Efficacy of intradermal methylene blue injection for donor site pain relief: a randomised trial protocol

Riddhi U Solanki[1] M Hospital Admin, M Human Disease Genetics, **Ashvi U Solanki***[2] MS General Surgery, **Kausar A Fakih**[2] MS General Surgery, **Sandhya S Iyer**[2] PhD MA

[1] Department of Public Health, Indian Institute of Public Health, Gandhinagar, India
[2] Department of Surgery, Lokmanya Tilak Municipal General Hospital and Medical College, Mumbai, India

*Corresponding author email ashvi555@gmail.com

ABSTRACT

Background Pain at the donor site of skin grafts remains a significant clinical challenge. Existing pain management approaches include topical agents, oral analgesics, and local anaesthetics, yet optimal strategies remain elusive. Methylene Blue (MB), known for its analgesic potential in various pain types, is being evaluated in this trial for its safety and efficacy when injected intradermally at donor sites following skin grafting

Aim To evaluate the safety and efficacy of intradermal injection of MB for pain relief at the donor site in patients undergoing split-thickness skin grafts (STSG), in comparison to standard dressing without MB.

Methods This single-center, randomised, parallel-group, active-controlled trial will enrol 60 adult patients undergoing STSG. Participants will be randomised into two groups: Intervention group (n=30) which will receive an intradermal MB injection at the donor site, while the control group (n=30) will receive standard tulle gras dressing. The primary outcome of this study is degree of pain relief achieved with the use of MB in split-skin grafting, measured by changes in Visual Analog Scale (VAS) scores at different time points postoperatively at 5th, 7th, 15th and 30th days. Secondary outcomes will include the requirement of analgesia, staining of the donor site, donor site healing (epithelisation) rate, scar quality, and adverse effects reported. Data analysis will include comparisons between the groups postoperatively, using independent statistical tests to assess intervention effects.

Discussion This trial aims to validate MB as a novel, low-cost analgesic intervention in postoperative care. If effective, it could improve patient comfort and recovery with minimal additional risk and may inform the development of more effective pain management strategies for the patient population.

Keywords split-thickness skin grafts, methylene blue, visual analog scale, pain relief

For referencing Solanki RU et al. Efficacy of intradermal methylene blue injection for donor site pain relief: a randomised trial protocol. Journal of Wound Management. 2025;26(3):182-187.

DOI https://doi.org/10.35279/jowm2025.26.03.09

Submitted 4 May 2025, Accepted 12 June 2025

KEY MESSAGES

- This protocol outlines a randomised, parallel-group, active-controlled clinical trial evaluating the efficacy of intradermal methylene blue injections in managing post-operative pain at skin graft donor sites
- The primary objective of this study is to assess whether intradermal methylene injection provides superior and longer-lasting analgesia compared to conventional tulle gras dressing in patients undergoing split-thickness skin grafting.
- The trial will compare pain intensity using VAS scores over multiple time points, monitor the need for rescue analgesia, and assess safety and healing outcomes. Results will offer evidence on the analgesic benefits and potential for broader use of methylene blue in surgical pain management

INTRODUCTION

The management of postoperative pain is a crucial aspect of surgical care, especially in procedures such as skin grafting, where pain at the donor site can greatly affect recovery and patient satisfaction. Skin grafting involves the transplantation of skin and often the associated adnexal structures from one area of the body to another. The rationale for skin grafting involves harvesting skin from a donor site, which subsequently undergoes healing, and transferring it to a recipient site in need. Upon successful integration, skin grafts confer protection to wounds against environmental factors, pathogens, temperature fluctuations, and excessive water loss, akin to the function of normal skin.[1]

It is reported that the harvesting of a split-thickness skin graft (STSG), which includes both epidermal and dermal layers, results in a partial-thickness wound that necessitates careful pain control, wound management and sufficient healing time.[2] Many patients report greater pain, itching, and discomfort at the donor site compared to the recipient site.[3,4] Additionally, donor site healing is often limited by complications, such as infections, dyspigmentation and hypertrophic scarring.[5-7] These challenges can be further intensified by factors such as wound size, patient age, underlying comorbidities, and

other individual health variables. Thus, there is severe pain and morbidity associated with STSG. A systematic literature review on STSG donor-site morbidity reported that mean pain scores on the VAS ranged from 0.73 to 10.0 on Post Operative Day (POD) 1 as compared to 0.75 to 5.13 on POD 7.[8] Currently, there is no accepted guideline or standardised method for evaluating pain after skin graft procedures. Pain assessments are typically recorded over a broad postoperative period, ranging from as early as POD 1 to POD 14 or beyond. Patients undergoing graft harvesting are usually under general anaesthesia and receive pain relief during their hospital stay as well as after discharge. Among several studies that reported pain management strategies, treatments varied widely and included agents such as lidocaine, paracetamol (used alone or combined with pethidine or tramadol), bupivacaine, and ibuprofen with polyurethane foam dressings. This variation highlights the absence of a universally accepted approach to donor site pain management. Moreover, the effectiveness of these interventions remains elusive, and it is unclear which method provides the most consistent and sustained relief. Given the lack of clarity and standardisation, there is a pressing need to explore alternative pain control strategies, such as the use of methylene blue (MB) that may offer more effective and reliable outcomes.

Currently, MB is primarily recognised as a vasoconstrictor. It exerts this effect by downregulating nitric oxide (NO), a molecule that normally facilitates vascular smooth muscle relaxation, thereby inducing vasoconstriction. Clinically, it exerts analgesic effects through multiple mechanisms. Primarily, it inhibits NO production—a central mediator of pain and inflammation—by modulating soluble guanylate cyclase and nitric oxide synthase pathways.[9] Additionally, MB disrupts sensory nerve conduction by blocking sodium channels, leading to decreased excitability of nociceptors within the dermis and epidermis. This combined mechanism not only attenuates pain but also potentially reduces inflammation at the injection site.

Recent studies have proved this role of MB. Early postoperative pain relief was reported in studies using MB without notable adverse effects. In patients with chronic discogenic low back pain (CDLBP), intradiscal MB injections resulted in significant reductions in VAS and Oswestry Disability Index (ODI) scores at one, three and six months, although the effect diminished in most cases by one year.[9,10] For idiopathic pruritus ani, a condition marked by severe perianal itching, over 85% of patients experienced symptom relief after one or two MB injections.[11] In preclinical models of osteoarthritis, MB not only alleviated pain but also reduced inflammation and protected cartilage integrity.[12] These findings support MB's broad potential as an analgesic agent through mechanisms that include nitric oxide inhibition and anti-inflammatory action. While its pain-relieving effects are encouraging, especially in the early stages of recovery, further studies are needed to establish standardised dosing protocols and confirm long-term safety and efficacy.

STUDY AIMS AND OBJECTIVES

The study aims to evaluate the safety and efficacy of intradermal injection of MB for pain relief at the donor site in patients undergoing STSG, in comparison to standard dressing without MB. The specific objectives are as follows:

PRIMARY OBJECTIVE

To assess the effectiveness of intradermal MB in reducing donor site pain following STSG, as measured by VAS scores on postoperative days 5, 7, 15, and 30.

SECONDARY OBJECTIVES

- To compare the need for additional analgesia between the MB group and control group
- To evaluate the time to complete epithelisation at the donor site in both groups.
- To assess the incidence of adverse effects, such as allergic reactions, skin staining, and any signs of local or systemic toxicity
- To compare the quality of scarring at the donor site between the two groups at one month postoperatively

METHODS

Study setting

This clinical study will be conducted in the Department of General Surgery at Lokmanya Tilak General Municipal Hospital, located in Mumbai, India. The hospital is equipped with a dedicated surgical ward, minor and major operation theatres, and postoperative recovery units capable of managing patients undergoing elective and emergency skin graft procedures. The study site caters to a diverse population from both urban and rural backgrounds and receives a high volume of patients requiring reconstructive procedures, such as STSG. All patient interactions, including recruitment, intervention administration, follow-up and data collection, will take place within the hospital premises to ensure standardised protocol adherence and minimise data variability.

Study design

This study is a prospective, randomised, parallel-group, active-controlled clinical trial which will be conducted over a duration of 18 months. The design involves two arms: an intervention group receiving intradermal MB injection (1%) at the donor site post-skin harvest, and a control group receiving standard postoperative dressing (tulle gras) without MB. Patients will be randomised in a 1:1 ratio using computer-generated random numbers, with the allocation concealed through Sequentially Numbered Opaque Sealed Envelopes (SNOSE method).[13]

Due to the visual nature of MB staining, the study is open-label for patients and healthcare providers. However, pain assessment and outcome evaluation will be performed by trained, blinded evaluators to minimise detection and performance bias. The primary endpoint is the intensity of pain at the donor site, assessed using the VAS on postoperative days 5, 7, 15, and 30. Secondary endpoints include time to epithelisation, requirement for additional analgesia, adverse effects and scar quality.

All participants will receive standard postoperative care, including paracetamol (1g every 8 hours) as first-line analgesia. Opioids will be used as rescue medication if needed. The study is designed in accordance with the CONSORT 2025 guidelines to ensure methodological transparency and robustness.[14]

Sample size and participants

The sample size for this randomised controlled trial was calculated based on prior data indicating a clinically meaningful difference in postoperative pain scores (VAS) between patients receiving intradermal methylene blue and those treated with standard dressing. Assuming a mean difference of −0.82 (2.16–2.98) with standard deviations of 0.61 and 0.69 in the intervention and control groups respectively, and setting a two-sided alpha of 0.05 with 80% power, the minimum required sample size was estimated to be 18 patients (9 per group) to detect a significant difference using an independent samples t-test.[15]

To account for an anticipated dropout or attrition rate of approximately 20%, the total sample size has been increased to 60 participants, with 30 participants in each group (intervention and control arms). This adjustment ensures that the study remains adequately powered to detect meaningful clinical differences in the event of participant loss to follow-up or protocol non-compliance.

STUDY PROCEDURE

Eligible patients who meet the inclusion criteria will be admitted to the surgical ward and will undergo thorough preoperative evaluation. This will include baseline demographic data collection, clinical history and detailed physical examination. All participants will have their vital parameters assessed and be screened for comorbid conditions that could impact surgical outcomes or wound healing. Routine preoperative investigations include complete blood count, serum albumin levels, renal and liver function tests, and Body Mass Index (BMI), as nutritional status and systemic health play a critical role in postoperative recovery and wound healing.

Table 1. Eligibility criteria for participant selection

Inclusion criteria	Exclusion criteria
Male and female patients aged 18 to 65 years with healthy deep or superficial wounds, fit for STSG	Patients with history of known allergy to MB or any of the tulle grass dressings (test dose of MB)
Patients without any co-morbidities and/or controlled co-morbidities (mainly IHD and HTN controlled through medications)	Pregnant and lactating women, children, and immunocompromised patients
Patient willing for admission and giving consent	Medically unstable patients requiring ICU care

All patients will undergo STSG performed by the same experienced surgeon using a standardised technique. A Humby's knife will be used in every case to ensure consistent graft thickness and donor site wound uniformity. The donor

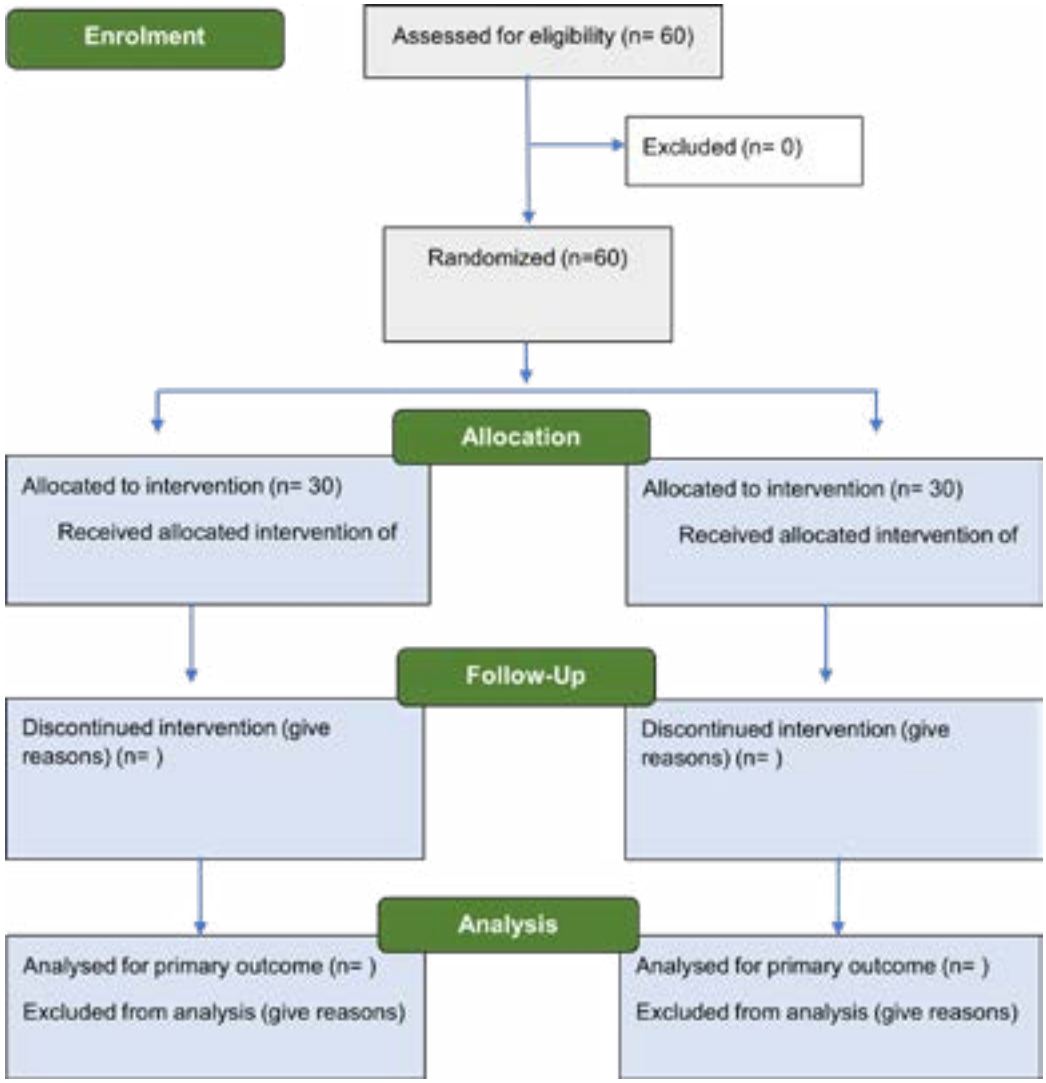

Figure 1. CONSORT 2025 flow diagram

site for all patients, regardless of group allocation, will be the anterolateral aspect of the thigh. Preoperatively, all patients will receive standard antibiotic prophylaxis as per institutional protocol to minimise the risk of surgical site infections. Postoperative analgesia will be provided in the form of paracetamol (1g every 8 hours), and patients experiencing intractable pain will be administered step-up analgesia with opioids according to clinical judgment.

Participants will be randomly assigned to one of two groups. In the intervention group, patients will receive an intradermal injection of MB (1% solution) directly at the donor site immediately after skin harvest. A preoperative test dose of MB will be administered intradermally over the forearm to rule out any hypersensitivity reactions. The injection at the donor site will be delivered using a 26-gauge needle, with a maximum volume of up to 5mL (typically 1mL per injection point in a grid-like pattern), ensuring even distribution across the donor area. The control group will receive no such injection and will receive only the standard tulle gras dressing.

The donor sites in both groups will be covered with non-adherent dressings and secured appropriately to minimise movement and contamination. Dressings will be evaluated and replaced only on postoperative days 5, 7, 15, and 30, in order to standardise observations and limit manipulation of the donor site, which could affect healing. Pain assessment will be conducted using the VAS at each dressing change, with patients being asked to report the intensity of pain experienced specifically during dressing removal. At one month postoperatively, patients will also be evaluated for scar quality using a standardised scar assessment tool. All interventions and assessments will be documented in a structured case record form (CRF) to maintain uniformity and accuracy in data collection

MANAGEMENT OF ADVERSE OUTCOMES

All patients will be closely monitored for adverse events, including local allergic reactions, prolonged staining, infection, or systemic effects. Any adverse event will be recorded in the CRF and reported to the institutional ethics committee. Participants will receive added analgesia of paracetamol, and by step up approach opioids in case of intractable pain at no additional cost, and follow-up will continue until resolution of the event.

OUTCOME MEASURES

Primary outcome

The primary outcome of this study is the level of postoperative pain experienced at the donor site following split-thickness skin grafting, assessed using the VAS. The VAS is commonly represented as a 100mm horizontal line anchored by descriptors at each end. The left end indicates 'no pain' (score of 0), while the right end represents 'worst pain imaginable' (score of 10). Patients denote their perceived pain intensity by placing a mark along the line. The distance in millimetres from the left endpoint to the patient's mark is then measured, yielding a numerical value corresponding to the reported pain level.[16]

Pain scores will be recorded at standardised intervals—postoperative days 5, 7, 15, and 30—during dressing changes. The primary comparison will be between the mean VAS scores of the intervention group (receiving intradermal MB) and the control group (receiving standard dressing) at each time point. The scores will be interpreted as shown in Table 2.

This outcome is intended to determine the efficacy of MB in providing sustained analgesia and reducing pain severity at the donor site, which is often a significant source of discomfort following graft harvesting.

SECONDARY OUTCOMES

Added analgesia: The need for additional analgesia will be assessed by monitoring and recording the administration of opioid medications beyond the routine postoperative paracetamol regimen. Patients reporting uncontrolled pain despite standard analgesia will be prescribed step-up medications at the discretion of the treating surgeon. The number and percentage of patients requiring such additional analgesia will be compared between the intervention and control groups, providing an indirect measure of the effectiveness of MB in minimising pain.

Epithelisation rate: The time taken for complete re-epithelisation of the donor site will be recorded in days. Dressings will be changed and the site evaluated on postoperative days 5, 7, 15, and 30. Complete epithelisation is defined as full closure of the wound without any residual raw area, exudate or crusting.[18] The endpoint will be the first postoperative day on which complete epithelisation is clinically confirmed. Comparison of mean epithelisation time between groups will help determine whether MB affects the healing process.

Scar quality: Scar quality at the donor site will be assessed one month postoperatively using a simple clinical grading system.[19] Scars will be classified as either normal (flat, non-hypertrophic and cosmetically acceptable) or hypertrophic (raised, thickened or erythematous). Assessment will be performed by a blinded evaluator based on visual inspection and palpation. This outcome aims to determine whether MB has any impact—positive or negative—on cosmetic outcomes and tissue remodelling

Adverse reactions: All participants will be monitored closely for potential adverse events, including allergic reactions (such as rash, swelling, redness at the injection site), prolonged staining of the skin, signs of local infection, or systemic side effects.[20] A preoperative test dose of MB will be administered intradermally over the forearm to screen for hypersensitivity. Any adverse events will be documented in the CRF and categorised by severity (mild, moderate, severe). Patients experiencing moderate to severe adverse events will be treated appropriately and, if necessary, withdrawn from the study.

Table 2. Interpretation of the VAS scores

Value	Interpretation
0–4mm	No pain
5–44mm	Mild pain
45–74mm	Moderate pain
75–100mm	Severe pain

DATA COLLECTION, MANAGEMENT AND STATISTICAL ANALYSIS

Data will be collected prospectively from all eligible patients enrolled in the study following informed consent. Baseline demographic data, comorbidities, operative details, donor site characteristics and pain scores will be recorded using a standardised CRF. Pain will be assessed using the VAS at predetermined intervals. Secondary outcomes include time to complete donor site healing, need for additional analgesia, and occurrence of adverse events. Data will be anonymised and entered into a secure, password-protected digital database by trained personnel. The security of the data and maintaining the backups will be ensured by the principal investigator. Statistical analysis will be conducted using SPSS (version 29). Continuous variables will be presented as mean ± standard deviation whereas categorical variables will be summarised as counts and percentages. Inter-group comparisons of VAS scores will be analysed using independent t-tests or Mann-Whitney U tests. A p-value of <0.05 will be considered statistically significant.

DISCUSSION

Donor site morbidity remains a significant concern in patients undergoing STSG, with pain at the donor site often exceeding that at the recipient site in terms of both intensity and duration. Effective and sustained analgesia at the donor site is critical to enhancing postoperative recovery, minimising opioid use, and improving overall patient satisfaction. Despite advancements in wound care and regional anaesthesia, there remains a need for safe, cost-effective, and easily administered methods to manage donor site pain.[21] MB, traditionally used as a diagnostic dye and antimicrobial agent, has recently emerged as a potential agent for neurochemical pain modulation. Its mechanism is believed to involve reversible inhibition of peripheral nerve endings and reduction in inflammatory cytokine release. MB acts as an inhibitor of nitric oxide synthase and guanylate cyclase pathways, both of which are involved in the propagation of pain signals. Additionally, it possesses oxidative and anti-inflammatory properties, which may contribute to local analgesic effects.[9, 22, 23]

A pre-clinical study conducted by Rosique et al[24] in 2017 demonstrated that intradermal injection of MB in male Wistar rats significantly attenuated the progression of necrosis in the stasis zone of burn wounds. This finding suggests a potential antioxidant effect of MB, likely mediated by the reduction of oxidative stress and modulation of inflammatory responses. Notably, rats treated with MB exhibited significantly larger areas of re-epithelialisation compared to control animals, indicating accelerated wound healing.[24] Building on these findings, Beldi et al[25] reported in 2021 that intradermal administration of MB in ischemic perilesional areas similarly reduced tissue necrosis and promoted tissue viability, highlighting its potential utility as a supportive treatment in burn injuries.Beyond the context of burns, multiple studies have reported promising analgesic effects of MB in various surgical settings, including haemorrhoidectomy, hernia repair, thoracoscopic surgery and total knee arthroplasty, reinforcing its potential role as a novel agent for postoperative pain relief.[26-29]

However, current evidence is limited by small sample sizes, heterogeneity in dosing, and variations in outcome measurement. Few studies have employed a randomised controlled design, and very few have standardised the timing of administration, concentration of MB, or comparator analgesic protocols. Furthermore, concerns remain about potential adverse effects, such as local tissue irritation, methemoglobinemia and delayed wound healing, although these events appear to be rare and dose-dependent. This study protocol aims to address these gaps by employing a randomised, parallel-group, active-controlled design to rigorously assess the efficacy and safety of intradermal MB for donor site pain relief following STSG. Pain scores will be measured at multiple time points using a standardised scale, and secondary outcomes, such as healing time and adverse events, will provide a holistic assessment of the intervention's utility. If proven effective, intradermal MB could be integrated into routine surgical practice, especially in resource-limited settings where advanced pain control options may be unavailable. The low cost, ease of administration, and potential for long-lasting analgesia make it a feasible alternative or adjunct to traditional analgesics, including opioids and regional blocks. By providing high-quality clinical evidence, this trial may lay the foundation for further mechanistic studies and multicentric implementation trials evaluating MB as a novel analgesic agent in surgical pain management.

CONCLUSION

This randomised controlled trial will systematically investigate the analgesic potential of intradermal MB at STSG donor sites. If proven effective, the intervention could represent a safe, simple and affordable adjunct to standard pain management protocols, potentially improving postoperative recovery and patient satisfaction.

IMPLICATIONS FOR CLINICAL PRACTICE

If proven effective, intradermal MB could become a simple, low-cost adjunct for managing donor site pain following skin graft procedures.

This approach may reduce the need for systemic analgesics, particularly opioids, thus minimising related side effects and dependence risks.

Enhanced donor site pain control can lead to improved patient comfort, faster recovery, and better compliance with post-operative care.

Standardising the use of methylene blue could help establish new protocols for post-grafting pain management in surgical and burn units.

ETHICAL APPROVAL

The study protocol has been reviewed and approved by the Institutional Ethics Committee (IEC) of Lokmanya Tilak Municipal General Hospital and Lokmanya Tilak Municipal Medical College. The trial will be conducted in accordance with the principles of the Declaration of Helsinki (2013) and Good Clinical Practice guidelines. All participants will provide written informed consent prior to enrolment.

FUNDING PARTNERS

This research has not received any external funding from agencies in the public, commercial, or not-for-profit sectors.

AUTHOR CONTRIBUTIONS

At the time of submission (4 May 2025), one of the senior contributors who fulfilled the authorship criteria was inadvertently omitted from the author list. The other three authors have since realised this omission and, under the circumstances, would like the journal to consider a post-publication authorship correction.

The correction to the author list has been made in accordance with the assigned declaration (undated but received 4 August 2026) that appears here.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

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