

Enhancing Thyroidectomy Safety with Intraoperative Methylene Blue

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ABSTRACT

Background: Thyroidectomy requires meticulous identification and preservation of the parathyroid glands and recurrent laryngeal nerves (RLNs). Their small size, variable anatomy and close relationship to the thyroid make intraoperative identification challenging. This study evaluated topical methylene blue spraying as a simple adjunct for visualization of these critical structures and assessed postoperative calcium and vocal cord outcomes.

Methods: A prospective observational study included 50 patients undergoing open total thyroidectomy at K R Hospital, Mysore Medical College and Research Institute, over an 18-month period. One millilitre (10 mg) of 1% methylene blue was sprayed over the thyroid lobe and perilobar area before inferior pole ligation. Differential staining was assessed after 5 minutes. Primary outcomes were parathyroid and bilateral RLN identification. Secondary outcomes included serum calcium, postoperative hypocalcemia, vocal cord function and complications. Continuous variables were compared using a paired t-test and categorical variables using chi-square or Fisher's exact test; $p < 0.05$ was considered significant.

Results: Mean age was 45.50 ± 11.38 years and 90% of participants were female. At least one parathyroid gland per side was identified in 47 patients (94%), with bilateral identification in 45 (90%); the mean number identified was 2.54 ± 1.02 . Bilateral RLN identification was achieved in all 50 patients (100%), with adequate visual contrast in 48 (96%). Mean serum calcium decreased from 9.18 ± 0.56 to 8.64 ± 0.63 mg/dL (mean difference 0.54 ± 0.30 mg/dL; $t = 12.79$; $p < 0.001$). Biochemical postoperative hypocalcemia (< 8.0 mg/dL) occurred in 7 patients (14%), all reported as transient. No permanent hypocalcemia or postoperative vocal cord dysfunction was recorded. Preoperative calcium < 8.5 mg/dL was strongly associated with postoperative hypocalcemia (85.7% vs 3.8%; RR 22.29; $p < 0.001$). Hypocalcemia was also associated with age < 40 years (30.0% vs 0% in 40–50 years and 7.1% in > 50 years; $p = 0.025$).

Conclusion: Topical methylene blue spraying provided high rates of parathyroid and RLN visualization in this cohort and was not associated with documented dye-related adverse events. The observed hypocalcemia was transient, while low preoperative calcium and younger age were associated with increased risk. Larger controlled studies are required before the technique can be considered superior to standard thyroidectomy or established fluorescence and neuromonitoring technologies.

Keywords: Thyroidectomy; Methylene Blue; Parathyroid Gland; Recurrent Laryngeal Nerve; Hypocalcemia.

INTRODUCTION

Thyroidectomy is a commonly performed endocrine surgical procedure for benign and malignant thyroid disorders. Although contemporary thyroid surgery has a favorable safety profile, postoperative hypocalcemia and recurrent laryngeal nerve (RLN) injury remain important procedure-specific complications. Large series have demonstrated that clinically relevant morbidity persists even in experienced practice, emphasizing the importance of meticulous dissection and identification of critical structures.^[1-3]

The parathyroid glands are small structures that may resemble surrounding fat, lymph nodes or thyroid tissue.

Their location is variable, particularly for the inferior glands, because of their embryological migration.

Preservation depends not only on recognizing the glands but also on maintaining their vascular supply.^[1,4] Similarly, the RLN has variable relationships to the inferior thyroid artery and may branch before entering the larynx. These anatomical variations can increase the risk of injury during mobilization of the thyroid and dissection near Berry's ligament.^[3,5]

Routine visual identification of the RLN and careful capsular dissection remain fundamental principles of safe thyroidectomy. Intraoperative neuromonitoring can provide additional functional information, but it requires dedicated equipment and does not replace anatomical identification.^[6] Newer techniques such as near-infrared autofluorescence and indocyanine green angiography can assist parathyroid identification or perfusion assessment, but their availability may be limited in resource-constrained settings.

Methylene blue is a readily available thiazine dye with a long history of medical use. Previous reports have described preferential staining and subsequent washout of parathyroid tissue following topical application, while surrounding tissues remain stained for longer. The RLN may remain pale or white, producing a visual contrast against the stained operative field.^[7-9]

Studies cited in the thesis have reported improved parathyroid identification with methylene blue and have suggested that the technique may reduce the difficulty of recognizing small glands during thyroid surgery. However, reported outcomes vary according to concentration, volume, timing and operative technique.^[8-10] The present study therefore

evaluated a standardized topical protocol using 1 mL of 1% methylene blue during total thyroidectomy, with particular attention to parathyroid and RLN identification and postoperative complications.

MATERIALS AND METHODS

Study Design and Setting

This prospective observational study was conducted in the Department of General Surgery, K R Hospital, Mysore Medical College and Research Institute, Mysuru, India. Fifty patients undergoing open total thyroidectomy were enrolled over an 18-month period using purposive sampling according to predefined criteria. The study was conducted after permission from the institutional ethical committee, as documented in the thesis.

Eligibility Criteria

Patients aged 18–70 years who were planned for open total thyroidectomy and willing to provide informed consent were eligible. Exclusion criteria included previous thyroid surgery, methylene blue allergy, renal disease, glucose-6-phosphate dehydrogenase deficiency, pregnancy, concurrent monoamine oxidase inhibitor use, preoperative vocal cord dysfunction, Graves' disease and retrosternal goiter.

Operative Technique

All patients underwent open total thyroidectomy under general anaesthesia through a standard Kocher incision. Subplatysmal flaps were developed and the strap muscles were separated in the midline and retracted laterally. The thyroid lobe was mobilized medially and the middle thyroid vein was ligated. The superior pole was addressed before the application of the dye. Before inferior pole ligation, the lobe was deviated medially and 1 mL (10 mg) of 1% methylene blue solution was sprayed over the thyroid lobe and perilobar area, including the expected locations of the parathyroids, inferior thyroid vessels, RLN and adjacent soft tissues.

Five minutes after spraying, the operative field was assessed. Parathyroid glands were recognized by differential staining and rapid washout, returning toward their characteristic yellow appearance while adjacent thyroid and soft tissues remained blue. The RLN was sought as a pale or white cord-like structure against the blue background. Identification was performed in conjunction with standard anatomical dissection; methylene

blue was treated as an adjunct rather than a substitute for anatomical knowledge.

Outcome Assessment

The primary outcomes were successful identification of at least one parathyroid gland per side, bilateral parathyroid identification and bilateral RLN identification. Secondary outcomes included the number of parathyroid glands identified, adequacy of RLN visual contrast, preoperative and postoperative serum calcium, postoperative hypocalcemia, vocal cord function and other postoperative complications. Preoperative and postoperative indirect laryngoscopy was used to assess vocal cord mobility. Postoperative hypocalcemia was defined as serum calcium <8.0 mg/dL. The thesis reports that all patients received standard postoperative care and that symptoms/signs of hypocalcemia were recorded.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS. Continuous variables

were summarized as mean±standard deviation and categorical variables as frequencies and percentages. A paired t-test was used for preoperative versus postoperative serum calcium. Chi-square or Fisher's exact testing was used for categorical associations. Relative risk was calculated for selected risk categories. Statistical significance was set at $p < 0.05$.

RESULTS

Baseline Characteristics

The study included 50 patients. The mean age was 45.50 ± 11.38 years (reported range 25–71 years). The largest age groups were 30–40 years and 41–50 years, each comprising 32%. Females accounted for 45 patients (90%) and males for 5 (10%), giving a male-to-female ratio of 1:9. Multinodular goiter was the principal diagnosis (42, 84%), followed by papillary carcinoma (6, 12%) and follicular carcinoma (2, 4%). All patients were reported to be euthyroid preoperatively.

Variable	Category	n	%
Age	<30 years	4	8.0
Age	30–40 years	16	32.0
Age	41–50 years	16	32.0
Age	51–60 years	10	20.0
Age	>60 years	4	8.0
Sex	Female	45	90.0
Sex	Male	5	10.0
Diagnosis	Multinodular goiter	42	84.0
Diagnosis	Papillary carcinoma	6	12.0
Diagnosis	Follicular carcinoma	2	4.0

Table 1. Baseline demographic and clinical characteristics (N=50)

Intraoperative Identification

At least one parathyroid gland on each side was identified in 47 patients (94%), and bilateral identification was achieved in 45 patients (90%). The mean number of parathyroid glands identified per patient was 2.54 ± 1.02 . Bilateral RLN identification was achieved in all

50 patients (100%). Adequate visual contrast was reported in 48 cases (96%), and the nerve appeared distinctly white or pale against the blue background in the same proportion. In all 50 cases, the staining pattern was considered to facilitate RLN dissection.

Outcome	n	%
≥1 parathyroid gland identified per side	47	94.0
Bilateral parathyroid identification	45	90.0
Mean parathyroid glands identified	2.54 ± 1.02	—
Bilateral RLN identification	50	100.0
Adequate RLN visual contrast	48	96.0
RLN white/pale against blue background	48	96.0
RLN identification facilitated dissection	50	100.0

Table 2. Intraoperative identification outcomes with topical methylene blue

Serum Calcium and Hypocalcemia

Mean preoperative serum calcium was 9.18 ± 0.56 mg/dL and postoperative calcium was 8.64 ± 0.63 mg/dL.

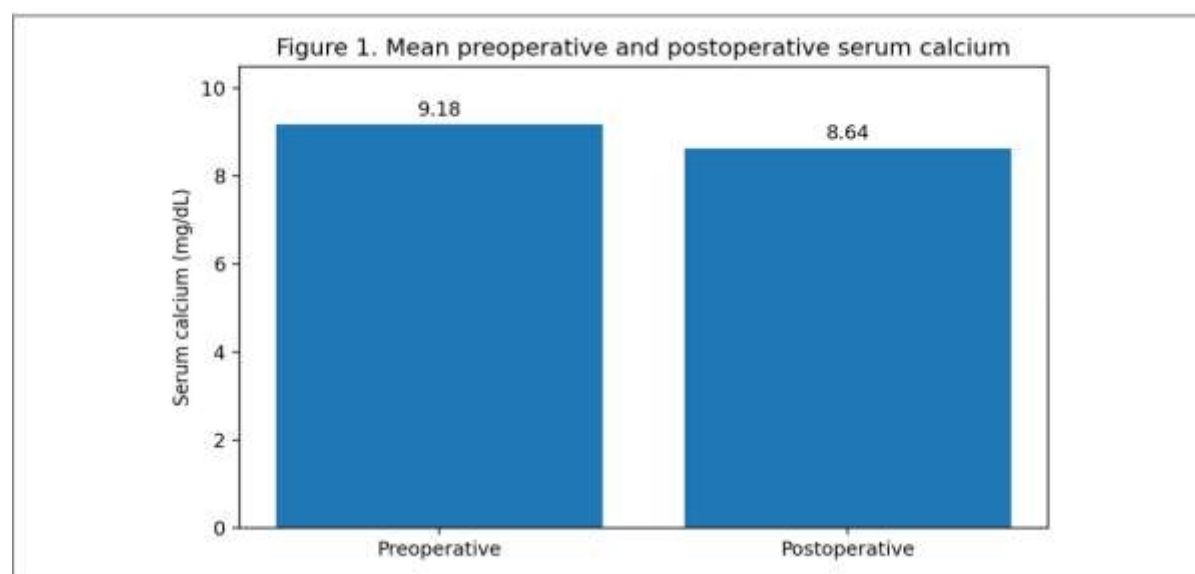
The mean reduction was 0.54 ± 0.30 mg/dL (95% CI 0.46–0.63), with a paired t-

statistic of 12.79 and $p < 0.001$. Postoperative biochemical hypocalcemia (< 8.0 mg/dL) occurred in 7 patients (14.0%; 95% CI 4.4–23.6%), while 43 patients (86%) did not meet the biochemical definition.

Measure	Preoperative	Postoperative
Mean $\pm$ SD (mg/dL)	9.18 ± 0.56	8.64 ± 0.63
Median (mg/dL)	9.20	8.73
Range (mg/dL)	8.00–10.00	7.00–9.60

Table 3. Serum calcium outcomes

Mean change: 0.54 ± 0.30 mg/dL; 95% CI 0.46–0.63; paired $t = 12.79$; $df = 49$; $p < 0.001$.

**Predictors of Postoperative Hypocalcemia**

Preoperative calcium was strongly associated with postoperative hypocalcemia. Among patients with preoperative calcium < 8.5 mg/dL, 6 of 7 (85.7%) developed hypocalcemia, compared with 1 of 26 (3.8%) among those with calcium > 9.0 mg/dL. The relative risk comparing these groups was 22.29 ($\chi^2 = 34.89$, $p < 0.001$).

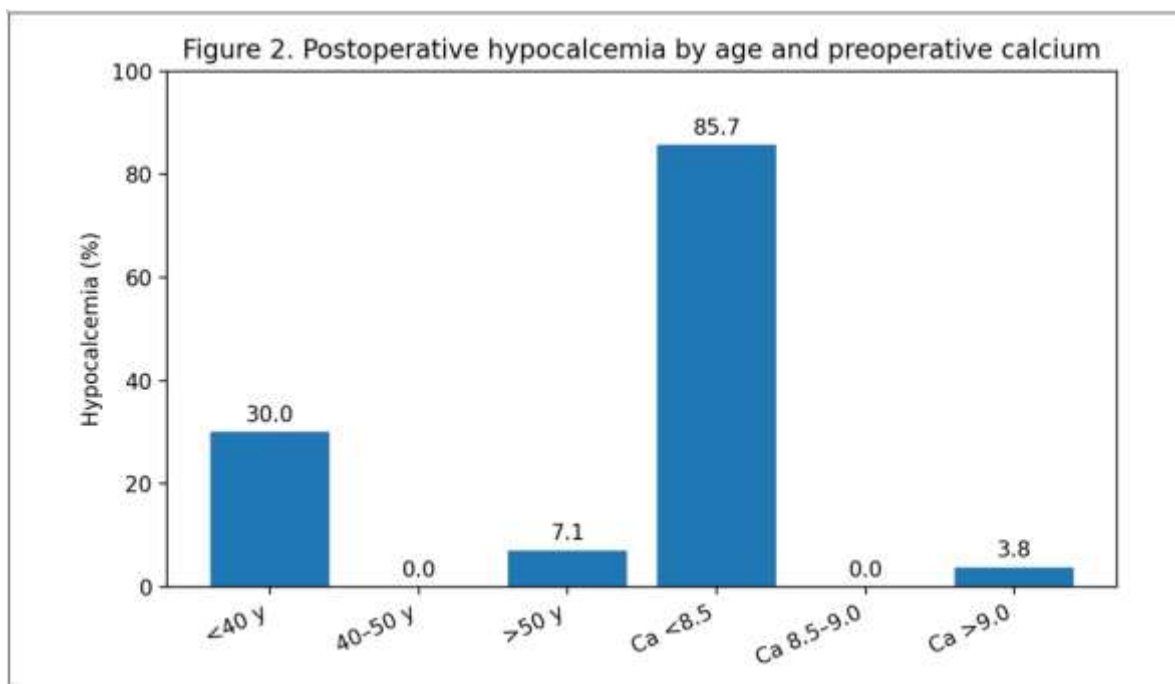
Age was also associated with hypocalcemia. Six of 20 patients aged < 40

years (30.0%) developed hypocalcemia, compared with none of 16 patients aged 40–50 years and 1 of 14 patients aged > 50 years (7.1%). The association was statistically significant ($\chi^2 = 7.40$, $df = 2$, $p = 0.025$). Diagnosis was not significantly associated with hypocalcemia: 5/42 patients with benign multinodular goiter (11.9%) and 2/8 patients with malignancy (25.0%) developed hypocalcemia (Fisher's exact $p = 0.310$; OR = 2.47).

Variable	Category	Hypocalcemia n (%)	No hypocalcemia n (%)	p value
Age	< 40 years	6 (30.0)	14 (70.0)	0.025
Age	40–50 years	0 (0.0)	16 (100.0)	
Age	> 50 years	1 (7.1)	13 (92.9)	
Diagnosis	Benign (MNG)	5 (11.9)	37 (88.1)	0.310*
Diagnosis	Malignancy	2 (25.0)	6 (75.0)	
Preop calcium	< 8.5 mg/dL	6 (85.7)	1 (14.3)	< 0.001
Preop calcium	8.5–9.0 mg/dL	0 (0.0)	17 (100.0)	
Preop calcium	> 9.0 mg/dL	1 (3.8)	25 (96.2)	

Table 4. Associations with postoperative hypocalcemia

*Fisher's exact test. Relative risk for preoperative calcium < 8.5 mg/dL versus > 9.0 mg/dL = 22.29.



Vocal Cord Function and Overall Complications

All 50 patients had intact preoperative vocal cord function and all remained intact postoperatively according to the thesis data. No temporary or permanent vocal cord dysfunction

was documented. The overall postoperative complication rate was 14.0%, consisting entirely of transient hypocalcemia. No permanent hypocalcemia, RLN injury or other postoperative complication was recorded.

Outcome	n	%	95% CI
Transient hypocalcemia	7	14.0	4.4–23.6
Permanent hypocalcemia	0	0.0	0.0–0.0
Transient vocal cord dysfunction	0	0.0	0.0–0.0
Permanent vocal cord dysfunction	0	0.0	0.0–0.0
Other complications	0	0.0	0.0–0.0
Overall complication rate	7	14.0	4.4–23.6

Table 5. Postoperative safety outcomes

DISCUSSION

The present prospective observational study evaluated a simple topical methylene blue protocol during total thyroidectomy. The principal findings were high parathyroid identification (94% for at least one gland per side; 90% bilateral), complete bilateral RLN identification (100%), a significant postoperative fall in serum calcium, and an overall complication rate of 14%, consisting solely of transient hypocalcemia. The findings are broadly consistent with the rationale for using a visual adjunct to supplement conventional anatomical dissection. At the same time, because the study had no control group and included only 50 patients, the observed outcomes cannot establish that

methylene blue itself caused the favorable complication profile.

Parathyroid Identification

The 94% rate of successful identification of at least one parathyroid gland per side is clinically relevant because preservation begins with reliable recognition of the gland. Akerstrom et al. described substantial anatomical variability in parathyroid location, helping explain why visual identification may be difficult during thyroidectomy.^[1] In the present study, the mean number of glands identified was 2.54 ± 1.02 and bilateral identification was achieved in 90% of cases. These results suggest that topical staining provided a useful additional visual cue without requiring specialized imaging hardware.

The thesis literature review cites previous methylene blue studies reporting identification rates in a broadly similar range. Sari et al. evaluated intrathyroidal methylene blue spraying and reported successful identification in approximately 88–92.9% of cases, while other studies described improvement in identification compared with conventional anatomical assessment.^[8-10] The current 94% rate is therefore within the range reported in the source literature. Importantly, differences in dye concentration, volume, timing of spraying, operative exposure and definitions of successful identification make direct numerical comparison between studies difficult.

The physiological basis proposed for the technique is differential uptake and washout of methylene blue. In the operative field, parathyroid tissue is reported to regain its yellowish appearance within several minutes while adjacent thyroid and soft tissue remain blue.^[7] This contrast may be particularly useful when the gland is small or surrounded by tissue of similar color. Nevertheless, staining should not be interpreted in isolation, and preservation of the vascular pedicle remains essential because identification alone does not guarantee functional viability.

Recurrent Laryngeal Nerve Identification

Bilateral RLN identification was achieved in all 50 patients, with adequate visual contrast in 96%. The nerve remained pale or white while surrounding tissues were stained, creating a recognizable visual contrast. RLN injury remains one of the most important feared complications of thyroid surgery because unilateral injury may cause persistent voice dysfunction and bilateral injury may compromise the airway. Large surgical series have documented measurable rates of RLN palsy despite modern techniques.^[3,11]

The present 0% postoperative vocal cord dysfunction rate is favorable compared with the rates reported in larger multicenter literature. However, the small sample size means that a zero-event result should not be interpreted as proof that the technique eliminates RLN injury. The upper confidence bound for a rare complication remains clinically meaningful when only 50 patients are observed. The absence of a control group also prevents attribution of the result to methylene blue rather than to surgeon experience, case selection or meticulous conventional dissection.

Routine RLN identification remains the foundation of safe surgery. Randolph and colleagues emphasized the role of electrophysiologic monitoring as an adjunct to visual identification rather than as a replacement for anatomical dissection.^[6] The present study did not use IONM, and therefore the findings should be interpreted as evidence that topical methylene blue can provide an additional visual cue in settings where IONM is unavailable, rather than as evidence that methylene blue is superior to neuromonitoring.

Postoperative Hypocalcemia

A statistically significant decrease in serum calcium was observed after surgery, from 9.18 ± 0.56 to 8.64 ± 0.63 mg/dL. This finding is compatible with transient postoperative impairment of parathyroid function following manipulation or temporary devascularization. Seven patients (14%) met the study definition of biochemical hypocalcemia, and all were reported as transient. Rosato et al., in a large multicenter series of 14,934 patients, reported transient hypocalcemia in 19.7% and permanent hypocalcemia in 0.9%.^[2] The 14% rate in the present cohort is numerically lower than that benchmark, although differences in patient selection, definitions and follow-up prevent a direct efficacy comparison.

The strong association between low preoperative calcium and postoperative hypocalcemia was one of the most important observations. Patients with preoperative calcium <8.5 mg/dL had an 85.7% incidence of postoperative hypocalcemia compared with 3.8% among those with calcium >9.0 mg/dL, corresponding to a relative risk of 22.29. The thesis discussion relates this finding to the possibility of reduced preoperative calcium reserve or underlying vulnerability of parathyroid function. Clinically, this association supports careful preoperative calcium assessment and closer postoperative monitoring in patients entering surgery with lower calcium values.

The age association was also statistically significant, with the highest hypocalcemia rate in patients younger than 40 years. This result should be considered hypothesis-generating rather than definitive because the number of patients in each subgroup was small. The thesis itself notes that the observation differs from some previous literature and that mechanisms related to parathyroid vascular anatomy or calcium homeostasis remain uncertain. Larger studies

are required to determine whether age independently predicts hypocalcemia after adjustment for diagnosis, operative complexity and biochemical variables.

Comparison with Alternative Technologies

Modern thyroid surgery increasingly incorporates near-infrared autofluorescence, indocyanine green angiography and IONM. These technologies can provide additional information but require equipment, training and institutional infrastructure. The present technique uses a low-volume topical dye and a short observation period, making it potentially attractive where advanced imaging systems are unavailable. The thesis review cites studies comparing methylene blue with more technologically intensive approaches and describes comparable clinical outcomes in some cohorts, while also acknowledging that newer technologies may identify more glands or provide functional information.

The potential advantage of methylene blue should therefore be framed as accessibility and simplicity rather than proven superiority. A technique that improves visualization without adding substantial complexity may be particularly useful during training and in resource-limited hospitals. However, the dye should complement rather than replace systematic anatomical dissection, vascular preservation and, where indicated, IONM.

Safety and Limitations

No adverse reaction attributable to topical methylene blue was documented in this cohort. This is reassuring, although a sample of 50 patients is insufficient to establish the incidence of uncommon dye-related adverse events. The study also has several methodological limitations: single-center design, small sample size, purposive sampling, lack of a control group, observational design and limited ability to detect rare RLN or permanent parathyroid complications. In addition, the study does not provide an independent assessment of interobserver identification accuracy, nor does it compare methylene blue directly with autofluorescence, ICG or IONM.

The reported findings also include a data point that should be verified against the original case records before journal submission: the eligibility criterion is stated as 18–70 years, whereas the observed age range is reported as 25–71 years. This discrepancy is retained here rather than silently altering the thesis data. The manuscript should be finalized only after the

source dataset is checked and the correct value is confirmed.

Clinical Implications and Future Research

The study supports the feasibility of topical methylene blue as an adjunct to visual identification of parathyroid glands and RLNs during total thyroidectomy. Its principal practical value may lie in settings where advanced visualization systems are not readily available. Future randomized studies should compare standardized methylene blue protocols with conventional surgery and, where feasible, with autofluorescence or IONM. Studies with larger samples and longer follow-up should also evaluate permanent hypoparathyroidism, functional parathyroid recovery, RLN outcomes and cost-effectiveness. Standardization of dye concentration, volume, application site and timing would improve comparability across studies.

CONCLUSION

In this prospective cohort of 50 patients undergoing total thyroidectomy, topical application of 1 mL of 1% methylene blue was associated with high rates of parathyroid and bilateral RLN identification. Parathyroid identification of at least one gland per side was achieved in 94% of cases, bilateral identification in 90%, and bilateral RLN identification in 100%. Postoperative hypocalcemia occurred in 14% of patients and was reported as transient, with no postoperative vocal cord dysfunction or permanent hypocalcemia documented. Lower preoperative calcium and younger age were significantly associated with postoperative hypocalcemia. These findings support topical methylene blue as a feasible, inexpensive visual adjunct, but larger controlled studies are needed to establish comparative effectiveness and to determine its role alongside established technologies.

Declarations

Ethics Approval

The study was approved by the Institutional Ethics Committee of Mysore Medical College and Research Institute.

Informed Consent

Written informed consent was obtained from all participants.

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Conflict of Interest

No conflict-of-interest.

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