



· 论 著 ·

甲状旁腺细针穿刺Diff-Quik染色在甲状腺癌手术中的临床应用

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[摘要] **背景与目的:** 甲状腺癌是常见的内分泌系统恶性肿瘤, 外科根治手术是治疗甲状腺癌尤其是分化型甲状腺癌的重要手段, 术中保护甲状旁腺对患者意义重大。既往术中穿刺快速检测可准确鉴别术中可疑淋巴结, 有助于甲状旁腺的保护及原位保留。探讨甲状旁腺细针穿刺Diff-Quik染色应用于甲状腺癌手术中, 是否可降低术后低血钙及低甲状旁腺素(parathyroid hormone, PTH)的发生率。**方法:** 纳入40例2018年10月—2020年1月上海市浦东医院收治的甲状腺癌患者, 随机分为2组, 对照组按照常规手术进行。细针穿刺组在淋巴结清扫过程中, 如遇到可疑的甲状旁腺, 由手术主刀医师对肉眼判断的可疑甲状旁腺进行细针穿刺, 立即行Diff-Quik染色, 如病理学检查结果显示为甲状旁腺组织或者非淋巴结予以保留。术后对穿刺吸取物行H-E染色以比较Diff-Quik染色诊断的可靠性。所有对照组和细针穿刺组患者术后第2天查血清钙及PTH, 术后第1、3、6个月门诊随访复查血清钙及PTH。**结果:** 利用Diff-Quik染色法, 快速从病理学层面诊断甲状旁腺联合淋巴结的排除避免术中误切甲状旁腺, 原位保留甲状旁腺的准确率达100.0%, 高于对照组的88.2%。两组患者术前与术后3个月、术后6个月的血清钙及PTH水平差异无统计学意义($P_{1a}=0.076$, $P_{2a}=0.088$, $P_{1b}=0.070$, $P_{2b}=0.082$); 两组患者术后2 d, 术后1个月的血清钙水平及PTH均低于术前($P_{3a}=0.001$, $P_{4a}=0.006$, $P_{3b}=0.007$, $P_{4b}=0.016$)。细针穿刺组患者术后2 d、术后1个月、术后3个月的血清钙及PTH水平高于对照组($P_{5a}=0.001$, $P_{6a}=0.017$, $P_{5b}=0.032$, $P_{6b}=0.045$)。对照组患者术前、术后6个月的血清钙及PTH水平与细针穿刺组比较差异无统计学意义($P_{7a}=0.802$, $P_{8a}=0.675$, $P_{7b}=0.857$, $P_{8b}=0.696$)。**结论:** 甲状腺癌术中使用细针穿刺可疑甲状旁腺Diff-Quik染色有助于原位保留甲状旁腺, 降低术后低血钙及低PTH的发生率。

[关键词] 甲状腺癌手术; Diff-Quik染色; 血清钙; 甲状旁腺

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Clinical application of fine needle puncture Diff-Quik staining for the differentiation and protection of parathyroid glands in thyroid carcinoma surgery YUAN Hao¹, JIANG Xiaofei¹, YOU Qinghua², ZHU Hongbo², YE Huiying², WU Wen¹, QIAN Fengyuan¹, ZHONG Ming¹, LI Yongping¹ (1. Department of Breast Surgery, Pudong Hospital of Shanghai, Shanghai 201399, China; 2. Department of Pathology, Pudong Hospital of Shanghai, Shanghai 201399, China)

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[Abstract] **Background and purpose:** Thyroid cancer is a common malignant tumor of endocrine system. Radical surgery is an important method for the treatment of thyroid cancer, especially differentiated thyroid cancer. It is of great significance to protect parathyroid gland during operation. In previous studies, rapid detection of intraoperative puncture can accurately identify intraoperative suspicious lymph nodes, which is helpful to the protection and *in situ* preservation of parathyroid. The purpose of this study was to explore the feasibility of the fine needle puncture Diff-Quik staining for the differentiation and protection of parathyroid glands in thyroid carcinoma surgery. **Methods:** Clinical data of 40 papillary thyroid cancer patients at Pudong Hospital of Shanghai between Oct. 2018 and Jan. 2020 were collected. The patients were randomly divided into two groups. In the control group, the parathyroid glands were identified with the naked eye by the chief surgeon according to experience. In the experimental group, if

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suspicious parathyroid gland was encountered during lymph node dissection, the surgeon in charge of the operation performed fine needle puncture on the suspected parathyroid judged by naked eyes, made cell smear, and immediately performed Diff-Quik staining. Blood calcium and parathyroid hormone (PTH) of all the patients were measured on the second day after operation. Blood calcium and PTH were followed up at 1, 3 and 6 months after operation. **Results:** Using Diff-Quik staining method to quickly diagnose parathyroid glands from the pathological level, could avoid miscutting the parathyroid glands during the operation, and the accuracy rate of parathyroid gland preservation *in situ* reached 100.0%, which was higher than the accuracy rate (88.2%) of the naked eye to identify parathyroid glands. There was no significant difference in the blood calcium and PTH levels between the patients of the two groups before the operation, 3 months and 6 months after operation ($P_{1a}=0.076$, $P_{2a}=0.088$, $P_{1b}=0.070$, $P_{2b}=0.082$); the patients of the two groups were 2 days after the operation and 1 month after the operation. The blood calcium and PTH levels of the patients were lower than those before the operation, the differences were statistically significant ($P_{3a}=0.001$, $P_{4a}=0.006$, $P_{3b}=0.007$, $P_{4b}=0.016$). The blood calcium and PTH levels of the experimental group were higher than those of the control group 2 days, 1 month and 3 months after operation ($P_{5a}=0.001$, $P_{6a}=0.017$, $P_{5b}=0.032$, $P_{6b}=0.045$). The blood calcium and PTH levels of the control group before and 6 months after the operation were compared with the experimental group, and the differences were not statistically significant ($P_{7a}=0.802$, $P_{8a}=0.675$, $P_{7b}=0.857$, $P_{8b}=0.696$). **Conclusion:** The fine needle puncture Diff-Quik staining could improve the accuracy and specificity of parathyroid differentiation and achieve parathyroid protection.

[Key words] Thyroid cancer surgery; Diff-Quik staining; Blood calcium; Parathyroid glands

甲状腺癌是常见的内分泌系统恶性肿瘤，在中国的发病率呈明显上升趋势，被列入增长速度最快的恶性肿瘤之一^[1]。其中分化型甲状腺癌超过90%，外科根治手术是治疗甲状腺癌尤其是分化型甲状腺癌的重要手段，然而术中常常会发现疑似甲状旁腺但又难以确认的组织，尤其是位置变异性大、定位性差的下甲状旁腺，常与淋巴结、脂肪组织混淆，术中误切甲状旁腺最终会诱发术后短暂性或永久性低钙血症^[2-3]。医学统计显示，甲状旁腺的术中永久性损伤发生率0.0%~20.0%，短暂性功能不全发生率为0.3%~49.0%^[4]。甲状旁腺的误切或损伤，使供血微循环受阻，导致低钙血症和甲状旁腺功能衰退进而导致一系列的并发症，很大程度上降低了患者的生活质量^[5]。因此，有效提高甲状旁腺的术中保护对甲状腺癌患者意义重大^[6-7]。目前术中鉴别和保护甲状旁腺的方法除了根据解剖位置和临床经验判断外，还有术中部分切除后冰冻切片^[8]、静脉注射亚甲蓝^[9]、亚甲蓝创面喷洒^[10]、术中CT扫描^[11]、甲状旁腺特异性荧光^[12]、细针抽吸后检测甲状旁腺素(parathyroid hormone, PTH)水平^[13]等方法。笔者在既往临床研究^[14]发现，术中穿刺快速检测鉴定淋巴结的准确率为100.0%，鉴定甲状旁腺的准确率为93.1%。证明了术中穿刺快速检测可准确鉴别术中可疑淋巴结，有助于甲状旁腺的保

护及原位保留，但未进一步研究术中穿刺快速检测是否可以降低术后低血钙及低PTH的发生率。本研究探索术中细针穿刺后快速Diff-Quik染色细胞学检测方法对甲状腺癌根治术中甲状旁腺原位保留的临床疗效，以及对术后血清钙和PTH水平的影响。

1 资料和方法

1.1 一般资料

选择2018年10月—2020年1月上海市浦东医院收治的40例初诊甲状腺乳头状癌(papillary thyroid carcinoma, PTC)患者纳入本研究，女性38例，男性2例，平均年龄(40.91±12.42)岁(表1)。本研究获得上海市浦东医院伦理委员会的批准，所有患者或家属均充分告知并签署知情同意书。

1.2 方法

将预期进行甲状腺癌手术的患者随机分为两组，由同一组手术医师施行手术。对照组患者在手术过程中由手术医师根据经验肉眼鉴别甲状旁腺，根据其形态、位置、有无血供等初步判断，予以原位保留，其余非甲状旁腺组织行清扫术。细针穿刺组患者在签署知情同意后进入研究，术中淋巴结清扫过程中，如若遇到可疑的甲状旁腺，由手术医师对肉眼判断的可疑甲状旁腺进行

细针穿刺,应用配套22号针头的5 mL无菌注射器进行原位穿刺,注射器预留1 mL空气,倾斜45°突破可疑结节包膜,随后推进0.1~0.2 mm,注意注射器针头不可贯通可疑的甲状旁腺,保持一定负压从不同方向抽吸5~6次,保持负压状态退出针头。快速排空针管,将针头内的吸取物正压吹至载玻片,做细胞涂片,3 min内放入固定液,固定5~20 s,取出后立即行Diff-Quik染色,由两名经验丰富的病理科医师镜下诊断。如病理学检查结果为甲状旁腺组织则予以保留,淋巴结等非甲状旁腺组织则予以切除。术后对穿刺吸取物行H-E染色,由两名经验丰富的病理科医师镜下诊断,以验证Diff-Quik染色和H-E染色具有相同的诊断准确性。利用术中Diff-Quik染色时间快、不需要切除部分甲状旁腺等优点,快速诊断鉴别保护甲状旁腺功能。所有对照组和细针穿刺组患者术后第2天查血清钙及PTH,术后第1、3、6个月门诊随访复查血清钙及PTH。

表1 40例PTC或甲状腺微小乳头状癌患者临床特征
Tab. 1 The clinical characteristics of 40 PTC/PTMC patients

Characteristics	Fine needle puncture group (N=20)	Control group (N=20)
Gender		
Male	1	1
Female	19	19
Age/year	34-71	36-74
Type of operation		
A	11	12
B	4	4
C	5	4
Pathological diagnosis		
PTC	18	19
PTMC	2	1
pTNM stage		
T ₁₋₂ N ₀ M ₀	12	12
T ₃ N ₀ M ₀	2	2
T _x N ₁ M ₀	6	6

A: Thyroid lobectomy + regional cervical lymph node dissection; B: Total thyroidectomy + regional cervical lymph node dissection; C: Total thyroidectomy + radical lymph node dissection; PTC: Papillary thyroid carcinoma; PTMC: Papillary thyroid microcarcinoma

1.3 统计学处理

采用SPSS 22.0统计软件对数据进行分析,计量资料以 $\bar{x} \pm s$ 表示,采用*t*检验;计数资料以率表示,采用 χ^2 检验。 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 术中Diff-Quik染色鉴别甲状旁腺的阳性率

细针穿刺组手术过程中,手术医师肉眼共发现51个可疑甲状旁腺,均行细针穿刺后Diff-Quik染色,病理学诊断结果显示,其中45个为甲状旁腺,另外5个为淋巴结,1个为脂肪组织,术后病理学诊断与Diff-Quik染色病理学诊断结果完全一致。肉眼鉴别甲状旁腺的阳性率为88.2%,利用术中Diff-Quik染色诊断甲状旁腺和淋巴结,排除淋巴组织,其余非淋巴组织予以保留,从而达到原位保留甲状旁腺率为100.0%。

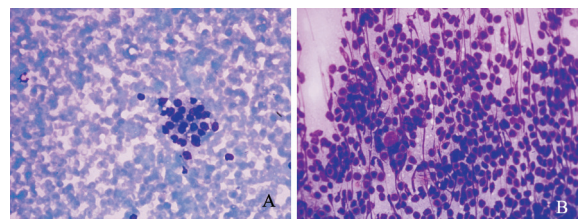


图1 甲状旁腺和淋巴结Diff-Quik染色

Fig. 1 Parathyroid glands and lymph node Diff-Quik staining

A: Parathyroid glands; B: Lymph node

2.2 两组患者手术前后血清钙、PTH水平及术后低血钙、低PTH的发生率

两组患者的术前与术后3个月、术后6个月的PTH水平比较,差异无统计学意义($P_{1b}=0.070$, $P_{2b}=0.082$)。两组患者术后2 d、术后1个月的PTH水平低于术前($P_{3b}=0.007$, $P_{4b}=0.016$)。细针穿刺组患者术后2 d、术后1个月的PTH水平高于对照组($P_{5b}=0.032$, $P_{6b}=0.045$)。细针穿刺组患者术前、术后3个月、术后6个月的PTH水平与对照组比较,差异无统计学意义($P_{7b}=0.857$, $P_{8b}=0.696$,表2)。

表2 两组患者手术前后血清钙、PTH水平

Tab. 2 Calcium and PTH levels of the two groups of patients

Item	Time				
	Before surgery	Two days after surgery	One month after surgery	Three months after surgery	Six months after surgery
Serum total calcium $c_B/(mmol \cdot L^{-1})$					
Fine needle puncture group	2.986	1.974	2.102	2.815	2.979
Control group	2.895	1.297	1.986	2.703	2.835
PTH $c_B/(pmol \cdot L^{-1})$					
Fine needle puncture group	7.687	4.879	5.154	6.934	7.657
Control group	7.811	3.936	4.795	7.032	7.559

两组患者的术前与术后3个月、术后6个月的血清钙水平比较差异无统计学意义 ($P_{1a}=0.076$, $P_{2a}=0.087$)。两组患者术后2 d、术后1个月的血清钙水平低于术前 ($P_{3a}=0.001$, $P_{4a}=0.006$)。细针穿刺组患者术后2 d、术后1个月的血清钙水平高于对照组 ($P_{5a}=0.001$, $P_{6a}=0.017$)。对照组患者术后3个月、术后6个月的血清钙水平与细针穿刺组比较, 差异无统计学意义 ($P_{7a}=0.802$, $P_{8a}=0.675$)。

细针穿刺组患者术后2 d、术后1个月的低血钙发生率低于对照组 ($P_{1c}=0.034$, $P_{1d}=0.003$)；术后3个月、术后6个月的低血钙发生率与对照组比较差异无统计学意义 ($P_{1e}=0.034$, $P_{1f}=0.003$)。细针穿刺组患者术后2 d、术后1个月的低PTH发生率低于对照组 ($P_{2c}=0.034$, $P_{2d}=0.003$)。术后3个月、术后6个月的低PTH发生率与对照组比较, 差异无统计学意义 ($P_{2e}=0.034$, $P_{2f}=0.003$, 表3)。

表3 两组患者低血钙、低PTH发生率情况的比较

Tab. 3 Comparison of incidence of hypocalcemia and hypoparathyroidism between two groups

[n(%)]

Time after operation in different groups	Fine needle puncture group (N=20)	Control group (N=20)	χ^2	P value
Hypocalcemia				
Two days	6 (30)	11 (55)	4.485	0.034
One month	3 (15)	6 (30)	3.296	0.049
Three months	1 (5)	1 (5)	1.343	0.478
Six months	1 (5)	1 (5)	1.343	0.478
Hypoparathyroidism				
Two days	6 (40)	10 (50)	5.012	0.029
One month	3 (15)	7 (35)	4.526	0.385
Three months	0 (0)	2 (10)	1.743	0.178
Six months	0 (0)	2 (10)	1.743	0.178

3 讨 论

临床认为预防甲状旁腺术中损伤和误切是降低术后甲状旁腺功能减退以及低钙血症发生风险的主要方法^[15]。除了熟悉甲状腺、甲状旁腺

的解剖特征, 提高术中辨认能力以及手术精确性之外, 一些甲状旁腺显露技术被逐渐应用于甲状腺癌根治术^[16]。Prosst等^[17]利用氨基乙酰丙酸光敏化甲状旁腺后行腔镜下微创甲状旁腺切除术, 在术中经D-LIGHT照射可显示甲状旁腺。

Kuriloff等^[18]在甲状旁腺手术中采用亚甲蓝溶液静脉滴注,更适用于甲状旁腺瘤的定位。2014年,Tian等^[19]利用纳米碳示踪淋巴结,保护甲状旁腺。随着对患者术后生活质量重视度的提高,Adler等^[20]及Lorente-Poch等^[21]相继提出了最大可能原位保留甲状旁腺的理念,不再争论保留几个甲状旁腺的问题,倡导珍视每一颗甲状旁腺。在甲状腺癌根治手术进程中,若要保证淋巴结清扫彻底,往往会导致甲状旁腺误切,诱发甲状旁腺功能减退甚至是永久性甲状旁腺功能减退,患者需长期进行补钙治疗,严重影响患者的生活质量^[22]。相反,若要尽可能保护甲状旁腺,则会导致淋巴结清扫不彻底,诱导甲状腺癌复发甚至转移。因此,提高甲状旁腺和淋巴结的术中辨认是目前临床医师面临的重大挑战^[23-24]。Patel等^[25]的观察性分析发现,亚甲蓝定位甲状旁腺是目前相对安全可靠的方法。在甲状旁腺定位中,亚甲蓝染色法的灵敏度为92.3%,特异度为56.8%^[26]。除此之外,纳米碳作为淋巴示踪剂在甲状腺癌术中定位亦有广泛应用,定位淋巴结的准确率为60.5%~88.4%,灵敏度为92.9%^[27-29]。

本研究采用术中穿刺技术,由病理科医师参与,利用Diff-Quik染色法,快速从病理学层面“鉴别”甲状旁腺,通过术后H-E染色验证Diff-Quik染色法的可靠性,并且排除淋巴组织,佐证非淋巴组织(图1)保留,避免术中误切甲状旁腺,原位保留甲状旁腺准确率达100.0%,远高于肉眼鉴别甲状旁腺88.2%的准确率,也高于亚甲蓝染色法、纳米碳染色法等其他术中鉴别甲状旁腺的方法。本研究结果表明,细针穿刺组术后低血钙发生率低于对照组,低PTH发生率也低于对照组,证实术中穿刺技术Diff-Quik染色法在甲状腺癌术中的应用可以降低术后低血钙和低PTH的发生率,术中保护甲状旁腺更为准确、快速。该方法可有效提高甲状旁腺术中辨认的准确性、保障甲状旁腺的原位保留,改善患者的术后生活质量。

本研究也存在一定的局限性,单中心、样本量小、甲状腺侧叶加峡部切除的患者比例较高,可能

对研究结果存在一定影响。该技术是否能够更好地指导临床实践并广泛推广,需要大样本、多中心随机对照临床试验,最好选择甲状腺全切的患者,并且提高术中穿刺吸取细胞的有效性和细胞病理学的质控,这样才能更好地应用于临床。

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