Normal Values of Gallbladder Ejection Fraction Using ^{99m}Tc-sestamibi Scintigraphy after a Fatty Meal Formula

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Abstract

Background & Aims. Sincalide, in conjunction with cholescintigraphy, is necessary for the diagnosis of chronic acalculous cholecystitis. However sincalide is not widely available. This study investigates the use of a commercially available formula as an inexpensive alternative to sincalide, containing a sufficient and known amount of fat to cause gallbladder contraction, and to determine normal gallbladder ejection fraction (GBEF) values. **Methods**. We studied 36 patients aged 51.7±10.9 years with body mass index 26.7± 5.2 who were referred for ^{99m}Tc-sestamibi myocardial perfusion imaging. They did not have any abdominal symptoms, or history of abdominal disease and were not taking any medication known to affect the biliary tract. All were prescreened with a hepatobiliary ultrasonography to exclude any abnormality. After 6 hours fasting, 20 mCi of ^{99m}Tc-sestamibi was injected intravenously at rest and 90 minutes later the subjects ingested a test meal (10 g fat). GBEF was calculated at 30 and 60 minutes after fatty meal ingestion. **Results**. GBEF at 30 minutes and at 60 minutes after fatty meal ingestion were 69.54±21.04% and 84.26±11.41%, respectively. GBEF did not differ significantly between men and women. There was no statistically significant correlation between BMI and GBEF. No significant difference was noticed in GBEF between obese, overweight and normal weight patient groups. **Conclusion**. Lower limit of normal GBEF values was 27.46% at 30 min and 61.44% at 60 min using a standard fatty meal. It is possible to report the results of a GBEF measurement after fatty meal in terms of the percentile rank, compared with subjects without biliary disease.

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Key words

Gallbladder ejection fraction - chronic acalculous cholecystitis - fatty meal - cholescintigraphy

Introduction

Evaluation of gallbladder contraction has proven useful in the diagnosis of chronic acalculous cholecystitis (CAC). Because the imaging diagnosis of chronic cholecystitis relies heavily on the finding of cholelithiasis, surgeons are reluctant to operate without objective confirmation of disease (1). The diagnosis of chronic acalculous cholecystitis generally has been based on the finding of an abnormal gallbladder ejection fraction (GBEF) diagnosed by cholescintigraphy before and after stimulation of gallbladder (GB) contraction with intravenous octapeptide of cholecystokinin (CCK-8: Sincalide; Kinevac; Braco diagnostics)(2-4). However, CCK-8 is not widely available and alternative methods for evaluating GB contraction have become necessary (5). Fatty meals have been used to stimulate GB contraction and possibly represent not only a more physiological stimulus but also are less expensive than Sincalide infusion (4,5). Numerous different fatty meals (including dried egg yokes, a corned beef and cheese sandwich with milk, half and half milk, whipping cream, whole milk, Lipomul, lactose-free fatty-meal, corn oil emulsion) have been used (2,5-9). The methodology was different accor-ding to the different fatty meal used for gallbladder stimulation. Fatty meals have never been well standardized, and the fat content of the same commercial meal may vary (4). ^{99m}TC-labeled iminodiacetic acid (IDA) derivatives especially ^{99m}Tc-mebrofenin are used routinely for cholescintigraphy. These tracers are taken up by hepatocytes through a carrier-mediated non-sodium-dependent membrane transport mechanism which is also shared by bilirubin. ^{99m}Tc-IDAs are secreted into the bile providing excellent visualization of the biliary tract.

^{99m}Tc-methoxy-methylpropyl isonitrile (sestamibi) has been in use for myocardial perfusion imaging (MPI).

Normally about 20% of the injected dose of ^{99m}Tc-sestamibi is taken up by the liver and secreted into the bile. Thus the gallbladder is well visualized after injection of ^{99m}Tc-sestamibi (10).

The purpose of this study was to investigate the use of a commercially available formula, containing sufficient and known amount of fat to cause gallbladder contraction, and to determine normal GBEF values using this supplement.

Methods

Study population

We studied 36 patients (18 men and 18 women) aged 33-87 years (mean: 51.7±10.9 years) with body mass index (BMI) from 16.9 to 38.7 kg/m^2 (mean: 26.7± 5.2) who were referred for ^{99m}Tc-sestamibi MPI. Of the 36 patients, 9 (25%) were classified as obese (BMI=30), 12 (33/33%) as overweight (25≥BMI<30), and the remaining 15 (41.67%) as being of normal weight (18.5≥BMI<25). The subjects had no abdominal symptoms, history of hepatobiliary and gallbladder disease, diabetes mellitus, abdominal surgery, or family history of hepatobiliary disease and were not taking any medication known to affect the biliary system. Other medications had been discontinued at least 2 days before the study. All were prescreened with a GB and liver ultrasonography to exclude any abnormality. Patients gave their written consent to participate in the study which was approved by the local Ethical Committee.

Radiopharmaceutical

In our study, we used ^{99m}Tc-sestamibi instead of ^{99m}Tc-mebrofenin to determine the amount of radioactivity in the gallbladder before and after fatty meal ingestion for calculation of GBEF.

Fatty meal

We prepared 120 ml of fatty meal (10 g fat) using a commercially available formula (Humana) as a cholecystagogue to stimulate GB contraction.

Scintigraphy protocol

All 36 subjects underwent stress/rest myocardial perfusion imaging using a 2-day protocol starting with a MPI examination after stress and continued the next day with rest MPI. In the rest phase, after 6 hour fasting, 740-925 MBq of ^{99m}Tc-sestamibi was injected intravenously and 90 minutes later the subjects ingested the test meal (10 g fat). The subjects were encouraged to eat quickly. Sixty-second anterior images from the abdomen before the fatty meal ingestion, as well as at 30 and 60 minutes after end of fatty meal ingestion were acquired. The images were obtained in the supine position using a large-field-of-view gamma-camera (E.CAM; Siemens) equipped with high-resolution, low-energy, parallel hole collimator. The images were stored in a 128×128 matrix in the computer.

GBEF calculation

On the computer display, all regions of interest were drawn towards the GB and adjacent liver (Fig.1). After background and decay correction, GBEF was calculated at 30 and 60 minutes after fatty meal ingestion using formula below:

GBEF (%) = (net GB counts before fatty meal)-(net GB counts at 30 or 60 min)/(net GB counts before fatty meal)

We also calculated another variable (average GBEFs) for the estimation of GB function as an algebric mean of GBEFs at 30 and 60 minutes.

Statistical analysis

Statistical analysis was made using SPSS software (version 11.5). Univariate statistics are expressed as mean± standard deviation (SD). To evaluate the effects of gender on the gallbladder contractility, the mean of GBEF in the male patient and female patient groups were compared using an independent sample *t* test. We studied the effect of BMI on GBEF with linear regression and Pearson correlation. One way ANOVA was used for comparison between multiple groups with Tukey HSD test as post Hoc analysis. A *p* value of less than 0.05 was considered statistically significant.

Results

GBEF at 30 minutes after fatty meal ingestion ranged from 15.1 to 100% (mean±SD: 69.54±21.04%). GBEF at 60-minute ranged from 40.44 to 100% (mean±SD: 84.26±11.41%). Mean ± SD of average GBEFs was 76.90±14.99 (range (range, 30.63 -95.97). GBEF in our 36 subjects showed a gaussian distribution. Therefore the 95% confidence interval using mean-2 SD showed a lower limit of normal GBEF values 27.46% at 30-min, 61.44% at 60-min and 46.92% with average GBEFs. Fig.2 shows the percentile rank versus the GBEFs.

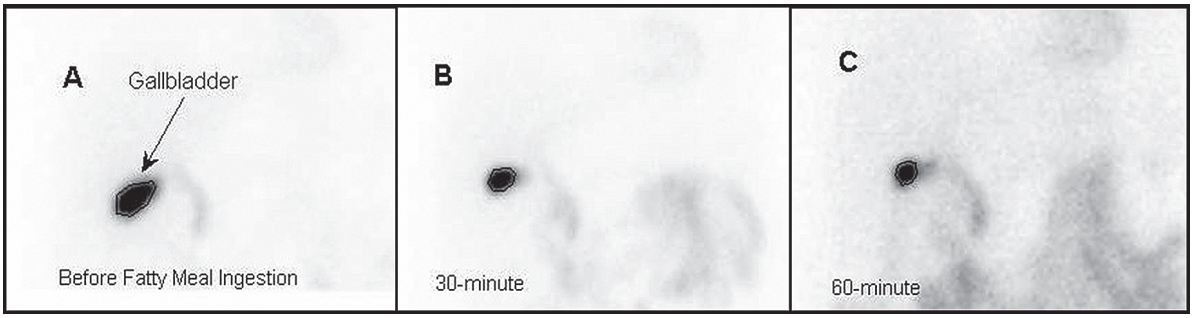

Fig.1 Anterior ^{99m}Tc-sestamibi images from the liver and gallbladder before fatty meal ingestion(A), 30 minutes after fatty meal ingestion(B) and 60 minutes after fatty meal ingestion(C).

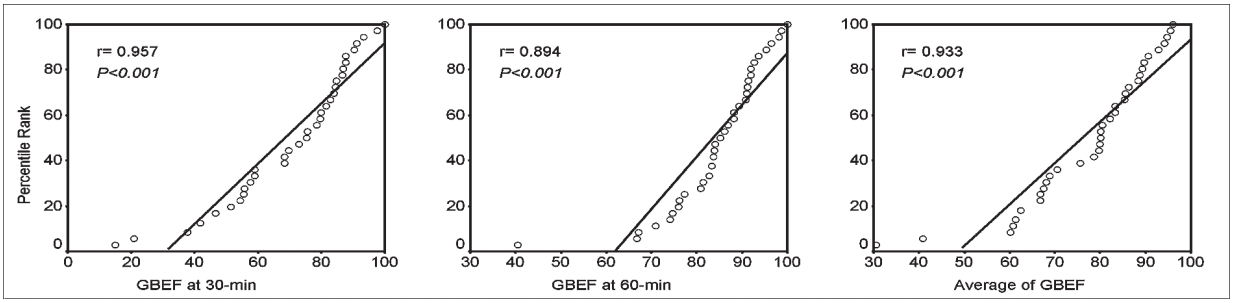

Fig.2 Percentile rank for gallbladder ejection fration (GBEF) at 30 minutes and at 60 minutes after fatty meal ingestion as well as for average GBEFs.

Using linear regression, we estimated the percentile rank in our patients:

- for GBEF at 30 min: percentile= $(1.33 \times GBEF_{30})$-41.1;
- for GBEF at 60 min: percentile= $(2.29 \times GBEF_{60})$-141.8;
- for average of GBEFs: percentile= $(1.82 \times GBEF_{a})$-88.7.

GBEF at 30 minutes, 60 minutes and the average of GBEFs did not differ significantly between men and women (Table I). There was no statistically significant correlation between BMI and GBEF (at 30 minutes: p=0.40, r=0.14, at 60 minutes: p=0.20, r=0.21). On-way ANOVA analysis and Tukey HSD test showed no significant difference in GBEF between obese, overweight and normal weight patient groups (Table II).

Table I Mean±SD of gallbladder ejection fraction(GBEF) after fatty meal ingestion and average GBEFs in different patient groups based on BMI

GBEF	Normal weight BMI<25	Overweight 25=BMI<30	Obese BMI=30	p value (ANOVA)
GBEF at 3min.	70.06±18.62	60.86±25.59	80.22±13.68	0.111
GBEF at 60min.	83.06±9.43	82.31±16.10	88.85±5.04	0.384
Average GBEFs	76.56±12.11	71.59±20.03	84.53±8.16	0.147

Table II Mean ± SD of gallbladder ejection fraction (GBEF) after fatty meal ingestion and average GBEFs based on gender

GBEF	Females	Males	p value
GBEF at 30min.	71.74±20.57	67.33±21.85	0.537
GBEF at 60min.	84.51±13.44	84±9.33	0.896
Average GBEFs	78.13±15.87	75.67±14.41	0.629

Discussion

Measurement of GBEF using cholescintigraphy helps surgeons preoperatively in their clinical decision to establish who has CAC and who may benefit from cholecystectomy (11,12). A wide variety of fatty meals have been used as an alternative to CCK-8 to evaluate GB contraction. These methods are often not standardized and do not have established normal values. The supplement that we used in this study is a common formula which is easily available in many countries. It contains sufficient fat (10 g) to produce GB contraction. Stone et al. suggested that 4 g of fat does not result in gallbladder contraction and at least 10 g of fat are required to produce good GB emptying (13). Normal values for fatty-meal cholescintigraphy also likely depend on the content of the meal, amount of fat in the meal, and the methodology used. The gastric-emptying rate may also affect GBEF normal values. The longer latent period using fatty meals as compared to the CCK-8 is probably the result of time taken for release of endogenous CCK (14). GBEF has been measured at 30, 60, 90, or 120 min after the meal, with varying GBEF values (2). Therefore, it is essential to report GBEF value with a time reference, such as GBEF at the end of 30, 60, 90, or 120 min after the meal.

In the present study, we demonstrated that the gender or BMI did not affect the GBEF value. There was no significant difference between women and men or between patients with normal weight, overweight and obese.

Many different methods are used to establish normal ranges and identify individuals requiring treatment. In studies involving a large sample of patients in whom the frequency distribution of population data resembles a gaussian curve, it is traditional to use mean - 2 SDs or 95% confidence limits to set the lower limit of the normal range. Data in our 36 subjects showed a gaussian distribution. Therefore the 95% confidence interval using mean-2 SD showed a lower limit of normal GBEF values. However, observed values in the lower tail of the distribution especially at 30 min may be quite unstable. A wide range of GBEFs was noticed in the 36 subjects. Although this confidence range is valid for our data which follow a gaussian distribution, problems sometimes are seen in clinical medicine due to the overlap between healthy subjects and patients. So it may be more appropriate to use percentile rank methodology to convey the clinical import of a GBEF measured with a fatty meal (2). Fig.2 shows the percentile rank versus the GBEFs. We could estimate the percentile rank in our patients using linear regression and the formula mentioned above. For example, a patient with a measured GBEF of 40% at 30 min

would be reported in the 12th percentile compared with healthy subjects. Therefore, it is possible that instead of an absolute cut-off, the percentile rank of the patient's GBEF is reported, and the clinician then uses other clinical information to assess the importance of GBEF in designing therapy.

The measured 60 min GBEFs in our study were higher than those reported by Shafer et al. for 7 healthy volunteers (mean±SD: 31 ±11%; range: 16-42%, lower threshold 60-min GBEF of 16%)(15). Krishnamurthy et al. studied 13 healthy subjects using ^{99m}Tc-hepatic iminodiacetic acid cholescintigraphy by stimulation of half-and-half milk (2). They reported that mean ± SD of GBEF at 60 min was 53.6±20.2%. But probably because the number of subjects in their study was too small, GBEFs did not have a Gaussian distribution. They also suggested using a percentile ranking for reporting results (2). Ziessman et al. studied 17 healthy volunteers and suggested that the lower limit of normal for GBEF at 60-minute is 33% using a lactose-free food supplement (5). Others reported GBEF 24-95% at 60 min in healthy subjects by half-and-half milk, and an average normal GBEF 70% at 75 min by heavy whipping cream (8,9). Different results in different studies may be due to: a relatively small sample size, a different fatty meal and different methodology (including radiopharmaceuticals). We used the Humana formula as a fatty meal (each 100 grams contain 28.1 g fat, 24.1 g carbohydrate, 12 g protein, and 517 Kcal). Our prepared test meal (120 ml) contained 10 g fat, 8.6 g carbohydrate, 4.3 g protein, and 184 kcal.

Another reason may be the more rapid gastric emptying that occurs in the upright position than in the supine position, which may stimulate earlier production of endogenous CCK and greater GB emptying during the study acquisition time (5). In our study, the subjects moved around between three imaging periods, whereas in many of the previous studies patients underwent a dynamic acquisition of images and they had to lie still in the supine position throughout the study.

Bartel et al. (9) studied 30 patients with abdominal pain and reported that using Receiver-Operating-Characteristics (ROC) analysis, the 60 min GBEF results in the highest diagnostic accuracy for CAC based on histological or clinical follow-up data. In that study, the area under the ROC curve of 0.963 is significantly better than that for the 30 min GBEF (9). Because we did not acquire dynamic images after fatty meal ingestion and with the assumption that average GBEFs in one hour time period may be better than a definite 30 or 60 minutes time point GBEFs, we reported normal range for average GBEFs as well. However it should be proved in future studies that this average GBEF is preferred to 30 or 60 minutes time point GBEFs.

Based on our study, the Humana formula may represent a useful alternative to CCK-8, because it is a well-defined meal that is easy to prepare at virtually any department of nuclear medicine and is inexpensive. The use of CCK-8 has some disadvantages. There is current shortage of the compound and it may be unavailable commercially (2,4,8). Furthermore, CCK-8 administered as a rapid bolus produces supra-physiological levels of CCK in the circulation (15). This may account for its tendency to produce nausea and abdominal pain in healthy individuals (4).

Study limitations. An arbitrary cut-off level may be chosen and tested using ROC analysis to determine positive and negative predictive values as well as accuracy of the GBEF measurements in a mixed sample of healthy subjects and patients. We could not use this methodology, because our current data included only subjects with no biliary disease. To avoid the radiation exposure to normal volunteers, we studied a group of patients suspected with coronary artery disease who had been referred for myocardial perfusion imaging. As these patients had normal GB ultrasonography and had no biliary disease, they can be considered as normal subjects.

Conclusion

We have described a lower limit of normal GBEF values using a simple, standard fatty meal (10 g of fat prepared from Humana formula) instead of Sincalide. Also we provided regression equations for the estimation of percentile rank of the GBEFs. It is possible to report the results of a GBEF measurement after fatty meal in terms of the percentile rank compared with healthy subjects.

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