Evaluation of the diagnostic utility of the aminotransferase/lactate dehydrogenase ratio for the suspension of tissue specimens during thyroid surgery for the identification of parathyroid tissue

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Abstract. Identification of the parathyroid glands during surgery is crucial for preventing postoperative hypoparathyroidism. Kikumori et al. reported that the aspartate aminotransferase (AST)/lactate dehydrogenase (LDH) ratio for the saline suspension of a suspicious tissue can differentiate parathyroid tissue from other tissues. The aim of this study was to evaluate the utility of this method and investigate the appropriate time for measurement. We obtained 465 tissue specimens during thyroidectomy of 102 patients with papillary thyroid carcinoma (PTC), and 422 specimens (129 parathyroid, 92 PTC, and 201 other tissues) with measurable AST and LDH were analyzed. Small pieces of the tissues were immersed in saline and sent for measurement of AST and LDH. The assay was performed immediately after thyroidectomy for 245 specimens (the same-day group) and during the next morning for the remaining 177 specimens (the next-day group). The accuracy of diagnosing parathyroid tissue was significantly better in the same-day group than in the next-day group. A cut-off value of 0.18 gave the best diagnostic precision, with an area under the receiver operating characteristic curve of 0.95 and 88.7% sensitivity and specificity in the same-day group. When the cut-off value was set to 0.20, the specificity for excluding carcinomatous tissues was 100%. When measured on the day of the surgery, the AST/LDH ratio for the saline suspension of the surgical specimens is useful for discriminating parathyroid tissues from other tissues. This method can be utilized at most hospitals where intraoperative frozen sections or rapid parathyroid hormone assays are not available.

Key words: Parathyroid tissue, Bilateral thyroidectomy, Hypothyroidism, Biochemical confirmation, Tissue immersion

PERMANENT HYPOTHYROIDISM is a well-known and significant complication of thyroid surgery. The overall incidence of permanent hypoparathyroidism after total thyroidectomy with central compartment neck dissection for differentiated thyroid carcinoma has been reported to be 19–25% [1, 2]. Patients require a lifetime prescription of vitamin D with or without calcium supplements. To avoid permanent postoperative hypoparathyroidism, locating the parathyroid glands during surgery is crucial for preserving the gland in situ or auto-transplanting the parathyroid tissue in muscular pockets. During thyroid surgery, the parathyroid glands are traditionally and mainly identified macroscopically by surgeons; the accuracy greatly depends on the surgeon’s experience. Alternatively, pathological diagnosis of frozen sections [3], intraoperative measurement of parathyroid hormone (PTH) in the suspension of suspicious tissues [4], and detection of the parathyroid using a near-infrared imaging system [5] have also been reported. However, these methods have low general versatility due to the need for pathologists or special equipment. Kikumori et al. reported that the aspartate aminotransferase (AST)/lactate dehydrogenase (LDH) ratio for saline in which trace amounts of suspicious tissues were suspended accurately differentiated parathyroid tissue from other tissues, and they proposed this method to be
whether the measured samples other than the parathyroid washouts of the needles used for preoperative aspiration biopsy. Furthermore, the timing of the AST and LDH measurements was unclear, and it was also unclear whether the measured samples other than the parathyroid tissues were pathologically identified. The aim of this study was to evaluate the utility of the AST/LDH ratios in discriminating parathyroid tissue from other tissues using surgical specimens that were pathologically confirmed. In addition, we studied the effect of the time between tissue sampling and the assessment of the diagnostic accuracy.

**Materials and Methods**

We evaluated 465 tissue specimens obtained from 102 patients with papillary thyroid carcinoma (PTC) who underwent total thyroidectomy between December 2019 and April 2020 at our institution. This study was approved by our institutional ethical review board, and the patients provided informed consent.

Although we planned to measure AST and LDH levels immediately after thyroid surgery, some measurements were performed according to the working time of laboratory personnel. Thus, there were two groups based on the time of measurement: the same-day group and the next-day group. Table 1 provides data on the 422 tissue specimens with measurable AST and LDH levels out of the pathologically confirmed 465 tissue specimens. The specimens macroscopically suspected to contain parathyroid tissue were sent for intraoperative frozen section evaluation, and they were minced with fine scissors and transplanted into muscular pockets after confirmation [7]. Tiny pieces of approximately 1 mm³ of the parathyroid tissues were processed the same way as the other tissues described below. Tiny pieces of approximately 1 mm × 2 mm of the other tissues in or around the thyroid were collected after thyroidectomy. They were cut into two pieces each. One piece was sent for pathological diagnosis of the tissue, while the other was immersed in 500 μL of saline. The immersions were sent to the biochemical laboratory at our institution to measure AST and LDH levels. AST and LDH levels were measured using a standard automatic analyzer (cobas® 8000; Roche Diagnostics, Tokyo, Japan). The measurements were performed immediately after surgery for 257 specimens (the same-day group), while the remaining 208 specimens were kept immersed in saline and stored in a refrigerator at 4°C until the measurements were performed the next morning because of the laboratory working time (the next-day group). If AST and LDH levels were below the measurement limits (<2 IU/L and <5 IU/L for AST and LDH, respectively), they were excluded as unmeasurable. We analyzed the AST/LDH ratios of various tissues and set a cut-off value for the diagnosis of parathyroid tissue. We also compared the diagnostic accuracy of the same-day and next-day measurements. The cut-off value for diagnostic precision was set as the value at which the sensitivity and specificity were equal. We confirmed the precision using the receiver operating characteristic (ROC) areas under the curves (AUC) for the same-day and next-day groups. We used Stat Flex version 6.0. (Artec/Japan) software for these analyses.

**Results**

AST and LDH levels and the AST/LDH ratio for distinguishing the parathyroid gland from the other tissues in the same-day and next-day groups are summarized in Table 2. AST and LDH levels were below the assay limit for 12 tissue specimens (4 parathyroid, 4 adipose tissues, 3 thyroid gland, and 1 PTC) and 1 parathyroid tissue specimen in the same-day group and for 10 tissue specimens (1 thymus, 3 adipose tissues, 1 normal lymph node, 1 thyroid gland, 1 metastatic lymph node, and 3 TPC) and 28 tissue specimens (7 parathyroid, 1 thymus, 5 adipose tissues, 4 normal lymph nodes, 2 thyroid gland, 1 metastatic lymph node, and 8 TPC) in the next-day group, respectively. In the same-day group, both AST and LDH levels were below the assay limit for 1 parathyroid tissue specimen. In the next-day group, both AST and LDH levels were below the assay limit for 7 tissue specimens (1 adipose tissue, 1 normal lymph node, 1

| Table 1 | Tissues of the specimens and the time of the measurements |
|---------|----------------------------------------------------------|
|         | Same day | Next day |
| Number of cases | 60 | 42 |
| Analyzed specimens | | |
| Parathyroid gland | 77<sup>a</sup> | 52<sup>a</sup> |
| Thymus | 9 | 8 |
| Adipose tissue | 41 | 28 |
| Normal lymph node | 14 | 10 |
| Thyroid gland | 54 | 37 |
| Metastatic lymph node | 13 | 16 |
| Papillary thyroid carcinoma | 37 | 26 |
| Total | 245 | 177 |

<sup>a</sup> 2 parathyroid tissues each were examined for each of 17 cases. <sup>b</sup> 2 parathyroid tissues each were examined for each of 10 cases. All tissues were confirmed histologically.
thyroid gland, 1 metastatic lymph node, and 3 TPC). Forty-three tissue specimens, including 12 tissue specimens in the same-day group and 31 tissue specimens in the next-day group, were excluded from the analysis. The parathyroid tissues showed significantly higher AST/LDH ratios than the other tissues in the same-day and next-day groups ($p < 0.01$ for each comparison in both groups) (Table 3). In the same-day group, the diagnostic accuracy was very good at 0.95 for AUC, 0.18 for the cut-off value, and 88.7% for the sensitivity and specificity (Fig. 1a and 1b). When the cut-off value was set to 0.20, the diagnostic accuracy for the parathyroid and malignant tissues (PTC and metastatic lymph nodes) was also excellent: AUC = 0.98, sensitivity = 85.7%, and specificity = 100% (Fig. 2a and 2b). However, the diagnostic accuracy was much lower in the next-day group than in the same-day group (AUC = 0.80; sensitivity and specificity = 67.3%) (Fig. 3a and 3b). AST level in the next-day group was 15.4 ± 14.2 IU/L, which was close to the value of 15.4 ± 16.6 IU/L in the same-day group. However, LDH level in the next-day group was 60.0 ± 73.1 IU/L, which was significantly lower than the value of 103.5 ± 87.4 IU/L in the same-day group ($p < 0.01$).

### Table 2

| Group   | N   | AST (IU/L) | LDH (IU/L) | AST/LDH |
|---------|-----|------------|------------|---------|
| Same-day| 77  | 23.3 ± 16.5| 83.3 ± 67.4| 0.29 ± 0.09|
|         | 9   | 10.0 ± 6.0 | 130.7 ± 74.3| 0.07 ± 0.02|
|         | 41  | 7.9 ± 5.4  | 92.2 ± 73.8 | 0.10 ± 0.07|
|         | 14  | 9.8 ± 10.4 | 148.9 ± 152.6| 0.07 ± 0.02|
|         | 54  | 18.1 ± 24.1| 118.6 ± 111.7| 0.14 ± 0.06|
|         | 13  | 9.7 ± 7.1  | 115.6 ± 63.9 | 0.08 ± 0.03|
|         | 37  | 8.8 ± 8.0  | 108.2 ± 66.4 | 0.08 ± 0.04|
| Next-day| 52  | 22.0 ± 17.0| 44.9 ± 65.3 | 0.09 ± 0.02|
|         | 8   | 8.8 ± 6.3  | 59.9 ± 56.1 | 0.26 ± 0.17|
|         | 28  | 10.6 ± 11.8| 56.1 ± 57.8 | 0.28 ± 0.21|
|         | 10  | 10.0 ± 4.7 | 70.2 ± 62.1 | 0.22 ± 0.17|
|         | 37  | 16.6 ± 13.8| 64.9 ± 66.7 | 0.57 ± 0.88|
|         | 16  | 7.4 ± 5.2  | 41.3 ± 36.2 | 0.27 ± 0.17|
|         | 26  | 14.5 ± 13.8| 94.9 ± 117.4| 0.37 ± 0.37|

PT, parathyroid gland; TM, thymus; Adipose, adipose tissue; N-LN, normal lymph node; Thy, thyroid; m-LN, metastatic lymph node; TPC, thyroid papillary carcinoma

### Table 3

|                  | The same-day group | The next-day group |
|------------------|--------------------|--------------------|
|                  | n     | AST/LDH ratio | p-values* | n     | AST/LDH ratio | p-values* |
| PT               | 77    | 0.29 ± 0.09   |           | 52    | 0.90 ± 0.62   |           |
| TM               | 9     | 0.07 ± 0.02   | <0.01     | 8     | 0.26 ± 0.17   | 0.03     |
| Adipose          | 41    | 0.10 ± 0.07   | <0.01     | 28    | 0.28 ± 0.21   | <0.01    |
| N-LN             | 14    | 0.07 ± 0.02   | <0.01     | 10    | 0.22 ± 0.17   | 0.01     |
| Thy              | 54    | 0.14 ± 0.06   | <0.01     | 37    | 0.57 ± 0.88   | <0.01    |
| m-LN             | 13    | 0.08 ± 0.03   | <0.01     | 16    | 0.27 ± 0.17   | <0.01    |
| TPC              | 37    | 0.08 ± 0.04   | <0.01     | 26    | 0.37 ± 0.37   | <0.01    |

PT, parathyroid gland; TM, thymus; Adipose, adipose tissue; N-LN, normal lymph node; Thy, thyroid; m-LN, metastatic lymph node; TPC, thyroid papillary carcinoma

*: Mann-Whitney U test

### Discussion

In this study, (1) the AST/LDH ratio for the saline suspension of the parathyroid tissue was significantly higher than that for other tissues, and (2) the diagnostic accuracy of the AST/LDH ratio in discriminating parathyroid tissue from other tissues was better in the same-day group than in the next-day group.

The reason for the high AST/LDH ratio of the parathyroid gland remains unclear, but it may be related to energy metabolism. The adult parathyroid gland consists of chief cells, eosinophilic cells, and adipocytes [8-10]. Eosinophilic cells contain a large number of mitochondria [9]. The distribution of several eosinophilic cells in the parathyroid glands can result in high mitochondrial
AST content, leading to high AST levels in the sample. As the parathyroid gland has a large distribution of eosinophilic cells and is rich in mitochondria, the metabolic pathway mainly uses the tricarboxylic acid cycle rather than glycolysis; therefore, LDH level might be lower in the parathyroid gland than in other tissues.

The reason for the difference in diagnostic accuracy associated with the difference in measurement time could be the inactivation of LDH. LDH is deactivated with time and decreases when specimen measurement is delayed [11]. In our study, we did not compare LDH levels on the same and next day in the same samples. However, the LDH levels in the next-day group were significantly lower than those in the same-day group, and the next-day measurement of LDH includes this problem. However, the present method is mostly used to differentiate parathyroid tissue from other tissues during thyroid surgery to allow the preservation of parathyroid glands or transplantation of the tissue if resected. The most important issue is distinguishing parathyroid tissue
from PTC. In the present study, the AST/LDH ratio had a specificity of 100% when the AST/LDH ratio was >0.20. Therefore, the above-mentioned fact is not a limitation for the clinical application of the method.

Kikumori et al. reported that the optimal cut-off value was 0.27, with 100% sensitivity and specificity for the parathyroid and other tissues in their study [6]. Applying this cut-off value to our study, in the same-day group, the sensitivity was 64.9% and the specificity was 98.7%. One of the reasons for the difference is the inactivation of LDH due to the difference in the time of measurement. In the present study, total thyroidectomy was performed in all cases, and although specimens were collected immediately after surgery, it took some time to measure AST and LDH. LDH may have been inactivated to some extent. Another possible reason is the difference in the preparation of the specimens. Kikumori et al. measured the suspensions of surgical specimens and washouts of the needles used for preoperative aspiration biopsy. The present study used only the immersion solutions (500 μL saline) of the tissues taken from the surgical specimens. This might have affected the measured and cut-off values. However, the accuracy for differentiating parathyroid tissue from other tissues in or around the thyroid gland was sufficiently good; it was especially excellent for discriminating parathyroid tissue from cancerous tissue, given its 100% specificity.

The present study was not intended for an intraoperative rapid diagnosis of parathyroid tissue. However, with the measuring device used, the results of AST and LDH measurements can be obtained within approximately 20 minutes. Considering the sample transportation time from the operating room to the laboratory, it may take approximately 30 minutes in total, which is acceptable for an intraoperative diagnosis. In addition, FUJIFILM NX500® can be used to measure these items within 10 minutes, and such a measuring device may enable faster intraoperative diagnosis. Therefore, this method would be useful in institutes where intraoperative frozen section diagnosis or rapid parathyroid hormone assay is not available.

This study confirmed the report by Kikumori et al. that the AST/LDH ratio of the suspension of tissue specimens can be used to distinguish parathyroid tissues from other tissues. However, it has some limitations. As the appropriate cut-off value depends on various conditions, it is necessary to establish the same for each institute. If the cut-off value is to be evaluated in a multicenter study, it will be important to standardize the surgical procedure and sample collection, as well as the measurement time.

In conclusion, we confirmed that the AST/LDH ratio for the suspension of tissue specimens can be used to distinguish parathyroid tissues from other tissues. This diagnostic method is easily acceptable in most hospitals and should be valuable, especially in institutes without pathologists or facilities for measuring the parathyroid hormone concentrations.

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