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A PROSPECTIVE STUDY OF USE OF METHYLENE BLUE SPRAY DURING THYROIDECTOMY FOR IDENTIFICATION OF RECURRENT LARYNGEAL NERVE AND PARATHYROID GLANDS

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ABSTRACT

Thyroidectomy, the second most common endocrine surgical procedure after diabetes-related surgery, carries significant risk of injury to the recurrent laryngeal nerve (RLN) and parathyroid glands, which can result in permanent dysphonia or hypocalcaemia. Intraoperative methylene blue spray exploits the avascular Schwann sheath of the RLN rendering it unstained and the dense lymphovascular supply of parathyroid glands that enables rapid dye uptake and washout, offering a simple, low-cost adjunct for safe thyroid surgery.

Aim: To evaluate the utility and safety of intraoperative methylene blue spray in identifying the RLN and parathyroid glands during thyroidectomy.

Materials and Methods: A prospective observational study was conducted on 35 patients undergoing thyroidectomy at HSK Hospital between September 2023 and September 2025. After superior pole ligation, 1 ml of 1% methylene blue solution was sprayed over the thyroid lobe and perithyroidal area. Pre- and post-operative indirect laryngoscopy was performed and serum calcium levels were measured. Statistical analysis used paired t-test and chi-square test; $p < 0.05$ was significant.

Results: The study included 35 patients (88.6% female, 11.4% male). Both the RLN and parathyroid glands were successfully identified in 91.4% ($n=32$) of cases; in 8.6% ($n=3$), the RLN was identifiable but parathyroid identification was unsuccessful. The RLN remained unstained in 100% of cases. Post-operative indirect laryngoscopy confirmed normal vocal cord mobility in all 35 patients, with no RLN palsy. A statistically significant post-operative decline in serum calcium was noted (8.88 ± 0.46 vs. 8.36 ± 0.59 mg/dL; $p < 0.001$); however, no patient developed clinical hypocalcaemia. No adverse effects attributable to methylene blue were recorded.

Conclusion: Intraoperative methylene blue spray is a safe, simple, inexpensive, and effective technique for identification of the RLN and parathyroid glands during thyroidectomy. It is a valuable adjunct to safe thyroid surgery, particularly where intraoperative neuromonitoring is unavailable.

Keywords: Methylene Blue Spray, Thyroidectomy, Recurrent Laryngeal Nerve, Parathyroid Glands, Hypocalcaemia, Intraoperative Identification.

INTRODUCTION

Thyroid disorders constitute one of the most prevalent endocrine problems worldwide, second only to diabetes mellitus.

In recent decades, a noticeable rise in both benign and malignant thyroid diseases — attributed to improved diagnostic modalities, increased health awareness, and genuine increases in disease prevalence — has led to a corresponding increase in the number of thyroidectomies performed globally. Thyroid surgery has consequently become a frequently performed procedure in general surgical practice [1,2]. Despite being a routine operation, thyroidectomy poses significant technical challenges owing to the complex anatomy of the neck and the close proximity of critical structures, particularly the parathyroid glands and the recurrent laryngeal nerve (RLN), whose injury can result in



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serious postoperative morbidity and profoundly affect quality of life [3,4]. Post-thyroidectomy hypocalcaemia remains one of the most common complications, primarily resulting from inadvertent removal, devascularisation, or trauma to the parathyroid glands [5,6]. Transient hypocalcaemia is relatively common, while permanent hypoparathyroidism — though less frequent — can be debilitating and requires lifelong calcium and vitamin D supplementation. Similarly, injury to the RLN can lead to hoarseness, aspiration, respiratory distress, and in bilateral cases, life-threatening airway obstruction, with even temporary nerve palsy significantly impacting a patient's social and professional life [7,8].

Conventional methods rely predominantly on visual identification using anatomical landmarks, which can be unreliable in the presence of anatomical variations, distorted planes due to inflammation or malignancy, large goitres, or revision surgeries. Several adjunctive techniques have been explored — including intraoperative nerve monitoring, parathyroid autofluorescence, frozen section biopsy, optical coherence tomography, and intraoperative parathyroid hormone measurement [9] — but many are expensive, time-consuming, require specialised equipment, or are unavailable in resource-limited settings. Intraoperative nerve monitoring, whilst improving anatomical identification of the RLN, does not entirely prevent nerve injury, and its cost and technical requirements limit accessibility [10,11]. Parathyroid gland identification is further challenged by their diminutive size, variable anatomical distribution, and morphological resemblance to lymph nodes and adipose tissue [12,13].

Methylene blue is a well-established dye used in medicine for over a century, with properties including rapid tissue uptake and differential washout that have made it valuable in various surgical applications. Topical application of methylene blue spray to the surgical field offers a simple, cost-effective, and widely available means of real-time visual differentiation of critical structures without significantly prolonging operative time [14,15]. The dye exploits the avascular Schwann sheath of the RLN — which prevents dye uptake, leaving the nerve unstained — and the dense lymphovascular supply of parathyroid

glands, which allows rapid initial staining followed by washout and restoration of yellow colour within minutes [16,17,18]. Given the increasing demand for safe thyroid surgery and the need for practical techniques that reduce postoperative complications, this study aims to assess the effectiveness and safety of topical methylene blue spray in identifying and preserving the parathyroid glands and RLN during thyroidectomy.

MATERIALS AND METHODS

This was a prospective observational study conducted on 35 patients who underwent thyroidectomy at HSK Hospital between September 2023 and September 2025. All patients undergoing thyroidectomy at the institution were eligible for inclusion, while those with pre-operative RLN palsy or a history of previous thyroid surgery were excluded. The sample size of 35 was calculated at a 95% confidence level and 10% relative precision based on a reported parathyroid preservation rate of 91% with methylene blue spray, yielding an estimated minimum of 32, rounded to 35.

Pre- and post-operative indirect laryngoscopy was performed in all patients to assess vocal cord mobility. Patients were placed supine with the neck extended. A low collar incision was made, superior and inferior subplatysmal flaps were developed, strap muscles were separated, and the thyroid lobe was bluntly dissected free and rotated medially. After ligation of the superior pole and before ligation of the inferior pedicle, the thyroid lobe was deviated medially and 1 ml (10 mg) of 1% methylene blue solution was sprayed over the thyroid bed and perithyroidal tissue. All surrounding tissues stained blue; the RLN remained white (unstained) by virtue of its avascular Schwann sheath. Within approximately 3–7 minutes post-spray, the parathyroid glands washed out the dye and regained their original yellow colour, while thyroid tissue retained the stain for approximately 15–20 minutes. Pre- and post-operative serum calcium levels were recorded in all patients and compared using a paired t-test. Qualitative data were expressed as frequencies and percentages; chi-square test was used for categorical comparisons. A p-value < 0.05 was considered statistically significant. Post-operative complications and any adverse effects of methylene blue were systematically documented.

RESULTS

Table 1: Demographic Profile and Type of Surgery (n = 35)

Variable	Category	Frequency	Percent (%)
Sex	Female	31	88.6
	Male	4	11.4
Age Group (Years)	21–30	9	25.7
	31–40	8	22.9
	41–50	6	17.1

	51–60	6	17.1
	Above 60	6	17.1
Type of Surgery	Left Hemithyroidectomy	12	34.3
	Right Hemithyroidectomy	11	31.4
	Total Thyroidectomy	12	34.3
Total		35	100.0

Table 2: Pre-operative Diagnosis Distribution (n = 35)

Diagnosis	Frequency	Percent (%)
Left Colloid Goitre	10	28.6
Multinodular Goitre of Bilateral Lobes	6	17.1
Right Colloid Goitre	6	17.1
Right Multinodular Goitre	2	5.7
Left Follicular Neoplasm	2	5.7
Right Follicular Neoplasm	2	5.7
Colloid Goitre of Bilateral Lobes	2	5.7
Left Hurtle Cell Neoplasm	1	2.9
Left Multinodular Goitre	1	2.9
Right Hashimoto's Thyroiditis	1	2.9
Right Hurtle Cell Neoplasm	1	2.9
Right Non-Toxic Colloid Goitre	1	2.9
Total	35	100.0

Table 3: Methylene Blue Application Outcome vs. Type of Surgery (n = 35)

Type of Surgery	RLN and Parathyroid Identified	Parathyroid Could Not Be Identified	Total	P-Value
Left Hemithyroidectomy	12 (100%)	0 (0%)	12	0.179
Right Hemithyroidectomy	11 (100%)	0 (0%)	11	
Total Thyroidectomy	9 (75%)	3 (25%)	12	
Total	32 (91.4%)	3 (8.6%)	35	

Table 4: Pre- and Post-operative Vocal Cord Mobility and Serum Calcium (n = 35)

Variable	Pre-Operative	Post-Operative	P-Value
Vocal Cord Mobility — Normal	35 (100%)	35 (100%)	—
RLN Palsy	0 (0%)	0 (0%)	—
Serum Calcium (Mean ± SD, mg/dL)	8.88 ± 0.46	8.36 ± 0.59	< 0.001*
Clinical Hypocalcaemia	—	0 (0%)	—

*Statistically significant (paired t-test)

Table 1 shows the study population comprised 35 patients with a clear female predominance (88.6%, n=31). The age distribution was broadest in the third decade (25.7%), consistent with the known epidemiological peak of thyroid disease in young to middle-aged adults. Left hemithyroidectomy and total thyroidectomy were each performed in 34.3% of patients, and right hemithyroidectomy in 31.4%, with no statistically significant association between age or sex and type of surgery ($p = 0.673$ and $p = 0.779$, respectively).

Table 2 demonstrates that the most common pre-operative diagnoses were left colloid goitre (28.6%), multinodular goitre of bilateral lobes (17.1%), and

right colloid goitre (17.1%), with benign conditions predominating across the cohort.

Table 3 presents the primary outcome. Methylene blue spray successfully identified both the RLN and parathyroid glands in 91.4% (n=32) of all cases. The RLN remained unstained in all 35 cases (100%). In the 8.6% (n=3) of cases where parathyroid identification failed, the RLN remained distinctly visible but the parathyroid glands could not be isolated — all three failure cases were from the total thyroidectomy group. The association between type of surgery and methylene blue outcome trended toward significance but did not reach it ($p = 0.179$). No significant association was found between age group and outcome ($p = 0.443$), diagnosis and

outcome ($p = 0.205$), or sex and outcome ($p = 0.202$).

Table 4 summarises the functional outcomes. Pre-operative vocal cord movement was within normal limits in all 35 patients (100%), and this was maintained in all 35 patients on post-operative indirect laryngoscopy, with zero cases of RLN palsy. A statistically significant decline in post-operative serum calcium was demonstrated (8.88 ± 0.46 vs. 8.36 ± 0.59 mg/dL; $t = 5.590$; $p < 0.001$); however, all values remained within or near the normal range and no patient developed clinical hypocalcaemia. No adverse effects attributable to methylene blue — including allergic reactions, toxicity, or wound complications — were observed in any patient, and there was no operative mortality.

DISCUSSION

Thyroidectomy carries inherent risk to two critical structures — the recurrent laryngeal nerve (RLN) and the parathyroid glands — whose iatrogenic injury results in lifelong morbidity. Despite advances in surgical technique and intraoperative monitoring, these complications remain a challenge, particularly in less experienced hands and resource-limited settings [3,4]. The present prospective study of 35 patients evaluated the utility of topical methylene blue spray as an intraoperative adjunct during thyroidectomy. The female predominance observed in this series (88.6%) was consistent with comparable published studies — Abdulrahman et al. [16] reported 91.7% female, Basavaraj et al. [19] 93.3%, and Matar et al. [17] 81.1% — reflecting the established epidemiological preponderance of thyroid disease in women. The study population spanned all adult age groups, predominantly in the third to fifth decades, consistent with the reporting of Matar et al. [17] (mean 38.36 years), Mohamed et al. [18] (mean 41.5 years), Ahmed et al. [20] (mean 40.75 years), and Mohamed and El-Hussieny [21] (mean 40.62 years). The most common diagnoses in the present series were left colloid goitre (28.6%), multinodular goitre of bilateral lobes (17.1%), and right colloid goitre (17.1%), with benign pathology predominating. Other published series predominantly performed total thyroidectomy [17,18,20], while the present study included all three types of resection, reflecting the broader clinical applicability of the spray technique.

The primary finding of this study was that methylene blue spray enabled identification of the RLN in 100% of cases and both the RLN and parathyroid glands in 91.4% of cases (Table 3). The RLN consistently appeared as an unstained white structure against the blue-stained background tissue — a consequence of its avascular Schwann sheath that prevents dye absorption [18,20]. The parathyroid glands washed out the dye within 3–5

minutes by virtue of their dense lymphovascular supply, regaining their characteristic yellow colour [16,17,18]. These findings are broadly corroborated across the literature: 100% RLN identification was reported by Abdulrahman et al. [16], Matar et al. [17], Basavaraj et al. [19], and Mohamed and El-Hussieny [21]; parathyroid identification rates ranged from 82% (Monib et al. [22]) to 100% (Abdulrahman et al. [16], Matar et al. [17], Basavaraj et al. [19]). The three failures of parathyroid identification in the present study were exclusively from the total thyroidectomy group, suggesting bilateral field involvement and greater tissue disruption pose greater challenges — consistent with the established higher risk of hypoparathyroidism following total thyroidectomy [5,6,12]. Washout kinetics were uniform across all published series: parathyroid glands de-stained in approximately 3–5 minutes [16,17,18,19,20,21,22], thyroid tissue at approximately 15 minutes, and surrounding perithyroid structures beyond 25 minutes — providing a time-sensitive operative window for safe dissection.

Post-operatively, all 35 patients demonstrated normal vocal cord mobility on indirect laryngoscopy, yielding a zero RLN palsy rate (Table 4). This is uniformly consistent with all published methylene blue spray series — Matar et al. [17], Basavaraj et al. [19], Mohamed et al. [18], Monib et al. [22], Mohamed and El-Hussieny [21], and Ahmed et al. [20] — all of whom reported zero RLN palsy in their methylene blue groups, in contrast with RLN injury rates of up to 2.22% in corresponding conventional surgery groups [17,20]. A statistically significant post-operative decline in serum calcium was noted ($p < 0.001$), reflecting expected physiological parathyroid perturbation; however, no patient developed clinical hypocalcaemia, consistent with Mohamed and El-Hussieny [21] who similarly reported zero clinical hypocalcaemia. Ahmed et al. [20] demonstrated a statistically significant reduction in hypocalcaemia with the spray technique compared to conventional surgery (6.7% vs. 26.7%; $p = 0.038$), and Matar et al. [17] reported a significantly shorter hospital stay attributable to lower hypocalcaemia rates. No adverse effects of methylene blue spray were recorded in any patient in the present study, a finding consistent across all published series [16,17,18,19,20,21,22]. Unlike intravenous methylene blue — associated with systemic effects including serotonin syndrome and haemolysis — the topical spray route avoids clinically significant systemic absorption [22]. Monib et al. [22] nonetheless recommend a preoperative skin test to exclude rare allergic reactions, and Ahmed et al. [20] confirmed no renal effects on post-operative creatinine monitoring. The technique requires no

special equipment, no intravenous administration, and no additional cost burden, making it highly applicable in resource-limited settings and as an adjunct for surgeons where intraoperative neuromonitoring is unavailable [14,15].

CONCLUSION

The present prospective study demonstrates that intraoperative methylene blue spray is a safe, simple, cost-effective, and reliable technique for the identification of the recurrent laryngeal nerve and parathyroid glands during thyroidectomy. In this cohort of 35 patients, the RLN was successfully identified as an unstained white structure in 100% of cases and both the RLN and parathyroid glands were distinctly identified in 91.4% of cases. The differential staining behaviour — parathyroid glands washing out the dye within 3–5 minutes, thyroid tissue retaining stain for approximately 15 minutes, and perithyroid structures beyond 25 minutes — provides a clear, time-sensitive operative window for safe dissection. No case of RLN palsy was encountered and all patients had normal post-operative vocal cord mobility. Post-operative serum calcium showed a statistically significant decline (8.88 ± 0.46 vs. 8.36 ± 0.59 mg/dL; $p < 0.001$), yet no patient developed clinical hypocalcaemia, underscoring the effectiveness of the technique in parathyroid preservation. No adverse effects related to methylene blue spray were recorded in any patient. The technique is particularly beneficial where intraoperative neuromonitoring is unavailable and is applicable across all types of thyroid resection. The findings support the routine adoption of intraoperative methylene blue spray as an adjunct to safe thyroid surgery; further prospective randomised controlled studies with larger sample sizes are recommended to consolidate the evidence base.

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