

Received: 2014.03.19
Accepted: 2014.05.21
Published: 2014.10.14

Application of Nano-Carbon in Lymph Node Dissection for Thyroid Cancer and Protection of Parathyroid Glands

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Source of support: Departmental sources

Background: The aim of this study was to explore a new method to identify and protect parathyroid glands in neck lymph node dissection for patients with thyroid cancer.

Material/Methods: One hundred patients with thyroid cancer underwent total thyroidectomy combined with central neck lymph node dissection. During the operation, 50 patients receiving nano-carbon suspension were included in the experiment group, and 50 patients without nano-carbon suspension were included in the control group. We compared changes in parathyroid hormone levels before surgery and at 48 h after surgery between the 2 groups and of serum Ca²⁺ level within 48 h after surgery, as well as postoperative parathyroid pathological and lymph node dissection results.

Results: Eight and 1 parathyroid glands were detected pathologically in the control and experimental group, respectively. Decrease in parathyroid hormone level at 48 h occurred in 7 patients in the control group and 1 patient in the experimental group. Hypocalcemia was found at 48 h after surgery in 10 patients in the control group and 2 patients in the experimental group.

Conclusions: Nano-carbon suspension can cause development of the thyroid gland and the central lymph node and a negative development of parathyroid glands. Careful identification and removal of black-stained lymphatic tissues in the process of total thyroidectomy with neck lymph node dissection can ensure a complete lymph node dissection and prevent parathyroid damage, thus effectively reducing the incidence of hypoparathyroidism.

MeSH Keywords: **Lymph Node Excision • Nanotubes, Carbon • Parathyroid Glands • Thyroid Neoplasms**

Full-text PDF: <http://www.medscimonit.com/abstract/index/idArt/890721>

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Background

Recently, the incidence of thyroid cancer has been increasing yearly, and lymph node metastasis can be found at an early stage [1]. Radical resection is still the best method of treatment because this disease is not sensitive to radiotherapy or chemotherapy. Insufficient intraoperative lymph node dissection easily causes cancer residue or relapse after surgery, and reoperation is required, and the excessive dissection may result in damage to the parathyroid glands and surrounding organs. The rate of erroneous parathyroid gland dissection is up to 16.4%, and the incidence of permanent hypoparathyroidism after neck lymph node dissection for thyroid cancer is 4%. The occurrence of hypoparathyroidism not only greatly reduces the life quality to seriously affect the outcome of surgery in patients with thyroid cancer, but also increases the risk of medical disputes. Therefore, protection of parathyroid glands is attracting the attention of more and more surgeons. A new technology is urgently needed in clinics to effectively reduce the incidence of postoperative hypoparathyroidism. In this study, application of nano-carbon suspension in neck lymph node dissection for thyroid cancer caused development of lymph nodes and negative development of parathyroid glands, which is conducive to complete lymph node dissection, increases the detection rate of lymph nodes during surgery, effectively avoids parathyroid damage, and improves the intraoperative identification and protection of parathyroid glands, thereby greatly decreasing the risk of thyroid cancer surgery.

Material and Methods

Clinical data

One hundred patients with thyroid cancer were selected randomly from those who were treated from April 2012 to October 2013 in our hospital. All the patients were given total thyroidectomy combined with central neck lymph node dissection by the same surgeon. Fifty patients (5 males and 45 females, mean age: 36.4 ± 2.5 (23–65) years) who received nano-carbon suspension during the operation were included in the experiment group and all were diagnosed with papillary thyroid carcinoma pathologically. Fifty patients (11 males and 39 females, mean age: 44.5 ± 5.8 (18–59) years) who did not use nano-carbon suspension were included in the control group, in which 46 cases were diagnosed with papillary carcinoma and 4 cases were diagnosed with follicular thyroid carcinoma pathologically. There were no statistically significant differences in age or sex between the 2 groups. Nano-carbon suspension was provided by Chongqing LUMMY Pharmaceutical Co., Ltd. Informed consent for all the materials and methods involved in the paper was obtained from the patients, and the study was approved by the Ethics Committee of our hospital.



Figure 1. Injection of nano-carbon into thyroid gland.



Figure 2. Development of thyroid gland (black-stained).

Procedures

All patients were given general anesthesia. The operation procedures were as follows: the heads of the patients were pulled back to expose the neck, and the neck suprasternal notch was identified as the bony landmark. A curved incision was made at the 2-finger distance above, and the skin flap was dissociated layer by layer to separate the thyroid gland and cervical anterior muscles, fully exposing the surgical site. In the experiment group, after the thyroid gland was exposed, nano-carbon suspension was injected into the thyroid gland from the top, middle, and lower point with a 1-mL syringe. About 0.2 mL of suspension was required per point and excessive volume was avoided by all means. Back-drawing should be performed while injecting to avoid mistakenly injecting into the blood vessel (Figure 1). The thyroid gland was gently pressed for 10 min until the gland was developed after completing injection (Figure 2). After 10–15 min, the lymph vessel and lymph node were developed (Figure 3), but parathyroid glands and

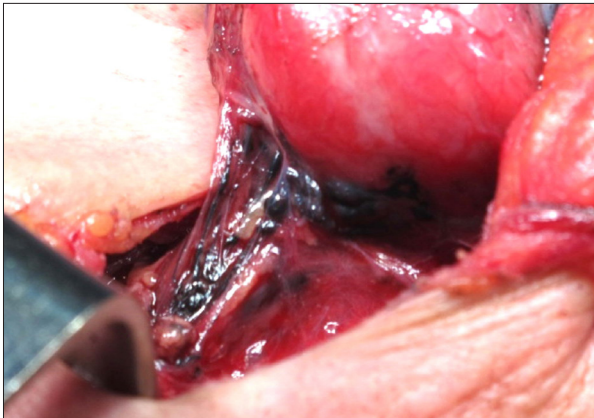


Figure 3. Development of lymph vessel and lymph nodes (black-stained).

recurrent laryngeal nerve were not developed (Figure 4). After exploring the thyroid gland of the affected side, removing the lobes with lesions, and conducting intraoperative frozen-section diagnosis, this condition was proved to be thyroid cancer. Afterwards, total thyroidectomy and central lymph node

dissection of the affected side were performed. If preoperative biopsy confirmed that the condition was thyroid cancer, nano-carbon suspension was directly injected, and then total thyroidectomy and central lymph node dissection of the affected side were performed. In the control group, the thyroid gland of the affected side was directly explored and the lobes with lesions were removed. After the results of intraoperative frozen-section diagnosis of thyroid cancer, the operation methods were the same as those of the experimental group. In both groups, the surgical site was precise and anatomical position was detailed; moreover, the parathyroid glands were protected, and the operation was completed by the same group of surgeons. The scope of lymph node dissection was as follows: from the upper bound (the upper pole of the thyroid gland) to the lower bound (the suprasternal fossa), from the external bound (the inner edge of the common carotid artery) to the inner bound (the median line of the neck), and the base (the esophagus and prevertebral fascia). The recurrent laryngeal nerves were exposed and protected after anatomy (Figure 5). En bloc resection of lymph node and adipose tissues in this region was performed. After lymph node dissection, the retained



Figure 4. Development of thyroid gland and un-developed parathyroid glands. (A) Development of thyroid gland (black-stained) and un-developed parathyroid glands (arrow). (B) Un-developed parathyroid glands (arrow). (C) Un-developed recurrent laryngeal nerve (yellow arrow) and parathyroid glands (black arrow).

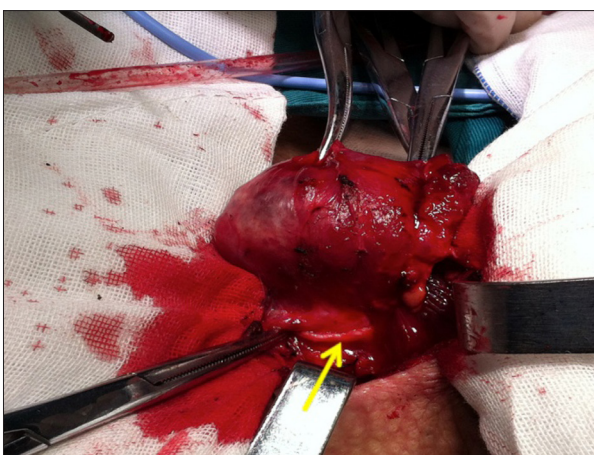


Figure 5. Undeveloped recurrent laryngeal nerve indicated by arrow.

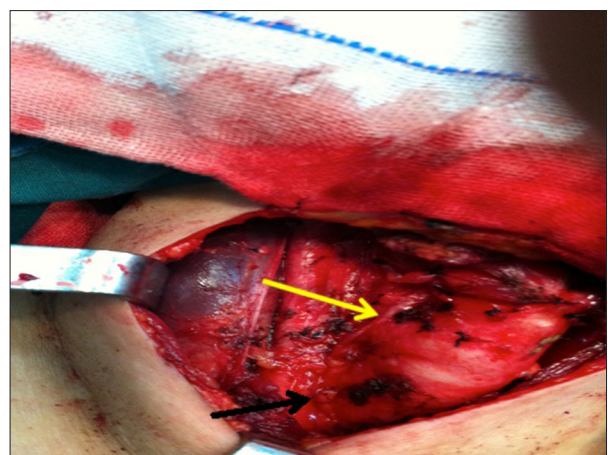


Figure 6. Recurrent laryngeal nerve (yellow arrow) and retained parathyroid glands (black arrow) after lymph node dissection.

Table 1. Number of lower PTH, lower serum Ca²⁺ and parathyroid glands.

Groups	n	Number of patients with temporary lower PTH	Number of patients with lower serum Ca ²⁺	Number of detected parathyroid glands
Control group	50	7 (14%)	10 (20%)	8 (16%)
Experimental group	50	1 (2%)	2 (4%)	1 (2%)
χ^2		4.89	6.09	6.83
<i>P</i>		0.027	0.014	0.009

Table 2. Comparison of PTH (pg/ml) and Ca²⁺ (mmol/L) levels before surgery, 48 h and 3 months after surgery between the 2 groups.

Groups	n	Before surgery		48 h after surgery		3 months after surgery	
		PTH	Ca ²⁺	PTH	Ca ²⁺	PTH	Ca ²⁺
Experimental group	50	33.45±5.11	2.31±0.39	29.66±4.27*	2.24±0.23*	31.41±3.32	2.27±0.31
Control group	50	32.66±6.78	2.29±0.26	23.21±3.61**	2.11±0.33**	27.45±4.61	2.18±0.33

* Compared with the control group, *P*<0.05; ** compared with the preoperative parameters of the same group, *P*<0.05.

parathyroid gland and recurrent laryngeal nerves can be displayed clearly (Figure 6).

Monitoring standard

The normal range of serum parathyroid hormone is 15–88 pg/ml, and <15pg/ml indicates hypoparathyroidism. The normal range of serum calcium is 2.08–2.80 mmol/L, and <2.08 mmol/L indicates hypocalcemia. If the serum parathyroid hormone at 3 months after surgery is still lower than this standard, the patient is considered to have permanent hypoparathyroidism. The clinical manifestations of hypoparathyroidism (e.g., tingling in the fingers and toes and around lips, tetany) were observed. Serum calcium, PTH, and pathological findings of parathyroid gland at 48 h after surgery in the 2 groups were recorded.

Statistical analysis

SPSS18.0 was used for analysis of postoperative parathyroid hormone, serum calcium, and pathological findings of parathyroid gland of the 2 groups. Measurement data are expressed as mean ± standard deviation ($\bar{x} \pm s$). The *t* test was used for inter-group comparison, and χ^2 test was used for inter-group comparison of count data. A difference of *P*<0.05 was considered statistically significant.

Results

No deaths occurred, and no toxic adverse effects were detected in the experimental group.

Hypothyroidism

Hypothyroidism was found in 7 patients in the control group (14%) and 1 patient in the experimental group (2%) at 48 h after surgery. The χ^2 test showed that the difference in the number of patients with decreased parathyroid hormone was statistically significant between the 2 groups. Permanent hypoparathyroidism was found in 2 patients in the control group and 1 patient in the experimental group at 3 months after surgery (Table 1).

Pathological findings of parathyroid gland

Eight (16%) and 1 (2%) parathyroid glands were detected in the control group and the experimental group, respectively. χ^2 test indicated that the difference in the number of detected parathyroid glands was statistically significant between the 2 groups (Table 1).

Decrease of serum calcium

Postoperative hypocalcemia was found in 10 patients (20%) of the control group and 2 patients (4%) of the experimental group at 48 h after surgery (Table 1). All the patients with decreased serum calcium exhibited varying degrees of symptoms such as tingling in the fingers and toes and around lips, and tetany. A postoperative 3-month follow-up visit was performed and the serum Ca²⁺ and PTH levels in the 2 groups before surgery and at 48 h and 3 months after surgery are shown in Table 2. In the experimental group, the serum Ca²⁺ and PTH levels before surgery were not different from those after surgery, and the postoperative serum Ca²⁺ was maintained at a

higher level. In the control group, the mean levels of postoperative serum PTH and Ca^{2+} were lower than those of the experimental group. The *t* test indicated that the difference between the 2 groups in serum PTH and Ca^{2+} level at 48 h was statistically significant and was not statistically significant at 3 months after surgery.

Lymph node dissection

A total of 135 and 116 lymph nodes were dissected in the experimental group and control group, respectively. Among them, metastases of 118 and 68 lymph nodes were found, with the positive rate of 87.4% and 58.6%, respectively.

Discussion

In most patients with thyroid cancer, lymph node metastasis is very common and can be found in the early stage. The application of total thyroidectomy combined with lymph node dissection of the affected side is currently increasing. However, more serious postoperative complications, including parathyroid injuries, limit the development of this surgery. Therefore, protection of parathyroid glands during surgery is receiving more attention from surgeons. Patients with temporary hypoparathyroidism may exhibit hypocalcemic tetany, which can manifest as tingling in the fingers and toes and around lips and has a lower impact on patients, while the patients with permanent hypoparathyroidism may have anxiety and depression, and sometimes even show confusion of consciousness and other mental symptoms. Patients with severe tetany may also exhibit convulsions, abdominal pain, and nausea or vomiting, thus seriously affecting quality of life and creating danger of medical disputes. Patients with permanent hypoparathyroidism need life-long calcium and vitamin D supplementation. Moreover, in case of absence of parathyroid hormone, vitamin D greatly increases the amount of excreted urinary calcium and easily induces kidney stones. The incidence of hypoparathyroidism in China and internationally is reported at 6.9–46% [2] and 14–31% [3,4], respectively. Therefore, identification and functional protection of parathyroid glands is important and should be paid attention to during total thyroidectomy combined with neck lymph node dissection.

At present, accurate identification of parathyroid glands and protection of blood supply during surgery can reduce the incidence of postoperative hypoparathyroidism. However, the position of thyroid glands (especially the inferior parathyroid gland) has great variability, and the number of individuals is significantly different. Additionally, the size and appearance of parathyroid glands are also obviously different and difficult to distinguish visually [5–7]. For mistakenly cut parathyroid glands, only autotransplantation of parathyroid glands

can be adopted for recovery. If the parathyroid glands are found to be mistakenly cut during the operation, the samples should be collected for intraoperative frozen section, and the remaining tissues should be transplanted into sternocleidomastoid once the sections above are proved to be the tissues of parathyroid glands. However, Kihara et al. [8] showed that autotransplantation of parathyroid glands did not completely restore the normal function. So far, there is no effective method to stain and position the parathyroid glands. Currently, the common dyes used in China include methylene blue and patent blue. Sari et al. [9] found that the parathyroid glands fade within 3 min, which is more rapid than that for thyroid tissues and lymph nodes after spraying methylene blue solution in the region around the thyroid gland. Related Chinese studies [10] have reported that methylene blue can be used for positioning of sentinel lymph nodes in thyroid glands, but it can stain both the parathyroid glands and lymph nodes, thus causing confusion between them and leading to mistakenly cutting parathyroid glands. Nano-carbon suspension as the lymph tracer is the only approved lymph tracer for marketing and has been applied clinically. Its granules have a mean diameter of 150 nm and have lymphatic tropism. Because the gap of capillary endothelial cells is 20–50 nm, and the gap of lymphatic capillary endothelial cell is 120–150 nm with basement-membrane hypoplasia, nano-carbon suspension is swallowed by macrophages and then transferred, retained, or gathered in the lymph nodes after injected into the local tissues of tumors to dye the lymph node black, thus achieving intravital staining of draining lymph nodes in the tumor region and achieving the purpose of lymph node tracing. Nano-carbon cannot enter the blood capillaries, avoiding the disadvantage of staining a large number of tissues. Nano-carbon as a lymph tracer has been used for treatment of gastric, breast, and colorectal cancer. Jiang et al. [11] used nano-carbon to trace sentinel lymph nodes in papillary thyroid carcinoma, and reported that the accuracy was 60.5–88.4% and the sensitivity was 92.9%. Application of nano-carbon suspension in lymph node dissection for thyroid cancer brings convenience to complete neck lymph node dissection. Black-stained lymph nodes can be found during surgery, and even the lymph nodes <1 mm in diameter embedded in adipose tissues can also be developed, avoiding omission of smaller and even metastatic lymph nodes. During central lymph node dissection, only the black-stained region is required to be removed, and the removal of unstained tissues should be paid attention to; moreover, parathyroid glands, recurrent laryngeal nerves, and others can be preserved due to negative development.

In this study, the thyroid glands and lymph nodes were developed but the parathyroid glands were not developed after nano-carbon was used in thyroid cancer surgery. Decrease of parathyroid hormone occurred in 7 patients of the control group (14%) and 1 patient of the experimental group (2%). Hypocalcemia

occurred in 10 patients of the control group (20%) and 2 patients of the experimental group (4%) at 48 h after surgery. At 3 months after surgery, 2 patients of the control group and no patients of the experimental group still exhibited hypocalcemia after the parathyroid gland's own compensation. This suggests that parathyroid gland damage was significantly reduced in the experimental group, which received the nano-carbon suspension.

Conclusions

The incidence of thyroid cancer is gradually increasing, making central lymph node dissection more important [12]. Application of nano-carbon suspension (lymphatic tracer) in central lymph

node dissection for thyroid cancer can result in an improvement of detection rate of lymph nodes (the positive rate of lymph nodes was 87.4% in the experimental group and only 58.6% in the control group) and help to reduce the incidence of false-negative findings. Through identification and protection of parathyroid glands using negative development technique, the incidence of postoperative decrease of serum calcium and lower parathyroid hormone is reduced, thus effectively decreasing complications and avoiding the occurrence of medical disputes. Use of nano-carbon in total thyroidectomy and central lymph node dissection is a new method to identify and protect the parathyroid glands and deserves clinical application. However, because of the small number of patients in the present study, this method needs further research.

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