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An easy and reproducible semi-automatic method for the evaluation of ^{99m}Tc-galactosyl human serum albumin

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^{99m}Tc-galactosyl human serum albumin (^{99m}Tc-GSA) scintigraphy is a new method for evaluating liver function. This scintigraphy is useful for evaluating the severity of liver diseases. The indices evaluating hepatic function include the receptor index (LHL15) and the index of blood clearance (HH15). These indices are calculated on the basis of the regions of interest (ROIs) for both whole liver and heart, and are susceptible to the ROIs over heart and liver. At present, there is no standard method for determining ROIs. We attempted to establish a standard method which shall not be subject to inter and/or intra operator variation. A computer program to determine heart and liver ROIs semi-automatically was developed. Ten patients (12 instances) were studied with ^{99m}Tc-GSA, and HH15 and LHL15 were calculated on the basis of the ROIs obtained manually and semi-automatically by 3 different operators independently. Blood sampling and gamma-counting yielded blood clearance data. The ICG R15 was compared with each index in 34 patients. The time needed for ROI determination was reduced from 2–3 minutes for the manual method to 0.5–0.8 minutes for the semi-automatic method. The % coefficients of variation (% CVs) of HH15 and LHL15 were improved in the order manual-inter observer (M-inter), manual-intra observer (M-intra) and semi-automated-inter observer (SA-inter); % CVs of HH15 were 2.26% for M-inter, 1.55% for M-intra and 0.07% for SA-inter, and % CVs of LHL15 were 2.29% for M-inter, 0.46% for M-intra and 0.07% for SA-inter. The correlation of HH15 and LHL15 among M-inter, M-intra and SA-inter was good. Comparison of indices obtained by manual and semi-automatic methods with blood clearance data obtained by blood sampling and gamma-counting showed good correlations and no significant differences. The comparison with ICG R15 showed that HH15 and LHL15 by the semi-automated method gave better correlation that that by the manual method. A newly developed semi-automated method improved data processing time and deviation of indices in ^{99m}Tc-GSA studies. This method should substitute for manual ROI determination.

Key words: liver function, ^{99m}Tc-GSA, semi-automatic method

INTRODUCTION

HEPATIC RECEPTOR IMAGING with ^{99m}Tc-galactosyl human serum albumin (^{99m}Tc-GSA) is a new method for the evaluation of liver function.[1–6] This technique is based on the specific binding of asialoglycoprotein to the asialo-glycoprotein receptor located on the plasma membrane of liver cells.[7–9] The indices used in this method are the receptor index (LHL15) and the index of blood clearance (HH15). These indices are calculated on the basis of the regions of interest (ROIs) for whole liver and heart,[1–6] but there is no standard method for determining ROI. Indices may vary according to the operator. The reason should come from the fluctuation of ROIs of liver and heart. We therefore attempted to establish an easy and reproducible standard method which reduced inter-operator variation.

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Table 1 Deviation of Tc-99m GSA hepatic function indices deviation

	Manual inter-observer	Manual intra-observer	Semi-automatic inter-observer
HH15	2.26% (0.77–6.53)*	1.55% (0.41–2.69)	0.07% (0–0.26)
LHL15	2.98% (0.79–8.92)	0.46% (0.20–1.24)	0.07% (0–0.30)

*: Mean of % CV (range of % CV) was shown as a representative of the deviation.

Table 2 Correlation coefficients of hepatic indices among gamma-camera methods

	Manual inter-observer	Manual intra-observer	Semi-automatic inter-observer
Manual inter-observer	—	0.995 (LHL15)**	0.991 (LHL15)**
Manual intra-observer	0.996 (HH15)*	—	0.990 (LHL15)**
Semi-automatic inter-observer	0.992 (HH15)*	0.991 (HH15)*	—

*: Correlation coefficient of HH15. **: Correlation coefficient of LHL15.

METHODS

Measurement of the receptor index and index of blood clearance

^{99m}Tc-GSA (185 MBq) was injected iv, and dynamic images were recorded with the patient supine under a large field of view gamma camera with a low-energy, all-purpose, parallel hole collimator. Computer acquisition of gamma camera data was started just before injection, and stopped 30 minutes later. Blood sampling and gamma counting were performed at 3 and 15 minutes.

Data processing

I. A semi-automatic method of making ROIs of heart and liver

We made a computer program for determining ROIs of heart and liver employing the concept of automated contour mapping. The area overlapped by the heart and liver was omitted from the heart ROI.

The following process was applied.

1. A heart image was made by early images.
2. A large square ROI (tentative ROI) was made on the heart area by manually on the process 1 image.
3. A large square field of whole liver ROI (tentative ROI) was made manually.
4. A liver image was made by the 5% (of the maximum liver count) liver contour in the square liver field (process 3).
5. The overlapped area (liver and heart ROIs) was subtracted from the square heart area (process 2) using the 5% liver contour image (process 4).
6. A heart ROI was made by 45% (of the maximum heart count) heart contour in the remnant heart area which subtracted liver contour (the image of process 5).

This program was made by C programming on a Unix system.

In these processes, only making the square tentative ROIs on heart and liver was performed manually. After processes 2 and 3, all processes were performed automatically. The time activity curves for heart and liver, receptor index (LHL15) and index of blood clearance (HH15) were calculated automatically. The receptor index (LHL15) was calculated by dividing the radioactivity of the liver ROI by the radioactivity of the liver and heart ROIs 15 minutes after injection. The index of blood clearance (HH15) was calculated by the dividing the activity of the heart ROI at 15 minutes by that at 3 minutes.

The values for fixed thresholds of liver (5%) and heart (45%) were determined by comparing with manually determined data. The threshold of heart ROI was shifted from 60% to 20% in 5% step. And the correlations of HH15 values with these variable thresholds to manually determined HH15 were calculated. The HH15 value with a 45% threshold gave the minimal regression coefficient. So we tentatively took 45% as a heart ROI threshold. With the liver ROI threshold, we made liver ROIs shifting the threshold from 50% to 5% in 5% step. The shapes of liver contours with these variable thresholds were visually compared with manually made liver contours. The shape of a liver contour with a 5% threshold gave the nearest image to a manually made liver contour. Then, we shifted the liver threshold from 10% to 1% in 1% step. The LHL15 values with these thresholds were calculated and compared with a manually determined LHL15 value. There was no significant differences in regression coefficients with 10 % to 1% thresholds, ranging 0.989–0.990, so we tentatively took 5% as the liver threshold.

II. A manual method of making ROIs of heart and liver.

After making heart and liver ROIs manually, the calculation was carried out by the semi-automatic method.

Patients

Ten patients with various liver diseases were studied. Seven metastatic liver tumor patients (2 from stomach and

Table 3 Correlation between B-HH15 by blood counting and HH15 by various gamma-camera methods

	Slope	*Y* intercept	Coefficient (range)
Manual inter-observer	0.777	0.237	0.924* (0.871–0.940)
Manual intra-observer	0.781	0.226	0.934* (0.926–0.940)
Semi-automatic inter-observer	0.806	0.201	0.912* (0.911–0.912)

*: Statistically significant, $p < 0.0001$.

Table 4 Correlation coefficients to ICG R15

		Slope	*Y* intercept	Coefficient
HH15	Manual	0.766	0.466	0.585*
	Semi-automatic	0.825	0.445	0.629*
LHL15	Manual	−0.286	0.972	0.535**
	Semi-automatic	−0.562	0.955	0.661*

*: Statistically significant, $p < 0.0001$. **: Statistically significant, $0.0001 < p < 0.001$.

5 from colon) and 3 primary liver tumor patients (2 hepatocellular carcinomas and 1 cholangiocarcinoma) were included in this study. Two patients (1 metastatic liver tumor and 1 hepatocellular carcinoma) were repeatedly studied, and 12 instances were included in this study.

With regard to the comparison with an indocyanine green test (ICG), 34 patients (36 instances) including 7 of the ten patients mentioned above were studied. Nineteen metastatic liver tumor patients (15 from colon, 2 from stomach, 1 from esophagus and 1 from breast) and 15 primary liver tumor patients (13 hepatocellular carcinomas and 2 cholangiocarcinomas) were evaluated by means of the correlation with liver function obtained by ^{99m}Tc-GSA (HH15 and LHL15) and ICG R15.

Evaluation of the results

1. Inter- and intra-operator variation

Inter-operator variation was assessed by data processing and obtaining on the index of blood clearance (HH15) and receptor index (LHL15) from 3 different operators. Intra-operator variation was assessed by 3 runs by an operator on separate days.

2. Index of blood clearance (B-HH15) obtained by blood sampling

Blood sampling and gamma-counting were performed at 3 and 15 minutes, and the index of blood clearance (B-HH15) was calculated (radioactivity of blood at 15 minutes was divided by that at 3 minutes). This B-HH15 by blood sampling and gamma-counting was obtained as a standard. The indices of blood clearance (HH15) calculated by gamma camera methods (manual ROI and semi-automatic ROI) were compared with B-HH15 by blood counting.

3. Comparison of receptor index and index of blood clearance with a liver function test (ICG R15)

The receptor index (LHL15) and the index of blood clearance (HH15), obtained both by manual and semi-automatic methods, were compared with an indocyanine green test (ICG). ICG R15 values were available in 34 patients (36 instances). ICG tests were conducted within 7 days after ^{99m}Tc-GSA studies.

Statistical analysis

To compare the variations, the percentage coefficient of variation (% CV) was used as an index. The % CV was calculated by standard deviation divided by the mean value, then multiplied by 100. A simple linear regression test was performed and Pearson's correlation coefficient was obtained.

RESULTS

ROIs determination time by the manual method was 2–3 minutes, and the semi-automatic method was 0.5–0.8 minutes. Table 1 shows the % CV of HH15 and LHL15 obtained by the gamma-camera method. The % CV both of HH15 and LHL15 became smaller with the manual method inter-observer, manual method intra-observer and semiautomatic method inter-observer. The % CVs for the manual method inter-observer were 2.26% (range 0.77–6.53%) for HH15 and 2.98% (range 0.79–8.92%) for LHL15. In some patients, % CVs were greater than 5%. The % CVs for the manual method intra-observer were 1.55% (range 0.41–2.69%) for HH15 and 0.46% (range 0.20–1.24%) for LHL15. These variations were not as large as that for inter-observers, but the % CVs for the semi-automatic method inter-observer were 0.07% (range 0–0.26%) for HH15 and 0.07% (range 0–0.30%) for LHL15. These variations were the smallest for all testing categories. Table 2 shows the correlation coefficient among values for the inter- and intra-observers with manual methods and values for the inter-observer with the semi-automatic method. The correlations among values obtained by the 3 methods were good, ranging 0.990–0.996.

Table 3 shows the correlation between B-HH15 ob-

tained by blood counting and that by gamma-camera method. The correlation coefficients, slopes, and *y* intercepts of the 3 methods were essentially the same, and correlation coefficients were over 0.9.

The correlations between ICG R15 and indices obtained by gamma-camera methods are shown in Table 4. The correlation of the semi-automatic method is better than that of the manual method. Correlations of HH15 and ICG R15 were 0.585 by the manual method and 0.629 by the semi-automatic method. Correlations of LHL15 and ICG R15 were 0.535 by the manual method and 0.661 by the semi-automatic method.

DISCUSSION

The liver function indices; HH15 and LHL15, fluctuate with inter-operator variation. The variation may come from the fluctuation of ROIs of liver and heart. This experience prompted us to make an easy and reproducible (with a very low deviation) method for determining ROIs for the heart and liver. The method consisted of 3 steps: 1) make liver ROI by means of a fixed threshold (5% of the maximum liver count), 2) subtract overlapped liver area from heart area, and 3) make the heart ROI by means of a fixed threshold (45% of the maximum heart count). We determined these values in comparison with manually determined HH15 and LHL15 data.

The % CV was reduced in the order of inter-operator with the manual method, intra-operator with the manual method and inter-operator with the semi-automatic method. The % CV of our semi-automatic method was dramatically reduced to 0.07% CV. The results were satisfactory with small inter- and intra-operator deviations. This method was easy and reproducible. The correlations of liver indices obtained by gamma-camera methods with HH15 obtained by blood counting were similar for manual and semi-automatic methods. The correlation of liver indices obtained by gamma-camera methods with ICG R15 was better with the semi-automatic method than that with the manual method. These data indicated that the semi-automatic ROI making method was superior to manual ROIs making method.

Many authors have recently published various liver function parameters or indices obtained by means of ^{99m}Tc-GSA liver scintigraphy.[2,10–12] These parameters were calculated on the basis of liver and heart ROIs. There is still no standard method for determining ROIs of the heart and liver, so that each hospital develops its own protocol. This makes it difficult to compare the values obtained at different hospitals. Indeed, the results in our hospital were produced by one operator. If the operator changes, the values may change. To standardize the method is very important.

In conclusion, the semi-automatic ROI method for ^{99m}Tc-GSA is an easy and reproducible method for calculating HH15 and LHL15 with an extremely low inter-observer variation. This method should replace the manual method for making ROIs of the heart and liver.

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