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Normal Gallbladder Ejection Fraction in Normal Thai Volunteers by Ultrasound and Intravenous Injection of Amino Acid

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ABSTRACT

Background: Biliary dyskinesia is one of clinical entity of motility disoders that associated with abdominal pain. Gallbladder ejection fraction (GBEF) has been used to select patient who will benefit from cholecystectomy. No normal GBEF in Thai population has been studied previously.

Objectives: To determine normal GBEF using rapid amino acid infusion and ultrasound in Thai healthy volunteers.

Patients and Methods: Ultrasound measurements of gallbladder volume before and after amino acid infusion were done in fasting healthy volunteers at 15 minutes interval for 6 times. The GBEF was calculated by the ellipsoid method and analyzed for normal limit.

Results: Twenty-seven volunteers were recruited, but only twenty subjects were eligible for analysis. There were 10 male and 10 female with mean age ± SD of 35.9 ± 9.0 years. (a range of 23-50 years). Two subjects were excluded due to incidental taking of some medication that may affect the GBEF at the time of GBEF study. The mean GBEF ± SD of this group was 60.3 ± 16.7% and the 90th percentile of lower limit of normal GBEF was 42%. Subset analysis for gender and age did not show any difference of the GBEF.

Conclusions: Ultrasound measurement of GBEF with amino acid infusion is simple to perform and is readily available in general hospitals. The normal GBEF in healthy Thai people was ≥ 42% in this study. In order for this value to be used as normal value, the measurement must be strictly followed our protocol.

Key words : gallbladder ejection fraction, gallbladder dyskinesia

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BACKGROUND

Biliary dyskinesia is defined as the presence of biliary colic symptoms without cholelithiasis, and suggests the potential of chronic inflammation[1-3]. Motility disorder of the gallbladder has been postulated to produce recurrent biliary pain. Cholecystectomy is usually performed to treat this condition but only some patients respond. Gallbladder ejection fraction (GBEF) has been used in some studies to select the patient who will benefit from cholecystectomy.

Cholescintigraphy with intravenous administration of cholecystokinin (CCK) is commonly used in the assessment of GBEF, however its usefulness in general is limited by the need of fairly complex equipment and radiation exposure[4-8]. Ultrasound (US) can measure the gallbladder volume and GBEF[9,10]. CCK is not widely available, so some alternative stimulations to contract gallbladder have been developed. Rapid amino acid infusion leads to gallbladder contraction mediated by endogenous CCK release is one of the options[11-13]. The role of amino acid infusion (AA) in the measurement of GBEF has been validated in several studies and the combination of US with amino acid infusion was validated in one report[14-17].

OBJECTIVES

The aim of this study was to determine normal GBEF by using rapid amino acid infusion and US in Thai healthy volunteers.

PATIENTS AND METHODS

The study protocol has been approved by the Ethic Committee of Faculty of Medicine, Prince of Songkla University. Written informed consents were obtained from all participating volunteers. All the subjects recruited were healthy volunteers with the age between 18-65 years without any symptoms or underlying diseases, normal physical examinations, no gallstone by US examination and normal laboratory tests encompassing CBC, BUN, Cr, electrolytes, LFT, and FBS.

In the previous studies, the mean ± SD of %GBEF in normal subjects was 59-74% ± 12-16[4,8]. The number of subjects needed in this study to achieve 95% confidence level was calculated. Sixteen subjects are required, so 20 subjects are set for this study to compensate for some dropouts that may occur.

GBEF Study Protocol

After fasting for at least 8 hours, triplicate measurements of gallbladder volume were done by ultrasound at baseline and at 15 minute-intervals for 90 minutes after the infusion of amino acid mixture. The amino acid (10% Aminovan®) 200 ml containing 20 grams of amino acid was given within 10 minutes.

Ultrasound Technique

The real-time ultrasonographic machine (SIEMENS SONOLINE Antares, Software Ver. 2.0 . 173) with a 3.5-MHz convex transducer was used. The transducer was initially placed in sagittal plane of the right upper quadrant of abdomen with the subject in left lateral decubitus position and was oriented until the greatest length of the gallbladder was obtained and the image was frozen to determine its length. Then the transducer was rotated 90˚ to obtain the cross section image of gallbladder. The probe was positioned until the greatest transverse and anteroposterior dimensions were obtained and recorded. Occasionally supine position was used and in some situation where gallbladder lying high under the right subcostal border, deep-held inspiration was necessary to obtain optimal measurement. (Figure 1A-1C)

The GB volume was calculated using the ellipsoid method with the formula

$$V = X / 6 (L \times W \times H)$$

where V is the volume, L is the length, W is the width, H is the height of the gallbladder and the value of the constant X / 6 is 0.52.

Measurement of the gallbladder ejection fraction

The gallbladder ejection fraction (% GBEF) was calculated by the following formula, % GBEF = (pre - post AA GBV)/ pre AA GBV) × 100, where pre AA GBV is the fasting pre-amino acid infusion gallbladder volume and post AA GBV is the smallest post-amino acid infusion residual gallbladder volume within 90 minutes interval.

Statistical Analysis

The demographic data of the subjects were analyzed by descriptive statistics. The GBEF data are expressed as mean ± SD and normal curve plotting for skewness of the GBEF data was done. The difference of GBEF between male and female subjects and, between the subjects with age < 40 years and age ≥ 40

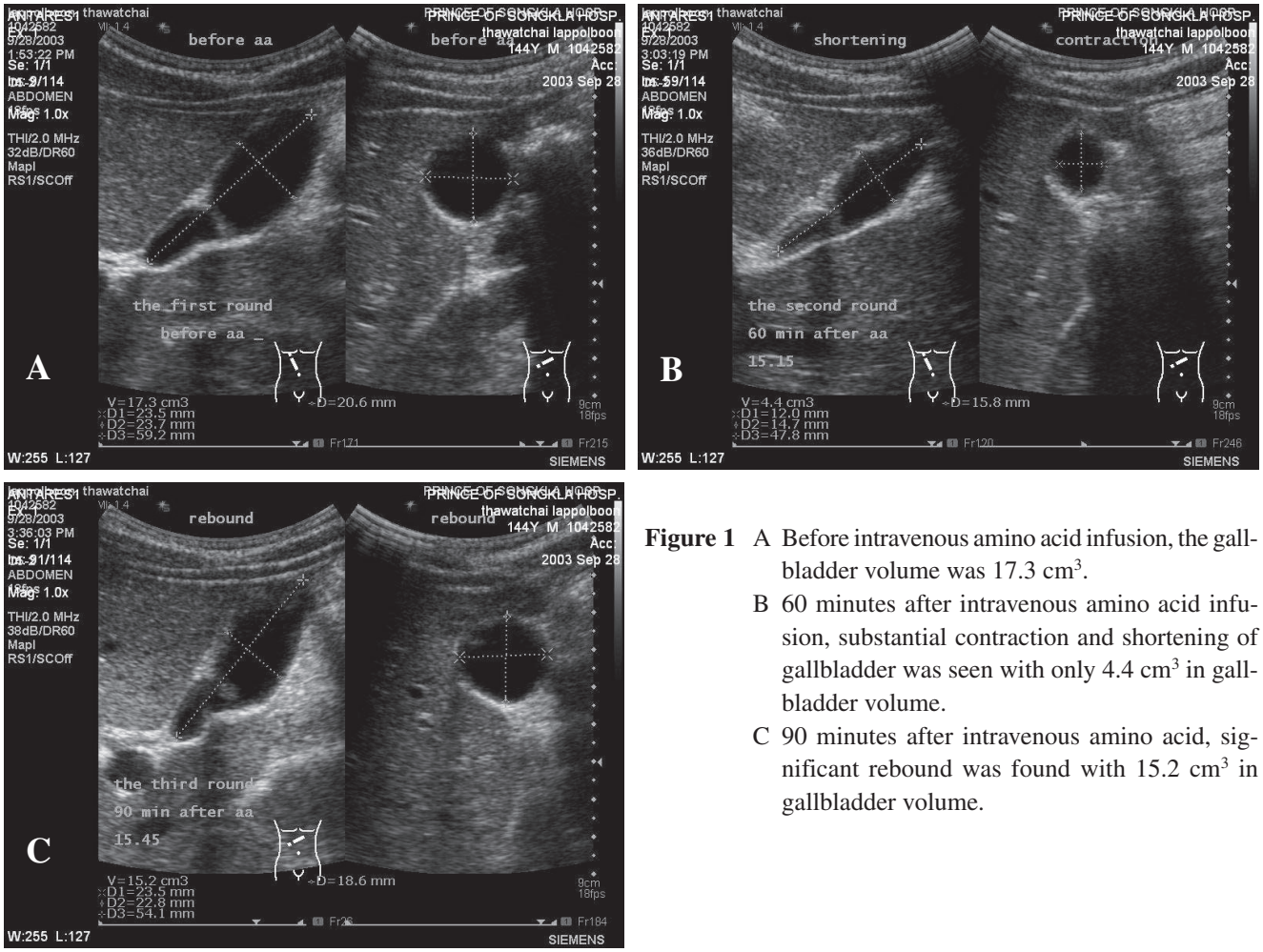

Figure 1 A Before intravenous amino acid infusion, the gallbladder volume was 17.3 cm^3.
B 60 minutes after intravenous amino acid infusion, substantial contraction and shortening of gallbladder was seen with only 4.4 cm^3 in gallbladder volume.
C 90 minutes after intravenous amino acid, significant rebound was found with 15.2 cm^3 in gallbladder volume.

years were assessed by Student's t-test. The normal range of GBEF is calculated by using 90th percentile. All the statistic analysis were done by using SPSS for Window version 11.

RESULTS

Twenty-seven volunteers were recruited but only twenty-one subjects actually completed the study and twenty subjects were included in the analysis. Of those seven who were excluded, four with abnormal liver function test, one with unsuspected pregnancy, one unvisualized gallbladder and one with paradoxically dilated gallbladder.

There were 10 male and 10 female with the mean age ± SD of 35.9 ± 9.0 years and a range of 23 to 50 years. The mean age ± SD of male was 33.7 ± 10.2 years with a range of 23-50 years and that of female was 38.1 ± 7.5 years with a range of 27-49 years. During the study, each of 3 subjects had just taken one medication included antihistamine (loratadine), oral contraceptive pills (Gynera), and vitamin (B1-6-12) respectively.

The time interval from baseline to maximum gallbladder contraction varied from 15 minutes to 75 minutes after amino acid infusion (Table 1).

The mean GBEF of the whole group was 55.9% ± 20.8% (X ± SD). The Means GBEF of the male, female, subject with age < 40 years, and age ± 40 years were shown in Table 2. The difference of GBEF between these subgroups classified by sex and age were analyzed by Student's t-test and showed no statistically significant difference.

Two subjects (number 11 and 16) were taking some medication that potentially may affect the GBEF. The analysis of the GBEF data excluding these two subjects showed mean GBEF ± SD of 60.3 ± 16.7%. Normal curve plotting of the GBEF data showed slightly deviated to the right (skewness = -.568), so the 90th percentile was used to determine the range of

Table 1 Demographic data, medication, adverse symptoms, time to maximum contraction and GBEF.

No.	Sex	Age (yr.)	Weight (kg.)	Height (cm.)	BMI (kg/m^2)	Medication	Symptom During AA	Time to Max. Contraction	GBEF (%)
1	male	23	55.0	168	19.5	none	none	45 min	69.0
2	male	23	51.5	165	18.9	none	none	60 min	73.0
3	male	24	62.0	167	22.2	none	none	30 min	69.0
4	male	24	53.0	165	19.5	none	none	60 min	61.7
5	male	29	67.6	175	22.1	none	none	75 min	57.6
6	male	38	53.5	165	19.7	none	dizziness	30 min	53.5
7	male	42	76.0	165	27.9	none	dizziness	30 min	88.4
8	male	42	62.5	167	22.4	none	none	45 min	58.2
9	male	42	55.0	165	20.2	none	none	45 min	74.9
10	male	50	54.0	167	19.4	none	none	30 min	23.9
11	female	27	48.0	156	19.7	antihistamine	none	30 min	12.5
12	female	30	70.5	158	28.2	none	none	30 min	80.6
13	female	31	53.0	155	22.1	none	dizziness	15 min	63.5
14	female	35	60.0	146	28.2	none	flushing	45 min	57.4
15	female	35	62.3	164	23.2	none	none	60 min	42.4
16	female	41	67.6	159	26.7	pills	none	45 min	20.0
17	female	43	61.2	156.5	25.0	none	none	30 min	52.2
18	female	43	66.0	156	27.1	none	none	15 min	77.0
19	female	47	53.3	150	23.7	none	none	45 min	53.0
20	female	49	53.0	150	23.6	vitamin	none	45 min	30.8

Table 2 GBEF data of subgroups categorized by sex and age.

	% GBEF			
	Minimum	**Maximum**	**Mean**	**Std. Deviation**
Total	12.5	88.4	55.9	20.8
Male	23.9	88.4	62.9	17.1
Female	12.5	80.6	48.9	22.7
Age < 40 years	12.5	80.6	58.2	18.3
Age ≥ 40 years	20.0	88.4	53.1	24.4

normal GBEF. (Figure 2) The lower limit of normal GBEF in our population is 42 per cent.

Four subjects developed some symptoms during intravenous infusion of the amino acid, three with dizziness and one with flushing but no treatment or termination of the study were required.

DISCUSSION

In 1983, Krishnamurthy *et al.*[8] studied gallbladder contraction by cholescintigraphy with CCK infusion in healthy volunteers and showed that nobody had GBEF < 35%. Yap *et al.*[4] reported in 1991that normal GBEF was > 40%. Our study showed that GBEF in healthy volunteers assessed by ultrasound and amino acid infusion was ≥ 42%. This value is higher than that of Krisnamurthy *et al*. but is quite similar to that of Yap *et al*. These discrepancies may due to different population, technique of measuring GBEF and GB stimulation used in the studies.

The effects of gender, female sex hormone during the menstrual cycle, pregnancy, and aging on gallbladder contraction were previously studies in a large number reports. Decreased gallbladder contraction was

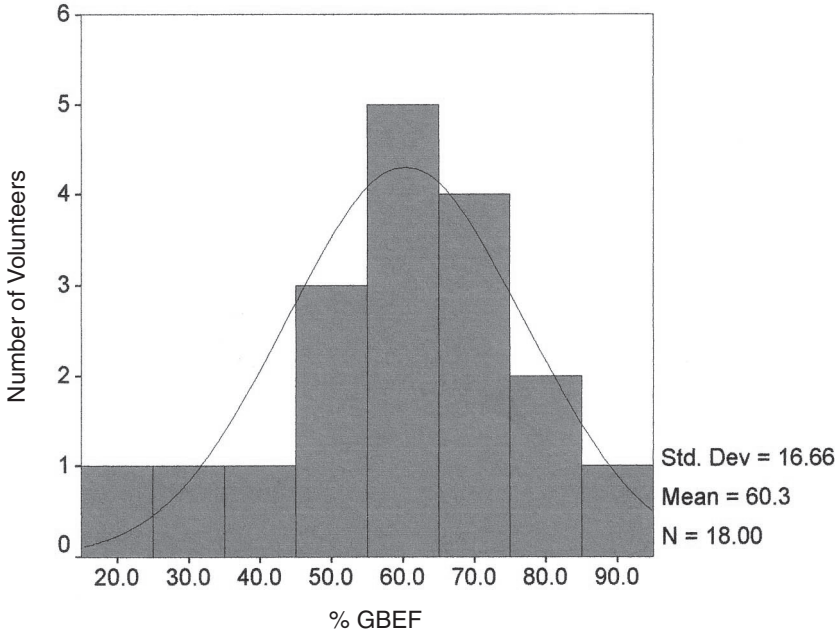

Figure 2 The graph showed the distribution of % GBEF for the whole group.

observed in pregnancy, which showed markedly decrease in the late pregnancy than in the early stage[18]. Female sex hormone, aging and gender did not significant influence gallbladder contraction[17,19]. In 1985, Khalil *et al.*[20] found higher fasting and fat meal stimulated CCK concentration in the plasma of older volunteer compare with younger subjects but the gallbladder contraction and the rate of gallbladder emptying was not significantly different between the two groups. In our study, sex and age had no effect on the gallbladder contraction.

One subject in our study showed paradoxical dilatation of the gallbladder in response of amino acid infusion suggesting asymptomatic gallbladder dyskinesia in this subject. This finding is clinically important since patients with non-specific pain may have coexisting asymptomatic gallbladder dyskinesia. The recommendation for surgery in these population may not be helpful.

Several methods for the study of gallbladder emptying had been employed in the past. Cholecystokinin cholecystography was the initial technique[21,22]; however, the precision was less than optimal due to the limitation of two-dimensional image, associated with exposure to unacceptable dose of radiation. Cholescintigraphy with CCK infusion was the other method to assess the extent of the gallbladder emptying but it was unable to determine the gallbladder size[4-8]. The most recent method is ultrasound assessment with computer software to calculate gallbladder volume. Amino acid infusion to stimulate gallbladder contraction is readily available. This ultrasound assessment of GBEF with amino acid infusion is more likely to be widely available in general hospitals. The normal GBEF by this technique with amino acid infusion described in this study is ≥ 42%. In order to adopt this value as normal in any particular hospital, the assessment technique must be strictly similar to this study. However, there is limitation of this technique in some situations where bowel gas obscuring the gallbladder or gallbladder is lying high in the subcostal region.

In conclusion, GBEF in healthy Thai people was ≥ 42% with age and sex had no effect on gallbladder contraction.

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