Chirurgia (2023) 118: 291-301
No. 3, May - June
Copyright© Celsius
http://dx.doi.org/10.21614/chirurgia.2023.v.118.i.3.p.291

The Role of Methylene Blue Spray to Keep Parathyroid Gland and Recurrent Laryngeal Nerve Safe During Thyroidectomy

Ahmed Mohamed Kamal Ahmed, Ramy Mikhail Nagiub, Ayman Magdy Boutros, Joseph Ellia Fahmy Hermina*

General Surgery Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

***Corresponding author:**
Joseph Ellia Fahmy Hermina, MD
General Surgery Department
Faculty of Medicine
Ain Shams University, Cairo, Egypt
E-mail: Joe_asu@live.com

Rezumat

Rolul spray-ului cu albastru de metilen pentru a menține glanda paratiroidă și nervul laringian recurent în siguranță în timpul tiroidectomiei

Context: Principalele complicații postoperatorii ale tiroidectomiei sunt hipoparatiroidismul şi paralizia nervului laringeu recurrent (RLN). Albastrul de metilen, care este atât un medicament cât şi un colorant, este sigur şi uşor de obținut şi poate fi utilizat pentru a evita complicațiile în timpul tiroidectomiei. *Obiective:* Scopul acestui studiu este de a evalua identificarea intraoperatorie a structurilor anatomice importante, respectiv glandele paratiroide şi nervul laringeu recurrent, prin pulverizarea de albastru de metilen. De asemenea, studiul evaluează siguranța acestei tehnici în comparație cu alte metode, eficiența şi fezabilitatea.
Metode: Acest studiu este un studiu de cohortă prospectiv efectuat în perioada 1 august 2021-31 iulie 2022 în Spitalul El-Demerdash. Studiul include pacienții diagnosticați cu guşă polinodulară, nodul tiroidian solitar sau guşă toxică la care tratamentul conservator medicamentos a eşuat şi care au primit indicația de tiroidectomie şi pacienți cu suspiciune de malignitate sau guşă retrosternală.
Rezultate: S-au practicat 60 tiroidectomii, dintre care 54 au fost totale şi 6 au fost hemitiroidectomii. Timpul necesar pentru a spăla colorantul de pe glanda tiroidă a fost de 15 până la 25 de minute, pentru glanda paratiroidă a fost de 4 până la 7 minute, iar pentru nervul laringeu recurent (RLN) a fost aproape de 0 până la 1 minut. Acest lucru a indicat faptul că RLN nu a absorbit colorantul. În grupul de control, doi pacienți au prezentat simptome şi semne de hipocalcemie, unul dintre aceştia având semne de tetanie, 8 pacienți au prezentat hipocalcemie pe analizele de laborator, doi pacienți au prezentat disfonie şi alți doi pacienți au prezentat

Received: 24.11.2022
Accepted: 22.03.2023

dispnee, dintre care unul a necesitat traheostomie temporară. În grupul intervențional, doar un pacient a prezentat simptome şi semne de hipocalcemie, niciunul nu a prezentat semne de tetanie, doi pacienți au avut hipocalcemie pe analizele de laborator, un pacient a prezentat disfonie, iar alți doi pacienți au prezentat dispnee, fără a fi necesară traheostomia.
Concluzii: Pulverizarea albastrului de metilen este o metodă sigură, utilă şi potrivită pentru protejarea glandelor paratiroide şi a nervului laringeu recurent în timpul tiroidectomiei, cu reducerea consecutivă a complicațiilor postoperatorii. De asemenea, utilizarea albastrului de metilen favorizează identificarea glandelor paratiroide şi a RLN în timpul intervenției chirurgicale, în ciuda faptului că unii chirurgi consideră căutarea paratiroidei şi a RLN o manevră cu efecte dăunătoare care creşte incidența leziunilor.

Cuvinte cheie: albastru de metilen, tiroidectomie, paratiroidă, nervul laringeu recurent

Abstract

Background: The main postoperative complications of thyroidectomy are hypoparathyroidism and recurrent laryngeal nerve (RLN) palsy. Methylene blue, which is a drug and dye, is safe and easy to get, and we can use it to avoid complications during thyroidectomy. Objectives: We aim to assess that we can spray Methylene blue to allow the identification of important structures intraoperative which are the parathyroid gland and recurrent laryngeal nerve. Also, to evaluate that this technique is safe, effective, technically feasible, and less dangerous than other techniques.
Methods: Our study is a prospective cohort study of patients, patients who presented with simple nodular goiter, solitary thyroid nodule, controlled toxic goiter, and failed medical treatment with the indication for thyroidectomy, suspected malignancy, and retrosternal goiters at El-Demerdash Hospital from the period of August 2021 to August 2022. In the specified study period of 1 year from 1/8/2021 to 31/7/2022,
Results: Sixty patients underwent thyroidectomy 54 underwent total while 6 only underwent hemithyroidectomy. The time taken to wash out the dye over the thyroid gland was 15 to 25 minutes, the parathyroid gland was 4 to 7 minutes and the recurrent laryngeal nerve (RLN) was almost 0 to 1 minute and this indicated that the RLN almost did not take the dye. In the controlled group, two patients had symptoms and signs of hypocalcemia only one of them had signs of tetany, 8 patients had laboratory hypocalcemia, two patients suffered from a change of voice and another two patients suffered difficulty breathing one of which needed temporary tracheostomy while in the interventional group, only one patient had symptoms and signs of hypocalcemia no one had signs of tetany, two patients had laboratory hypocalcemia, one patient suffered from the change of voice and another two patients suffered the difficulty of breathing but no one needed a tracheostomy tube.
Conclusions: It is safe, useful, and suitable to use Methylene blue spraying to keep the parathyroid gland and RLN during thyroidectomy, which led us to reduce the percentage of incidence of postoperative complications. Also, it helped us to find the parathyroid gland and RLN during the surgery, despite some surgeons seeing that searching for the parathyroid and RLN can lead to harmful effects and increase the incidence of injury.

Key words: methylene blue, thyroidectomy, parathyroid, recurrent laryngeal nerve

Introduction

The second most prevalent endocrine condition is thyroid disease following diabetes mellitus with increasing numbers of complications and indications for thyroid surgeries (1).

Thyroidectomy is one of the most frequent operations performed in iodine-deficient regions and one of the most surgical interventions in endocrine surgery (2,3).

Thyroid surgeries can be performed with lower morbidity and nearly 0% mortality in highly specialized hospitals as the complications of thyroidectomy are greatly increased by the extent of dissection and greatly reduced by the experience of the main surgeon. Thus, proper identification of anatomy and understanding of the pathology together with proper training are the keystones of proper and safe thyroidectomy (4,5).

The most common thyroid surgery to be done in different thyroid diseases is thyroidectomy and the most frequent complications are temporary or permanent hypoparathyroidism and temporary or permanent recurrent laryngeal nerve injury (6).

Hypoparathyroidism leads to postoperative hypocalcemia either temporary with an incidence of 18 % or permanent hypocalcemia with an incidence rate of 1.9% which can occur due to direct injury to the gland itself or its blood supply (7).

Postoperative hypocalcemia can prolong the patient's hospital stay and affect the patient's physical and psychological health (8).

Recurrent laryngeal nerve identification can be done by its anatomical relationship with the inferior thyroid artery at the tracheo-esophageal groove. Anatomical variations and pathology of the thyroid gland make it difficult to identify recurrent laryngeal nerve leading to dysphonia, loss of voice, or even aspiration, while bilateral injury leads to dyspnea and life-threatening airway obstruction Consequently, identifying the parathyroid gland and recurrent laryngeal nerve should be routinely done for all thyroid surgeries (9).

Methylene blue, which is a medication and dye that has been used for more than a century, is safe and readily available. A previous case series found that methylene blue spray on the surgical field was absorbed by the parathyroid gland faster than the surrounding perithyroidal area (10).

Methylene blue is safe and easy to get and use. A previous case series found that methylene blue spray on the surgical field was absorbed by the parathyroid gland leading to washing out of the dye faster than other perithyroidal areas (11). Due to its rapid absorption by the gland than perithyroidal areas methylene blue spray can specify the parathyroid gland. Also, we can identify the recurrent laryngeal nerve by spraying it after ligation of the superior pole at the tracheo-esophageal groove before ligation of the inferior pole during thyroidectomy as the recurrent laryngeal nerve appears as an unstained structure while the dye is absorbed by the parathyroid and the perthyroid areas (12).

Other methods for parathyroid gland identification during thyroid surgeries include a partial biopsy of the gland for pathological examination, intravenous methylene blue injection, and fine needle aspiration for an analysis of parathyroid hormone levels (13).

Thus, in the present study, we aimed to examine whether spraying methylene blue during thyroid surgeries allowed us to safely identify RLN and parathyroid glands, which helps us to get a safer thyroidectomy than the normal technique.

Aim of the Work

We aim to assess that Methylene blue spraying is a new step that can allow the identification of both the parathyroid gland and the recurrent laryngeal nerve, and to evaluate that this technique is safe, effective, technically feasible, and less dangerous than other techniques in order to achieve a safe thyroidectomy with fewer complications in comparison to complications we get from the conventional technique. Moreover, we also aimed to investigate any postoperative side

effects of methylene blue to prove that spraying is safe and easy to apply along with the assessment of the sensitivity and specificity of methylene blue spray in recognition of the parathyroid gland and RLN during thyroidectomy.

Patients and Methods

This is a Comparative prospective cohort study in Ain Shams University Hospital (Eldemerdash) and Ain Shams Specialized Hospital.

Study Population

Patients with indication for thyroidectomy at El-Demerdash Hospital and Ain Shams Specialized Hospital from the period of August 2021 to August 2022.

Inclusion Criteria:

1. We included in this study all patients who presented with simple nodular goiter, solitary thyroid nodule, controlled toxic goiter, and failed medical treatment with the indication for thyroidectomy, suspected malignancy, and retrosternal goiters.
2. Patients willing to undergo surgery under general anesthesia without any preoperative complications or major diseases.
3. Belonging to either gender, 18-60 years of age were included.

Exclusion Criteria:

1. Methylene blue allergies.
2. Renal impairment.
3. Major cardiac diseases.
4. G6PD deficiency.
5. Pregnant patients.
6. Having received monoamine oxidase inhibitors (MAO inhibitors) such as tranylcypromine, isocarboxazid, phenelzine
7. Patients who refused to participate in the study.
8. Presence of preoperative cord dysfunction.
9. Unfit for general surgery.

Sample Size

Sixty patients.

Sampling Method

A randomized method with comparison between two groups.

Ethical Considerations

All participants signed informed consent to participate after explaining the objective of the study and the side effects that may occur. We maintained the privacy of the participants and confidentially of the data by:

- Each patient had a special file with a code number.
- All pictures were taken only for sites of surgery covering the face.
- All results of the research are for scientific purposes.
- Participants will neither be charged nor paid to be part of this study.
- Patients' data will be deleted with complete confidentiality.

This study was approved by IRB 00006379 Ain Shams Medical School Surgery Department.

Study Tools

Methylene blue spray which we can get at a cheap price from the pathology department. In addition, it will be sterile and ready to use intra-operatively.

Study Procedures

All patients underwent thyroidectomy following the standard thyroidectomy technique which includes:

- First, we identified the site of the incision, two fingers above the sternal notch. The two ends of the incision are curved to follow Langer lines and must be symmetrical. Skin and subcutaneous tissues are sharply divided by scalpel and the platysma is clearly identified and then divided with cautery.
- The investing layer of deep fascia was opened in the midline between the

anterior jugular veins from the thyroid cartilage above to the suprasternal notch below. Strap muscles are then elevated and retracted to allow delivery of the gland.

- To allow more medial mobilization of the gland, ligation, and division of the middle thyroid vein was then done by blunt dissection the superior pole was skeletonized. Dissection of the superior pole from the trachea was done using the right-angled clamp from medial to lateral, the superior thyroid vessels were ligated and divided. The recurrent laryngeal nerve should next be located in the tracheoesophageal groove or lateral to the trachea in relation to the inferior thyroid artery.

All identified parathyroid tissue should be preserved in its native blood supply. The superior parathyroid gland was usually found after mobilization of the superior pole of the thyroid. The lateral aspect of the thyroid gland superior to the inferior thyroid artery usually revealed a fat pad where the parathyroid can be found.

During the final steps of the thyroidectomy, the lobe was dissected away from the trachea under constant exposure and preservation of the RLN. The same technique was done on the other lobe in total thyroidectomy.

Sixty patients participated with an indication for thyroidectomy. After consenting, the patients were divided randomly into two groups:

1. Group 1 (control group);
   Included 30 cases established with the conventional technique. And we will follow up on postoperative complications of hypocalcemia and symptoms of RLN injuries. Investigations will be done (parathyroid hormone postoperative, serum Ca level 24 hours then follow up every 24 hours till patient discharged). Then, we will calculate the percentage of patients that will get postoperative complications.
2. Group 2 (interventional group);
   Included 30 cases established with the methylene blue spraying technique. in this group, we will apply methylene blue spray to identify RLN which will not be stained with dye, and to identify the parathyroid gland which will absorb the dye faster than other perithyroidal areas.

After 3 to 5 mins, it will get back to its yellow color. So, we will identify it and avoid injury to the gland. Also, we will apply a small well-estimated histopathological sample from the small part which will become yellow first to prove that it is a parathyroid tissue. Thyroidectomy will be performed with safe RLN and parathyroid gland. Then we will follow up the patients with the same investigations postoperative to calculate the percentage of patients who will develop complications. In addition, we will follow up on any side effects of the dye.

We will make a comparative study of the percentages of complications between the two groups. Also, the sensitivity and specificity of methylene blue spray to recognize the parathyroid gland.

Data prospectively collected included

- Demographics (age and gender).
- Type of surgery (Total thyroidectomy or Hemi thyroidectomy).
- Clinical data (signs and symptoms).
- Laboratory and imaging results.
- Histopathology.
- Operative data: intraoperative complications, local identification of parathyroid and RLN in Group 1. Identification of parathyroid and RLN by washing out the dye in Group 2.
- Postoperative data and follow-up.

We collected the data from all 60 patients and then divided the data into two groups regarding using methylene blue spray or not. We compared the results of all collected data from the 2 groups.

Statistical Analysis

Data were collected, revised, coded, and entered into the Statistical Package for Social Science (IBM SPSS) version 25. The qualitative data

were presented as numbers and percentages while quantitative data were presented as mean, standard deviations, and ranges. Comparison between different groups regarding categorical variables was tested using the Chi-square test. When more than 20% of the cells had an expected count of less than 5, correction for chi-square was conducted using Fisher's exact test or Monte Carlo correction test. Significance level (p-value): $p > 0.05$ Insignificant, $p \leq 0.05$ Significant (95% level of confidence).

Results

As regards demographic data of both groups and all 60 patients, the mean age was 40.75 ± 9.16 years old. Fifty-one (85.0%) of all cases were women and only nine (15.0%) were men.

As regards the type of thyroidectomy, fifty-four (90%) underwent total thyroidectomy while only six (10.0%) underwent hemi-thyroidectomy.

As regards medical history, eleven patients were hypertensive on medical treatment. Three patients were diabetic (one of them was on insulin and 2 on oral hypoglycemic drugs). Five patients were hypertensive and diabetic on oral treatment. One patient was diagnosed to have ITP (Idiopathic thrombocytopenic purpura) on medical treatment and received platelets pre-operatively. Only one patient was asthmatic. The remaining 39 (65%) patients were medically healthy.

About thyroid imaging; the commonest TI-RADS score in neck ultrasound was TI-RADS III (26 patients) and TI-RADS IV (22 patients), followed by TI-RADS V in 8 patients while TI-RADS II in only 4 patients (*Table 1*).

Regarding the intra-operative data: The usage of Methylene Blue Spray (the dye) in Group 2 (the interventional group) showed that:

1. The time taken to wash out the dye over the parathyroid gland was ~5.65 ± 0.54 minutes, in the range of 4 to 7 minutes.
2. The time taken to wash out the dye over the thyroid gland was ~19.10 ± 2.87 minutes, in the range of 15 to 25 minutes.
3. The time taken to wash out the dye over the recurrent laryngeal nerve (RLN) was ~0.51 ± 0.16 minutes, which was almost 0 to 1 minute and this indicated that the RLN almost did not take the dye.

In Group 1 (the control group), the para-

Table 1. The differences in the demographic data, medical history, types of thyroidectomy, TI-RADS score in imaging, and Bethesda score in the biopsy

		Control No.=30	Intervention No.=30	Test v.	P v.	Sig.
age	Mean ± SD	39.4 ± 8.8	42.1 ± 9.4	-1.145	0.257	NS
	range	21-60	25-60			
sex	female	24(80%)	27(90%)	1.176	0.278	NS
	male	6(20%)	3(10%)			
Comorbidities	No	20(66.7%)	19(63.3%)	2.65	0.754	NS
	DM	2(6.7%)	1(3.3%)			
	HTN	5(16.7%)	6(20%)			
	DM+HTN	2(6.7%)	3(10%)			
	Asthma	0 (0%)	1(3.3%)			
	ITP	1(3.3%)	0 (0%)			
U/S	TIRADS II	1(3.3%)	3(10%)	6.063	0.109	NS
	TIRADS III	12(40%)	14(46.7%)			
	TIRADS IV	15(50%)	7(23.3%)			
	TIRADS V	2(6.7%)	6(20%)			
FNABC	Besthesda I	1(3.3%)	7(23.3%)	6.195	0.045	S
	Bethesda II	22(73.3%)	20(66.7%)			
	Besthesda III	7(23.3%)	3(10%)			
Surgery	Total	27(90%)	27(90%)	0.000	1	NS
	Hemi	3(10%)	3(10%)			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
*:Chi-square test; •: Independent t-test

thyroid and the RLN were locally identified by inspection and dissection and that showed:

1. The parathyroid gland could be identified in 21 (70%) of the patients in group 1, while it could not in 9 patients (30%). Thus, 30% of patients in Group 1 could have a parathyroid injury.
2. The RLN could be identified in 28 (93.3%) of the patients in group 1, while it could not in only two patients (6.7%). Thus, 6.7% of patients in Group 1 could have RLN injury.

Signs and Symptoms of Postoperative Hypocalcemia

1. Two patients had symptoms and signs of hypocalcemia as perioral paresthesia, finger numbness, and muscle cramps in group 1 (control group). But, only one patient had these signs and symptoms in group 2 (interventional group).
2. Only one patient of the two patients in group 1 that had symptomatic hypocalcemia had signs and symptoms of tetany but in controlled attacks, while no one had signs of tetany in group 2.
3. No one in both group had any other signs and symptoms like confusion, seizure, and delirium.

Signs and Symptoms of Postoperative Recurrent Laryngeal Nerve (RLN) Injury

1. Two patients had voice changes in group 1, while only one in group 2.
2. Two patients had difficulty breathing in group 1. And also, two patients in group 2.
3. Only one patient had dyspnea and needed temporary tracheostomy in group 1 due to choking and a drop of O2 (but exhibited improvement later and the tracheostomy tube was closed by the ENT department). No one needed tracheostomy or had dyspnea postoperative in group 2.

Regarding the signs and symptoms of postoperative side effects of Methylene Blue spray used in group 2 only (urine color changes, local reaction, GIT symptoms, and increased creatinine level), no patients had any complications of the dye, which indicated that the dye was safe with no side effects.

Postoperative Calcium Levels (Total Serum Ca, Ionized Ca)

1. In group 1 (control group), 8 (26.7%) patients had low Ca levels in both total serum Ca and Ionized Ca, the rest 22 (73.7%) had normal Ca levels.
2. In group 1 the mean of total serum Ca levels was 8.37 ± 0.79 mg/dL, ranging from 6.3 to 9.6 mg/dL (Normal levels (N); 8.5-10.3 mg/dL). The mean of ionized Ca levels was 4.29 ± 0.39 mg/dL, ranging from 3.1 to 4.8 mg/dL (N: 4.3-5.2 mg/dL).
3. On the other side, in group 2 (interventional group), only two patients (6.7%) had low Ca levels in both total serum Ca and Ionized Ca, the rest 28 (93.3%) had normal Ca levels.
4. In group 2 the mean of total serum Ca levels was 8.71 ± 0.39 mg/dL, ranging from 7.7 to 9.8 mg/dL (Normal levels (N); 8.5-10.3 mg/dL). The mean of ionized Ca levels was 4.53 ± 0.29 mg/dL, ranging from 3.7 to 5.1 mg/dL (N: 4.3-5.2 mg/dL).
5. Comparison between the two groups in

Table 2. The differences between the two groups in postoperative Ca level and ranges.

Labs Post-operative		Controlled group No. = 30	Interventional Group No.= 30	Test value	P value	Sig.
S. Ca Total	Mean ± SD range	8.37±0.79	8.71±0.39	-2.125	0.038	S
		6.3-9.6	7.7-9.8			
Ionized Ca	Mean ± SD range	4.29±0.39	4.53±0.29	-2.681	0.010	S
		3.1-4.8	3.7-5.1			

P-value > 0.05: non-significant, P- value < 0.05: significant, p-value <0.01: highly significant.

total Ca levels showed that 8 patients in group 1 had hypocalcemia and only two patients in group 2 who had a Significant P- value in *Table 2* which indicated that there was a true difference between the two groups and the number of patients who had postoperative hypocalcemia reduced after using intra-operative methylene blue spray.

Postoperative Parathyroid Hormone Levels (PTH)

1. The mean of PTH levels in group 1 was 41.43 ± 14.79 pg./mL, which ranged from 11 to 67 pg./mL, while the mean of PTH levels in group 2 was 44.87 ± 13.44 pg./mL, which ranged from 18 to 66 pg./mL (N: 12 to 65 pg./mL).
2. Twenty-eight (93.3%) patients had normal PTH levels in group 1, but only one had low levels and another one had high levels of PTH. In group 2, 29 (96.7%) patients had normal PTH levels, but only one had a high level of PTH.

Postoperative Albumin Level

1. The mean Albumin level in group 1 was 3.89 ± 0.41 g/dL, which ranged from 3 to 4.5 g/dL, while the mean Albumin level in group 2 was 3.65 ± 0.41 g/dL, which ranged from 3 to 4.5 pg./mL (N: 3.4 to 5.4 g/dL).
2. Twenty-two (73.3%) patients had normal Albumin levels in group 1 but 8 (26.7%) had low levels. In group 2, 27 (90%) patients had normal Albumin levels but 3 (10%) had low levels.

Postoperative Phosphorus (P) Levels

1. The mean Phosphorus level in group 1 was 3.26 ± 0.52 mg/dL, which ranged from 2.3 to 4 mg/dL, while the mean Phosphorus level in group 2 was 3.69 ± 0.78 mg/dL, which ranged from 2.2 to 6.1 pg./mL (N: 3.4 to 5.4 g/dL).
2. Twenty-seven (90%) patients had normal Phosphorus levels in group 1 but 3 (10%) had low levels. In group 2, 25 (83.3%) patients had normal Phosphorus levels but only one (3.3%) had low levels, while 4 patients (13.3%) had high levels (in these 4 patients 3 had pre-operative high P levels).

Postoperative Creatinine Levels (Kidney Function)

1. All patients had normal postoperative creatinine levels in both groups with a mean range of ~0.78 ± 0.19 mg/dL in group 1 and a mean range of ~0.85 ± 0.21 mg/dL. Four patients had low creatinine level 2 in each group and that was due to overhydration only so we reduced the IV fluids and got back to normal levels.
2. No patients induced an increase in creatinine levels in either group, so the methylene blue did not affect the kidney function.

In our study, localization, and identification of the glands was possible in 93.3% as we applied small well-estimated histopathological samples taken in group 2 from the small part which got washout of the dye first to prove that was a parathyroid tissue which showed; 18 of the cases (60%) confirmed as parathyroid tissue while 12 of the cases (40%) were not confirmed. Of those 12 cases which were not confirmed as parathyroid tissue shows; 10 cases were fatty tissue (83.3%) while 2 cases were lymph nodes (LN) (16.7%).

Discussion

Although thyroidectomy was first done in 1791, to date there is no safe technique to perform it except by proper identification of anatomy which needs great experience and surgical skills, the reason which lies at the basis of our study. The main aim of this study is to try to find an easy, safe, and available technique to identify the important structures mainly recurrent laryngeal nerve and para-thyroid glands, and to avoid their injuries.

Postoperative hypoparathyroidism leading

to hypocalcemia is a common complication and is becoming a major area of research. Many factors are considered to increase the incidence of post-thyroidectomy hypocalcemia. Parathyroid gland injury or injury to its blood supply during thyroidectomy is the most important factor that leads to hypocalcemia. Thus, finding how to avoid the injury remains uncertain.

Postoperative RLN palsy is also a well-known and serious complication and may lead to a lot of morbidities. The main factor which leads to RLN palsy is RLN injury during thyroidectomy. Thus, finding how to avoid the injury remains uncertain.

Methylene blue in spray form is a new way to identify the parathyroid gland and the RLN during thyroidectomy, and few researchers have trials to find how the role of spray and its sensitivity and effect during thyroidectomy but results are still unsatisfactory.

In our study, the main findings as regards the methylene blue spray are that it is sensitive to the parathyroid gland and can specify it because of rapid absorption and washing out of the dye by the gland faster than other tissues. Also, we can identify the RLN as an unstained structure in the field after spraying. So, we can identify the RLN and the parathyroid gland using methylene blue spray.

The time it takes to wash out the dye of the parathyroid glands ranged from 4 to 7 min, while the surrounding thyroid tissue ranged from 15 to 20 min, while recurrent laryngeal nerve did not take the dye at all because it took only 0.1 min to washout the dye.

These differences in wash-out time between the parathyroid glands and surrounding thyroid tissues are the result of the lymphovascular pattern. Parathyroid glands are rich in lymphovascular supply. While recurrent laryngeal nerve remains unstained due to its covering by Schwann sheath. That is why we can determine the role of methylene blue spray to keep the parathyroid gland and recurrent laryngeal nerve safe during thyroid surgeries by assessing the hypocalcemia (resulting from hypoparathyroidism) and recurrent laryngeal nerve injury.

Assessment of Postoperative Hypoparathyroidism

Postoperative hypocalcemia resulting from hypoparathyroidism is a main complication affecting patients' life and prolonging hospital stays and expenses. In our study, there is a clear difference between the two groups as in group 2 (the interventional group with methylene blue spray) only two cases had hypocalcemia (6.7%) while in group 1 (conventional method) there were eight cases which had transient hypocalcemia (26.7%). The known incidence of transient hypoparathyroidism ranges from 7% to 46%.

All research dealing with thyroidectomy and staining of parathyroid glands was performed through intravenous and/or intra-arterial methylene blue injections.

Assessment of Postoperative RLN Injury

Another main complication of thyroidectomy greatly affects patients' life is recurrent laryngeal nerve injury. Consequently, recurrent laryngeal nerve identification during operation has greatly reduced the incidence of permeant palsy unfortunately injuries still occur. Thus, we need another method to help in recurrent laryngeal nerve identification so, in our study we used methylene blue spraying after the ligation of the superior pole in the tracheoesophageal groove and before the ligation of the inferior pole. The recurrent laryngeal nerve did not stain and appeared as an unstained structure in the groove.

In group 2 (interventional group) no patients suffered from recurrent laryngeal nerve palsy while in group 1 there was one patient who complained of bilateral vocal cord immobility.

Recurrent laryngeal nerve assessment can be achieved in many ways as the use of intramuscular vocal cord electrode which is used nowadays for the identification of recurrent laryngeal nerve especially in difficult, malignant, and recurrent cases as it helps in measuring the function of recurrent laryngeal nerve in the field during thyroidectomy. Other

methods include nerve stimulation and concurrent palpation of the cricoarytenoid muscle and vocal cord assessment by fiberoptic laryngoscopy (14).

Randolph et al. tested the laryngeal palpation technique using RLN stimulation in 449 thyroid and parathyroid surgeries. In a group of patients, laryngeal palpation and laryngeal Electromyography (EMG) recordings were compared during intraoperative RLN stimulation. In this study, there was no permanent RLN paralysis. There was one case of temporary RLN paralysis due to neural stretch that improved 6 weeks postoperatively (temporary paralysis rate: 0.2% of patients) (15).

Calò et al. examined patients who had intra-operative neuromonitoring and conducted a study between June 2007 and December 2012, 2034 consecutive patients who underwent thyroidectomy. They compared patients who had IONM and patients who had standard thyroidectomy with only visualizing recurrent laryngeal nerve alone; 993 were operated on with neuromonitoring, while 1041 were with nerve visualization alone.

In patients with neuromonitoring, 28 (2.82%) recurrent laryngeal nerve injuries were observed, 21 (2.11%) transient, and 7 (0.7%) permanent. In patients with RLN visualization alone, 23 (2.21%) nerve injuries were observed, with 17 (1.63%) transient and 6 (0.58%) permanent. Differences were not statistically significant. Therefore, the main cornerstone to avoid recurrent laryngeal nerve injury is nerve visualization during thyroidectomy. Although neuromonitoring helped Calò to identify the nerve, especially in difficult cases, according to the study it did not decrease nerve injuries compared with visualization alone (16).

Our study not only aimed to identify the parathyroid glands during thyroidectomy but also recurrent laryngeal nerve. We aimed to see the parathyroid gland and assess the role of methylene blue spray to keep parathyroid safe during thyroid surgeries but we could also assess the role of the spray to keep recurrent laryngeal nerve safe. Also, we could confirm the methylene blue specifying to the gland by the samples taken for histopathology.

The intravascular techniques ensure only parathyroid gland visualization with a lot of complications so not used anymore.

The Gremillion study showed that IONM did not decrease surgery time in total and hemithyroidectomy in 119 surgeries without a significant decrease in the number of recurrent laryngeal nerve palsy but it increased the cost of surgery by $ 387 (17).

Our study did not use any expensive tools or materials as methylene blue is a cheap dye and freely available and prepared in our microbiology unit in the Clinical Pathology Department in Ain Shams University Hospitals, where it is packed in spray bottle concentrated in 100ml alcohol 70% and sterilized so no additional cost or financial burden on the patient or the hospital.

Conclusion

After comparing the results of two groups that underwent thyroidectomy by two techniques, one using the methylene blue spray and the second group using the conventional technique without spraying, the study had the following conclusions:

- Methylene blue spraying technique is safe, efficient, available, and easy to use which helped us to keep the parathyroid gland and RLN safe during thyroidectomy and that led us to reduce the percentage of incidence postoperative complications. Also, it helped us to identify the parathyroid gland and RLN during the surgery, although some surgeons do not recommend visualizing the recurrent laryngeal nerve and the parathyroid as they think it may lead to an increased possibility of their injury others see that visualizing the nerve and the parathyroid glands will decrease the stress during operation
- Finally, in our study, we showed that spraying methylene blue intraoperatively can be effective in visualizing the recurrent laryngeal nerve and the

parathyroid and there is no need for injecting it.
- We presume that our study can help future studies with a larger number of cases by different surgeons to confirm the efficacy and accuracy of this method.

Conflicts of Interests and Source of Funding

Authors state no conflict of interest and no funding involved.

Ethical Statement

This study was approved by IRB 00006379 Ain Shams Medical School Surgery Department. Also, all participants signed informed consent to participate after explaining the objective of the study and the side effects that may occur.

References

1. Myneni R, Chawla HV, Grewal AS, Vivekanandan G, Ndakotsu A, Abubacker AP, et al. Thyroxine Replacement for Subfertile Females With Subclinical Hypothyroidism and Autoimmune Thyroiditis: A Systematic Review. Cureus. 2021;13(8):e16872.
2. Bellantone R, Lombardi CP, Bossola M, Boscherini M, De Crea C, Alesina P, et al. Total thyroidectomy for management of benign thyroid disease: a review of 526 cases. World J Surg. 2002;26(12):1468-71.
3. Bron LP, O'Brien CJ. Total thyroidectomy for clinically benign disease of the thyroid gland. Br J Surg. 2004;91(5):569-74.
4. Bliss RD, Gauger PG, Delbridge LW. Surgeon's approach to the thyroid Gland: surgical anatomy and the importance of technique. World J Surg. 2000;24(8):891-7.
5. Oertli D, Udelsman R. Complications in thyroid and parathyroid surgery (Chapter 17). In: Oertli, Udelsman (Eds.). Surgery of the Thyroid and Parathyroid Glands. 2nd ed. New York: Springer-Verlag Berlin Heidelberg; 2007. p. 217–221.
6. Bewick J, Pfleiderer A. The value and role of low dose methylene blue in the surgical management of hyperparathyroidism. Ann R Coll Surg Engl. 2014; 96(7):526-9.
7. Pattou F, Combemale F, Fabre S, Carnaille B, Decoulx M, Wemeau JL, et al. Hypocalcemia following thyroid surgery: incidence and prediction of outcome. World J Surg. 1998;22(7):718-24.
8. Reeve T, Thompson NW. Complications of thyroid surgery: how to avoid them, how to manage them, and observations on their possible effect on the whole patient. World J Surg. 2000;24(8):971-5.
9. Randolph GW, Kobler JB, Wilkins J. Recurrent laryngeal nerve identification and assessment during thyroid surgery: laryngeal palpation. World J Surg. 2004;28(8):755-60.
10. Sari S, Aysan E, Muslumanoglu M, Ersoy YE, Bektasoglu H, Yardimci E. Safe thyroidectomy with intraoperative methylene blue spraying. Thyroid Res. 2012;5(1):15.
11. Piromchai P, Juengtrakool T, Laohasiriwong S, Kasemsiri P, Ungarereevittaya P. The sensitivity and specificity of methylene blue spray to identify the parathyroid gland during thyroidectomy. PeerJ. 2019;7:e6376.
12. Basavaraj PK, Patil RS, Panduranga Rao SU, Panicker R. Study on the Use of Methylene Blue Spray in Identification of Recurrent Laryngeal Nerve During Thyroid Surgery. JCDR. 2018;12(5):MC01-MC04.
13. Anton RC, Wheeler TM. Frozen section of thyroid and parathyroid specimens. Arch Pathol Lab Med. 2005;129(12):1575-84.
14. Sun H, Tian W, Jiang K, Chiang F, Wang P, Huang T, et al. Clinical guidelines on intraoperative neuromonitoring during thyroid and parathyroid surgery. Ann Transl Med. 2015;3(15):213.
15. Randolph GW, Kobler JB, Wilkins J. Recurrent laryngeal nerve identification and assessment during thyroid surgery: laryngeal palpation. World J Surg. 2004;28(8):755-60.
16. Calò PG, Pisano G, Medas F, Pittau MR, Gordini L, Demontis R, et al. Identification alone versus intraoperative neuromonitoring of the recurrent laryngeal nerve during thyroid surgery: experience of 2034 consecutive patients. J Otolaryngol Head Neck Surg. 2014;43(1):16.
17. Gremillion G, Fatakia A, Dornelles A, Amedee RG. Intraoperative recurrent laryngeal nerve monitoring in thyroid surgery: is it worth the cost? Ochsner J. 2012;12(4):363-6.