Does Local Injection of Methylene Blue allow Safe parathyroid gland preservation during Total Thyroidectomy?

Ahmed Zidan,1 MD; Taher H. Elwan,1,2 MD; Ahmed M. Nawar1

Abstract

Objectives: To evaluate surgical and biochemical outcome of local methylene blue (TT-MB) injection during subcapsular thyroidectomy in preservation of parathyroid glands.

Patients and Methods: The study include 88 patients divided into two groups. The study GP include 44 patients assigned for TT-MB and control GP include 44 patients that undergo CTT. All patients underwent clinical and radiological workup and estimation of serum parathyroid hormone (PTH) and serum total and ionized calcium (Ca). Operative procedure entails injection of 1-3 ml MB (0.8 mg/ml) in thyroid subcapsular layer and the procedure was repeated till completion of thyroidectomy.

Study outcomes: included frequency of para-thyroid gland (PTG) localization, frequency and extent of hypo-parathyroidism (HPT) as judged by estimated serum PTH on 1st and 6th postoperative day (POD1 & POD6) in relation to preoperative level and the frequency and extent of disturbed serum calcium levels. The outcomes were compared versus outcome of the control group of patients had TT without the use of MB.

Results: Dissection was easy in 38 cases (86.36%), more difficult in 6 cases (13.63%). One study and 5 control patients had HPT on POD6 and no study patient, while two control patients continued suffering hypoparathyroidism with significant difference in favor of study group.

Conclusion: Subcapsular MB injection during subcapsular thyroidectomy facilitates safe thyroid dissection sparing PTG with easy successful dissection rate of 86.36%. Despite of decreased serum PTH, its extent was minimal and only 1 patient developed transient HPT.

Keywords: Methylene blue, Local injection, subcapsular thyroidectomy, Hypoparathyroidism, Hypocalcemia.

Introduction

Total thyroidectomy (TT) is worldwide treatment option for multiple thyroid disease states [1] and is the recommended management for thyroid carcinoma. [2] However, the main barriers for short-stay TT are hemorrhage, bilateral recurrent laryngeal nerve palsy causing respiratory compromise and hypocalcaemia. [3]

Parathyroid gland (PTG) failure is the most common postoperative (PO) complication after TT [4] and permanent hypoparathyroidism (HPT) is its problematic long-term complication [5] with an incidence varying from 30% to 60% and this causes significant morbidity and increased costs. [6] Recently, PO subclinical HPT was documented as a more common coincidence, even with the use of harmonic scalpel for dissection instead of classic dissection. [7]

Measurements of serum total and ionized calcium were strongly recommended in immediate and long-term follow-up of TT patients, but ionized calcium is more reliable than total calcium measurement in the immediate PO period. [8] Parathyroid hormone (PTH) assay performed 1-hour post-TT can be helpful in predicting PO hypocalcaemia and in decision-making for safe discharge of thyroidectomy patients as day-care cases [9] which is the target of modern surgery. [4]

Methylene blue (MB) is used for tissue staining for identification or localization during thyroid surgical procedures. [10] MB marking during TT allowed marking of the plane of dissection and better identification of thyroid subcapsular plane, the wash-out time of parathyroid glands was less than three minutes but for thyroid glands was more than 15 minutes, the differences in time are due to the lympho-vascular pattern of the tissues, the lympho-vascular structure of parathyroid glands is extremely dense. This peculiarity of the tissue is vital for immediate wash out of methylene blue staining. [12]

Hypothesis

Since the position of the parathyroid gland is variable, especially the inferior ones; using injections in the fascia spaces is important to delineate the thyroid from the parathyroid. Local injection of saline- methylene blue (MB) in the interface plane between capsule of the thyroid gland and the expected parathyroid gland allow save dissection without injuring the parathyroid glands (PTG).

Setting

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Patients and Methods

The study includes 88 patients divided into two groups. The study GP include 44 patients assigned for TT-MB and control GP include 44 patients that undergo CTT.

The study protocol was approved by the Local Ethical Committee and only patients who signed written informed consents were enrolled in the study. The current study started since January 2013 till January 2018. Patients prepared for
total thyroidectomy without lymph node dissection were enrolled in the study. Exclusion criteria included carcinoma or suspicious of carcinoma, recurrent thyroid enlargement, any condition associated with disturbed serum calcium, and pregnancy. Also, patients had vitamin D insufficiency with serum level of <75 nmol/L [11] were excluded from the study.

All patients eligible for inclusion underwent complete clinical examination, indirect laryngoscopy for assessment of vocal cord mobility, radiological workup and lab investigations including complete blood count, fasting and postprandial blood glucose, liver and kidney function tests and serum parathormone (PTH), calcium (total & ionized) and 25-OH vitamin D levels.

**Preparation of Methylene blue solution**
Sterile methylene blue solution 2% was used for preparation of fluid to be injected; 20 ml of 2% solution were diluted in 500 ml of 0.9% normal saline to prepare a solution of 0.8 mg/ml; using a sterile 3-ml syringe with a 26-gauge needle, 3 ml of the prepared solution will be ready for injection.

**Operative procedure**
1-3 ml methylene blue was injected in the plane between thyroid capsule and the yellow fat enclosing the parathyroid gland which induce swelling in the tissue and spacing between the yellow fatty tissue and the thyroid gland (*Figs. 1a,b,c*) Then, dissection was performed with bipolar electrocautery along the margin of the thyroid gland on the marked blue line (*Fig.1d*) until the superior parathyroid glands were exposed and visualized. This means that all yellow tissue separated from the capsule of the gland the procedure was repeated till completion of subcapsular thyroidectomy and wound was closed with bilateral suction drain. (*Figs.1a,b,c,d*).

**Study outcome**

**Primary outcome**
Frequency of intraoperative PTG localization, Frequency and extent of HPT as judged by estimated serum PTH on 1st and 6th postoperative day (POD1 & POD6) in relation to preoperative level and Frequency and extent of disturbed serum total and ionized calcium estimated on POD1 and POD6. HPT was defined as serum PTH of <10 pg./ml on POD1 or POD2 [13] and was considered transient if normal serum PTH level was regained [14] The obtained outcomes were compared versus outcome of a similar number of patients had total thyroidectomy conventionally without the use of dye guide as a control group.

**Study power**
Considering the probability of type-I error (alpha cut-off) at 5%, probability of type-II error (beta cut-off) at 20% and the incidence of HPT after conventional TT is 17.5%; [15] the calculated number of patients/ groups must be 40 patients. [16]

**Statistical analysis**
Obtained data were presented as mean ±SD. Results were analyzed using paired t-test and One-way ANOVA Test. Statistical analysis was conducted using the IBM SPSS (Version 23, 2015) for Windows statistical package. P value <0.05 was considered statistically significant.

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![Fig.1a](image1a.png) ![Fig.1b](image1b.png) ![Fig.1c](image1c.png) ![Fig.1d](image1d.png)

*Fig 1: Fig1a- mobilization of upper pole of the gland, B- sub capsular injection of MB dye, C- identification of the plane between TG and PTG, D-completion of dissection with PTG preservation.*
Results

Table 1. Patients’ demographic data.

<table>
<thead>
<tr>
<th>Data</th>
<th>Control</th>
<th>Study</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59.1±3.5</td>
<td>58.8±3.1</td>
<td>0.711</td>
</tr>
<tr>
<td>Sex; M:F</td>
<td>27:17</td>
<td>23:21</td>
<td>0.389</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.6±3.6</td>
<td>28.6±2.6</td>
<td>0.167</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD; POD: Postoperative day; P value indicates the significance of difference between both groups.

MB injection allowed proper marking of the plane of dissection and identification of the thyroid subcapsular plane perfectly with easy satisfying dissection and PTG localization in 38 cases (86.1%), while dissection was more difficult in 6 cases (13.9%), while PTG localization was more difficult in 13 (29.5%) cases of the control group.

Serum total and ionized calcium levels and the Ionized/total Ca ratio showed non-significant differences between both groups at preoperative, POD1, and POD6 estimations. However, POD1 serum total Ca levels were significantly lower in patients of both control (p=0.004) and study (p=0.032) groups compared to preoperative levels. At POD6, serum total Ca levels were lower compared to preoperative (p=0.004 & 0.084) and POD1 (p=0.209 & 0.164) in control and study patients, respectively. Estimated serum ionized Ca level in patients of study group showed non-significant difference between the three samples (p=0.069 & 0.526 versus preoperative and p=0.285 versus POD1 level). On contrary, in control patients, POD1 levels were significantly lower (p=0.007) than preoperative levels, while POD6 levels were non-significantly lower than preoperative (p=0.526) and POD1 (p=0.285) estimates. Consequently, ionized/total Ca ratio showed non-significant difference between the three samples of patients of each group (Table 2).

Preoperative serum PTH showed non-significant (p=0.344) difference between patients of both groups. POD1 serum PTH levels significantly (p<0.001) decreased in patients of both groups compared to preoperative levels with significantly (p=0.014) lower levels in control versus study patients. Moreover, the percentage of decrease in PTH levels on POD1 was significantly (p=0.007) lower in study versus control patients. On POD6, serum PTH levels were re-increased in all patients, but were still significantly lower levels compared to preoperative (p<0.001) level. POD6 were significantly higher compared to POD1 levels in control (p<0.001) and study (p=0.009) patients. The percentage of decrease PTH level at POD6, in relation to preoperative level, was significantly (p<0.001 & 0.007) lower in patients of control and study patients, respectively with significantly (p=0.013) lower percentage of decrease in study versus control patients (Table 2, Fig. 2).

Table 2. Lab findings of patients of both groups estimated at POD1 and POD6 compared to preoperative estimates.

<table>
<thead>
<tr>
<th>Data</th>
<th>Control</th>
<th>Study</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Ca level</td>
<td>11.41±2.93</td>
<td>11.63±2.22</td>
<td>0.699</td>
</tr>
<tr>
<td>(mg/dl) Preop.</td>
<td>10.65±3.18*</td>
<td>11.31±2.75*</td>
<td>0.303</td>
</tr>
<tr>
<td>POD1</td>
<td>11±2.71</td>
<td>11.53±2.15*</td>
<td>0.311</td>
</tr>
<tr>
<td>POD6</td>
<td>4.75±0.37</td>
<td>4.78±0.42</td>
<td>0.687</td>
</tr>
<tr>
<td>Ionized Ca level</td>
<td>4.65±0.42*</td>
<td>4.7±0.57</td>
<td>0.072</td>
</tr>
<tr>
<td>(mg/dl) Preop.</td>
<td>4.71±0.55</td>
<td>4.77±0.67</td>
<td>0.666</td>
</tr>
<tr>
<td>POD1</td>
<td>0.456±0.171</td>
<td>0.429±0.102</td>
<td>0.368</td>
</tr>
<tr>
<td>POD6</td>
<td>0.499±0.227</td>
<td>0.453±0.18</td>
<td>0.291</td>
</tr>
<tr>
<td>Ionized/total Ca</td>
<td>0.468±0.183</td>
<td>0.429±0.106</td>
<td>0.221</td>
</tr>
<tr>
<td>ratio Preop.</td>
<td>35.2±9.7</td>
<td>37±8.35</td>
<td>0.344</td>
</tr>
<tr>
<td>PTH (pg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preop.</td>
<td>22.93±14.36*</td>
<td>30.12±12.61*</td>
<td>0.014</td>
</tr>
<tr>
<td>POD1 Level</td>
<td>36.67±30.53</td>
<td>20.17±25.43</td>
<td>0.007</td>
</tr>
<tr>
<td>POD6 Level</td>
<td>29.14±11.52*</td>
<td>33.65±8.68**</td>
<td>0.041</td>
</tr>
<tr>
<td>% of change</td>
<td>18.24±20.4*</td>
<td>9.65±9.39*</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Data are presented as mean ±SD; POD: Postoperative day; P value indicates the significance of difference between both groups; *: indicates the significance of difference versus preoperative value; †: indicates the significance of difference versus POD1 value.
On POD1, estimated serum PTH defined HPT in 13 patients; 11 control and 2 study with significantly (p=0.033) lower frequency in study versus control group. On POD6, 6 patients were still hypo-parathyroid; 5 in control and only one in study group with significantly (p=0.048) lower frequency in favor study group (Fig. 3). Throughout follow-up period 4 of these 6 hypothyroid patients; one in study and 3 in control group regained their serum PTH, while the remaining two control patients still suffering hypothyroidism.

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**Fig 2:** Mean POD1 and POD6 serum PTH levels estimated in patients of both groups compared to preoperative levels.

**Fig 3:** Frequency of patients developed HPT.
Discussion
Conventional total thyroidectomy (CTT), when indicated, carries a definite risk for injury of PTG. The outcome of 44 patients had CTT (Control group) showed the development of HPT, in 11 patients (25%) developed manifest HPT with serum PTH of <10 pg/ml on POD1 with concomitant significantly decreased serum total and ionized calcium in relation to preoperative estimates, till POD6 whenever 6 regained serum PTH of >10 pg/ml and were maintained on calcium supplemental therapy.

(Ca-ST) till regeneration of near normal calcium and PTH levels. Two of the other five patients continued suffering HPT for a frequency of 11.8% among patients developed manifest HPT, while three patients had recovered.

These findings go in hand with that recently documented by Vasileiadis et al. [17] who retrospectively studied files of 2556 patients who underwent CTT and reported incidental parathyroidectomy (IP) in 18.3% with significantly higher risk of postoperative (PO) biochemical and symptomatic hypocalcemia than non-IP patients and concluded that patients with malignancy especially with tumor >10 mm, extrathyroidal extension, and lymph node metastasis are at higher risk of IP and PO symptomatic hypocalcemia and these patients should be adequately treated.

Moreover, Su et al. [18] reported that auto-transplantation is an effective strategy for restoration of PTG function, but transient HPT is positively correlated to the number of auto-transplanted PTG during TT. Also, Luo et al. [19] retrospectively found PTG remaining in situ after CTT was significantly inversely associated with development of transient and/or protracted HPT and PO hypocalcemia and was identified as an independent risk factor for these events. On contrary to Su et al., [18] Luo et al. [19] reported that auto-transplantation of PTG would not affect serum calcium level in the long term.

All patients developed manifest HPT, control and study, received Ca-ST started on POD1, in line with early use of Ca-ST, previous studies documented that PO calcium supplementation effectively prevents symptomatic [20] and biochemical hypocalcemia [21] after TT and is proved to be a safe and cost-effective method that can be applied without routine laboratory assessment. [20-22]

Subcapsular thyroidectomy with methylene blue marking (TT-MB), relied on local injection of diluted MB to provide 0.8 mg/ml normal saline and only 1-3 ml was injected per site. Moreover, MB injected in the subcapsular space allowed excision of tissues retained MB with the excised thyroid gland, this minimal dose and local injection of MB had safeguarded against documented complications secondary to MB systemic absorption [23,24] that were documented with the use of larger doses [25] or intravenous route [25,26] and also points to safety of the concentration used as it coincided with doses documented in literature. [27]

TT-MB is associated with acceptable preservation of serum calcium; total and ionized, and PTH and despite of the detected decreased levels in comparison to preoperative levels, the extent of decrease was lower than in patients of the control group. Moreover, two patients developed manifest HPT on POD1, but only one patient failed to restore normal PTH level on POD6 but did not develop permanent HPT with significant difference compared to control patients. These data point to the possibility of evaluation of outcome of TT using a combination of estimation of serum calcium and PTH. Similarly, Sitges-Serra et al. [27] reported that combining both serum calcium and PTH estimations may guide medical treatment and monitoring of post-thyroidectomy prolonged HPT and predicts the likelihood of recovery of the parathyroid function with >90% accuracy.

The MB marking during TT allowed marking of the plane of dissection and perfect identification of thyroid subcapsular plane with easy satisfying dissection in 38 cases (86.1%) and concomitant faster surgery. Such success rate could be attributed to the marking color that allowed easier visualization of the plane of dissection, and to the space occupying effect of the injected fluid which allowed opening of the plane for dissection.

These data go in hand with studies previously used MB during thyroidectomy where Sari et al. [12] reported different wash-out times for MB after its spraying during thyroidectomy where recurrent laryngeal nerve and arteries were not stained, PTG were washed out of the blue stain within three minutes, thyroid tissue wash-out time was ≥15 minutes; peri-thyroid muscles, tendinous and lipid structures wash-out time was ≥25 minutes and concluded that the differential wash-out time of MB allowed safe preservation of PTG and recurrent laryngeal nerves during dissection for thyroidectomy. Also, Candell et al. [28] and Haciyanli et al. [29] found blue dye injection is a safe and effective method of localizing PTG. Moreover, Salman et al. [30] documented that preoperative intra-arterial MB infusion appears to be an effective and safe method for localization of ectopic mediastinal parathyroid adenomas and allows rapid identification during thoracoscopic resection. Recently, Hillary et al. [31] reported that PTG especially enlarged glands fluoresce from MB more intensely than thyroid glands and this auto-fluorescence may aid PTG detection.

In line with the space-occupying effect of injected fluid, Choi et al. [32] reported that procedure of subcapsular saline injection effectively spared the parathyroid gland during thyroidectomy and compared with non-subcapsular saline injection patients and significantly reduced the frequency of transient and permanent HPT.

Conclusion
Local MB subcapsular injection during TT facilitates safe thyroid dissection sparing PTG with easy successful dissection rate of 86.1%. Despite of decreased serum PTH, its extent was minimal and only 2 patients developed manifest HPT, but no patient developed permanent HPT.

References


5. Ito Y, Kihara M, Kobayashi K, Miya A, Miyauchi A: Permanent hypoparathyroidism after completion total


