The Role of Methylene Blue in Identification of Parathyroid Glands during Total Thyroidectomy

Mohamed Ibrahim Abd El Aziz Elzanaty, MSc; Ahmed Elgendy, MD; Waleed El Sherpiny, MD; Ayman Elnemr, MD; Hossam Ramadan, MD
Surgical Oncology Unit, General Surgery Department, Faculty of Medicine, Tanta University, Tanta, Egypt

Introduction: Methylene blue (MB) spray was used to identify the parathyroid gland's location. The parathyroids are capable of absorption of the blue staining and regaining their original yellow color in three minutes, however, other tissues take more time.

Aim of work: To evaluate safety and efficacy of MB spray in identifying parathyroid glands during total thyroidectomy.

Patients and methods: This randomized prospective study included 30 patients aged ≥18 years with various thyroid diseases undergoing total thyroidectomy using MB spray for parathyroid gland detection. Findings were compared with 30 patients who underwent total thyroidectomy using conventional methods without MB spray.

Results: No substantial variations existed regarding identification of parathyroids and recurrent laryngeal nerves. There was only one case that developed transient hypo-calcemic symptoms in group I and another case in group II that improved with medical treatment. There were no any recorded cases that suffered from post-operative recurrent laryngeal nerve injury symptoms, wound infection, seroma or haematoma collection. The mean value of postoperative ionized calcium levels was 4.32 ± 0.76 mg/dl and of total calcium levels was 8.47±1.55 mg/dl. The mean value of postoperative parathyroid hormone levels was 19.51 ± 5.64 pg/ml.

Conclusion: MB spraying is technically viable and secure but has no significant role in preservation of the parathyroid glands throughout thyroidectomy, with no value in minimizing the incidence of postoperative hypocalcemia. Meticulous dissection is the fundamental component for conservation of the parathyroid glands throughout thyroidectomy.

Key words: Methylene blue spray, parathyroid glands, identification, total thyroidectomy.

Introduction

Thyroid disorders are the second most common endocrine condition after diabetes mellitus, and thyroidectomy is frequently performed in iodine-deficient regions such as Indonesia, Malaysia, and Vietnam.[1] Post-thyroidectomy hypocalcemia, often caused by direct injury to the parathyroid glands or their vascular supply, adversely affects physical and psychological well-being, prolongs hospitalization, and may present with peri-oral paresthesia, fingertip numbness, or a positive Chvostek's sign.[2] Accurate intraoperative identification of parathyroid glands is essential to reduce this complication.[3]

Methylene blue (MB) spray is a simple, accessible technique for gland localization.[4] Parathyroids rapidly absorb MB and revert to their yellow color within 3 minutes, compared to 15 minutes for thyroid tissue and over 25 minutes for adipose and muscle, likely due to their rich lymphovascular network. 1 First described for parathyroid staining by Klopper et al. in 1966, MB replaced toluidine and trypan blue due to their teratogenic risks.[5] MB is inexpensive, widely available, and has been used safely for over a century.[6]

Advanced methods such as autofluorescence, indocyanine green (ICG) fluorescence, carbon nanoparticles, 5-aminolevulinic acid (5-ALA) photodynamic detection, optical coherence tomography, fine-needle aspiration with parathyroid hormone assay, and frozen section analysis achieve high specificity and sensitivity but are costly, time-consuming, or unavailable in many centers.[3,7]

Ectopic parathyroid glands, occurring in ~15% of individuals due to aberrant embryologic migration, are a major cause of persistent or recurrent disease after surgery. 3 Inferior glands are more prone to ectopia, commonly found in the thymus (30%), anterior mediastinum (20%), thyroid (20%), or thyrohyoid ligament (15%), while superior glands may occur in the tracheoesophageal groove (45%), retroesophageal space (20%), or posterior mediastinum (15%). Resection of ectopic glands >6 cm below the clavicle may require a thoracic approach, while higher glands are accessible cervically. Rare sites include pericardium and intramuscular locations in MEN1. Supernumerary glands and ectopia occur in up to 46% of hyperplasia cases.[8]

Postoperative hypoparathyroidism is defined as serum iPTH <15 pg/mL, corrected calcium <8.0 mg/dL (2.0 mmol/L), or ionized calcium <1.1 mmol/L, with or without symptoms.[9] Transient hypoparathyroidism resolves within 6–12 months, occurring in ~10% of patients, while permanent cases are reported in 4–43%.[10] Symptoms vary with

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severity and onset, ranging from paresthesia and muscle spasms to neuropsychiatric disturbances, cardiac arrhythmias, laryngospasm, bronchospasm, and seizures, all of which can be life-threatening.[11]

Aim of work: This study aims to evaluate the safety and efficacy of MB spray in parathyroid identification during total thyroidectomy.

Patients and methods

Design and population

This randomized prospective study was conducted on 30 patients aged ≥ 18 years old, of both sexes, with thyroid disease subjected to total thyroidectomy (thyroid cancer as "papillary, follicular and medullary" toxic multinodular goiter, goiter with compressive symptoms, toxic adenomas, Graves' disease not responding to medical treatment.

We compared the findings with 30 patients who underwent total thyroidectomy with conventional methods (without MB spray in the identification of parathyroid gland) in Oncology unit, General surgery Department, Tanta University Hospitals.

Participants with presence of preoperative cord dysfunction confirmed by indirect laryngoscope and patients with serum calcium (Ca) < 8.5 mg/dl were excluded from the study.

Assessments

All subjects underwent complete history, clinical examinations, laboratory tests [Full blood picture, random blood sugar, kidney and liver function testes, thyroid hormone levels (triiodothyronine (T3), thyroxine (T4), serum thyroid stimulating hormone (TSH)) and parathyroid hormone and serum calcium (Total and ionized) levels] and radiological investigation [Ultrasound on the neck, ultrasound-guided FNA cytology from the thyroid gland lesion to establish tissue diagnosis and endoscopic evaluation of vocal cords to assess the mobility].

Operative procedure

All cases were performed unoler general anesthesia with endotracheal intubation. Total thyroidectomy was performed according to the standard technique with the aid of MB spray to detect parathyroid glands throughout surgical procedure to prevent their injury. The patient was deposited in a supine position. A shoulder roll or gel pad was positioned at the acromion process of the scapula level to aid in neck extension. **(Fig. 1A)** A collar incision was used. Methylene blue (In ampules of 4 ml (0.1%) concentration) was sprayed upon the thyroid lobe and perilober area after ligation of middle thyroid vessels and controlling of upper pole and delivery of thyroid lobe. Washout period for parathyroid glands was under 3 minutes, although for thyroid glands it exceeded 15 minutes. Nevertheless, RLN doesn't absorb stain whatsoever. Parathyroid glands were located, as well as efforts were undertaken to preserve sufficient blood flow to each while displacing the gland from the thyroid lobe. Careful terminal branches of the inferior thyroid artery and vein dissection and ligation were performed to safeguard arteries feeding parathyroid glands. **(Figs. 1B,1C)**

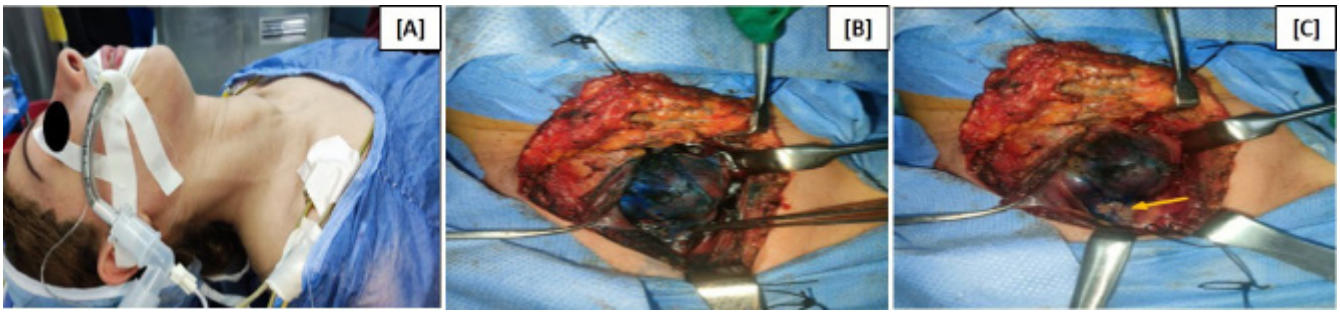

Fig 1: (A)The patient position, (B) thyroid lobe after immediate spraying with methylene blue showing the parathyroid glands staining bluish (Did not appear) and (C) washed out parathyroid glands after 5 min (Marked by the yellow arrow).

Post-operative follow up

All patients were followed closely postoperatively in ward twice daily with close observation of their respiratory symptoms, tone of voice, drain content and amount and manifestation of any complication such as Hypocalcemia, Hematoma collection and infection. Postoperative evaluation of serum calcium and parathyroid hormone level after 6 hours and in the 2nd day.

Statistical analysis

Statistical analysis was accomplished employing SPSS v28 (IBM©, Armonk, NY, USA). Shapiro-Wilks test and histograms had been utilised to assess data distribution normality. Quantitative parametric data had been displayed as mean and standard deviation (SD) and had been inspected by unpaired student t-test. Quantitative non-parametric data had been displayed as the median and interquartile range

(IQR) and had been determined via Mann Whitney-test and Wilcoxon signed ranks test to compare between two periods. Qualitative parameters had been displayed as frequencies and percentages (%) and explored utilising the Chi-square test or Fisher's exact test when applicable. A two-tailed P value < 0.05 was deemed statistically significant.

Results

No significant variation existed as regards recognition of parathyroid glands and recurrent laryngeal nerve. **(Table 1)**

Regarding the postoperative complications, 1 (3.3%) patient had hypocalcemic symptoms in both groups, 2 (6.6%) patients had asymptomatic hypocalcemia in group I while 3 (10.0%) in group II, 0 (0.0%) patient had recurrent laryngeal nerve injury symptoms in both groups, 0 (0.0%) patient had wound infection in both groups, 0 (0.0%) patient had seroma in both groups, 0 (0.0%) patient had haematoma collection in both groups, 0 (0.0%) patient had allergic reaction to the methylene blue in both groups. **(Table 2)**

Regarding the pathology of resected specimen, 9 (30.0%) patients had multi-nodular goiter in group I while 10 (33.3%) patients had multi-nodular goiter in group II, 2 (6.7%) patients had papillary thyroid cancer with LN dissection in both groups, 6 (20.0%) patients had papillary thyroid cancer without LN dissection in group II while 5 (16.6%) patients had papillary thyroid cancer without LN dissection in group II, 3 (10.0%) patients had follicular thyroid cancer in both groups, 4 (13.3%) patients had grave's disease with failed medical treatment in group I while 3 (10.0%) patients had Grave's disease with failed medical treatment in group II, 3 (10.0%) patients had toxic nodular goiter in group I while 4 (6.7%) patients had toxic nodular goiter in group II, 1 (3.3%) patient had hashimoto's thyroid disease with compression symptom in group I while 2 (6.7%) patient had hashimoto's thyroid disease with compression symptom in group II, and 2 (6.7%) patients had thyroid adenoma in group I while 1 (3.3%) patients had thyroid adenoma in group II. **(Table 3)**

The postoperative ionized Ca levels mean value was 4.32 ± 0.76 mg/dl and total Ca levels was 8.47±1.55 mg/dl. The postoperative PTH levels mean value was 19.51 ± 5.64 pg/ml. **(Table 4)**

Cases presentation

Case 1: Male patient aged 38 years old with Grave's disease with failed medical treatment. **(Fig. 2)**

Case 2: Female patient aged 50 years old with multi-nodular goiter. **(Fig. 3)**

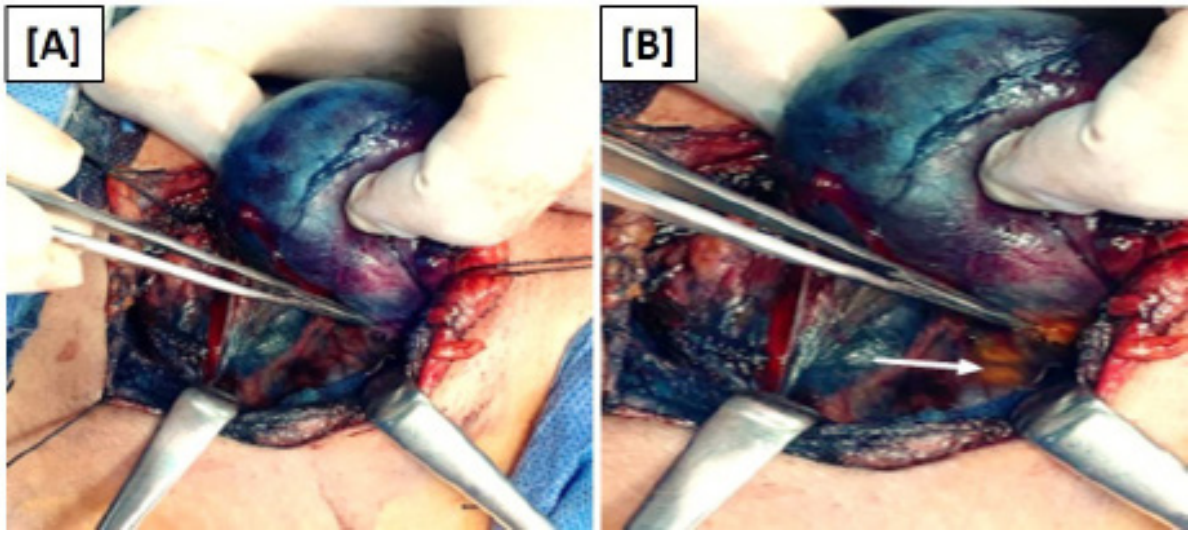

Fig 2: (A) Immediately after spraying methylene blue, and (B) After 3 mins.

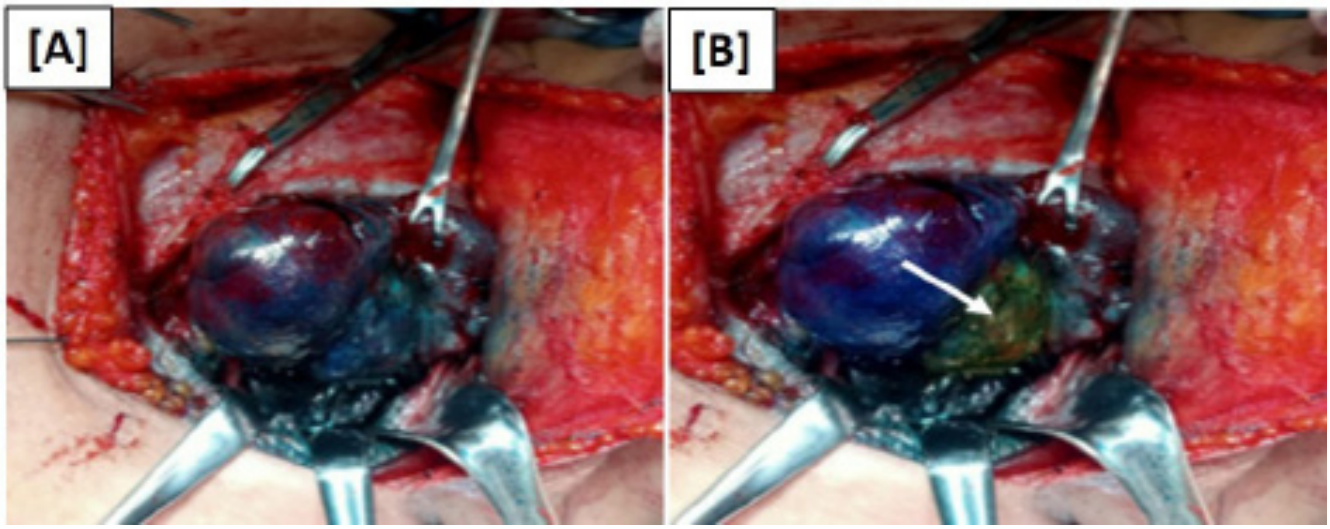

Fig 3: (A) Immediately after spraying methylene blue, and (B) After 3 mins.

Table 1: Comparison between the two studied groups according to identification of parathyroid glands and recurrent laryngeal nerve

		Group I (n=30)	Group II (n=30)	χ^2	P
Identification of parathyroid glands	Identified	25 (83.3%)	24 (80.0%)	0.111	0.739
	Non-Identified	5 (16.6%)	6 (20.0%)		
Identification of recurrent laryngeal nerve	Identified	30 (100.0%)	29 (96.7%)	1.017	^{FE}p= 1.000
	Non-Identified	0 (0.0%)	1 (3.3%)		

Data are presented as frequency (%).

Table 2: Post-operative complications of the studied patients

	Group I (n=30)	Group II (n=30)
Hypocalcemic symptoms	1 (3.3%)	1 (3.3%)
Asymptomatic hypocalcemia	2 (6.6%)	3 (10.0%)
Recurrent laryngeal nerve injury symptoms	0 (0.0%)	0 (0.0%)
Wound infection	0 (0.0%)	0 (0.0%)
Seroma	0 (0.0%)	0 (0.0%)
Haematoma collection	0 (0.0%)	0 (0.0%)
Allergic reaction to the methylene blue	0 (0.0%)	0 (0.0%)

Data are presented as frequency (%). LN: lymph node.

Table 3: Pathology of resected specimen and post-operative complications of the studied patients

		Group I (n=30)	Group II (n=30)
Multi-nodular goiter		9 (30.0%)	10 (33.3%)
Papillary thyroid cancer	**With LN dissection**	2 (6.7%)	2 (6.7%)
	Without LN dissection	6 (20.0%)	5 (16.6%)
Follicular thyroid cancer		3 (10.0%)	3 (10.0%)
Grave's disease with failed medical treatment		4 (13.3%)	3 (10.0%)
Toxic nodular goiter		3 (10.0%)	4 (6.7%)
Hashimoto's thyroid disease with compression symptom		1 (3.3%)	2 (6.7%)
Thyroid adenoma		2 (6.7%)	1 (3.3%)

Data are presented as frequency (%). LN: lymph node.

Table 4: Postoperative ionized and total Ca and PTH levels of the studied patients

	N=30
Postoperative Ionized Ca (mg/dl)	4.32 ± 0.76
Z	1.986*
P_0	0.047*
Postoperative total Ca levels	8.47 ± 1.55
Z	2.511*
P_0	0.012
Postoperative PTH levels (pg/ml)	19.51 ± 5.64
Z	1.280
P_0	0.200

Data are displayed as mean ± SD. * Significant p value <0.05, p0: p value for comparing between preoperative and postoperative, Z(p_0): the standardized value (Z-score) to compare against critical values from the standard normal distribution, PTH: parathyroid hormone, Ca: Calcium.

Discussion

Problems after thyroidectomy are prevalent; variables contributing to morbidity from thyroid surgery consist of the degree of resection, reoperation for completion, surgeon patient volume, as well as the surgeon's proficiency; careful dissection is essential for reducing problems. Postoperative hypoparathyroidism incurs both medical and economical ramifications due to prolonged hospital stays and elevated expenses.[3]

MB dye is easily accessible, although it presents some problems, particularly when administered intravenously.[3] Consequently, we sought to assess the diagnostic efficacy of employing a MB spray for the detection of parathyroid glands throughout thyroidectomy to mitigate injuries and subsequent difficulties.

In the present study, the postoperative calcium and parathyroid hormone (PTH) levels were analyzed separately for both groups. In Group I (MB spray), the mean postoperative ionized calcium level was 4.32±0.76 mg/dl, total calcium was 8.47±1.55 mg/dl, and PTH was 19.51±5.64 pg/ml. In Group II (Conventional dissection), the corresponding values were 4.09 ± 0.96 mg/dl, 7.95±2.10 mg/dl, and 16.48±5.79 pg/ml, respectively. Although the values tended to be slightly higher in the MB group, no statistically significant differences were observed between the two groups. These findings indicate that MB spray did not provide a clear advantage in maintaining postoperative calcium or PTH levels. Kamal et al.[12] reported higher mean values of 1-day postoperative serum calcium (8.84 ± 0.98 mg/dl) and early postoperative PTH (68.84 ± 20.39 pg/ml), while Monib et al. 3 observed hypocalcemia in 18% of patients (6% symptomatic), with calcium levels ranging from 3.5 to 5.6 mg/dl (Mean 4.8 ± 0.6 mg/dl), which is comparable to the ranges observed in our study.

Orban et al.[13] highlighted that The preoperative serum calcium levels weren't substantially different between both groups. Postoperative blood calcium levels were dramatically reduced in both groups; however, no notable difference was observed in serum calcium levels between the two groups. Abdalla et al.[14] revealed 1-day postoperative serum calcium and post operation 4h parathyroid hormone among studied cases. The mean value of 1-day postoperative serum calcium, post operation 4h parathyroid hormone was 8.84±0.98 and 68.84±20.39, respectively.

In the present study, the mean postoperative PTH level was 19.51 ± 5.64 pg/ml in Group I (MB spray) and 16.48 ± 5.79 pg/ml in Group II (Conventional), with no statistically significant difference between both groups. Monib et al.[3] reported postoperative PTH values ranging from 2 to 52 pmol/L (22.2±10.1), which are in line with our findings.

In the present study, there was one case that developed transient hypocalcemic symptoms, postoperative serum calcium ranged from 3.4 to 4.6 mg/dl and two cases showed asymptomatic hypocalcemia.

In agreement with our results about pathology of resected specimen, Abdalla et al.[14] found that multinodular goiter was the most common pathology (54.5%), followed by papillary thyroid carcinoma (25.5%) and lastly, solitary thyroid nodule which represented 20%.

In the present study, it was found that no significant variations existed between the two groups regarding identification and parathyroid gland was 25 (83.3%) in group I while was 24 (80.0%) in group II, and identification and Recurrent laryngeal nerve was 100% in group I while 96.7% in group II. Orban et al.[13] reported that the histological examination of the excised thyroid glands in his research revealed follicular adenoma, multinodular goiter, and colloid

goiter, with no significant variations seen amid both groups. Monib et al.[3] highlighted that Postoperative histological analysis indicated 94% (N=47) of patients had multinodular goitre (MNG), whereas 6% (N=3) were diagnosed with papillary cancer. Thyroid gland specimens revealed parathyroid glands in six individuals (12%), of which four individuals (8%) had one gland, and two individuals (4%) had two glands each.

Regarding the recognition of parathyroid glands was deemed successful at a rate of 83.3% when a minimum of three glands were detected and maintained. No statistically significant variations regarding identification of parathyroid glands between both groups.

Monib et al.[3] showed that identification of parathyroid glands was 82% of instances, with no postoperative-related or MB-associated complications.

In the present study, regarding the postoperative complications, 1 (3.3%) patient had hypocalcemic symptoms, 2 (6.6%) patients had asymptomatic hypocalcemia. Sari et al.[1] found that the occurrence of transitory hypoparathyroidism was just 5%. Only three instances had temporary hypocalcemia, lasting no more than three days.

In the present study we found that no cases had allergic reaction to the methylene blue spray. Abdalla et al.[14] found that there were no recorded adverse events or allergic manifestations to the MB spray. Regarding hypocalcemia, only 5% of the studied cases developed hypocalcemia. Falk et al.[15] observed temporary hypoparathyroidism developed in 27.8%, presenting as transitory hypocalcemia, which was well controlled with dietary supplements of vitamin D and calcium.

In the present study, we found no recurrent laryngeal nerve injury symptoms. Erbil et al.[16] indicated that RLN palsy manifested in 1.8% of their patients.

In the present study, we found that no cases had allergic reaction to the methylene blue spray. Abdalla et al.[14] found that there were no recorded adverse events or allergic manifestations to the MB spray. Regarding hypocalcemia, only 5% of the studied cases developed hypocalcemia.

Falk et al.[15] observed that temporary hypoparathyroidism happened to 27.8% of their subjects, mostly presenting as transitory hypocalcemia, which was well controlled with vitamin D and calcium oral supplements.

In the present study, as regards hospital stay ranged from 1.0 – 4.0 days with a mean ± SD of 2.30 ± 0.75 days. Monib et al.[3] showed that the duration of hospital stays varied from one to three days (1.4±0.8), throughout patients received clinical evaluations, calcium level monitoring, and therapy for hypocalcemia. Ahmed et al.[17] revealed that the duration of hospital stays varied from one to three days (1.5±0.7), during which individuals received clinical evaluations, calcium level monitoring, and therapy for hypocalcemia.

Limitations

Limitations of the work involved sample size being quite small. The work had been in a single center. Hence, it is recommended that further research is srequired to study the changes in PTH and Vit D levels with long follow up period.

Conclusion

Methylene blue spraying is technically feasible and safe but has no significant role in preservation of parathyroids throughout thyroidectomy, with no value in minimizing the incidence of postoperative hypocalcemia. Meticulous dissection is the fundamental component for the preservation of parathyroid glands during thyroidectomy.

List of abbreviations

5-ALA: 5-aminolevulinic acid.
Ca: Calcium.
CSC: Corrected serum calcium.
FNA: Fine-needle aspiration.
ICG: Indocyanine green.
iPTH: Intact parathyroid hormone.
IQR: Interquartile range.
LN: Lymph node.
MB: Methylene blue.
MEN1: Multiple endocrine neoplasia type 1.
MNG: Multinodular goiter.
PTH: Parathyroid hormone.
RLN: Recurrent laryngeal nerve.
SD: Standard deviation.
T3: Triiodothyronine.
T4: Thyroxine.
TSH: Thyroid-stimulating hormone.

Statements & declarations

Generative artificial intelligence statement

Generative AI and AI-assisted technologies were not used in the preparation of this work.

Ethical consideration

The work had been conducted from October 2022 to April 2024 following approval from the Ethics Committee Tanta University Hospitals, Tanta, Egypt (Approval code 36013/10/22). Each subject provided informed written consent.

Conflict of interest statement

The authors have no conflicts of interest or financial

disclosures that could bias or are relevant to the research or information in this paper. The authors have no conflicts of interest to declare.

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Data availability statement

The data supporting this study's findings are not publicly available due to information that could compromise the privacy of research participants, but they are available from M.E. upon reasonable request.

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