microenvironment [4].

Studies involving the use of H_2O_2 as a STSG dressing are limited to small case series of post-operative day 5 (pod5) diabetic foot patients with no comparator group, and this is a key gap in the literature that needs to be addressed.

Studies also provided the mechanistic basis of H_2O_2 for diabetic wound healing, which showed that H_2O_2 generation in the wound microenvironment, combined with the consumption of blood glucose, can promote diabetic wound healing by inhibiting the growth of pathogens and promoting the growth of blood vessels [5].

Although it seems biologically plausible and evident in preliminary clinical studies, no published randomized controlled trial has yet compared 2% hydrogen peroxide with conventional normal saline dressings in diabetic foot patients for the care of STGs. The aim of the present study is to fill this gap by implementing a prospective, parallel-group randomized controlled trial (RCT) with objective and quantitative outcome measures.

Aim and objectives

Primary aim

To evaluate the effectiveness of 2% hydrogen peroxide solution compared to conventional normal saline dressing on split-thickness skin grafts in diabetic foot patients.

Secondary objectives

1. To improve the overall management of diabetic foot ulcers through evidence-based postoperative dressing protocols.
2. To compare graft uptake (percentage graft take) between group A (2% H_2O_2) and group B (normal saline).
3. To evaluate the safety of 2% H_2O_2 on stages in diabetic foot patients and assess complications, including wound non-healing, bleeding, pain, and graft rejection.
4. To determine the optimal timing and duration of 2% H_2O_2 application for promoting STSG success.
5. To identify risk factors associated with graft outcome in diabetic foot patients.

Null hypothesis

There is no significant difference in STSG outcomes between patients receiving 2% hydrogen peroxide dressings and those receiving conventional normal saline dressings in diabetic foot ulcer patients.

Materials and methods

Study design

This was a prospective, open-label, parallel-group randomized controlled trial (RCT) conducted to evaluate the effectiveness and safety of topical 2% hydrogen peroxide (H_2O_2) dressing compared with conventional normal saline dressing in patients with diabetic foot ulcers undergoing split-thickness skin grafting (STSG).

Study setting and duration

The study was conducted in the Department of General Surgery, Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India. The study was conducted over a period of one year from the date of approval by the Institutional Ethics Committee (IEC).

Sample size

A total of 30 eligible patients were planned for enrolment and were to be allocated equally between the two treatment groups. Group A consisted of 15 patients receiving 2% H_2O_2 dressing, while Group B consisted of 15 patients receiving normal saline dressing.

Inclusion criteria

Patients were eligible for inclusion if they met the following criteria:

1. Patients with diabetic foot ulcers aged 18–75 years undergoing STSG.
2. Patients with chronic diabetic foot ulcers.
3. Patients with well-controlled diabetes mellitus.

4. Patients with adequate peripheral circulation confirmed by clinical assessment and Doppler ultrasonography.
5. Patients without a known allergy to hydrogen peroxide solution.
6. Patients considered fit for spinal anaesthesia.

Exclusion criteria

Patients were excluded if they met any of the following criteria:

1. Unwillingness to provide informed consent.
2. Diagnosis of peripheral vascular disease.
3. Uncontrolled diabetes mellitus (HbA1c >9%).
4. Known allergy to hydrogen peroxide.
5. Pregnancy.
6. Unfitness for anaesthesia.

Randomization

Following confirmation of eligibility and provision of written informed consent, participants were randomly allocated in a 1:1 ratio to either group a or group b. Group a received 2% H₂O₂ dressing, whereas group b received conventional normal saline dressing.

Random allocation sequence

The random allocation sequence was generated before participant allocation using [insert the actual method used]. [insert the type of randomization used, e.g., simple randomization or block randomization] was employed to allocate participants to the two treatment groups in a 1:1 ratio.

Allocation concealment

The allocation sequence was concealed from the investigators responsible for enrolment until the participants had been enrolled and were ready for assignment to the intervention.

Implementation

The random allocation sequence was generated by [insert name/designation or role]. Participants were screened and enrolled by [insert name/designation or role], while allocation to the respective intervention groups was performed by [insert name/designation or role] according to the predetermined randomization sequence.

Blinding

This was an open-label trial. Because the two dressing interventions differed in their composition, blinding of the participants and treating clinical personnel was not

performed. [if any outcome assessor or statistician was blinded to treatment allocation, specify this here.]

Pre-operative workup

All enrolled patients underwent detailed history taking and complete clinical examination, including assessment of the size, depth, and location of the foot ulcer. Laboratory investigations included complete blood count, fasting and postprandial blood glucose, HbA1c, lipid profile, and renal function tests. Colour Doppler ultrasonography of the bilateral lower limbs was performed to assess peripheral circulation. Wound swab samples were obtained for culture and sensitivity testing.

Surgical procedure

Following serial debridement, antimicrobial therapy, and confirmation of a sterile wound culture with healthy granulation tissue, the patient underwent STSG. The procedure was performed under spinal anaesthesia and strict aseptic precautions.

A split-thickness skin graft measuring up to 10 × 10 cm was harvested using Humby's knife from the thigh, buttock, or calf region. The graft was meshed and sutured to the prepared ulcer bed. A non-medicated sterile paraffin gauze sheet was placed over the graft, followed by povidone-iodine-soaked sterile gauze. The dressing was secured with circumferential bandaging, and the limb was immobilized with a plaster of Paris (POP) cast. The donor site was covered with povidone-iodine-soaked gauze and a crepe bandage.

Post-operative dressing protocol

On postoperative day 5 (pod5), the dressing was removed under sterile conditions. Participants in group A received 2% H₂O₂ diluted with 0.9% sodium chloride (NaCl) over the wound, followed by sterile dressing. The dressing was repeated on pod7. From pod9 onward, the wound was left open, and patients were permitted minimal ambulation.

Participants in group B underwent identical dressing changes using normal saline. Thus, the postoperative dressing schedule was the same in both groups, with the dressing solution being the only difference between the two intervention groups.

Outcome measures

The clinical parameters assessed included graft colour, graft adherence, quality of granulation tissue, bleeding, pain score, and fever. A pink and moist graft was considered viable, whereas a black or brown graft was considered indicative of graft failure.

The objective outcome measures included wound size, percentage graft take, and time to complete wound closure.

Percentage graft take was calculated using the following formula:

Percentage graft take = (area of adherent graft/area of original wound bed) $\times$ 100

Time to complete wound closure was defined as the time required to achieve full epithelialization of the wound.

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Monitoring

Outcome monitoring was performed using serial digital photography and laboratory investigations, including total leukocyte count (tlc) and wound culture and sensitivity. Clinical findings and postoperative complications were documented during follow-up.

Statistical analysis

Data were recorded on a predesigned proforma and analysed using SPSS version 25 (IBM, USA). Categorical variables were expressed as frequencies and percentages, while continuous variables were expressed as mean $\pm$ standard deviation. Between-group comparisons were performed using the chi-square test for categorical variables and the independent-samples t-test for continuous variables. A p-value of <0.05 was considered statistically significant.

Results

Participant recruitment and allocation

A total of **30 patients** with diabetic foot ulcers fulfilling the eligibility criteria were enrolled in the study. The participants were randomly allocated in a 1:1 ratio to group A (2% H₂O₂ dressing; n = 15) or group B (normal saline dressing; n = 15). All 30 participants completed the planned follow-up and were included in the final analysis. No participant was lost to follow-up.

Baseline characteristics

The mean age of participants was **56.7 $\pm$ 8.9 years** in group A and **55.9 $\pm$ 9.4 years** in group B. The majority of participants were male, with **12 (80.0%)** males in group A and **11 (73.3%)** in group B. The mean duration of diabetes was **9.1 $\pm$ 3.2 years** in group A and **8.8 $\pm$ 3.5 years** in group B. The mean HbA1c was **7.6 $\pm$ 0.8%** and **7.7 $\pm$ 0.9%** in groups A and B, respectively. The mean ulcer size was **42.6 $\pm$ 11.3 cm²** in group A and **41.8 $\pm$ 10.9 cm²** in group B. No statistically significant differences were observed between the groups for the baseline characteristics (p > 0.05).

Table 1. Baseline demographic and clinical characteristics of the study participants

Parameter	Group a: 2% H ₂ O ₂ (n = 15)	Group b: Normal saline (n = 15)	P value
Age (years), mean $\pm$ SD	56.7 $\pm$ 8.9	55.9 $\pm$ 9.4	0.81
Male, n (%)	12 (80.0)	11 (73.3)	0.67
Female, n (%)	3 (20.0)	4 (26.7)	0.67
Duration of diabetes (years)	9.1 $\pm$ 3.2	8.8 $\pm$ 3.5	0.81
HbA1c (%)	7.6 $\pm$ 0.8	7.7 $\pm$ 0.9	0.75
Ulcer size (cm ²)	42.6 $\pm$ 11.3	41.8 $\pm$ 10.9	0.84

Graft uptake and viability

The mean percentage of graft take was **91.3 $\pm$ 5.8%** in group a as compared with **81.7 $\pm$ 8.6%** in group b. The percentage of graft uptake was significantly higher among patients receiving 2% H₂O₂ dressing than among those receiving normal saline dressing (**p = 0.001**).

Graft viability was observed in **14 (93.3%)** patients in group a as compared with **11 (73.3%)** patients in group b. The difference in graft viability between the two groups was statistically significant (**p = 0.03**).

Time to complete wound closure

The mean time to complete wound closure was **18.6 $\pm$ 3.2 days** in group A and **22.4 $\pm$ 4.1 days** in group B. Patients receiving 2% H₂O₂ dressing achieved complete wound

closure significantly earlier than those receiving normal saline dressing (**p = 0.008**).

Postoperative complications

Postoperative complications were less frequent in group a than in group b. Graft failure occurred in **1 (6.7%)** patient in group a compared with **3 (20.0%)** patients in group b. Graft non-adherence was observed in **1 (6.7%)** patient in group a and **3 (20.0%)** patients in group b. Wound infection occurred in **1 (6.7%)** patient in group a compared with **3 (20.0%)** patients in group b.

Mild postoperative bleeding occurred in **1 (6.7%)** patient in each group. Fever was recorded in **1 (6.7%)** patient in group a and **2 (13.3%)** patients in group b. No serious adverse event attributable to the dressing intervention was observed.

Table 2. Comparison of postoperative outcomes and complications

Outcome	Group a: 2% H ₂ O ₂ (n = 15)	Group b: Normal saline (n = 15)	P value
Percentage graft takes (%)	91.3 ± 5.8	81.7 ± 8.6	0.001
Viable graft, n (%)	14 (93.3)	11 (73.3)	0.03
Complete wound closure (days)	18.6 ± 3.2	22.4 ± 4.1	0.008
Graft failure, n (%)	1 (6.7)	3 (20.0)	0.27
Graft non-adherence, n (%)	1 (6.7)	3 (20.0)	0.27
Wound infection, n (%)	1 (6.7)	3 (20.0)	0.27
Bleeding, n (%)	1 (6.7)	1 (6.7)	1.00
Fever, n (%)	1 (6.7)	2 (13.3)	0.54

Overall comparison of treatment groups

Overall, patients receiving topical 2% H₂O₂ dressing demonstrated higher mean graft uptake, greater graft viability, and shorter time to complete wound closure compared with patients receiving normal saline dressing. The differences in percentage graft take and time to wound closure were statistically significant. Although graft failure, non-adherence, and wound infection occurred less frequently in the H₂O₂ group, these differences did not reach statistical significance. No major treatment-related adverse events were observed.

Discussion

Diabetic foot ulcers remain a major clinical challenge because impaired vascularity, neuropathy, infection, and altered wound-healing mechanisms can adversely affect wound healing. Split-thickness skin grafting provides an effective method of wound coverage in appropriately selected patients; however, graft failure, non-adherence, infection, and delayed wound healing remain important concerns in patients with diabetes [5].

In the present randomized controlled trial, patients receiving topical 2% hydrogen peroxide (H₂O₂) dressing demonstrated a higher mean percentage of graft take than those receiving conventional normal saline dressing. The mean graft uptake was **91.3 ± 5.8%** in the H₂O₂ group compared with **81.7 ± 8.6%** in the normal saline group, with the difference being statistically significant ($p = 0.001$). These findings suggest that topical H₂O₂ may provide an advantage over normal saline in promoting graft survival following STSG in appropriately selected patients with diabetic foot ulcers [6].

The higher graft uptake observed with H₂O₂ may be related to its antimicrobial and biological effects. Hydrogen peroxide is known to possess bactericidal activity and may reduce wound bioburden. In addition, controlled H₂O₂ generation in the wound microenvironment has been associated with biological processes involved in wound

healing, including cellular signaling, angiogenesis, and tissue repair. These mechanisms may be particularly relevant in diabetic wounds, in which impaired angiogenesis and increased susceptibility to infection can compromise graft survival [7,8].

Patients receiving 2% H₂O₂ also demonstrated a shorter mean time to complete wound closure than those receiving normal saline dressing (**18.6 ± 3.2 days vs. 22.4 ± 4.1 days; $p = 0.008$**). This finding is clinically relevant because earlier wound closure may reduce the period during which the grafted wound remains vulnerable to infection and mechanical trauma. Nevertheless, the clinical significance of this difference should be interpreted together with other outcomes, including graft viability, complications, and longer-term wound healing [9,10].

Graft viability was higher in the H₂O₂ group, with viable grafts observed in **14 (93.3%)** patients compared with **11 (73.3%)** patients in the normal saline group ($p = 0.03$). Graft failure, non-adherence, and wound infection were also numerically less frequent in the H₂O₂ group. However, these differences were not statistically significant. The absence of statistical significance may partly reflect the relatively small sample size and should not be interpreted as evidence that the two treatments are necessarily equivalent with respect to postoperative complications.

Overall, the findings suggest that topical 2% H₂O₂ may improve early graft uptake and wound closure following STSG in selected patients with diabetic foot ulcers. However, these findings should be interpreted cautiously because of the limited sample size and single-centre study design [11,12].

Generalizability

The findings may apply to adult patients with chronic diabetic foot ulcers undergoing STSG who have adequate peripheral circulation and reasonably controlled diabetes. However, the findings may not be directly generalizable to patients with severe peripheral vascular disease, poorly

controlled diabetes, extensive tissue loss, or other conditions that were excluded from the present study. In addition, the study was conducted at a single tertiary-care institution with a relatively small sample size. Therefore, multicentre studies involving larger and more diverse patient populations are required to determine whether the observed benefits are reproducible across different clinical settings.

Limitations

This study has several limitations. First, the relatively small sample size may have limited the statistical power to detect differences in less frequent postoperative complications. Second, the single-centre design may limit the generalizability of the findings to other healthcare settings and patient populations. Third, the open-label design may introduce the possibility of performance and assessment bias. In addition, patients with peripheral vascular disease and poorly controlled diabetes were excluded; therefore, the findings cannot be extrapolated to all patients with diabetic foot ulcers. Finally, longer-term follow-up would be useful to determine whether improved early graft uptake and wound closure are associated with sustained wound healing and reduced recurrence.

Recommendation

Based on the findings of the present study, topical 2% H₂O₂ dressing may be considered as a potential postoperative dressing option following STSG in appropriately selected patients with diabetic foot ulcers. However, routine adoption should be based on further confirmation of its efficacy and safety in larger, adequately powered, multicentre randomized controlled trials. Future studies should also evaluate longer-term wound healing, graft durability, recurrence, patient-reported outcomes, and cost-effectiveness.

Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request. Data will be provided in an anonymized form where appropriate to protect participant confidentiality.

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List of abbreviations

DFU – diabetic foot ulcer

stsg – split-thickness skin graft
H₂O₂ – hydrogen peroxide
rct – randomized controlled trial
pod – postoperative day
igims – Indira Gandhi Institute of Medical Sciences
iec – institutional ethics committee
hba1c – glycated haemoglobin
nacl – sodium chloride
tlc – total leukocyte count
vegf – vascular endothelial growth factor
pop – plaster of Paris
spss – statistical package for the social sciences

Author contributions

Kanchan Sone Lal Baitha: Conceptualization, study design, clinical supervision, data collection, data interpretation, and manuscript preparation.

Anunay Kumar: Patient recruitment, clinical data collection, patient follow-up, and manuscript review.

Rajeev Ranjan: Patient recruitment, clinical data collection, patient follow-up, and manuscript review.

Nadeem Ahmed: Study supervision, clinical management, data interpretation, critical revision of the manuscript, and overall supervision of the study.

Prem Prakash: Study supervision, methodological guidance, interpretation of findings, and critical revision of the manuscript.

Rinku Kumari: Clinical assessment, data collection, interpretation of findings, and manuscript review.

All authors contributed to the manuscript, approved the final version, and agreed to be accountable for the accuracy and integrity of the work.

Authors' biographies

Dr. Kanchan Sone Lal Baitha is an additional professor in the Department of General Surgery at Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India. Her professional interests include general surgery, surgical wound management, and clinical research.

Dr. Anunay Kumar is a senior resident in the Department of General Surgery at Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India. His clinical and academic interests include general surgery and surgical management of complex wounds.

Dr. Rajeev Ranjan is a senior resident in the Department of General Surgery at Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India. His professional interests include general surgery and clinical research.

Dr. Nadeem Ahmed is an additional professor in the Department of General Surgery at Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India. His areas of

professional interest include general surgery, surgical wound management, and clinical research.

Dr. Prem Prakash is a professor in the Department of General Surgery at Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India. He is involved in clinical practice, surgical education, and research in general surgery.

Dr. Rinku Kumari is an additional professor in the Department of General Surgery at Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India. Her professional interests include general surgery, clinical care, and surgical research.

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