Thyroid segmentation using perithyroidal halo layer on ^{99m}Tc-pertechnetate thyroid SPECT/CT: An easy and reliable method for accurate quantification of thyroid activity

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Abstract

Objective: In previous fluorine-18-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) studies, tumor segmentation using peritumoral halo layer (PHL; SegPHL) was shown to be reliable and accurate segmentation method in various malignant tumors. We found that the halo layer also was observed on the ^{99m}Tc pertechnetate ($^{99m}TcO_4$) thyroid single photon emission computed tomography (SPECT)/CT. In the present study, we attempted to apply thyroid segmentation using the perithyroidal halo layer (PTHL; SegPTHL) on $^{99m}TcO_4$ thyroid SPECT/CT and compared SegPTHL with CT-based thyroid segmentation (SegCT). **Subjects and Methods:** A total of 33 patients (19 females, 14 males; mean age, 46.91±15.7 years old) were enrolled in this study. For SegCT, three-dimensional volume of interest (VOI) of the thyroid was generated via multiple 2-dimensional regions of interest (ROI) along the thyroid margin on transaxial CT images that were manually drawn slice by slice. The PTHL was easily identified by an abrupt increase in layer thickness with minimal or mild distortion of the main thyroid contour, and the thyroid margin for SegPTHL was determined at the innermost portion of PTHL. An automated VOI generation for SegPTHL was performed using the Q.Volumetrix software. The correlation and reliability tests were performed between the quantification parameters of SegPTHL and SegCT. **Results:** The PTHL threshold adjusted according to maximal SUV of thyroid were similar to the results of previous SegPHL studies of ^{18}F-FDG PET/CT. A good correlation was observed between the thyroid volumes of SegCT and SegPTHL r= 0.725; P<0.0001), although the thyroid volume of SegPTHL was slightly larger than that of SegCT (P= 0.0017). The % thyroid uptake (TcTU), total lesion activity (TLA), and mean standardized uptake value (SUVmean) of SegPTHL correlated well with those of SegCT (r=0.9877, 0.9883, 0.9875, respectively; P< 0.0001). No significant error was observed between the parameters (i.e., TcTU, TLA, and SUVmean) of SegPTHL and SegCT. **Conclusions:** Thyroid segmentation PTHL may be a useful method for reliable quanti-fication of thyroid uptake, because the SPECT/CT parameters of SegPTHL were strongly correlated with those of SegCT, as well as the process of SegPTHL is easier and faster than that of SegCT.

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Introduction

Technetium-99m pertechnetate ($^{99m}TcO_4$) has been used for the nuclear imaging of thyroid. Although radioactive iodine can be more physiologic for the evaluation of thyroid hormone synthesis than $^{99m}TcO_4$, $^{99m}TcO_4$ is the most commonly used tracer for thyroid scintigraphy because of its short half-life, absence of beta emission, lower cost, and ready availability [1-3]. Thyroid scintigraphy with % thyroid uptake of $^{99m}TcO_4$ (TcTU) can provide unique information for the differential diagnosis of thyrotoxicosis. High TcTU strongly suggests Graves' disease, and low TcTU may indicate destructive thyroiditis [3, 4]. However, accurate measurement of TcTU may be virtually impossible on planar thyroid scintigraphy. Although a region of interest (ROI) is required to be drawn over the thyroid on planar thyroid scintigraphy, the ROI always includes non-thyroid background activities. Therefore, background activity correction must be performed using the subtraction of background counts from counts over thyroid [3, 5]. However, it may be impossible to draw a perfect background ROI for the background subtraction, because the exact background activity over thyroid ROI cannot be determined on planar thyroid scintigraphy.

Single photon emission computed tomography/computed tomography (SPECT/CT) can provide three-dimensional information presented as cross-sectional slices in a patient. Sin-gle photon emission computed tomography images allow an accurate volume of interest (VOI) of thyroid and may not include non-thyroid background activities that are located anterior and posterior to the thyroid [6]. Furthermore, the CT data of SPECT/CT correction of attenuated and scattered radiations, which is essential for accurate radioactivity quantification [7]. Standardized uptake value (SUV) also can be acquired like positron emission tomography (PET).

The quantification of thyroid uptake using SPECT/CT may be a promising field in nuclear medicine; however, there have been only few reports with regard to thyroid quantification using SPECT/CT [8, 9]. Although an accurate radioquan-tification in each pixel of thyroid SPECT/CT is possible due to the application of attenuation and scatter corrections, the main issue is a method for accurate segmentation of thyroid, which an essential part for TcTU measurement using SPECT/ CT. If a VOI for thyroid is too large or too small, the TcTU may be overestimated or underestimated. Previous researchers segmented the thyroid using a combined CT image of SPECT/CT [8, 9]. They drew multiple ROI along the thyroid contour slice by slice from the upper tip to the lower pole to generate a VOI for thyroid on transaxial CT images. This CT-based VOI generation method may be accurate for the asses-sment of TcTU on SPECT/CT image. However, the process is time-consuming and effortful, because an observer must manually and carefully draw multiple ROI to generate a VOI for a thyroid. Approximately 40 transaxial ROI may be requ-ired to generate a VOI for an entire thyroid [8].

While the accurate segmentation method for thyroid uptake on SPECT/CT image has not been sufficiently investigated, the segmentation methods for tumoral uptake on fluorine-18-fluorodeoxyglucose (^{18}F-FDG) PET/CT image have been studied to a considerable degree [10]. Jun et al. (2015) reported the presence of a peritumoral halo layer (PHL), which was observed between tumor activity and background on ^{18}F-FDG PET/CT [11]. Using the 10 step-color scale and specific window level setting, the distinct layer was easily identified by an abrupt increase in layer thickness with minimal or mild distortion of the main tumor contour [11]. The segmented tumor extent by the PHL method was far more approximate to the pathologic extent than the tumors segmented by fixed threshold methods [11-13]. Another advantage of the PHL method is the availability of auto-segmentation program, because the exact % threshold of tumor margin can be easily determined by PHL. Thus, the segmentation process using PHL method can be more convenient than visual segmentation [11, 12].

We speculated that the shape of thyroid on $^{99m}TcO_4$ SPECT/CT might be analogous to that of the tumor on ^{18}F-FDG PET/CT. Therefore, we hypothesized that the PHL method could be applied to segment the thyroid on SPECT/CT image. In the present study, we attempted to apply thyroid segmentation using perithyroidal halo layer (PTHL; SegPTHL) on SPECT/CT. We performed reliability tests for the TcTU, total lesion activity (TLA), and SUV measured by SegPTHL and CT-based segmentation (SegCT).

Subjects and Methods

Patients

The present retrospective study was approved by the institutional ethical review board and performed in accordance with the tenets of the Declaration of Helsinki. From August to September 2020 (6 weeks), 37 consecutive patients who had undergone thyroid SPECT/CT and laboratory tests for thyroid hormones were included in this study. Of the 37 patients with thyroid disease, 4 were excluded for the following reasons: (1) severe misalignment between the SPECT image and the CT image so that correction was impossible (n=1), (2) severely uneven uptake on the SPECT image so that the segmentation was impossible (n=1), (3) left thyroid lobectomy state (n=1) and (4) thyroid hormones checked in another institution (n=1). A total of 33 patients (19 females, 14 males; mean age, 46.91±15.7 years old) were finally enrolled in this study.

Sensitivity measurement study for thyroid SPECT/CT

The thyroid SPECT/CT images were acquired using the gamma camera with SPECT/CT system by installing the low energy high resolution sensitivity collimators (GE Healthcare, TiratHacamel, Israel). For the calculation of the quantitative parameters such as %TcTU, SUV), and TLA, the SPECT/CT scanner was calibrated to the same dose calibrator through a sensitivity study as follows. First, $^{99m}TcO_4$ (185MBq) was mixed in a uniform petri dish (a diameter of 10cm and a length of 5cm) filled with water to cover the bottom of the dish by 2-3mm. Second, after rotating the gantry such that detector surface was perfectly horizontal and facing up, the petri dish was placed at the center of the detector using the holder with negligible attenuation. Third, the system sensitivity, the conversion factor for radioactivity from counts/second, was measured for 10min with checking the background radiation. The sensitivity was calculated using the following equation:

$$Sensitivity = \frac{Total\ counts\ number \times EXP[\ln(2) \times \frac{(Acqusition\ start\ time - Measured\ time)}{Isotope\ half\ life\ time}]}{Acqusition\ duration \times Measured\ activity}$$

To increase the accuracy of the sensitivity, the sensitivity was measured three times and determined as 6.2382 counts/minute per MBq (168.6 counts/minute per μCi).

Thyroid SPECT/CT acquisition

All patients underwent thyroid SPECT/CT. After $^{99m}TcO_4$ injec-tion (approximately 185MBq) to patients, the remnant $^{99m}TcO_4$ radioactivity in the syringes was measured using the same dose calibrator. At 20min after $^{99m}TcO_4$ injection, the planar thyroid images were acquired for 1 min. Then, using the gamma camera with SPECT/CT system, the thyroid SPECT images were acquired for 1min with 360-degree continuous mode and the photon peak was set at 140keV with a 20% window (126-154keV). Next, non-enhanced CT was performed using the following parameters: tube voltage of 120kVp, tube current of 30-100 mA (automatic mA), beam collimation of 10mm (=0.625×16), pitch of 0.938:1, coverage time of 22.1sec, and tube rotation time of 0.5sec.

The SPECT images were reconstructed using ordered-subset expectation maximization (OSEM) algorithm with 2 thickness was 4.42mm and an image matrix was 128×128. For quantitation, the triple corrections, CT-based attenuation correction, scatter correction using lower energy peak of 120KeV with 10% window (114-126KeV) and resolution recovery were applied to the SPECT images on the software (Q. Volumetrix MI, GE Healthcare). In addition, a post-reconstruction low-pass filter (Butterworth filter with a cut-off frequency of 0.48 and an order of 10) was used.The reason for using the Butterworth filter was not only to reduce noise to realize better images, but also to enable segmentation, since segmentation would be impossible due to excessive noise. It was determined that analysis in a universal setting like this would be generally easily available. Since other previous studies [8, 9] also used the Butterworth filter, the Butterworth filter was used to ensure continuity with other previous studies.

The CT images were reconstructed using an adaptive statistical iterative reconstruction algorithm (ASiR, GE Healthcare) into 1.25 mm thick transaxial slices and an image matrix of 512×512.

CT-based thyroid segmentation

For SegCT, three-dimensional VOI of the thyroid were generated via multiple 2-dimensional ROI along the thyroid margin on transaxial CT images that were manually drawn slice by slice. The generated VOI were covered the whole thyroid volume. Then, SegCT parameters, which were the thyroid volume, the maximum SUV (SUVmax), the mean SUV (SUVmean), the TLA and the TcTU were obtained using quantitative SPECT/CT software (Q. Volumetrix MI, GE Healthcare).

Thyroid segmentation using PTHL

Following previous studies using the PHL method for tumor segmentation on ^{18}F-FDG PET/CT [11-13], a distinct layer between thyroid activity and background was identified on SPECT image and we termed that layer the PTHL. For the segmentations using PTHL (SegPTHL), SPECT window level and color scale were set as follows:

1. SPECT contrast window level was set in SUV (g/mL) units;

2. The top value of the window level was set to SUVmax of thyroid, and the bottom value was set to 0;

The top value was automatically set to the highest SUV in the scanned image. The SUV of the thyroid gland was the usually highest, but if the SUV of the salivary gland was higher, the SUVmax of the thyroid gland was measured using VOI and the top value was corrected with that value.

3. Color scale was set to a 10-step color scale using Step10 of the software (Q. Volumetrix MI, GE Healthcare);

4. The thyroid margin was determined using PTHL;

The PTHL was identified by an abrupt increase in layer thickness with minimal or mild distortion of the main thyroid contour in magnified axial or coronal images. Using the PTHL setting described above, each color layer represents 10% of the SUVmax. The margin of thyroid is the innermost point of the PTHL.

5. Automated VOI generation using PTHL;

An automated VOI generation was easily enabled using Q. Volumetrix software with setting the functional threshold to 100%. If an observer clicked the point of margin of thyroid activity (i.e., the innermost point of PTHL), three-dimensional VOI was automatically drawn along the innermost portion of the PTHL, which was considered as the boundary of thyroid activity.

An example of identification of PTHL and SegPTHL is shown in Figure 1. The SegPTHL parameters (i.e., thyroid volume, SUVmax, SUVmean, TLA, and TcTU) were then measured.

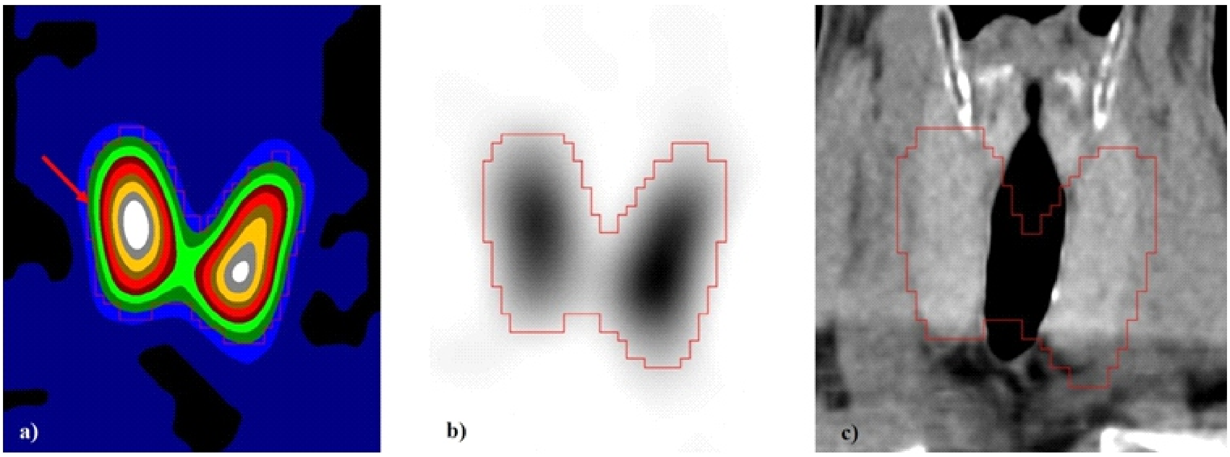

Figure 1. Volume of interest generated by the perithyroidal halo layer method.a) Method for generating a VOI for SegPTHL. When the SPECT contrast window level is set properly (i.e., top value=SUVmax; bottom value=0) and the 10 step color scale was applied, an observer can identify the hottest cores (white portion of the centers of both lobes), multiple thyroidal layers, PTHL (light blue layer), and background activity (dark blue area). The thyroid activities are composed of the hottest cores and multiple thyroidal layers. PTHL is located between the thyroid activity and background activity. The margin of thyroid is determined at the innermost point of PTHL (red arrow). When the functional threshold was set to 100% (i.e., the VOI includes higher activity than the activity of the clicked point), the VOI of SegPTHL using Q.Volumetrix software can be automatically generated after just one click of the mouse button at the innermost point of PTHL (red boundary). b) Coronal view of thyroid SPECT using an inverse grey scale (window level: top value=SUVmax; bottom value=0). The VOI of SegPTHL is drawn along the margin of thyroid (red boundary). c) Coronal view of CT. The generated VOI of SegPTHL (red boundary) is thought to be similar with CT margin of thyroid SPECT/CT, although SegPTHL is slightly larger than SegCT.

Statistical analysis

The sex and age among the disease status groups were compared using Chi-square test and Kruskal-Wallis test. Weighted Kappa test was performed to evaluate inter-observer agreement between the two nuclear medicine physicians for PTHL threshold. Pearson's correlation coefficients and Bland-Altman plots were used to evaluate the correlation and reliability between SPECT/CT parameters (i.e., thyroid volume, TcTU, TLA, and SUVmean) of the SegPTHL and SegCT. Wilcoxon signed rank-test was performed to compare the thyroid volumes between SegPTHL and SegCT. Relationships between SUVmax of thyroid and PTHL thres-hold were evaluated by Spearman's rank correlation. Med-Calc® for Windows (MedCalc Software, Mariakerke, Belgium) was used for all statistical analyses.

Ethical approval and consent to participate

The present study was approved by the institutional review board of Chungnam National University Sejong Hospital (IRB No: 2021-10-014), and informed consent was waived due to the retrospective study design. All procedures involving human participants were performed in accordance with the ethical standards of the institutional research committee and with the Helsinki declaration as revised in 2013 and its later amendment.

Results

Patient characteristics

A total of 33 patients were enrolled in our study. Two expert endocrinologists determined the final diagnosis of thyroid disease: thyroiditis (n=13) including chronic thyroiditis in 11 patients, a patient with painless thyroiditis and a patient with subacute thyroiditis; initially diagnosed or recurred Graves' disease (n=8); thyroid nodule with euthyroid state (n=6); and treated Graves' disease but not in remission (n=6). There were no significant differences in sex and age among the disease groups (P=0.893 and P=0.872, respectively).

Inter-observer agreement for the determination of PTHL

Of the 33 enrolled patients, the inter-observer agreement was very good (k=0.873). There were inter-observer disagreements for the PTHL threshold in only 3 patients (Table 1).

PTHL threshold change according to SUVmax

In the 37 patients who underwent thyroid SPECT/CT, SegPTHL was available in 36 patients. In only one patient, the PTHL could not be identified because of severely uneven and low uptake on the SPECT image. The PTHL thresholds ranged from 20% to 50% of SUVmax of thyroid. As the SUVmax of thyroid increased, the PTHL threshold was sig-nificantly decreased (σ=-0.748, P<0.0001; Figure 2). The SUVmax of thyroid ranged from 14.3 to 681.6 (median=84.8; interquartile range=50.1~201.6).

Table 1. *Inter-observer agreement test for determination of % threshold for SegPTHL*

Observer B	Observer A			
	20%	**30%**	**40%**	**50%**
20%	23	3		
30%		3		
40%			2	
50%				2

k=0.873

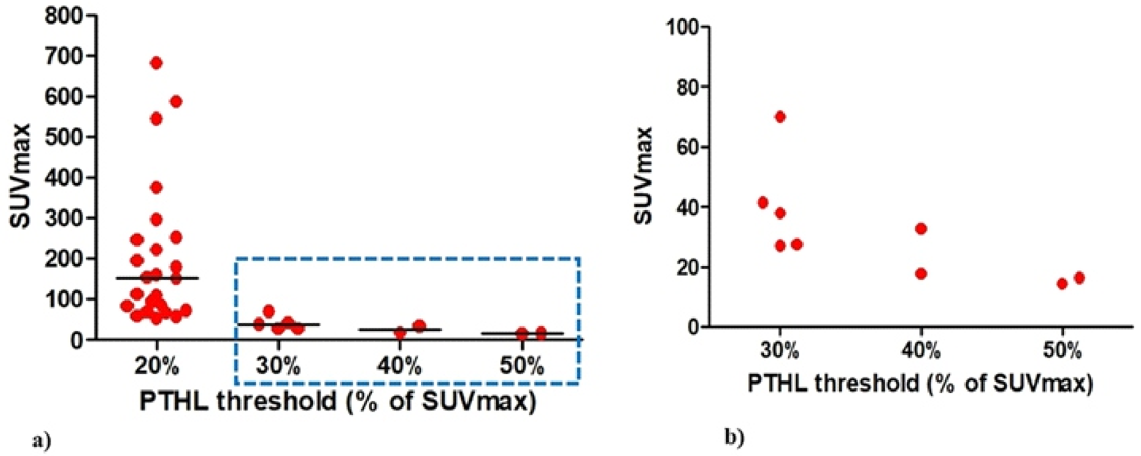

Figure 2. Relationship between SUVmax and PTHL threshold. Unlike the SUVmax of malignant tumor of ^{18}F-FDG PET/CT, the range of thyroid SUVmax of ^{99m}Tc-pertechnetate SPECT/CT (SUVmax=14.3~681.6) is much wider than that of tumor of ^{18}F-FDG PET/CT. PTHL threshold was inversely correlated with SUVmax (a: σ=-0.748, P<0.0001). b) Magnified image in the blue dotted box.

Comparison, correlation, and reliability tests of thyroid volume between SegCT and SegPTHL

Among the 37 patients, only one patient had severe misalignment between the SPECT image and the CT image that could not be corrected automatically and manually, so it was impossible to measure parameters other than thyroid volume by the SegCT.

In the 33 patients finally enrolled, the thyroid volumes were measured by both SegCT and SegPTHL. The median thyroid volume of SegCT was 30.2mL (range=10.2~114.1mL; interquartile range=24.2~43.5mL), and the median thyroid volume of SegPTHL was 41.0mL (range=16.8~76.9mL; interquartile range=37.0~51.3mL). The thyroid volume of SegPTHL was significantly larger than that of SegCT (P=0.0017). On visual inspection of both SegPTHL and SegCT, the prominent difference was observed around the isthmus (Figure 3). A good correlation was observed between the thyroid volumes of SegCT and SegPTHL (r=0.725; P<0.0001; Figure 4a). On the Bland-Altman plot, there was no proportional or divergent error between the thyroid volumes of SegCT and SegPTHL (bias=-7.5mL; limits of agreement=-33.4~19.4mL; Figure 4b).

Correlation and reliability tests for TcTU, TLA, and SUVmean between SegCT and SegPTHL

The SUVmax of SegCT was the same as that of SegPTHL in all enrolled cases. The TcTU, TLA, and SUVmean of SegPTHL were excellently correlated with those of SegCT (TcTU: r=0.9877, P<0.0001, Figure 5a; TLA: r=0.9883, P<0.0001, Figure 5b; SUVmean: r=0.9875, P<0.0001; Figure 5c). No proportional or divergent error was observed between the parameters (i.e., TcTU, TLA, and SUVmean) of SegCT and SegPTHL (TcTU: bias=-0.64, limits of agreement=-2.05~0.78, Figure 5d; TLA: bias=-395.4, limits of agreement=-1323.3~532.6, Fi-gure 5e; SUVmean: bias=1.1, limits of agreement=-16.6~18.8, Figure 5f).

Comparison of uptake parameters between Graves' disease and thyroiditis

Among the 33 enrolled patients, there were 8 patients with initially diagnosed or recurred Graves' disease and 13 patients with thyroiditis. TcTU, TLA, SUVmax, and SUVmean in patients with overt Graves' disease were significantly higher than those in patients with thyroiditis (Table 2). The area under curve (AUC) of receiver operating comparison analysis was not different between parameters (i.e., TcTU, TLA, and SUVmean) of SegCTand SegPTHL (TcTU: AUC of SegCT=0.952, AUC of Seg PTHL=0.962, P=0.4795; TLA: AUC of SegCT=0.952, AUC of SegPTHL=0.962, P=0.4795; SUVmean: AUC of SegCT=0.933, AUC of SegPTHL=0.942, P=0.4795).

Discussion

The major finding of our study is that PTHL is present on $^{99m}TcO_4$ thyroid SPECT/CT image like PHL of ^{18}F-FDG PET/CT. The main feature of PTHL was similar to that of PHL. The PTHL is located between thyroid activity and background, and an abrupt increase of layer thickness was also observed in PTHL on thyroid SPECT/CT image (Figure 1). Perithyroidal halo layer was naturally adjusted as the SUVmax of thyroid changed (Figure 2). The SPECT/CT parameters (i.e., TcTU, TLA, and SUVmean) using SegPTHL also had strong correlations with those of SegCT, and there was no remarkable error on the Bland-Altman plot (Figure 5). Therefore, we suggest that SegPTHL may be used for accurate measurement of thyroid SPECT/CT parameters. Moreover, the segmentation process of SegPTHL can be more convenient than that of SegCT because multiple ROI drawings are not mandatory to generate an accurate VOI for thyroid. A VOI of SegPTHL can be easily generated using auto-segmentation software, which can define the area of higher activity than the determined threshold (Figure 1).

In the 1960s and 1970s, thyroid scintigraphy was an important test for the evaluation of thyroid nodules. Currently, the role of thyroid scintigraphy for the evaluation of malignant thyroid nodule has been reduced because the techniques of ultrasonography and fine needle aspiration cytology have gradually advanced [3]. However, TcTU has been used to differentially diagnose the cause of thyrotoxicosis for over five decades.

Theoretically, TcTU represents the percentage ratio of $^{99m}TcO_4$ uptake in thyroid to total administered dose [3] and is reflective of total radioactivity of thyroid. There are two conventional methods to obtain TcTU. One is measuring the TcTU using the gamma probe system, and the other is the gamma camera method using planar thyroid scintigraphy [5]. The TcTU measured by the gamma probe system, which measures the counts of the anterior neck and subtracts the counts of the thigh as background counts, may be overestimated because of the significant amount of extrathyroidal high radioactivity such as in saliva in the oral cavity and salivary glands [8]. The gamma camera method using planar thyroid scintigraphy may also have limitations for the accurate measurement of TcTU. Because the ROI overlying thyroid on planar scintigraphy always includes the background activity anterior and posterior to the thyroid, background correction must be performed using a subtraction of background counts from counts over thyroid [3, 5, 14]. However, it may be difficult to accurately define the background ROI for background subtraction, because the amount of background activity anterior and posterior to the thyroid on planar scintigraphy cannot be determined. In addition, if the subtracted background count is high, TcTU may be underestimated. For this reason, there have been several studies to obtain an accurate TcTU using SPECT/CT [8, 9, 15]. SPECT image allows separation of the overlying tracer accumulation from areas of interest [6]. In regard to TcTU, background subtraction is not needed in SPECT/CT imaging of thyroid, because an accurate VOI of thyroid may not include non-thyroid background activities. Also, SPECT/CT can be used to measure other parameters such as SUVmax, SUVmean, and TLA as well as TcTU [8, 9, 15, 16]. In previous reports for thyroid SPECT/CT, thyroid segmentation was performed using SegCT [8, 9, 15]. Although SegCT may be an accurate method for the segmentation of thyroid, it has several limitations. First, the SegCT can take a long time to

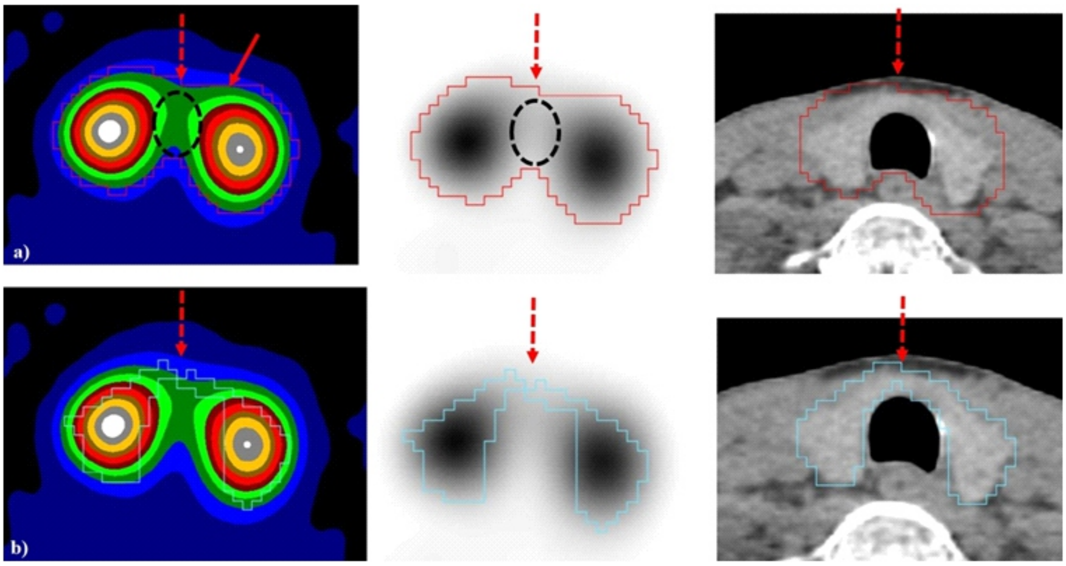

Figure 3. Visual comparison between SegPTHL and SegCT. a) Transaxial view of SegPTHL; b) Transaxial view of SegCT. PTHL is the light blue colored layer. Thus, the margin of thyroid is determined at the innermost part of blue colored layer (red arrow). Thyroid segmentation PTHL is slightly larger than SegCT. The difference is prominent around the isthmus (red dotted arrow). Because both lobes of thyroid are located very closely around the isthmus, the isthmic PTHL activities from both thyroid lobes may be joined into mild thyroidal activity (in the black dotted circle).

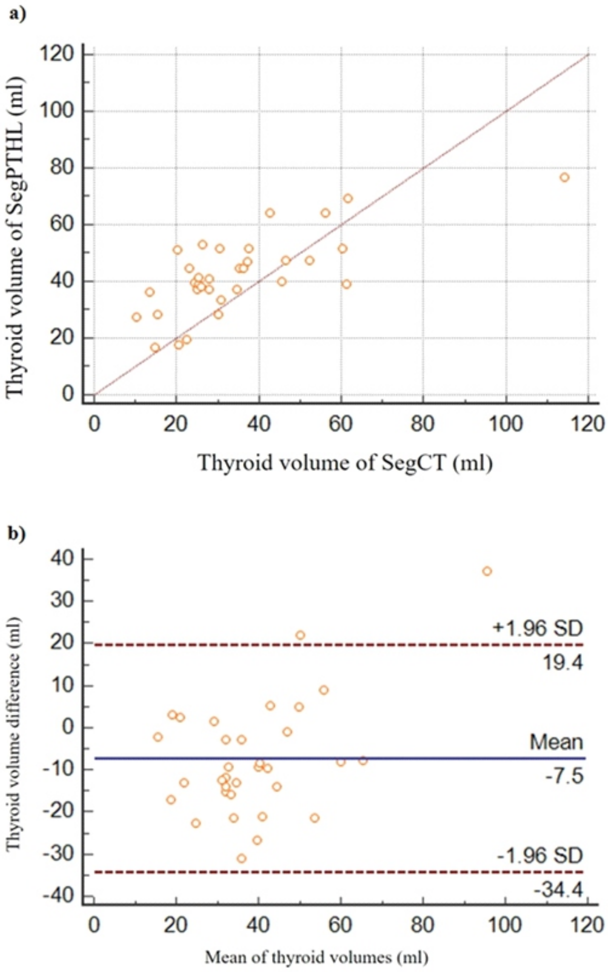

Figure 4. Correlation analysis and Bland-Altman plot between the thyroid volumes of SegPTHL and SegCT. a) The thyroid volume of SegPTHL was correlated well with that of SegCT (r=0.725; P<0.0001). b) There was no significant error between SegPTHL and SegCT on Bland-Altman plot.

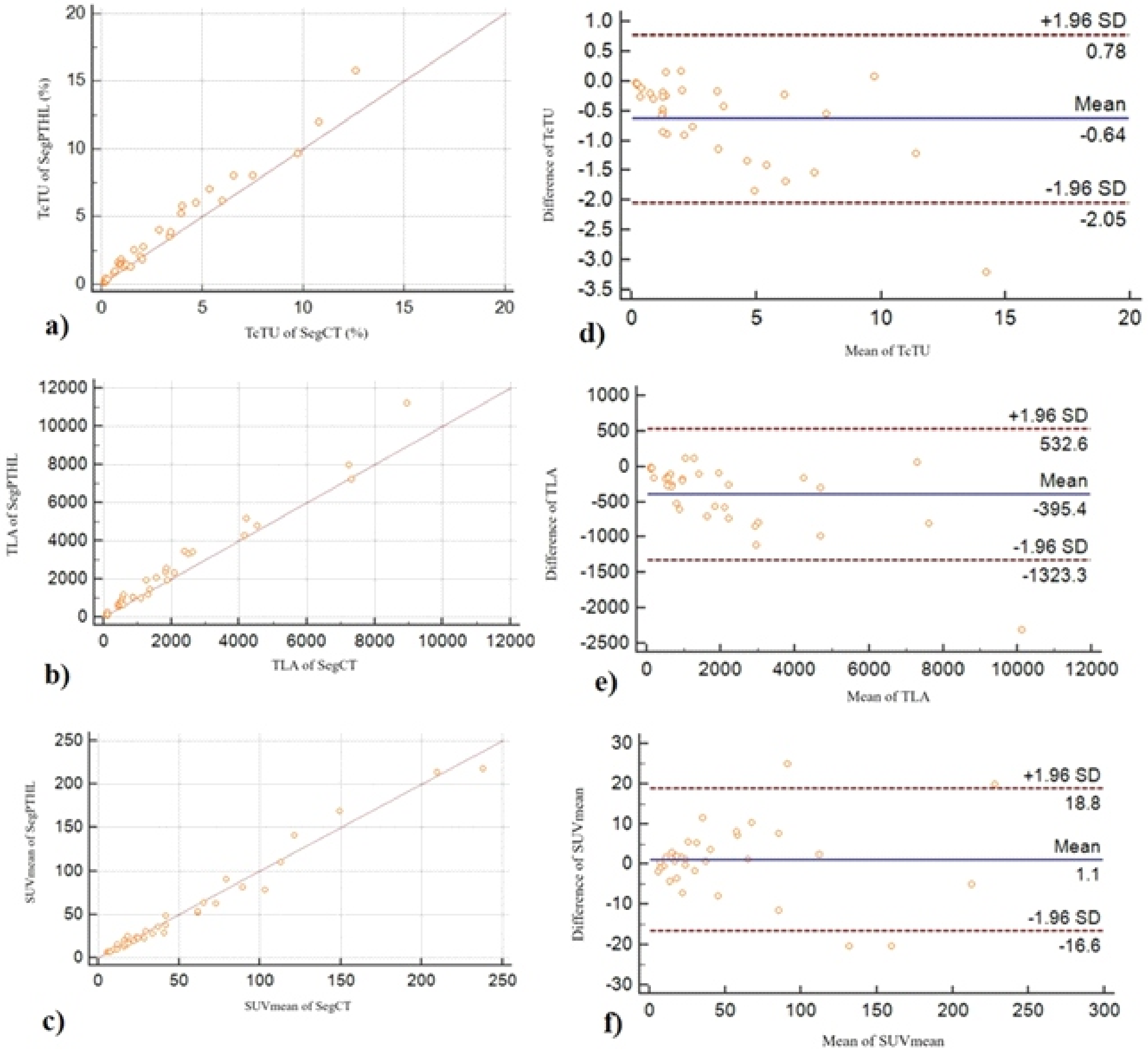

Figure 5. Correlation analyses and Bland-Altman plots of TcTU, TLA, and SUVmean between SegPTHL and SegCT. TcTU, TLA, and SUVmean of SegPTHL were highly correlated with those of SegCT (a): r=0.9877, P<0.0001; b) r=0.9883, P<0.0001; c) r=0.9875, P<0.0001). There was no significant proportional or divergence error between SegPTHL and SegCT (d-f).

Table 2. *Comparison of parameters of SegPTHL and SegCT between overt Graves' disease and thyroiditis*

	Overt Graves' disease (n=8)	Thyroiditis (n=13)	P values
TcTU of SegCT (range)	5.96% (2.88 ~ 12.6%)	0.81% (0.17 ~ 7.51%)	0.0002*
TLA of SegCT (range)	3351 (1817 ~ 8950)	543 (93 ~ 4540)	0.0002*
SUVmean of SegCT (range)	100.95 (41.40 ~ 237.80)	16.80 (5.10 ~ 149.30)	0.0004*
TcTU of SegPTHL (range)	7.56 % (3.53 ~ 15.8%)	1.32 % (0.19 ~ 8.05%)	0.0001*
TLA of SegPTHL (range)	4259 (1975 ~ 11250)	677 (115 ~ 4831)	0.0001*
SUVmean of SegPTHL (range)	95.85 (49.1 ~ 217.8)	16.8 (6.6 ~ 169.4)	0.0003*
SUVmax** (range)	274.05 (152.90 ~ 681.60)	53.00 (14.30 ~ 544.60)	0.0003*

*TcTU=$^{99m}TcO_4$ uptake; TLA=total lesion activity; SUVmean=mean standardized uptake value; SUVmax=maximum standardized uptake value; SegCT=CT-based segmentation; SegPTHL=segmentation using perithyroidal halo layer method *Statistically significant, ** The SUVmax of SegCT was the same as that of SegPTHL in all enrolled cases.*

generate a VOI because the multiple ROI for thyroid glands have to be drawn manually and carefully slice by slice on transaxial CT images. Second, non-contrast CT in SPECT/CT is used for the thyroid segmentation; the visual segmentation of SegCT is sometimes difficult because the CT attenuation value decreases when the iodine concentration of the thyroid is low, such as in diffuse thyroid disease. In this study, there were 5 cases (5/33; 15.2%) in which it was hard to distinguish the thyroid gland from its surrounding muscles (Figure 6). In addition, the SegCT of the lower thyroid portion were difficult because of artifacts caused by CT beam hardening at the clavicle level in 14 of 33 enrolled cases (14/33; 42.4%). In these cases, it took longer than 10min to perform CT segmentation alone. To overcome this beam hardening artifact, a higher tube current may be required, but radiation exposure to the patient may be increased. Third, the registration for alignment between CT and SPECT images may be necessary for more accurate quantitative analysis in cases with misalignment by patient movement that may occur during the examination. In our study, misalignments were observed in 3 cases. In two cases, the misalignments were corrected by manual rearrangement. However, in one case which showed severe misalignment, the correction could not eliminate the misalignment and this case had to be excluded because the parameters could not be measured by the SegCT.

In previous studies, tumor segmentation using the PHL method on ^{18}F-FDG PET/CT was reliable for measurement of tumor size and tumor volume of primary tumor in several cancers [11-13]. We applied this method for the segmentation of thyroid SPECT/CT. In our study, PTHLs were identified in most cases (36/37), and the inter-observer agreement was very good (k=0.873; Table 1). If the PTHL threshold can be determined on thyroid SPECT/CT image, the SegPTHL could be conveniently performed using an auto-segmentation program like the previous tumor segmentation studies using PHL on ^{18}F-FDG PET/CT [11-13]. Thus, the time required for SegPTHL could be much shorter than the time required for SegCT. With regard to the SPECT/CT parameters such as TcTU, TLA, and SUVmean, the parameters of SegPTHL were strongly correlated with those of SegCT (Figure 5), although the thyroid volume measured by the SegPTHL was slightly larger than those measured by the SegCT (Figure 3 and 4). The SPECT/CT parameters acquired by both SegPTHL and SegCT had similar diagnostic performance to distinguish Graves' disease from thyroiditis (Table 2). Although the number of patients in our study was small, the result was similar to those of a previous SPECT/CT study by Lee et al. (2016) [8]. Therefore, it is expected that SegPTHL can be used for the accurate quantification of thyroid SPECT/CT.

In the previous PHL studies for tumor segmentation on ^{18}F-FDG PET/CT, the PHL threshold for tumor segmentation was naturally adjusted as SUVmax of tumor changed [11-13]. In our PTHL study for thyroid segmentation on SPECT/CT, the result was similar to those of the previous PHL studies. The PTHL threshold (i.e., % of SUVmax in the point of thyroid margin) for thyroid segmentation was inversely correlated with the SUVmax of thyroid (Figure 2). However, there was a noteworthy difference on scatter gram compared with pre-vious PHL studies on ^{18}F-FDG PET/CT. The far wide range of SUVmax of thyroid was noted on scatter gram (Figure 2), and the PTHL threshold (i.e. % of SUVmax of thyroid) was relati-vely lower than that in previous PHL studies on ^{18}F-FDG PET/ CT (our PTHL study: 20%~50% of SUVmax; previous PHL studies: 20%~90% in thyroid [11], breast [13], or esophageal [12] cancer). Because the SUVmax of thyroid (range= 14.3~681.6 in our study) on

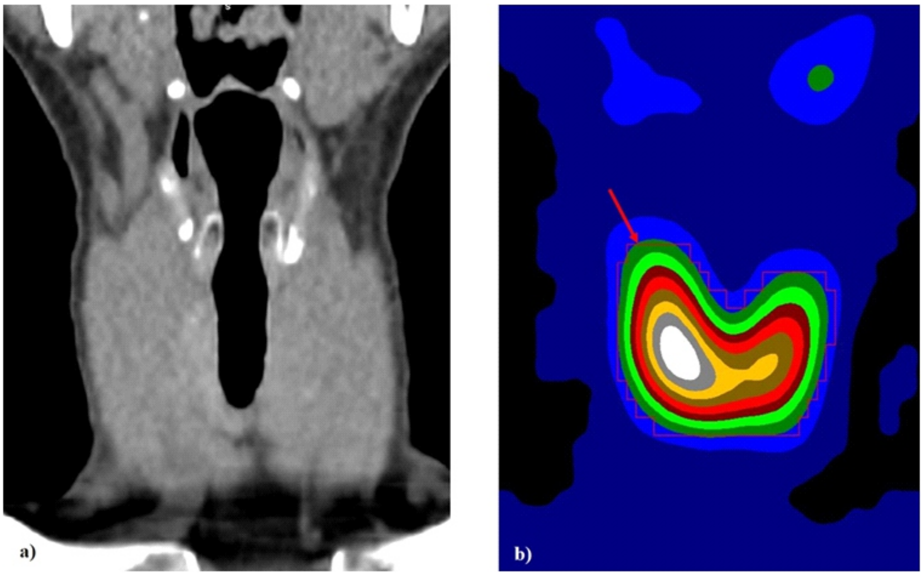

Figure 6. A 44-year-old female with thyroiditis. a) CT image of thyroid SPECT/CT. SegCT was difficult because the density of thyroid was similar with that of the surrounding neck muscles. b) PTHL image view. Thyroid could be easily segmented by PTHL method. PTHL was observed in the light blue-colored layer. Thus, the thyroid margin was deter-mined at the innermost portion of PTHL. SegPTHL (red boundary) was conveniently performed by just one click of the point of thyroid margin (red arrow).

threshold of most cases may be adjusted to the low % of SUVmax of thyroid [i.e. 20% of SUVmax; 24/33 (72.7%); Figure 2]. We consider that the fixed SUV threshold method should not be applied for accurate segmentation of thyroid activity on $^{99m}TcO_4$ SPECT/CT because of the wide range of SUVmax.

Regardless of the segmentation method, the important points are the accurate exclusion of extrathyroidal background activity around the thyroid and the inclusion of true thyroid activity in the segmented VOI. When comparing the VOI (i.e., thyroid volume) of both SegCT and SegPTHL, the VOI of SegPTHL was slightly larger than that of SegCT. Thus, we concluded that the VOI of SegPTHL could sufficiently include the true thyroid activity. The inclusion of absurd extrathyroidal background activity could also be easily avoided in SegPTHL, because PTHL was not included in the VOI of SegPTHL. Perithyroidal halo layer was located between the thyroid activity and background activity, and it could maintain the original thyroid contour. If we do not include PTHL in the process of segmentation of VOI for true thyroid activity, the removal of absurd extrathyroid background activity may occur. In the current study, the VOI of SegPTHL were mostly similar to the typical thyroid shape on maximum intensity projection and coronal images, except for 3 thyroiditis cases that showed uneven uptake.

In this study, the effective dose of CT was estimated to be 0.35mSv using the converting factor of 3.1mSv/mGy-cm [21]. In previous studies, the effective dose of CT was 1.12mSv, but since the tube current was 180mA [8, 15], it was higher than in this study using automatic tube current (30-100mA). It is expected that radiation exposure can be reduced if SPECT/CT is performed with a lower tube current that can allow for attenuation correction.

The current study had several limitations. First, the study design was a retrospective analysis. However, when segmenting the thyroid glands on SPECT/CT images, the two nuclear medicine physicians were blinded to the information about the subject's disease and laboratory data. Second, since a small number of subjects in a single center was included, it was difficult to analyze results by thyroid diseases, and it was impossible to obtain the normal range of each SPECT/CT parameter by SegPTHL. Therefore, further study including a larger number of subjects classified by thyroid disease is needed. Third, the comparisons of radioiodine uptake and TcTU in the same patients were not done as performed in previous study [8].

In conclusion, this is the first segmentation study using SegPTHL in thyroid SPECT/CT. Our results indicate that SegPTHL could be a useful method for reliable quantification of thyroid uptake, because the SPECT/CT parameters of SegPTHL were strongly correlated with those of SegCT. As well as the process of SegPTHL is easier and faster than that of SegCT.

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