Original Article

Intraoperative Identification of Parathyroid Tissue Using the Ratio of Aspartate Transaminase to Lactate Dehydrogenase

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Abstract

Introduction: Inadvertent devascularisation or removal of parathyroid glands (PT) during thyroidectomy necessitates their autotransplantation after Frozen section (FS). FS is time-consuming, tedious and expensive, disrupts the pathology department and is not universally available. This study aimed to determine the ratio of aspartate aminotransferase to lactate dehydrogenase (AST/LDH) of PT extracts to differentiate it from fat, thyroid, and lymph node (LN). **Methods:** This prospective study was conducted on all patients planned for any thyroid or parathyroid surgery. Intra-operatively, a 2.5 × 2.5 × 2.5 mm^3 of the devascularised or inadvertently removed PT was excised, minced in 2 mL normal saline and the supernatant fluid was sent to the standard lab for AST/LDH estimation. The minced tissue was sent for histopathological examination for confirmation. Thyroid, LN and fat samples were taken as controls and analysed similarly. Parathyroid adenoma (PTA) was analysed separately. **Results:** The mean AST/LDH ratios of PT (0.311 ± 0.176) were significantly higher than that of thyroid, fat and LN. A cut-off of ≥0.165 for PT had a sensitivity and specificity of 83.8% and 83.1% against thyroid tissue, 83.8% and 74% against fat, and 83.8% and 100% against LN. AST/LDH ratio of PTA was found to be 0.318. **Conclusion:** AST/LDH ratio can be a simple, reliable, less labour-intensive method of identification of PT and can be a replacement for FS. The high specificity to differentiate an LN is clinically relevant in central compartment lymph node dissections with a higher probability of inadvertent removal or devascularisation of PT.

Keywords: Alternative to frozen section, intraoperative identification, Parathyroid, parathyroidectomy, thyroidectomy

Introduction

Thyroidectomy is the most commonly performed endocrine surgical procedure. Post-thyroidectomy hypocalcaemia is a common and dreaded complication. Accidental removal of parathyroid glands (PT) is not uncommon and can occur in 1–15% of thyroidectomies.[1] The risk of inadvertent parathyroid excision (IPE) is even higher when central compartment lymph node dissection is carried out in a setting of thyroid malignancy, the incidence being 9% to as high as 28% and the PT can be easily confused with fat or lymph nodes.[2-4] However, it can also occur during difficult thyroid surgeries for benign conditions, especially thyroiditis.[5] Postoperative hypocalcaemia can also occur due to the devascularisation of PT. Devascularisation of a PT can be predicted by its darkening or by using Indo-cyanine green dye to look for its perfusion. The viability of such a PT can be checked by piercing its capsule with a needle or incising its capsule to illicit brisk red oozing which indicates continued arterial perfusion.[6,7]

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Autotransplantation (AT) of parathyroid glands is a standard procedure if IPE is identified intra-operatively or if a devascularised gland is found to be non-viable.[8] For successful AT, it is paramount to correctly identify PT and differentiate it from other tissues like fat, LN or thyroid nodules. Traditionally, surgeons identify PT macroscopically during thyroid surgery. Other methods of identification are the 'sink test', parathyroid hormone (PTH) assay washings, auto-fluorescence (AF) and indo-cyanine green fluorescence (ICG), among others.[9] However, the gold standard investigation for the intraoperative identification of PT is the FS. FS must be performed before AT.Click or tap here to enter text.[8,10] FS is also necessary to

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identify and confirm the diseased parathyroid gland during surgeries for pathologies related to PT like PTA. However, FS is time-consuming, tedious and expensive, requires highly trained manpower and disrupts the pathology department services.[11] It might also be unavailable at various centres. Intraoperative PTH (IOPTH) is also not universally available, and using AF and ICG to identify PT requires investment in the form of expensive equipment.

Kikumori *et al*.[12] demonstrated that the aspartate aminotransferase (AST)/lactate dehydrogenase (LDH) ratio in a tissue suspension could distinguish parathyroid tissues from other tissues.

As the facility to measure serum levels of AST and LDH is readily available in most centres, utilising the ratio of AST/LDH measured from tissue suspension, could act as a cost-effective, quick, universally available alternative to FS, obviating the need for skilled human resources. This method can also be used to identify PT using point-of-care biochemical analyser devices that are relatively inexpensive, easy to handle and can even be placed inside the operation theatre, hence reducing the turnaround time.[13] If this ratio can also distinguish normal PT from PTA, it could be an adjunct to IOPTH during surgeries for hyperparathyroidism. This study aimed to determine the AST/LDH ratio of PT, fat, thyroid and LN tissue extracts to differentiate PT from other tissues.

Materials and Methods

All patients who had planned for any thyroid or parathyroid surgery were included in the study. Patients in whom the PT was either devascularised or inadvertently removed and those who underwent parathyroidectomies were included. We thus had 68 patients, of whom 207 samples were obtained.

Intra-operatively, an attempt was made to localise all parathyroid glands. Any devascularised (change in colour or reduced perfusion with Indo-cyanine green fluorescence) or inadvertently removed PT was identified. Approximately $2.5 \times 2.5 \times 2.5$ mm^3 of the parathyroid tissue was excised. It was minced in 2 mL NS (using a micropipette), and the supernatant fluid was sent to the central laboratory to analyse AST and LDH [Figure 1a and b]. Thyroid, fat and LNs were also processed similarly. The minced tissue was sent for an FS or histopathological examination for confirmation. The devascularised glands were dealt with as per the change in their colour post-removal of the bit of tissue and capsular incision. All inadvertently removed parathyroid tissues were auto-transplanted in the sternocleidomastoid muscle. PTA obtained after focussed parathyroidectomies were analysed separately.

The central laboratory used Selectra Pro XL analyser, and Q-line biotech for biochemical analysis. AST and LDH values obtained were noted, and the AST/LDH ratio was calculated. After obtaining tissue confirmation from the histopathology report, the Receiver Operating Characteristic (ROC) curve was plotted to identify a suitable cut-off for the AST/LDH ratio to identify PT and differentiate it from thyroid, fat and LNs. Analysis was also performed for various thyroid and parathyroid pathologies. Statistical analysis was performed using IBM-SPSS version 22.0 (Chicago Inc, USA).

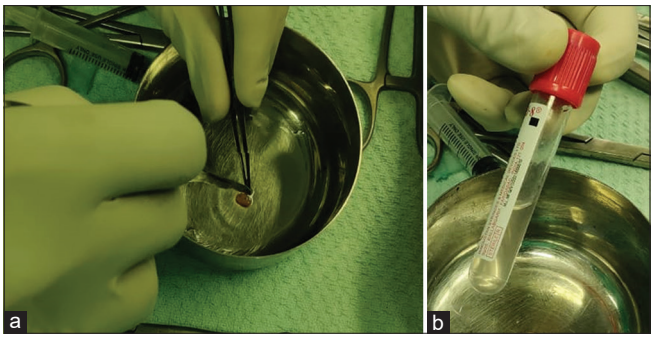

Figure 1: (a) Mincing of the inadvertently removed parathyroid and (b) obtaining a tissue suspension

Ethical aspect

The study was approved by the Institutional Ethical Committee of King George's Medical University, Lucknow vide Reference code: VII-PGTSC-IIA/P34 on 17 December 2021. Written informed consent was obtained from all patients after explaining the protocol of the study in the language of their understanding. The study was carried out in compliance with the Declaration of Helsinki 1964.

Results

Patient characteristics of the study population have been depicted in Table 1. AST and LDH levels were measurable in all samples. The mean of the AST/LDH ratio of various tissues was analysed [Table 2]. The mean AST/LDH ratios of PT was 0.311 ± 0.176 and was significantly higher than that of thyroid, fat and LN. A ROC curve of this ratio of PT was plotted against that of all other tissues, and the AUC (area under the curve) was found to be 0.888 [Figure 2a]. Using this curve, a cut-off of ≥ 0.165 was identified for PT which had an equal sensitivity and specificity of 83% [Figure 2b]. Comparing this cut-off individually, it was found that the sensitivity and specificity were 83.8% and 83.1% against thyroid tissue, 83.8% and 74% against fat, and 83.8% and 100% against LN [Table 3].

The mean AST/LDH ratio of different thyroid histopathology (benign, malignant nodules and thyroiditis) was analysed and found not to be statistically significant ($P = 0.877$, data not presented). AST/LDH ratio of PTA was found to be 0.318 ± 0.13. The ROC curve plotted between PT and PTA had an AUC of 0.569 and was statistically not significant [Figure 3a]. Furthermore, a moderate correlation (Spearman's rho coefficient: 0.334) was found between the AST/LDH ratio of PT and serum PTH levels. However, it was only near statistical significance ($P = 0.057$) [Figure 3b].

Discussion

Identification of PT is necessary for its in-situ preservation. After IPE, PT is identified by FS and then auto-transplanted

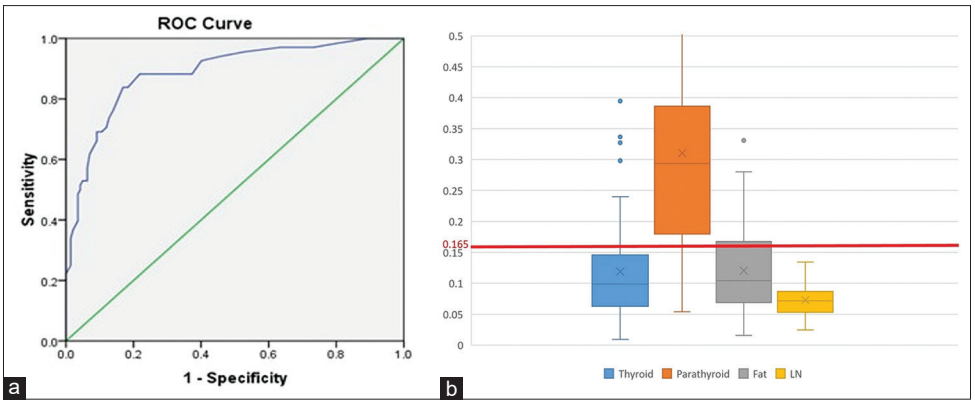

Figure 2: (a) ROC curve of AST/LDH ratio for differentiating parathyroid from other tissues (Area Under Curve = 0.888) (*P* value = 0.012) and (b) Box and whisker plot of AST/LDH ratio of thyroid, PT, fat and LN with the proposed cut-off

Table 1: Demographic data of the study population

Parameters	Results
Total number of patients	68
Total number of samples:	207
Thyroid	62 (29.96%)
Parathyroid	68 (32.8%)
Fat	50 (24.2%)
Lymph node	27 (13.04%)
Female: Male	8:1 (F=60; M=8)
Age	36.51±12.96 years (15-66)
Pre-operative diagnosis	
Solitary nodule thyroid	41 (60.3%)
Multinodular goitre	18 (26.4%)
Graves' disease	3 (4.4%)
Primary hyperparathyroidism	6 (8.9%)
Biochemical status	
Euthyroid	54 (79.4%)
Hypothyroid	7 (10.3%)
Hyperthyroid	7 (10.3%)
Surgery performed	
Hemithyroidectomy	41 (60.3%)
Total thyroidectomy	21 (30.9%)
Parathyroidectomy	6 (8.8%)
Histopathology report	
Benign	52 (83.9%)
TFND	38
FA	3
Thyroiditis	10
NIFTP	1
Malignant (DTC)	10 (16.1%)
PTC	7
FTC	3
Parathyroid adenoma	6

into the sternocleidomastoid muscle. However, this is time-consuming compared to the duration of the surgery, expensive, requires trained manpower and may not be universally available. Other methods to identify PT do exist but need expensive instruments and are not universally available. AST and LDH are common serology parameters that are available in most centres. This study aimed to identify PT using the ratio of AST to LDH of the tissue suspension. We found that the AST/LDH ratio of PT was significantly higher than other tissues, and a cut-off of ≥0.165 could identify PT with a sensitivity and specificity of 83% each. No significant difference in this ratio was found for different histopathologies of thyroid or parathyroid.

Kikumori *et al*.[12,13] observed that the mean AST/LDH ratio of tissue suspension of PT was 0.43 ± 1.19 and a cut-off of ≥0.27 could identify PT with a sensitivity and specificity of 100%. Different ratios in the current study can be explained by the different analysers and reagents used. In a subsequent study, using a point-of-care machine, Kikumori *et al*.[13] found a different cut-off of 0.48 for PT, highlighting the need for establishing a cut-off for each analyser and technique of analysis.

Kikumori *et al*. had a sensitivity and specificity of 100% in all three of their published studies, and this is in contrast to the present study, where a cut-off of ≥0.165 could identify PT with a sensitivity and specificity of 83% each. Several possible explanations exist for this. The sample size in the current study is larger, and the study population and analysers are different.[12-14] Also, Kikumori *et al*.[13] excluded those samples with AST and LDH values <10 IU/L, whereas no samples have been excluded in the present study. Also, as the patient's age increases, the adipocyte content in the PT increases.[15,16] This also explains the possible difference in cut-offs and the poor specificity of the cut-off against fat. The percentage of fat being unpredictable is one of the limitations of this estimation method. However, the cut-off of ≥ 0.165 for PT was 100% specific in differentiating it from LN. This is of practical importance as central compartment lymph node dissection has a high probability of IPE, especially of the inferior PTs, which, before AT, needs to be confirmed so as not to auto-transplant a tumour-laden LN. This method of identification of PT is therefore of clinical use in such scenarios.

Kikumori *et al*.[14] also analysed the AST/LDH ratio of benign hyperfunctioning PT. It was found that the ratio

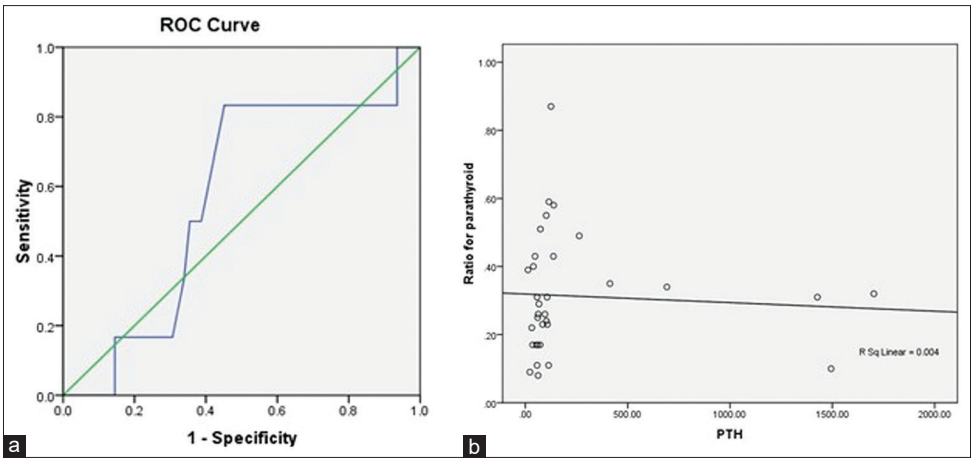

Figure 3: (a) ROC curve for differentiating parathyroid adenoma from normal parathyroids (Area Under Curve = 0.569) (*P* value = 0.58) and (b) Correlation between AST/LDH ratio of parathyroid and S. PTH levels (*P* = 0.057)

Table 2: Mean AST/LDH ratio of various tissue samples (*P*<0.001)

Tissue	Mean	Standard deviation	95% Confidence interval		Minimum	Maximum
			Lower bound	Upper bound		
Thyroid	0.121	0.085	0.098	0.140	0.010	0.400
Parathyroid	0.311	0.176	0.268	0.353	0.050	1.440
Fat	0.121	0.074	0.100	0.142	0.015	0.330
LN	0.073	0.028	0.062	0.084	0.024	0.134

Table 3: Sensitivity and specificity of the AST/LDH ratio of 0.165 for PT to differentiate it from other tissues. (*P*<0.001)

Tissue	Sensitivity	Specificity	AUC	95% Confidence interval	
				Lower bound	Upper bound
Thyroid	83.8%	83.1%	0.873	0.812	0.934
Fat	83.8%	74%	0.867	0.803	0.930
Lymph node	83.8%	100%	0.961	0.926	0.997
All tissues	83.8%	83.1%	0.888	0.839	0.937

was consistently higher for benign hyperfunctioning PT compared to other tissues and parathyroid carcinoma. In the present study, although the mean AST/LDH ratio was PTA was significantly higher than in other tissues, the difference between PTA and normal PT was insignificant. This could be because of the small sample size of PTA in the study population. If further comparison with a larger sample size identifies a significant difference between the diseased and normal PT, this method can act as an adjunct to intraoperative identification of the diseased PT and as an adjunct to intraoperative PTH.

The turnaround time for analysing the AST/LDH ratio in the central laboratory was around 19 mins, compared to 35 mins required for FS. This turnaround time can be further reduced by utilising point-of-care testing (POCT) devices, which can be placed in the operation theatre and are fully automated, obviating the need for specialised manpower. The cost of this kind of device would be approximately ₹15 lakhs ($ 20,000). This device can also measure serum electrolytes, lipid profile, renal parameters, cardiac markers and other parameters of liver function. Also, the cut-off for the device can be recalculated by utilising either FS or analysis in the central laboratory. This determination of the cut-off can be performed using up to 10 specimens of each tissue and should not be a major obstacle in setting up this system.[13,17]

The rationale for utilising the ratio of AST to LDH for PT identification is the fact that PT is a highly metabolically active gland with abundant mitochondria. Eosinophilic cells of PT contain many mitochondria, leading to high AST levels. Therefore, the metabolic pathway mainly uses the tricarboxylic acid cycle rather than glycolysis, leading to a lower LDH level in the PT than in other tissues. Utilising AST/LDH ratio is a novel method, especially in tissue suspensions; however, this has been utilised in other conditions. Isogai *et al*.[18] utilised the LDH/AST ratio in serum on the 7th post-admission day in patients with acute pancreatitis to predict pancreatic necrosis. AST/LDH ratio has also differentiated ischaemic

hepatitis from viral hepatitis. AST/LDH in the serum <1.5 indicates ischaemic hepatitis, thought to be due to the rapid and severe rise of LDH due to hypoperfusion.[19] In Obstetrics, Keiser *et al*.[20] demonstrated that LDH/AST ratio >22 during the third trimester can differentiate pregnancy-associated thrombotic thrombocytopenic purpura from pre-eclampsia/HELLP syndrome (haemolysis, elevated liver enzymes and low platelets).

This method of identification of PT is relatively inexpensive, more universally available, does not require skilled manpower, and can also be set up in the operation theatre. However, it has certain limitations. Oncocytic adenoma or carcinoma of the thyroid could also theoretically have a high AST/LDH ratio. This study contained no patients with such pathology and hence, this theoretical possibility could not be evaluated. Also, various isoforms of AST and LDH have been described in the literature.[21,22] The concentration of these isoforms in the mitochondria varies in normal tissue and various pathologies. The assays used in this study were mainly for cytosolic isoforms of these enzymes and, hence, might not be in line with the proposed theoretical principle of this method. Tissue handling during surgery, along with other factors like anaesthesia, vitamin C, alcohol, etc., is likely to impact the levels of absolute values or ratio of AST and or LDH; however, small differences in the amount of tissue analysed and slight changes in the dilution is partially negated by the fact that ratio is calculated instead of absolute values. The other limitations of the current study are a comparatively smaller sample size and a loss/destruction of PT tissue, albeit small, during the preparation of the tissue suspension, especially in cases where PT was only devascularised and not inadvertently removed. Further research with a larger sample size and using fine needle aspiration cytology washout for analysis can increase the scope of this method in clinical practice.

Conclusion

AST/LDH ratio can be a simple, reliable, less labour-intensive method of identification of PT and can be a replacement for FS. The high specificity to differentiate an LN is clinically relevant in central compartment lymph node dissections with a higher probability of IPE. Considering a short turnaround time, setting up a POCT device in the operation theatre provides this method with an edge compared to FS. Further research with a larger sample size, especially of PTA, and utilising fine needle aspiration cytology washout to estimate this ratio can expand the scope of this method in clinical practice.

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Author contribution

Dr Ganesh Bhat was responsible for sample and data collection, data analysis and drafting the article. Dr Rizhin Sooraj and Dr Ashwinee Rahalkar also contributed in sample and data collection. Dr Wahid Ali was incharge of the biochemical evaluation of the collected samples. Dr Chanchal Rana analysed all the frozen sections and histopathological specimens. Dr Pooja Ramakant reviewed the data periodically and helped in statistical analysis. Dr Anand Kumar Mishra guided in sample collection and final review of the article. Dr Kul Ranjan Singh helped conceptualise the study and reviewed and revised the final draft of the article.

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Conflicts of interest

There are no conflicts of interest.

Data availability

Data collected is provided in the supplementary material.

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