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ORIGINAL ARTICLE

95 **Significance of Microscopic Residual Tumor in Adenocarcinoma of Stomach and Esophagogastric Junction after Gastrectomy with D2 Lymphadenectomy**
Thammawat Parakonthun, et al.

103 **Surgical results of Laparoscopic Loop Duodenojejunal Bypass with Sleeve Gastrectomy (LDJB-SG) in Obese Asians (BMI ≥ 27.5 kg/m^2) with Type 2 Diabetes Mellitus (T2DM): A New Promising Bariatric and Metabolic Surgery**
Voraboot Taweerutchana, et al.

114 **Insulin Sensitivity and Clinical Profiles in CPP Girls Compared to Controls**
Maynart Sukharomana, et al.

123 **Improving International Standards in Surgical and Procedural Training through Comparative Operative Log Growth Charts**
Vannipa Vathanophas, Noel Jabbourb

127 **Identification of Uncommon Pathogenic Bacteria by MALDI-TOF Mass Spectrometry Using a Custom Library of Siriraj Hospital**
Kanda Ekcharoenkul, et al.

131 **One-Year Anatomical and Functional Outcomes of 302 Elevate Posterior and Apical Mesh Repairs**
Pattaya Hengrasmee, et al.

139 **Prevalence of *Helicobacter pylori* Infection in Patients with Perforated Peptic Ulcer in a Tertiary Hospital in Thailand: A Single Tertiary Hospital Study**
Suppadech Tunruttanakul, Jintana Wairangkool

145 **Ultrasound-Guided Thoracic Paravertebral Block for Controlling Pain in Percutaneous Radiofrequency Ablation of Hepatic Tumors: A Prospective Case Series**
Pawinee Pangthipampai, et al.

152 **The Association between Mammographic and Ultrasound Features and Histologic Grade in Invasive Ductal Carcinoma of the Breast**
Voraparee Suvannarerg, et al.

159 **Chemical Profiling of an Antipyretic Drug, Thai Herbal Harak Formula, by Liquid Chromatography Coupled with Quadrupole Time-of-Flight Mass Spectrometry**
Patcharamon Seubnooch, et al.

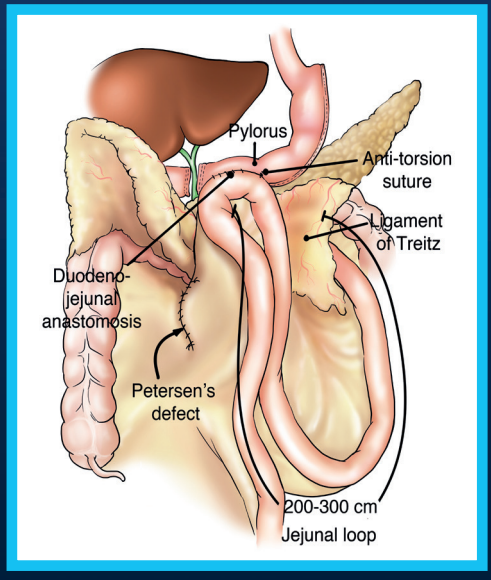
169 **Impact of ^{18}F-FDG PET/CT Scan on Management of Non-Small Cell Lung Cancer Patients in Contexts of University-Based Hospital in Thailand**
Achiraya Teyateeti, et al.

CASE REPORT

175 **Retrieval Analysis of Polyethylene in Total Knee Arthroplasty in Thailand: A Case Report**
Pakpoom Ruangsomboon, et al.

178 **Perforated Duodenal Ulcer after Laparoscopic Roux-en-Y Gastric Bypass for Morbid Obesity**
Voraboot Taweerutchana, et al.

182 **Posaconazole Induced Diffuse Lentigines: A Case Report**
Kittipoom Chinhiran, Charussri Leeyaphan



By Voraboot Taweerutchana, et al.

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Significance of Microscopic Residual Tumor in Adenocarcinoma of Stomach and Esophagogastric Junction after Gastrectomy with D2 Lymphadenectomy

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ABSTRACT

Objective: Microscopic residual tumor (R1 resection) may have a significant negative impact on prognosis of patients with adenocarcinoma of stomach and esophagogastric junction. Results of R1 resection on survival and recurrence patterns have not yet been thoroughly investigated in Thailand. The aim of this study was to study the oncological outcomes of R1 resection after curative intended surgery.

Methods: We retrospectively analyzed the medical records of patients who underwent curative intended surgery from 2005 to 2013 at Siriraj Hospital.

Results: 162 patients underwent curative intended gastrectomy with radical lymphadenectomy. 122 (75.3%) patients received R0 resection (non-residual group), 40 (24.7%) patients had microscopic residual tumor (residual group). Microscopic residual tumor was associated with tumor size (p=0.008), tumor extension (p=0.028), pT-stage (p=0.012), pN-stage (p=0.012), and AJCC/TNM stage (p<0.001). Large tumor size (≥5 cm.) and number of metastatic lymph nodes (≥3 nodes) were the factors contributing to higher rate of microscopic residual tumor on multivariate analysis (p=0.024 and 0.039, respectively). Median overall survival of non-residual and residual groups were 34.6 and 16.4 months (p=0.065). Median disease-free survival of non-residual and residual groups were 13.2 and 10.2 months (p=0.099). Distant metastasis was the most common site of recurrence in both groups. R1 resection was associated with worse 5-year survival rate (17.5% vs 34.0%, p=0.049).

Conclusion: Microscopic residual tumor was associated with larger tumor size and more number of metastatic lymph nodes. R1 resection resulted in worse prognosis. Distant metastasis was the most common site of recurrence, therefore, we might suggest systemic chemotherapy as an adjuvant treatment after curative intended surgery.

Keywords: Gastric cancer; adenocarcinoma of stomach; adenocarcinoma of esophagogastric junction; gastrectomy; D2 lymphadenectomy; positive margin; microscopic residual tumor; prognosis (Siriraj Med J 2018;70: 95-102)

INTRODUCTION

Gastric cancer is a common solid cancer in Thailand. The incidence is up to 5 cases per 100,000 people per year.[1] Gastric cancer is the second most common cause of cancer related death worldwide.[2] The highest incidence is in northeast Asia (Japan, Korea, and China). Intermediate incidences occur in Europe and South America.[2,3] Complete surgical resection is the potentially curative treatment of

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choice and offers a chance of cure. Although complete surgical resection (R0) was attempted in advanced cancer, the disease recurred in many patients.[4] Therefore, curative resection with perioperative chemotherapy has become the standard treatment option for the advanced disease.

In previous studies, positive resection margins (R1) have been associated with worse survival outcome in gastric cancer. The recurrence rate ranged from 5-20% and was associated with later stage of disease.[4-7] The previous study showed positive resection margins, were associated with higher American Joint Committee on Cancer (AJCC) stage[8], T-stage, N-stage, median number of positive nodes, diffuse Lauren type, and poorly differentiated tumors. They are a strong predictor of worse outcomes after curative gastrectomy.[4-7] Unfortunately, there is no standard treatment strategy after R1 resection.[9-13] In the past decades, adjuvant treatment strategies, including chemotherapy or chemo-radiothepy, have improved overall survival and recurrence rate, so it may be become the standard of care in the future.[14,15]

At the present time, we don't know the oncological outcomes of microscopic residual tumor after surgery in gastric and esophagogastric junction cancer patients in Thailand. The aim of this study was to define the oncological outcomes of curative intended resection with microscopic residual tumor in gastric and esophagogastric junction cancer patients.

MATERIALS AND METHODS

This study was approved by the Siriraj Institutional Review Board (Si 708/2016). The medical records of histopathologically proven adenocarcinoma of stomach and esophagogastric junction patients who had undergone curative intended gastrectomy with D2 lymphadenectomy from 2005 to 2013 at Department of Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand were retrospectively reviewed. Microscopic residual tumor (residual group) was defined as a presence of tumor cell at the proximal and/or distal resection margin on final histological examination or positive cytological examination of the peritoneal washing on final cyto-pathological report. Patients who underwent pathological negative surgical margin and cytological examination were classified as non-residual group. Patients with positive intra-operative frozen section who were re-resected to a negative margin were defined as a negative resection margin. The clinicopathological features and oncological outcomes of residual and non-residual tumors were used for comparison.

Patients' demographic data and tumor characteristics were recorded. Patient related variables were age and gender. Tumor related variables were tumor markers, primary tumor site, tumor extension, tumor size, tumor differentiation, presence of perineural and lymphovascular invasion, number of lymph node dissections and involvement, and TNM (Tumor-Node-Metastasis, according to the seventh edition of the American Joint Committee on cancer staging system[1]). Treatment related variables were the type of operation and postoperative complications. Treatments for microscopic residual tumor were also recorded. Overall survival was calculated from the first postoperative date to the date of death or the most recent contact data. Disease-free survival duration was calculated from the first date of operation to the date of recurrence.

Patients who underwent palliative gastrectomy or macroscopically identified residual tumor or patients who received neoadjuvant chemotherapy were excluded from this study.

Statistical analysis

All data was analyzed using the SPSS software version 18.0 (SPSS, Inc, Chicago, III). The demographic data or clinical data were presented using n%, mean±SD or median (min, max). Comparisons of patient characteristics between residual and non-residual groups were conducted using Chi square method for categorical variables or independent T-test for continuous variables. Kaplan-Meier method was used for survival curves demonstration. Predictive factors associated with microscopic residual tumor (R1 resection) were conducted using logistic regression analysis. A p-value less than 0.05 was considered statistically significant.

RESULTS

Patients and tumor characteristics

This study included 162 gastric and esophagogastric junction cancer patients who underwent curative intended gastrectomy with D2 lymphadenectomy. Of these, 40 (24.7%) patients had microscopic residual tumor. Nineteen (11.7%) patients had positive longitudinal (proximal and/or distal) resection margin, 20 (12.3%) patients had atypical or malignant cell on cytological examination from peritoneal washing fluid, one (0.6%) patient had positive both cytological and surgical resection margins. The baseline characteristics of patients and tumors were shown in Table 1. There was no significant difference in age, sex, serum CEA and CA19-9 levels, primary tumor location, type of operation, and number of dissected lymph nodes between groups. Patients in the residual group significantly had larger tumor size (5.0 vs 4.2 cm, p=0.008), and circumferential tumor involvement

TABLE 1. Patients and tumor characteristics (N=162)

Characteristics	Non-residual group (N=122)	Residual group (N=40)	*p*-value
Age (years)	60.8±13	60.1±14	0.763
Gender			0.469
Male	69 (56.6%)	20 (50%)	
Female	53 (43.4%)	20 (50%)	
CEA (ng/mL)	2.1 (0.2-13.4)	1.6 (0.2-81.91)	0.836
CA 19-9 (U/mL)	10.67 (0.6-208.9)	10.71 (0.6-945)	0.591
Primary tumor location			0.16
Esophagogastric junction	19 (15.8%)	6 (15%)	
Upper gastric portion	7 (5.8%)	4 (10%)	
Middle gastric portion	35 (29.2%)	7 (17.5%)	
Lower gastric portion	55 (45.8%)	17 (42.5%)	
Diffuse	3 (2.5%)	5 (12.5%)	
Anastomosis or Remnant stomach	1 (0.8%)	1 (2.5%)	
Tumor extension			0.028
Circumferential	26 (22.2%)	16 (40%)	
Non-circumferential	91 (77.8%)	24 (60%)	
Tumor size (cm)	4.2 (0.3-27)	5.0 (1-28)	0.008
Pathologic grade			0.117
Well differentiated	5 (4.2%)	0 (0.0%)	
Moderately differentiated	35 (29.2%)	7 (17.5%)	
Poorly undifferentiated	80 (66.7%)	33 (82.5%)	
Pathological T stage			0.012
T0	2 (1.6%)	0 (0%)	
T1a	4 (3.3%)	1 (2.5%)	
T1b	9 (7.4%)	0 (0%)	
T2	19 (15.6%)	3 (7.5%)	
T3	45 (36.9%)	8 (20%)	
T4a	34 (27.9%)	21 (52.5%)	
T4b	9 (7.4%)	7 (17.5%)	
Pathological N stage			0.012
N0	38 (31.1%)	5 (12.5%)	
N1	17 (13.9%)	3 (7.5%)	
N2	26 (21.3%)	6 (15%)	
N3a	23 (18.9%)	15 (37.5%)	
N3b	18 (14.8%)	11 (27.5%)	

TABLE 1. Patients and tumor characteristics (N=162) (Continued)

Characteristics	Non-residual group (N=122)	Residual group (N=40)	*p*-value
AJCC stage			<0.001
IA	10 (8.2%)	0 (0.0%)	
IB	13 (10.7%)	1 (2.5%)	
IIA	18 (14.8%)	3 (7.5%)	
IIB	16 (13.1%)	3 (7.5%)	
IIIA	16 (13.1%)	0 (0.0%)	
IIIB	27 (22.1%)	3 (7.5%)	
IIIC	19 (15.6%)	11 (27.5%)	
IV	3 (2.5%)	19 (47.5%)	
Lymphovascular invasion			0.055
Positive	61 (52.5%)	28 (70.0%)	
Negative	55 (47.5%)	12 (30.0%)	
Perineural invasion			0.077
Positive	85 (73.9%)	35 (87.5%)	
Negative	30 (26.1%)	5 (12.5%)	
Operation			0.244
Total gastrectomy	34 (27.9%)	16 (40.0%)	
Distal gastrectomy	70 (57.4%)	17 (42.5%)	
Esophagogastrectomy	18 (14.8%)	7 (17.5%)	
Dissected lymph nodes	32 (0-73)	30 (8-72)	0.991
Metastatic lymph nodes	3.5 (0-63)	10 (0-35)	<0.001
Postoperative complication			0.141
No complication	53 (43.4%)	8 (20.0%)	
Grade I	26 (21.3%)	16 (40.0%)	
Grade II	32 (26.2%)	12 (30.0%)	
Grade IIIa	4 (3.3%)	2 (5.0%)	
Grade IIIb	2 (1.6%)	1 (2.5%)	
Grade IVa	3 (2.5%)	1 (2.5%)	
Grade IVb	0 (0.0%)	0 (0.0%)	
Grade V	2 (1.6%)	0 (0.0%)	
Hospital stay (days)	11 (3-47)	13 (6-46)	0.017

Abbreviations: AJCC = American Joint Committee on cancer; CA 19-9 = Cancer antigen 19-9; CEA = Carcinoembryonic antigen.

(40.0% vs 22.2%, p=0.028). Microscopic residual tumor was associated with higher AJCC stage, pT-stage, pN-stage and median number of metastatic lymph nodes (p<0.001, 0.012, 0.012, <0.001, respectively). Tumors in the residual group had higher grade of tumor differentiation, percentage of lymphovascular and perineural invasion, although there was no statistically significant difference from the non-residual group (p=0.117, 0.055, 0.077, respectively). The locations of microscopic residual tumor were demonstrated in Table 2.

Adjuvant treatment after curative surgery

Seventy three (59.8%) patients in non-residual group underwent adjuvant treatment according to the tumor stage. Forty eight (39.3%) patients received adjuvant chemotherapy, 3 (2.5%) patients received adjuvant radiotherapy alone due to poor performance status, and 22 (18.0%) patients received adjuvant chemo-radiotherapy. Forty eight patients (39.3%) did not receive adjuvant treatment due to early state of cancer or poor performance status. One patient (0.8%) was lost to follow-up.

In the residual group, 32 (80.0%) patients received further treatment after surgery. Two (5.0%) patients underwent a second operation to correct the microscopic positive margin. Twenty one (52.5%) patients received adjuvant chemotherapy. Nine (22.5%) patients received chemo-radiotherapy. Eight (20.0%) patients required best supportive care. No patient received radiotherapy alone in this group.

Predictive factors of microscopic residual tumor

Predictive factors associated with microscopic residual tumor were shown in Table 3. Univariate analysis demonstrated that tumor size larger than 5 cm (OR 2.72, CI [1.28-5.74], p=0.009), metastatic lymph node more than 3 nodes (OR 3.94 CI [1.45-7.92], p=0.005), circumferential tumor involvement (OR 2.33 CI [1.08-5.03], p=0.031), and T3-4 tumor (OR 3.48 CI [1.15-10.51], p=0.027) were significantly associated with microscopic residual tumor. However, multivariate analysis identified tumor size (5 cm and over) and metastatic lymph nodes (3 nodes and over) as independent predictive factors of microscopic residual tumor.

TABLE 2. Site of microscopic residual tumor (N=40).

	Location of involved margin		
	Esophagus	Stomach	Duodenum
Proximal margin (N=9)	5	4	
Distal margin (N=7)		3	4
Both margins (N=3)	3	← →	3
Positive peritoneal washing cytology (N=21)	21		

TABLE 3. Predictive factors associated with microscopic residual tumor (N=162)

Factor	Univariate analysis Crude OR (95% CI)	*p*-value	Multivariate analysis Adjusted OR (95% CI)	*p*-value
Tumor size ≥5 cm.	2.72 (1.28-5.74)	0.009	2.54 (1.12-5.36)	0.024
Metastatic lymph node ≥3 nodes	3.94 (1.45- 7.92)	0.005	2.54 (1.04-6.19)	0.039
Circumferential involvement	2.33 (1.08-5.03)	0.031		
T-stage ≥T3	3.48 (1.15-10.51)	0.027		
Pathological grade	2.36 (0.96-5.80)	0.062		
Lymphovascular invasion	2.10 (0.98-4.53)	0.058		
Perineural invasion	2.47 (0.90-6.89)	0.084		

Recurrence pattern

With a median follow-up of 28.5 months for the non-residual group and 14 months for residual group, recurrence rate was 32.0% (39 patients) and 52.5% (21 patients) respectively (Fig 1). Distant metastasis was the most common pattern of recurrence or progression followed by peritoneal metastasis and loco-regional recurrence. Liver was the most common organ of distant metastasis. In the non-residual group, 27 (69.2%) patients suffered from recurrent disease at the distant sites. Peritoneal metastasis was found in 13 (33.3%) patients. Only 5 (12.8%) patients had loco-regional recurrence. In the residual group, recurrence diseases were identified mostly at the distant sites (13 patients, 61.9%) followed by peritoneal (8 patients, 38.1%) and loco-regional region

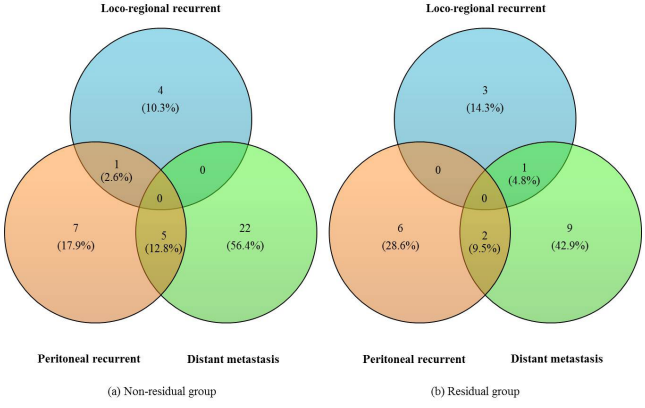


Fig 1. Recurrence pattern: **(a)** Non-residual group, **(b)** Residual group.

(4 patients, 19.0%).

Five of 9 (55.6%) patients, who had microscopic residual tumor cell at the proximal resection margin, suffered from disease progression. Distant metastasis was identified in 2 (40.0%) patients. One (20.0%) patient had recurrence at loco-regional and distant sites. One (20.0%) patient had peritoneal recurrence and only 1 (20.0%) patient had loco-regional recurrence.

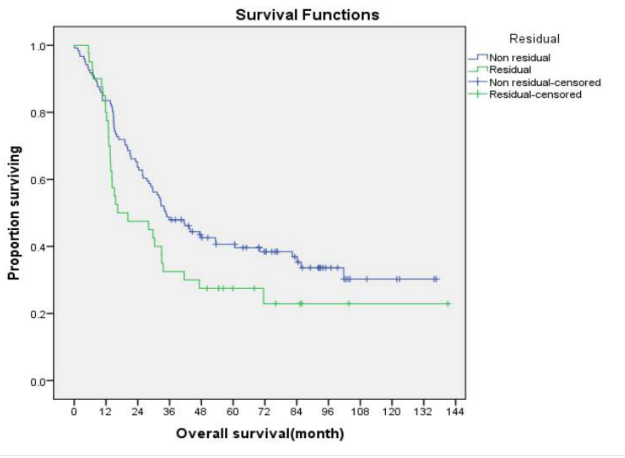
Five of 7 (71.4%) patients with positive distal resection margin had recurrent diseases. Peritoneum and distant organ were the most common sites of recurrence, 2 (40.0%) with distant metastasis, 2 (40.0%) with peritoneal metastasis, and 1 (20.0%) with peritoneal and distant metastasis. However, loco-regional region was not identified as a recurrent site in these patients.

Two of 3 (66.7%) patients with positive both proximal and distal resection margins suffered from disease progression. All of them had recurrent diseases at the peritoneum.

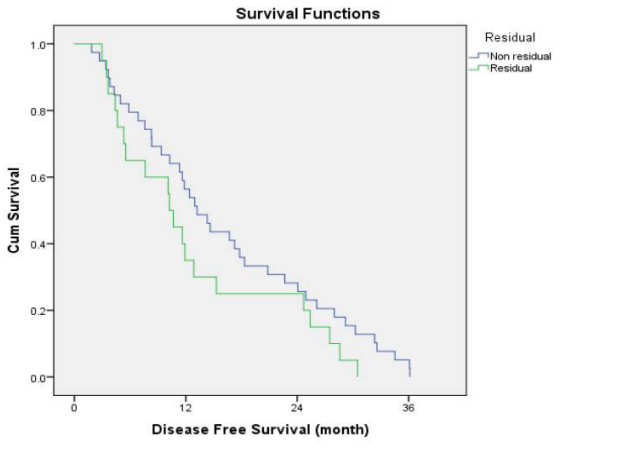
Recurrent diseases were detected in 12 of 21 (57.1%) patients who had positive cytological examination. Distant metastasis was the most common pattern of recurrence (6 patients, 50.0%) followed by peritoneal metastasis (3 patients, 25.0%). One (8.3%) patient had synchronous peritoneal and distant metastasis. Only 2 (16.7%) patients had loco-regional recurrence.

Survival analysis

Median overall survival and disease-free survival were not statistically different between residual and non-residual groups (Fig 2). The median overall survival time of residual group was 16.4 months, and that of non-residual group was 34.6 months (p=0.065). The median disease-free survival of residual and non-residual groups were 10.2 and 13.2 months, respectively (p=0.135). 5-year overall survival rate in residual group was 17.5%, comparing with 34.0% in non-residual group (p=0.049) (Table 4).



(a) Overall survival



(b) Disease-free survival

Fig 2. Survival curves: (a) Overall survival, (b) Disease-free survival.

TABLE 4. Overall survival of gastric cancer (N=162).

	Non-residual (N=122)	Residual (N=40)	*p*-value
Median overall survival (months) (95% CI)	34.6 (24.58-44.74)	16.4 (0-36.29)	0.065
3-year overall survival	58 (48%)	13 (33%)	0.099
5-year overall survival	41 (34%)	7 (17.5%)	0.049

DISCUSSION

This study is the largest series dealing with the effect of microscopic residual tumor on prognosis and recurrence for gastric and esophagogastric junction cancer patients after curative intended gastrectomy with D2 lymphadenectomy in Thailand.

The incidence of microscopic residual tumor in this study was 24.7%. Other large series have revealed that rate of positive resection margins ranges from 5% to 20%. Wang, et al,[4] reported 8.2% of 1,565 gastric cancer patients and Bickenbach, et al,[7] reported 4.5% of 2,384 gastric cancer patients with positive surgical margin. The incidence in this study was higher than other series because we described microscopic residual tumor as the presence of tumor cells on surgical resection margin and/or peritoneal washing fluid. Positive surgical margin, in this study, was 11.7%. This number was not different from other large series.[4-7]

From the previous study, the risk factors for loco-regional recurrence were various. McNeer, et al,[16] performed an autopsy study and reported the rate of local recurrence was 80% of gastric cancer patients who underwent gastric cancer surgery. Wang, et al,[4] reported a study of 1,565 gastric cancer patients in Taiwan and showed a loco-regional recurrence rate of 25% in 129 patients who had microscopic surgical margins. Similarly, Papachristou, et al,[17] reported a local recurrence rate of 25% in 257 patients. Bickenbach, et al,[7] reported an anastomosis recurrent rate of 16% among 2,384 gastric cancer patients. In this study, we found a loco-regional recurrence rate of 11.7% which comprised 14.3% in residual group and 10.3% in non-residual group. The loco-regional recurrence rate was not high especially in the residual group. However, the most common site of recurrence in both groups was distant metastasis. This finding, in this study, was the same as the previous series. The local recurrence mainly occurred in stage I or II disease. In contrast, distant metastasis and peritoneal recurrence were common in the more advanced stage. This might be explained by the results of an advanced disease and aggressive tumor biology.

The previous study showed that positive resection margin was associated with worse survival outcome. Cho, et al,[18] reported the study of advanced gastric cancer patients, who underwent extended (D2/D3) gastrectomy, which found that positive resection margin was associated with worse survival outcome in patients without metastatic lymph node. However, there was no significant difference in patients with metastatic lymph nodes. In this study, the median overall survival time of residual group was worse than non-residual group, but borderline statistical difference due to small sample size. The current study demonstrated that margin status was an independent factor for survival outcome in pT1-2 cancer patients with less than 3 metastatic lymph nodes.

This is the first study reporting the oncological outcomes of microscopic residual tumor in patients with adenocarcinoma of stomach and esophagogastric junction who underwent standard gastrectomy with D2 lymphadenectomy in Thailand. We reported not only the results of microscopic residual tumor cells at surgical resection margins, but also the free cancer cells in the peritoneal washing fluid. The limitation of our treatment standard was that we could not know the cytological report of peritoneal washing examination at the time of surgery. Therefore, we included the positive cytology in the residual group in this study. Nowadays there is no standard treatment strategy for microscopic residual disease at the surgical margin after standard resection. The common adjuvant treatments are chemotherapy and chemo-radiation. Some patients underwent resection at the positive resection margin aiming for loco-regional control. Regarding recurrence pattern in our study, the most common site of recurrence was distant metastasis whereas loco-regional recurrence was uncommon. We might propose that systemic treatment with chemotherapy,

might be a proper treatment strategy for microscopic residual tumor after standard surgical resection with radical lymphadenectomy. This also suggests the need for a prospective further long term study in the future. The selective bias of a retrospective study and small number of sample size were limitation in this study.

In conclusion, microscopic residual tumor was associated with large tumor size and more metastatic lymph nodes. Although patients underwent standard surgical resection with radical lymphadenectomy, microscopic residual tumor resulted in worse prognosis. Distant metastasis was the most common site of recurrence or disease progression. Therefore, we might suggest systemic chemotherapy as an adjuvant treatment after curative intended surgery.

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Surgical Results of Laparoscopic Loop Duodenojejunal Bypass with Sleeve Gastrectomy (LDJB-SG) in Obese Asians (BMI ≥ 27.5 kg/m^2) with Type 2 Diabetes Mellitus (T2DM): A New Promising Bariatric and Metabolic Surgery

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ABSTRACT

Objective: To demonstrate surgical outcomes, safety and complications in obese Asians (BMI > 27.5 kg/m^2) who underwent LDJB-SG in our center.

Methods: We retrospectively reviewed ninety-one patients who underwent LDJB-SG from October 2011 to March 2014. One-year surgical outcomes regarding the efficacy of weight loss, safety as well as complications of this procedure were demonstrated. Remission of T2DM and co-morbidities resolution after one year were also analyzed.

Results: The median duration of T2DM was 60 months and the median operative time was 140 min. Interestingly, the mean/median preoperative BMI, HbA1C and FPG levels dropped significantly from 30.7 kg/m^2, 8.9%, and 139.0 mg% to 23.7 kg/m^2, 6.2%, and 95.0 mg% respectively at 1 year after operation ($p<0.001$). Furthermore, 61.5% of patients who had completed 1 year follow-up showed complete diabetic remission and 92.3% experienced glycemic control (HbA1c<7% without any medication). The postoperative complications were intra-abdominal bleeding (4.4%), leakage (1.1%), stricture (3.3%) and port site hernia (2.2%).

Conclusion: LDJB-SG is a safe and feasible bariatric and metabolic surgery, which has demonstrated excellent outcomes in terms of weight reduction, co-morbidities resolution as well as glycemic control in short-term follow-up.

Keywords: Loop duodenojejunal bypass with sleeve gastrectomy; type 2 diabetes mellitus; Obese Asians (Siriraj Med J 2018;70: 103-113)

INTRODUCTION

Currently, changes in lifestyle, particularly overeating and less exercise, economic and social factors have mainly contributed to the increase in obesity around the world. It is estimated that about 2 billion individuals are encountering this problem.[1] Moreover, obesity is associated with an increased risk of death, and carries a significant risk of life-threatening complications such as type 2 diabetes mellitus (T2DM), coronary artery disease, hyperlipidemia, hypertension, obstructive sleep apnea and several cancers.[2]

According to WHO 1998 classification of weight status in adults, the definition of obesity [body mass index (BMI) >30 kg/m^2] is based on criteria derived from studies that involved European populations.[3] It has been proposed that there are differences between European and Asian populations in terms of the associations of BMI with body composition and health outcomes. Furthermore,

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Asian populations have also been manifested to have an elevated risk of serious co-morbidities at a relatively low level of BMI. Regarding these observations, it has been suggested that the BMI cut-off points for obesity should be lower for Asians than Europeans. The suggested BMI cut-off points for obese Asians is ≥ 27.5 kg/m^2.[3]

Type 2 diabetes mellitus has become the most serious life-threatening condition which is apparently related to obesity. Diet control, increased physical activity, weight loss and anti-diabetic medications are the basical standard treatment for T2DM. Nevertheless, many studies have shown that the results of these non-surgical treatments are ineffective in long-term observation. [4-7] On the contrary, several reports have demonstrated that the majority of obese diabetic patients who received bariatric surgery resulted in T2DM remission and/or improvement which was significantly more than patients who did medical therapy alone.[8-10]

Many bariatric and metabolic surgical procedures such as Laparoscopic Roux-en-Y Gastric Bypass (LRYGB) and Biliopancreatic Diversion with Duodenal Switch (LBPD-DS) have shown impressive weight loss and comorbid diseases resolution particularly in T2DM in both severely obese and mild to moderate obese patients from many recent studies.[11-16,20-22] However, the drawbacks of these procedures are the long-term complications including marginal ulcer, intractable dumping syndrome, internal hernia, malnutrition and even mortality in some patients. Regarding these eventful complications, we were the first group who invented a novel bariatric procedure called "Laparoscopic Loop Duodenojejunal Bypass with Sleeve Gastrectomy (LDJB-SG)" in October 2011. This procedure is theoretically more physiological and carries less surgical related complications. In addition, we previously reported our preliminary result of this novel procedure since March 2013, which was demonstrated to be safe, feasible and achieved excellent weight loss and glycemic control in T2DM patients at 6 months of follow-up.[23] The objective of this study is to demonstrate two-year surgical outcomes regarding the efficacy of weight loss, safety and complications as well as one-year surgical results of diabetic remission and co-morbidities resolution in obese Asians (BMI ≥ 27.5 kg/m^2) with T2DM who underwent LDJB-SG in our center.

MATERIALS AND METHODS

This study was approved by the Medical Ethics Committee and Institutional Review Board of E-Da Hospital. We retrospectively reviewed, from prospectively maintained database, ninety-one patients who underwent LDJB-SG in our hospital from October 2011 to January 2014. All of the patients underwent a routine preoperative work-up and were assessed by a specialized team including a surgeon, gastroenterologist, anesthetist, psychiatrist and dietician. The inclusion criteria was based on The Consensus Statement of International Federation for Surgery of Obesity and Metabolic Disorders-Asia Pacific Chapter Meeting 2011 (IFSO-APC 2011)[24] and The International Diabetes Federation (IDF) statement in the treatment of Obese Patients with T2DM[25] (age of 18-65 years and body mass index (BMI) ≥ 27.5 kg/m^2 with inadequately controlled T2DM). The patients who were younger than 18 or older than 65 years, BMI < 27.5 kg/m^2, psychiatric illness, liver cirrhosis, active malignancy condition, addiction to either drugs or alcohol, severe gastroesophageal reflux disease (grade C and D by Los Angeles Classification[27] base on endoscopic finding) and any duodenal ulcer or lesion from preoperative upper endoscopic evaluation were excluded from receiving this surgery.

The prospectively collected data included preoperative patient demographics such as gender, age, body weight (BW), BMI, waist circumference (WC), hip circumference (HC), waist/hip ratio (WHR), systolic blood pressure (SBP), diastolic blood pressure (DBP), obesity-related co-morbidities (diabetes mellitus, hypertension, hyperlipidemia, hyperuricemia, steatohepatitis), duration of diabetes, insulin or oral hypoglycemic agents (OHA) usage, fasting plasma glucose (FPG), glycosylate hemoglobin (HbA1C), C-peptide level, total cholesterol (CHOL), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), complete blood count (CBC) and uric acid (UA). Intra- and postoperative complications, operative time (OT) as well as length of hospital stay (LOS) were also recorded. Furthermore, preoperative esophagogastroduodenoscopy (EGD) and upper abdominal ultrasound (US) were performed in every patient. The concomitant cholecystectomy with LDJB-SG was done when the patient had gallstone from preoperative US and the hiatal hernia repair was also performed simultaneously when we found any hiatal hernia from preoperative EGD.

All the patients were followed up routinely at 1, 3, 6, 9 and 12 months after surgery. The postoperative collected data were BW, BMI, weight loss percentage (%EWL), WC, HC, WHR, blood pressure as well as the levels of FPG, HbA1C, C-peptide, CHOL, TG, HDL-C, LDL-C, AST, ALT, CBC, and UA. Moreover, EGD and US were also performed additionally at 12 months postoperatively in every patient.

The surgical outcomes in our study included

the efficacy of weight loss and co-morbidities resolution after operation. Additionally, we also demonstrated the remission of T2DM, which was based on the criteria of the American Diabetes Association (ADA) 2012.[26] Complete remission was stated if HbA1c < 6% and FPG < 100 mg/dl and partial remission was considered when HbA1C < 6.5% and FPG 100-125 mg/dl for at least 1 year's duration with the absence of anti-diabetic medication. If HbA1C < 7% without any anti-diabetic medication, it could be determined that glycemic control was achieved. When the FPG decreased more than 25 mg/dl or HbA1C decreased more than 1%, it was considered as glycemic improvement. In contrast, failure of diabetic control after operation was defined as no significant improvement or worsened glycemic indices or a requirement for additional diabetic medication. Hypertension resolution was documented if blood pressure < 130/80 mmHg without medication and the level of TG < 150 mg/dl and LDL-C < 100 mg/dl without medication were considered as hyperlipidemia resolution.[28]

Finally, we also analyzed the side effects, complications, morbidity and mortality of this operation. Early complications were described as those, which occurred within the 30 days after operation. Major complications were life threatening or required surgical intervention treatment. Furthermore, diagnosis and grading of erosive esophagitis were determined endoscopically based on the Los Angeles (LA) classification.[27]

Operative technique

LDJB-SG was performed laparoscopically under general anesthesia. The patient was placed in the supine position with both arms abducted. The surgeon and assistants position were on the right and left side of patient, respectively (Fig 1). Pneumoperitoneum was created using a long Veress needle technique and intra-abdominal pressure was maintained at 17 mmHg. We used five-port laparoscopic surgery to access the abdominal cavity. (Fig 2) The liver was retracted superiorly using T-shape liver suspension tapes in order to achieve good exposure of both stomach and duodenal area. Complete devascularization of the greater curvature of the stomach was done, 4 cm., from pylorus up to the left crus of the diaphragm, using 5 mm., LigaSure vessel sealing (Tyco, New Haven, CT, USA) followed by sleeve gastrectomy (SG) procedure over a 36-Fr., orogastric tube calibrator using 60 mm., laparoscopic linear tri-stapler ENDO GIA (Covidien, Mansfield, MA, USA). Black reloads were used for the first two firings while purple reloads were used for the subsequent firings toward the gastric fundus. After ensuring hemostasis, a stay suture was done at distal



Fig 1. Patient and Surgeon positioning.

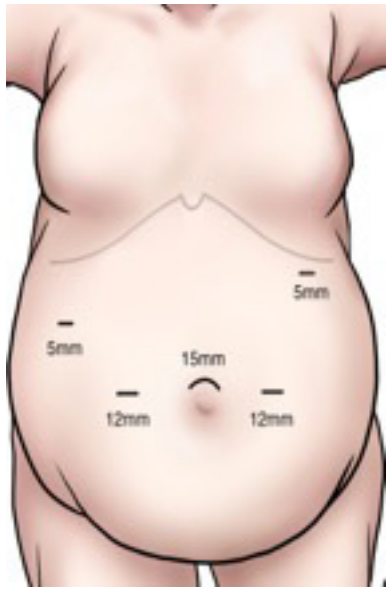


Fig 2. Ports position.

end of SG for counter-traction and better visualization of the first part of duodenum. The transection of the first part of duodenum was performed at 2 cm., away from pylorus using 45 mm., laparoscopic linear white stapler ENDO GIA (Covidien, Mansfield, MA, USA) then a 1.5 cm., side to side, antecolic, loop duodenojejunal anastomosis (totally hand-sewn continuous suture with 3-0 Monocryl, single-layer technique) was created at 200-300 cm., from the ligament of Treitz. The limb tailoring for duodenojejunostomy bypass depended on the patient's preoperative C-peptide level as follows: the limb length for any patient who had preoperative C-peptide level more than 2, between 1 and 2 and less

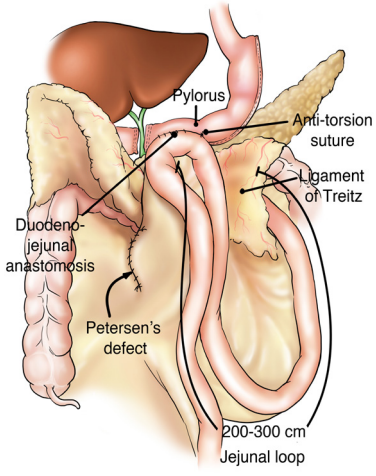


Fig 3. Laparoscopic Loop DuodenoJejunal Bypass with Sleeve Gastrectomy (LDJB-SG).

than 1 ng/ml were 200, 250 and 300 cm., respectively. An anti-torsion suture between proximal jejunum and antrum (2 cm., proximal to pylorus) was made then the Peterson's defect was closed with continuous suture. (Fig 3) A Jackson-Pratt drain was placed behind the duodenojejunal anastomosis and stapler line of SG.[23]

Early postoperative care

All the patients were extubated and transferred to the surgical ward after surgery. Deep-breathing exercises, early mobilization and adequate pain control administration were given in order to avoid postoperative pulmonary complications. A clear-liquid diet was started once patients were fully conscious. The patients were discharged when they could tolerate oral fluid, passed flatus and had no sign of tachycardia, which was usually by postoperative day 2-3. Patients were scheduled for follow-up in the outpatient clinic 1 week after discharge. They advanced to soft diet intake by the third week and solid diet intake after 1 month. Subsequent routine follow-ups were scheduled as mentioned previously.

Statistical Analysis

Patient data were collected prospectively and verified retrospectively. Descriptive results for categorical variables were presented as frequency and percentage (%) of subjects affected. Normally distributed continuous variables were demonstrated as means ± standard deviation (SD), whereas those variables without a normal distribution were showed as the median (interquartile range, IQR). The changes in general medical parameters and laboratory values were compared pre- and postoperatively using pair t-test, the Wilcoxon signed rank test and McNemar test when appropriate. Categorical variable frequencies were compared using Chi-square or Fisher exact test if small expected frequencies had been found. All statistical tests were calculated with SPSS statistical software (version 22.0; SPSS Inc., Chicago, IL). A p-value < 0.05 was considered statistically significant.

RESULTS

A total of ninety-one patients (64 female, 27 male) with T2DM were enrolled in this study and the overall patient's preoperative demographics, co-morbidities and operative data have been summarized in Table 1. The mean age was 46.6 years (SD=9.5 years, range 27-65 years). The preoperative median BMI was 32.0 kg/m^2 (range 27.5-47.3 kg/m^2). The median duration from the onset of T2DM was 60 months (range 1-240 months). There were 67 patients (73.6%) who had oral hypoglycemic agents (OHA) usage only, 2 patients (2.2%) had insulin injection usage only and 15 patients (16.5%) had both OHA and insulin injection usage. However, there were 7 patients (7.7%) who did not use any glucose-lowering medication. Regarding patient's co-morbidities, 43 patients (45.7%) had hypertension, 73 patients (80.2%) had hyperlipidemia, 74 patients (81.3%) had steatohepatitis, and 16 patients (17.6%) had hyperuricemia. The mean preoperative HbA1C was 8.7% (SD=1.9%) and the median preoperative FPG and C-peptide level were 146.0 mg% (range 64-400 mg%) and 2.5 ng/ml (range 0.4-12.3 ng/ml), respectively.

All of the patients underwent LDJB-SG successfully without any conversion to open surgery. The median operative time was 140 minutes (range 73-265 minutes) and duration of hospitalization was 2 days (range 1-7 days). There was no mortality in our study. Nevertheless, the postoperative complications (both early and late complications) were found as follows: 4 patients (4%) had intra-abdominal bleeding, 1 patient (1%) had sleeve gastrectomy (SG) leakage, 3 patients (3%) had SG stricture, 2 patients (2%) had port site hernia and 1 patient (1%) developed acute uncomplicated calculous cholecystitis. There was no dumping syndrome, internal hernia or marginal ulcer in our study.

In this study, all of the patients had at least 3 months of follow-up. Thirty-nine patients had completed one-year follow up. The comparison between preoperative and postoperative one-year surgical outcomes of these 39 patients, have been demonstrated in Table 2. The median % EWL was 82.3% (range 45.1-150.4%). The median BMI decreased significantly from 30.7 kg/m^2 (range 27.6-43.6 kg/m^2) preoperatively to 23.7 kg/m^2 (range 18.0-28.6 kg/m^2) at 1 year postoperatively (p=<0.001). The changes in mean/median WC, HC, WHR, SBP and DBP at 1 year in comparison to the preoperative data were also significant (p=<0.001). The mean/median preoperative

TABLE 1. The overall patient's preoperative demographics, co-morbidities and operative data.

Parameters	Mean (±SD) / Median (range)	N (%)
No. patients		91
Age (years)	46.6 (9.5)	
Gender (F/M)		64/27
Body Height (cm.)	163.7 (10.2)	
Body weight (kg.)	87.4 (16.3)	
BMI (kg/m^2)	32.0 (27.5-47.3)	
Co-morbidities		
Diabetes		91 (100%)
OHA only		67 (73.6%)
Insulin only		2 (2.2%)
OHA and insulin		15 (16.5%)
No OHA or insulin		7 (7.7%)
Hypertension		43 (45.7%)
Hyperlipidemia		73 (80.2%)
Steatohepatits		74 (81.3%)
Hyperuricemia		16 (17.6%)
Duration of Diabetes (months)	60.0 (1-240)	
HbA1C (%)	8.7 (1.9)	
FPG (mg/dl)	146.0 (64-400)	
C-peptide (ng/dl)	2.5 (0.4-12.3)	
Operative time (minutes)	140.0 (73-265)	
Hospital stay (days)	2.0 (1-7)	
Intraoperative complication		0
Conversion to open		0
Early postoperative complication		
Intra-abdominal bleeding		4
Leakage (SG part)		1
Infection		0
Late postoperative complication		
Port site hernia		2
Acute calculous cholecystitis		1
Stricture (SG part)		3
Dumping syndrome		0
Internal hernia		0
Marginal ulcer		0
Mortality		0

Abbreviations: BMI: Body Mass Index, OHA: Oral Hypoglycemic Agent, FPG: Fasting Plasma Glucose, SG part : Sleeve Gastrectomy part.

TABLE 2. One-year surgical outcomes after LDJB-SG in 39 patients who had completed one-year follow up.

Parameters	Preoperative Mean (±SD) / Median (range)	One year Mean (±SD) / Median (range)	p-value
General medical parameters			
Body weight (kg)	83.8 (15.3)	62.0 (10.9)	<0.001
BMI (kg/m^2)	30.7 (27.6-43.6)	23.7 (18.0-28.6)	<0.001
EWL (%)	-	82.3 (45.1-150.4)	<0.001
WC (cm.)	100.5 (11.5)	81.8 (8.3)	<0.001
HC (cm.)	108.7 (10.1)	94.0 (8.2)	<0.001
WHR	0.91 (0.15-1.03)	0.87 (0.78-0.98)	<0.001
SBP (mmHg)	133.4 (15.3)	120.0 (14.9)	<0.001
DBP (mmHg)	83.5 (11.0)	70.6 (12.1)	<0.001
Laboratory values			
FPG (mg/dl)	139.0 (102-317)	95.0 (78-211)	<0.001
HbA1c (%)	8.9 (1.8)	6.2 (0.9)	<0.001
C-peptide (ng/ml)	2.5 (0.4-11.3)	1.4 (0.6-3.4)	<0.001
TG (mg/dl)	149.0 (51-825)	81.0 (25-316)	<0.001
CHOL (mg/dl)	183. 0 (40.1)	170.4 (30.9)	0.119
HDL-C (mg/dl)	45.0 (32-180)	52.0 (39-94)	0.002
LDL-C (mg/dl)	96.3 (31.9)	90.3 (27.5)	0.547
AST (U/L)	30.0 (14-96)	28.0 (14-55)	0.879
ALT (U/L)	35.0 (10-150)	32.5 (13-63)	0.986
Uric acid (mg/dl)	6.4 (2.1)	4.9 (1.5)	<0.001
Hb (g/dl)	13.8 (9.9-17.3)	12.9 (8.0-15.7)	0.003
Hct (%)	40.0 (4.7)	37.3 (5.4)	0.001
WBC (x 10^3 cell/mcL)	7.2 (2.1)	7.4 (7.5)	0.867
Plt (x 10^3 cell/mcL)	245.5 (61.3)	229.5 (67.7)	0.130

Abbreviations: BMI: Body Mass Index, EWL: Excess Weight Loss, WC: Waist Circumference, HC: Hip Circumference, WHR: Waist-Hip Ratio, SBP: Systolic Blood Pressure, DSP: Diastolic Blood Pressure, FPG: Fasting Plasma Glucose, TG: Triglyceride, CHOL: Cholesterol, HDL-C: High Density Lipoprotein Cholesterol, LDL-C: Low-Density Lipoprotein Cholesterol, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, UA: Uric Acid, Hb: Hemoglobin, Hct: Hematocrit, WBC: White Blood Cell count, Plt: Platelet count

FPG, HbA1C and C-peptide level dropped significantly from 139.0 mg% (range 102-317 mg%), 8.9% (SD=1.8%) and 2.5 ng/ml (range 0.4-11.3 ng/ml) to 95.0 mg% (range 78-211 mg%), 6.2% (SD=0.9%), and 1.4 ng/ml (range 0.6-3.4 ng/ml), respectively at 1 year after operation (p=<0.001).

We found the mean/median of TG and UA level decreased significantly from 149.0mg/dl (range 51-825 mg/dl) and 6.4 mg/dl (SD=2.1 mg/dl) preoperatively to 81.0mg/dl (range 25-316 mg/dl) and 4.9 mg/dl (SD=1.5 mg/dl), respectively at 1 year postoperatively (p=<0.001). The level of HDL-C increased significantly from 45.0 mg/dl (range 32-180 mg/dl) preoperatively to 52.0 mg/dl (range 39-94 mg/dl) at 1 year postoperatively (p=0.002). In addition, the level of Hb and Hct preoperatively decreased significantly from 13.8 g/dl and 40.0% to 12.9 g/dl and 37.3%, respectively at 1 year after operation (p=0.003 and p=0.001 respectively). However, there were no significant changes in LDL-C, AST and ALT level at 1 year in comparison to preoperative data (p>0.05).

Regarding the result of co-morbidities resolution, the overall complete diabetic remission rate (CR) was 61.5%

(24/39 patients), partial diabetic remission rate (PR) was 20.5% (8/39 patients), glycemic control rate (GC) was 10.3% (4/39 patients) and glycemic improvement rate (GI) was 7.7% (3/39 patients). There was no failure of treatment in our study. (Table 3) Additionally, in subgroup analysis of these patients, we found that the CR was 80.0% (12/15 patients), PR was 6.7% (1/15 patients), GC was 6.7% (1/15 patients) and GI was 6.7% (1/15 patients) in the patients who had BMI ≥ 32.5 kg/m^2 (Group A). In the patients who had BMI < 32.5 kg/m^2 (Group B), the CR was 50% (12/24 patients), PR was 29.2% (7/24 patients), GC was 12.5% (3/24 patients) and GI was 8.3% (2/24 patients). (Table 4) Also, we found that the rate of hypertension and hyperlipidemia resolution were 69.2% (27/39 patients) and 89.7% (35/39 patients), respectively at 1 year after operation. (Table 3)

TABLE 3. The results of co-morbidities resolution at 1 year postoperatively.

Co-morbidities resolution (1 year postoperatively)	N (%)
Total of patients (completed follow-up)	39
Diabetic remission	
Complete remission (CR)	24/39 (61.5%)
Partial remission (PR)	8/39 (20.5%)
Glycemic control (GC)	4/39 (10.3%)
Glycemic Improvement (GI)	3/39 (7.7%)
Failure of treatment	0/39 (0%)
Hypertension resolution	27/39 (69.2%)
Hyperlipidemia resolution	35/39 (89.7%)

TABLE 4. Subgroup analysis of diabetic remission in both Group A and B.

Diabetic remission 1 year after surgery (Total 39 patients)	Group A (15 patients)	Group B (24 patients)	P value
Complete remission (CR)	12/15 (80.0%)	12/24 (50.0%)	0.686
Partial remission (PR)	1/15 (6.7%)	7/24 (29.2%)	
Glycemic control (GC)	1/15 (6.7%)	3/24 (12.5%)	
Glycemic Improvement (GI)	1/15 (6.7%)	2/24 (8.3%)	
Failure of treatment	0/15 (0%)	0/24 (0%)	

Group A: BMI ≥ 32.5 kg/m^2, Group B: BMI<32.5 kg/m^2

DISCUSSION

Bariatric surgery has been shown to be the most effective treatment for morbid obesity in terms of long-term weight loss and co-morbidities resolution compared to non-surgical treatment. The impact on the resolution of co-morbidities such as T2DM after bariatric surgery besides weight loss has contributed to the increasing use of the term "Metabolic Surgery". The improvement of glucose homeostasis experienced before weight loss occurs has drawn attention and proposed an expansion of the traditional indications for bariatric surgery to the lower BMI obese patients.[29,30] Interestingly, many studies have shown the excellent short- term results in regards to glycemic improvement and co-morbidities resolution in non-severely obese patients (BMI 30-35 kg/m^2) after bariatric surgery.[16-22] Generally, Asian populations have a higher percentage of body fat than do Europeans, and also have been shown to have an elevated risk of T2DM, hypertension, and hyperlipidemia at a relatively low level of BMI.[1] Regarding these concepts, presently, The International Diabetes Federation (IDF) statement in the treatment of Obese Patients with T2DM and The

consensus of International Federation for Surgery of Obesity and Metabolic Disorders-Asia Pacific Chapter Meeting 2011 (IFSO-APC 2011) suggest that Bariatric/GI Metabolic Surgery should be considered for the treatment of T2DM or metabolic syndrome for patients who are inadequately controlled by lifestyle alternations. Medical treatment for acceptable Asian candidates with BMI 30-35 kg/m^2 and Metabolic Surgery may be considered as a non-primary alternative to treat inadequately controlled T2DM, or metabolic syndrome, for suitable candidates with BMI ≥ 27.5 kg/m^2.[24,25]

In recent years, many bariatric and metabolic surgeries such as BPD-DS and LRYGB, have demonstrated impressive weight loss and comorbid diseases resolution in severely obese patients and excellent long-term glycemic control in mild to moderate obese patients with T2DM from many recent studies.[11-16,20-22] However, the drawbacks of these procedures are the long-term complications including marginal ulcer, intractable dumping syndrome, internal hernia, and malnutrition. Therefore, Laparoscopic Loop Duodenojejunostomy Bypass with Sleeve Gastrectomy (LDJB-SG) has been developed in our center in order to overcome these long-term eventful complications as well as demonstrate the surgical outcomes in regards to efficacy of weight loss and co-morbidities resolution, which are comparable to other bariatric and metabolic surgeries.[23]

Theoretically, LDJB-SG offers many advantages. The pyloric preservation may decrease the dumping phenomenon as well as the chance of marginal ulcer. The acid and intrinsic factor secretion would be maintained. Thus, iron, vitamin and protein deficiency should be less because some parts of the antrum are preserved. Moreover, one anastomosis and only Petersen's defect should have some advantages in decreasing the probability of postoperative anastomotic leakage and internal hernia as compared to LRYGB and BPD-DS.

LDJB-SG can result in weight loss and glycemic improvement through multiple mechanisms. The partial vertical sleeve gastrectomy in this procedure can produce efficient weight loss by reducing food/calorie intake (restrictive mechanism) and reducing the ghrelin, orexigenic hormone, level by removing the fundus of stomach which led to increase satiety.[31-34] Also, the glycemic improvement can be explained by hindgut hypothesis by 200-300 cm., jejunal bypass and foregut hypothesis by exclusion of the second part of duodenum. The hindgut hypothesis claims that the surgical rerouting of nutrients to the distal part of the small intestine results in increasing secretion and concomitant glucose-lowering effects of GLP-1[35,36] and the foregut hypothesis states that surgical bypass of foregut prevents the release of unidentified materials that induce a diabetogenic signal in susceptible individuals.[37,38]

This procedure has demonstrated excellent results in weight loss and is comparable to other bariatric procedures. Many studies have reported that the patients who undergo LRYGB, LSG, LAGB and BPD-DS typically experience an EWL of 60-70%, 33-85%, 40-50% and 60-76.7%, respectively at 1 year postoperatively. In our study, the overall median BMI preoperatively was significantly dropped from 31.2 kg/m^2 to 23.7 at 1 year postoperatively (p=<0.001). Furthermore, the median % EWL at 1 year after operation was 82.3% (range 45.1-150.4%) (Table 2), which were comparable to other studies. [11-16,20-22] Additionally, regarding IDF statement in the treatment of Obese Patients with T2DM, it states that surgery should be an acceptable option in people who have BMI ≥ 35 kg/m^2 with T2DM and BMI action points may be reduced by 2.5 kg /m^2 in Asians. Therefore, we aim to demonstrate the changes in BMI and % EWL in both patients who had BMI ≥ 32.5 kg/m^2 (15/39 patients, Group A) and BMI < 32.5 kg/m^2 (24/39 patients, Group B). In this subgroup analysis, we found that the median BMI of Group A and Group B were significantly decreased from 34.2 kg/m^2 (range 33.1-43.6 kg/m^2) and 29.3 kg/m^2 (range 27.6-32.1 kg/m^2) preoperatively to 23.9 kg/m^2 (range 18.0-28.6 kg/m^2) and 23.7 kg/m^2 (range 18.9-25.6 kg/m^2), respectively at 1 year after operation (p=0.018 and p<0.001, respectively). There was no statistical difference in median BMI between group A and B (p=0.152) at 1 year after operation (Fig 4). Furthermore, the median % EWL for Group A and Group B were 84.3 % (range 51.3-136.3%) and 81.4 % (range 45.1-150.4%), respectively at 1 year postoperatively without any significant difference between these two groups (p=0.658). Interestingly, these results have shown that although preoperative median BMI in Group A was higher than Group B initially, the postoperative median BMI at 1 year in Group A and B were ultimately almost equal (23.9 Vs. 23.7 kg/m^2).

In our study, LDJB-SG had also shown magnificent results in terms of glycemic control and diabetic remission. We found that the overall mean/median level of preoperative FPG, HbA1C and C-peptide were significantly decreased from 139.0 mg%, 8.9% and 2.5 ng/ml to 95.0 mg%, 6.2% and 1.4 ng/ml at 1 year postoperatively (p=<0.001). (Table 2) The overall diabetic complete remission rate (CR) was 61.5% (24/39 patients), partial remission rate (PR) was 20.5% (8/39 patients), glycemic control (GC) was 10.3 % (4/39 patients) and glycemic improvement (GI) was 7.7% (3/39 patients). There was no failure of treatment in our study. (Table 3) Additionally, in Group A analysis, we found that the diabetic complete remission rate (CR) 6

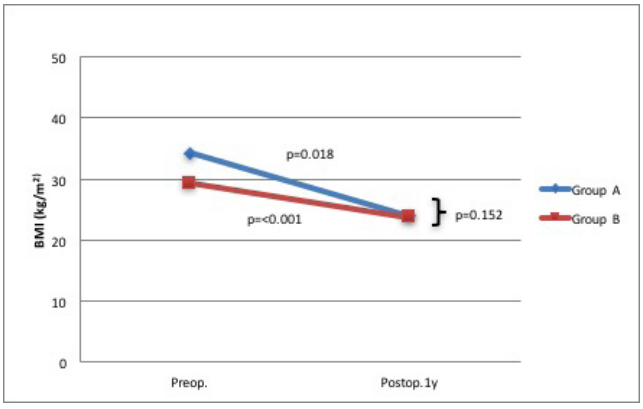


Fig 4. Change in median BMI of the patients who had preoperative BMI ≥ 32.5 kg/m² (Group A) and BMI < 32.5 kg/m² (Group B) at 1 year after operation.

80.0% (12/15 patients) (Table 4), which was comparable to other procedures.[11,14] Many studies had been reported the diabetic CR of LRYGB and LBPD with DS were 40-88% and 97-100%, respectively in the patients who had mean BMI ≥32.5 kg/m².[11-13,40,41] Furthermore, in Group B analysis, our results were also comparable to other procedures. Camilo et al.,[42] and Lee et al.,[16] reported the diabetic CR was 55.3% and 57%, respectively at 1 year after LRYGB in T2DM patients who have median/mean BMI<32.5 kg/m². Also, in regards to our previous study, LRYGB achieved 63.6% diabetic CR after 1 year in patients with mean BMI<32.5 kg/m².[17] Scopinaro et al.,[22] showed diabetic CR rate was 30% after LBPD in the T2DM patients with mean BMI<32.5 kg/m². We found that the diabetic CR was 50.0% (12/24 patients), PR was 29.2% (7/24 patients), GC was 12.5% (3/24 patients) and GI was 8.3% (2/24 patients) in Group B (Table 4). Interestingly, all of these findings meant there were 91.7% of T2DM obese patients (BMI<32.5kg/m²) who achieved glycemic control after LDJB-SG. These results have shown better outcome than another DJB-SG study. Navarrete et al., reported 4 (40%) patients who achieved glycemic control after Roux-en-Y DJB-SG in T2DM patients with an average BMI <32.5 kg/m².[43] Although the patients in group B achieved lesser CR rate than group A, their PR rate was higher than group A. Furthermore, there was no significant difference of overall diabetic remission (CR and PR) between groups A and B. (p=0.686) (Table 4) However, in our subgroup analysis of CR, group B had lower preoperative C peptide level than group A. This could elucidate that CR rate in group B was lesser than group A because they had poorer pancreatic endocrine reserve.

The excellent improvements of hypertension and hyperlipidemia after bariatric surgery, have been reported in many studies. The average of hypertension and hyperlipidemia resolution after LRYGB vs. LSG vs. BPD-DS were 75.4%, 93.6% vs. 71.7%, 61.0% vs. 81.3%, 99.5%, respectively.[11-14] These results were comparable to our study, in which we found the hypertension resolution rate was 69.2% and hyperlipidemia resolution rate was 89.7% at one-year after operation. (Table 3)

Regarding early postoperative complications in our study, we found that there were 5 patients (5.5%) who had early postoperative complications as follows: 4 patients (4.4%) had intra-abdominal bleeding, although only 1 out of these 4 patients underwent re-laparoscopy due to persistent dropping of her hematocrit despite the numbers of pack red cell replacements. Nevertheless, we found only old blood clot without any bleeding point could be detected intraoperatively. Postoperatively, the patient was stable and could be discharged 2 days after operation. One patient (1.1%) had leakage at sleeve gastrectomy part (near EG junction), which was treated initially by endoscopical stent placement. Unfortunately, the leakage still continued after the procedure. Thus, conversion to Roux-en-Y gastric bypass was performed in order to treat this problem. Postoperatively, the patient's clinical signs were improved and she was discharged without any further complication.

Late postoperative complications were found in 6 patients (6.6%) in this series, 2 patients (2.2%) developed incisional hernia at 1 year postoperatively at umbilical port (15 mm.), 1 patient (1.1%) developed uncomplicated acute calculous cholecystitis at 8 months postoperatively and 3 patients (3.3%) developed stricture at incisura angularis of the remnant stomach. Two of them, who had only mild to moderate stricture, were managed successfully by laparoscopic stricturoplasty and the last patient, who had severe stricture, was corrected successfully by conversion to LRYGB. There was no dumping syndrome, internal hernia, marginal ulcer or mortality in our study. Surprisingly, although anemia resulting from LDJB-SG should theoretically be less, we found the mean level of both Hb and Hct were significantly decreased from 13.8 g/dl and 40.0% preoperatively to 12.9 g/dl and 37.3%, respectively at 1 year postoperatively (p=0.003 and 0.001, respectively). Long-term close follow up is necessary in order to manage this anemic problem after LDJB-SG.

Thirty patients in our study completed upper abdominal U/S and EGD at 1 year postoperatively. We found 2/30 patients (6.6%) developed asymptomatic gallstone after LDJB-SG and watchful follow up was done in these patients. Additionally, in regards to Los Angeles (LA) classification for the endoscopic grading of reflux esophagitis, we found 22 patients (73.3%) had no reflux esophagitis and 8 patients (26.7%) had reflux esophagitis

grade A preoperatively. Postoperatively, we found 16 patients (53.5%) had no change of reflux esophagitis grading (14 patients for grade 0 to 0 and 2 patients for grade A to A). Meanwhile, there were 14 patients (46.5%) who had the alterations of reflux esophagitis grading after operation. Moreover, we discovered that there were 7 patients (23.3%) who developed reflux esophagitis grade B and 2 patients (6.7%) who had reflux esophagitis grade C at 1 year after operation. However, there was no reflux esophagitis grade D in this study. (Table 5) All of these patients did not have any severe reflux esophagitis symptom, while their symptoms could be controlled efficiently by oral proton-pump inhibitor medication. Furthermore, we also found 8/30 patients (26.6%) developed hiatal hernia at 1 year after operation. One out of eight patients had hiatal hernia preoperatively and despite repairing it intra-operatively, the patient still developed hiatal hernia postoperatively. Long-term close follow up is needed to clarify these problems after LDJB-SG.

TABLE 5. The results of EGD at 1 year after LDJB-SG.

EGD finding (Total 30 patients)	Preoperative N (%)	Postoperative N (%)
Hiatal hernia	1 (3.3%)	8 (26.6%)
Grading of reflux esophagitis		
0	22 (73.3%)	14 (46.7%)
A	8 (26.7%)	7 (23.3%)
B	0	7 (23.3%)
C	0	2 (6.7%)
D	0	0

EGD: esophagogastroduodenoscopy, Los Angeles (LA) classification for grading of reflux esophagitis (0= normal, A= One or more mucosal breaks < 5 mm in maximal length, B= One or more mucosal breaks > 5 mm, but without continuity across mucosal fold, C= Mucosal breaks continuous between > 2 mucosal folds, but involving less than 75% of the esophageal circumference, D= Mucosal breaks involving more than 75% of esophageal circumference)

CONCLUSION

Laparoscopic Loop Duodenojejunostomy Bypass with Sleeve Gastrectomy (LDJB-SG) is a safe and feasible bariatric and metabolic surgery for obese Asians with T2DM, which has demonstrated excellent surgical outcomes in terms of weight reduction, co-morbidities resolution as well as glycemic control after one-year follow up.

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Conflict of interest: All authors Voraboot Taweerutchana, Ming-Che Hsin, Po-Chih Chang, Chi-Ming Tai and Chih-Kun Huang declare that they have no competing interest and no relevant financial interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Insulin Sensitivity and Clinical Profiles in CPP Girls Compared to Controls

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ABSTRACT

Objective: This study aimed to compare obesity, insulin sensitivity, clinical, and metabolic parameters between CPP and age-matched control girls, with or without obese girls and to determine correlations between these factors.

Methods: CPP and control girls were recruited and were allocated into obese and non-obese groups. There were 3 groups of comparison including non-obese control girls (n=29), non-obese CPP girls (n=20), and obese CPP girls (n=6), because obese controls could not be recruited.

Results: Body mass index (BMI), body weight, and height percentiles were higher in obese CPP and non-obese CPP girls compared to control girls. Lipid profiles, glucose, insulin, the homeostasis model assessment-estimated insulin resistance (HOMA-IR), and the quantitative insulin sensitivity check index (QUICKI) were not different between all groups. Insulin and the HOMA-IR were positively but the QUICKI was negatively correlated with metabolic and anthropometric parameters mainly in the control group, while these parameters were significantly correlated with LH and FSH in the CPP group. From multivariate regression, insulin and the HOMA-IR were associated with reproductive indices in CPP and with fat mass in controls.

Conclusion: Insulin sensitivity and insulin resistance were related to metabolic factors in prepubertal stage, but were more associated with the reproductive status during pubertal advancement.

Keywords: CPP; insulin; HOMA-IR; QUICKI; obesity (Siriraj Med J 2018;70: 114-122)

INTRODUCTION

The onset of puberty is associated with activation of the hypothalamo–pituitary–gonadal axis, which is crucially dependent on sufficient energy stores and the amount of fat in the body.[1-3] Childhood obesity, higher body mass index (BMI), and adiposity are associated with earlier age at onset of puberty, increased diagnosed cases of central precocious puberty, earlier onset of menarche in girls[4-8], and increased cardiovascular risk during adulthood.[9,10] Moreover, some studies have reported that higher insulin levels were associated with an earlier age of menarche.[11-15] Additionally, high insulin secretion and low insulin sensitivity are related to increased fat mass in children who had a normal birth weight[10,16] and those who were small for gestational age.[17,18] Girls with CPP had significantly higher fat mass, lower insulin sensitivity, and adverse lipid profiles at the time of diagnosis compared with normal girls.[7] However, one study reported that girls with CPP did not have an adverse metabolic profile despite having a higher BMI compared with normal girls of the same age.[10]

To date, there have been limited data regarding adiposity, insulin, metabolic profiles, and body composition among girls with CPP. The causality between obesity and precocious puberty is still unknown and lipid profiles in girls with CPP are still controversial. The metabolic profiles

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and adiposity in girls with CPP need to be investigated in clinical practice to prevent adverse metabolic profiles and further complications of cardiovascular risk.

The objectives of this study were as follows: 1) to compare clinical and biochemical parameters, including insulin, fasting lipid profiles, fasting blood glucose, an index of insulin resistance (homeostasis model assessment-estimated insulin resistance, HOMA-IR), and an index of insulin sensitivity (quantitative insulin sensitivity check index, QUICKI) between girls with CPP and age-matched control girls, with subgroup analysis into obese and non-obese girls; 2) to determine correlations between insulin, HOMA-IR, and the QUICKI with clinical and biochemical parameters in CPP and control subjects; and 3) to determine multivariate regression analyses for insulin, HOMA-IR, and QUICKI in the CPP and control groups. In the transition from non-puberty to puberty, we hypothesized that clinical and biochemical parameters, insulin, an index of insulin resistance, and an index of insulin sensitivity might play different roles in the prepubertal and pubertal stages.

MATERIALS AND METHODS

Subjects

This study was a case-control study that was carried out from December 2011 to March 2013 at the Faculty of Medicine Siriraj Hospital. The methods and protocols in this study were approved by the Siriraj Institutional Review Board (Si 522/2011). They were in full compliance with International Guidelines for Human Research Protection, the Declaration of Helsinki, the Belmont Report, CIOMS Guidelines, and the International Conference on Harmonization in Good Clinical Practice. Written informed consent from all subjects and their parents or guardians was obtained. There were 2 groups of subjects, girls with CPP and age-matched controls. The CPP group consisted of girls who were diagnosed with CPP by pediatric endocrinologists. The inclusion criteria were 1) onset of breast development before 8 years, 2) advanced bone age; and 3) basal luteinizing hormone (LH) levels greater than 0.3 mIU/ml or a LH peak greater than 6 mIU/ml after gonadotropin-releasing hormone (GnRH) stimulation. The exclusion criteria were patients with a present or past history of the following: 1) diseases of the central nervous system, such as cerebral palsy, infection, chronically increased intracranial pressure, intoxication by substances, epilepsy, meningitis, and birth asphyxia; 2) diseases of the endocrinology system, such as hypothyroidism or hyperthyroidism, Cushing's syndrome, diabetes mellitus (DM), polycystic ovarian syndrome, congenital adrenal hyperplasia; and 3) those receiving hormonal therapy or other current medications.

All patients suspected of CPP had been scheduled to perform a GnRH stimulation test with a 12-hour overnight fast prior to the test day. At 07.30 to 08.00 am of the test day, 5 ml of fasting blood sample was collected from each patient to measure basal levels of LH, follicle-stimulating hormone (FSH), fasting blood glucose, insulin, triglyceride, total cholesterol, high-density lipoprotein (HDL)-cholesterol, and low-density lipoprotein (LDL)-cholesterol. After collection of blood, the GnRH stimulation test was immediately performed by using Diphereline® 0.1 mg injected subcutaneously, and blood samples were drawn every 20 minutes twice (at 20, and 40 minutes) to measure LH and FSH levels. Patients who had early pubertal features such as having breast budding, but did not fall within the criteria for diagnosis of CPP were classified as early puberty, but not CPP group (n=3) and these were excluded in the analysis. The control group consisted of healthy age-matched girl volunteers with a difference of no more than 2 months of age from the individual girls with CPP and who did not have the exclusion criteria. Subjects of the control group were advised to undergo a 12-hour overnight fast before the morning of the appointed day to collect clinical data and blood samples. Blood samples of 5 ml were collected in the control subjects for measurement of basal LH, FSH, fasting blood glucose, insulin, triglyceride, total cholesterol, HDL-cholesterol, and LDL-cholesterol. In both groups, the maximal age limitation of the subjects was 10 years. Subjects could withdraw from the project anytime, although there was no withdrawal of any subjects.

There were 26 CPP girls and 29 control girls. Obese subjects had a BMI standard deviation score (SDS) of more than 2 and non-obese subjects had a BMI SDS of 2 or less.[19] There were no subjects in the obese control group. Therefore, comparison between was made between non-obese control girls (n=29), non-obese CPP girls (n=20), and obese CPP girls (n=6).

Clinical parameters

The clinical data consisted of chronological age, birth weight, Tanner stage, and percentiles of systolic blood pressure (SBP), diastolic blood pressure (DBP), body weight (BW), height, and BMI. Percentiles of BW and height were assessed according to the National Standard Growth Curve of the Ministry of Public Health, Thailand Department of Health, Ministry of Public Health.[20] The BMI percentile was assessed according to the World Health Organization 2007 growth reference for children aged 5–19 years.[19] The sexual maturation

rate of the breasts and genitalia were assessed using the method of Marshall and Tanner.[21] A history of maternal age of menarche was collected from recall memory of the mothers of each subject. Data of reported paternal and maternal heights were used to calculate the mid-parental height by the formula: (paternal height+maternal height-13)/2.

Average daily caloric intake was assessed from a food record, which was recorded for 3 consecutive days prior to blood sample collection. Daily caloric intake was calculated by using the program INMUCAL-Nutrients version 1.0, which was developed by the Faculty of Public Health, Mahidol University. Body composition analysis was performed by using the total body composition analyzer TBF-300A (Tanita Corporation, Tokyo, Japan) to measure the percentage of body fat, fat mass, and fat-free mass.

Biochemical parameters

Levels of fasting blood glucose, insulin, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, basal LH, and basal FSH were measured by the central laboratory of Siriraj Hospital at the Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital. The methods of measurement were as follows: glucose by enzymatic (hexokinase) method; insulin, LH, and FSH by electrochemiluminescence immunoassay (ECLIA); and triglycerides, total cholesterol, HDL-cholesterol, and LDL-cholesterol by enzymatic colorimetric assays. Fasting plasma glucose and insulin levels were used to determine the level of insulin resistance using the HOMA-IR model, which was calculated as follows: fasting glucose (mg/dl)×fasting insulin (μU/ml)/405. The QUICKI was used to represent insulin sensitivity. This index is one of the most accurate surrogate indices for determination of insulin sensitivity[22], and was calculated as follows: 1/log fasting insulin (μU/ml)+log fasting glucose (mg/dl).

Statistical analysis

Clinical parameters including age, fat mass, and fat free mass are expressed as interquartile ranges representing as the 25th percentile (P25), median, and the 75th percentile (P75). Other data are expressed as the mean ± standard error of the mean (SEM). The Kolomonov–Smirnov test was used to test normality. To compare normally distributed data between non-obese control girls (n=29), non-obese CPP girls (n=20), and obese CPP girls (n=6), one-way analysis of variance (ANOVA) statistic followed by least significant difference post-hoc was used. To compare non-normally distributed data between these groups, a non-parametric test was used for analysis. To determine correlations between two factors, Pearson product-moment correlation was used to analyze normally distributed data and Spearman's rank correlation was used to determine non-normally distributed or ranked data. A p value less than 0.05 was considered as statistical significance. All data were analyzed by using SPSS (PASW) 18 (SPSS Inc., Chicago, IL).

RESULTS

Clinical characteristics of non-obese control, non-obese CPP, and obese CPP girls.

Clinical characteristics including age, fat mass, and fat-free mass of non-obese control, non-obese CPP, and obese CPP girls are shown in Table 1 as interquartile ranges (the P25, median, and P75).

Comparisons of clinical and biochemical parameters between non-obese control, non-obese CPP, and obese CPP girls.

Comparisons of clinical and biochemical parameters between non-obese control, non-obese CPP, and obese CPP girls are shown in Fig 1.

BW, BMI, and height percentiles, Tanner stage, and basal LH levels were significantly higher in non-obese

TABLE 1. Clinical characteristics of non-obese control, non-obese CPP, and obese CPP girls.
P25 = 25th percentile, Med = median, P75 = 75th percentile

Variables	Controls Non-obese (n=29)			CPP Non-obese (n=20)			CPP Obese (n=6)		
	P25	Med	P75	P25	Med	P75	P25	Med	P75
Age (year)	8.1	8.4	8.9	7.8	7.8	8.8	7.3	8.2	8.6
Fat mass (kg)	4.3	5.2	8.1	3.0	3.0	8.2	9.2	11.4	16.0
Fat-free mass (kg)	22.4	23.4	27.6	20.8	20.8	25.8	23.7	28.9	35.2

TABLE 2. Comparisons of clinical and biochemical parameters between non-obese control, non-obese CPP, and obese CPP girls.

Variables	Controls Non-obese (n=29) Mean±SEM	CPP Non-obese (n=20) Mean±SEM	CPP Obese (n=6) Mean±SEM
BW percentile	33.7±6.0	62.3±6.6**	98.0±0.9***,#
BMI percentile	40.8±6.5	64.9±6.0**	99.0±0.4***,#
Percent fat	18.6±1.8	18.9±1.6	30.0±1.9**,##
Height percentile	27.5±4.9	57.3±6.6**	77.7±11.4***
Tanner stage	1.3±0.1	2.9±0.1***	2.8±0.2***
Bone age (year)	-	10.9±0.2	11.0±0.3
Bone age difference from chronological age (year)	-	2.5±0.2	3.0±0.1#
SBP percentile	63.1 ±5.6	59.0 ±6.0	67.2±9.3
DBP percentile	68.9 ±4.4	53.9 ±5.5*	66.2±7.0
Average daily caloric intake (kcal)	1149.4±53.7	1311.4±71.1	1474.3±138.8*
Resting metabolic rate (kcal)	1080.4±25.5	1105.5±24.9	1283.3±84.8**,##
Birth weight (g)	3011.1±97.9	2952.3±104.3	3063.3±154.3
Maternal age of menarche (year)	13.7±0.6	12.5±0.3	12.7±1.0
Mid parental height (cm)	157.2±1.1	154.5±0.8	156.8±1.2
Paternal height (cm)	168.2±1.4	164.7±1.5	168.0±1.8
Maternal height (cm)	158.2±1.1	157.4±1.3	158.5±2.3
Total cholesterol (mg/dl)	175.3±6.3	171.7±8.6	188.5±16.8
HDL-cholesterol (mg/dl)	63.4±2.1	57.5±2.8	56.2±6.9
LDL-cholesterol (mg/dl)	96.7±5.2	102.3±5.3	115.9±13.1
Triglyceride (mg/dl)	76.0±6.3	78.8±8.2	73.3±32.7
Basal LH (mIU/ml)	0.3±0.1	2.0±0.6**	2.1±0.8*
Basal FSH (mIU/ml)	2.7±0.3	6.4±1.7**	4.1±0.7
Glucose (mg/dl)	82.2±1.1	81.6±1.2	82.7±3.6
Insulin (µU/ml)	10.9±1.7	11.5±2.2	13.9±2.2
HOMA-IR	2.3±0.4	2.3±0.4	2.8±0.4
QUICKI	0.36±0.008	0.35±0.006	0.33±0.01

*compared to control girls: *p<0.05, **p<0.01, ***p<0.001
#compared to CPP non-obese girls: # p<0.05, ## p<0.01

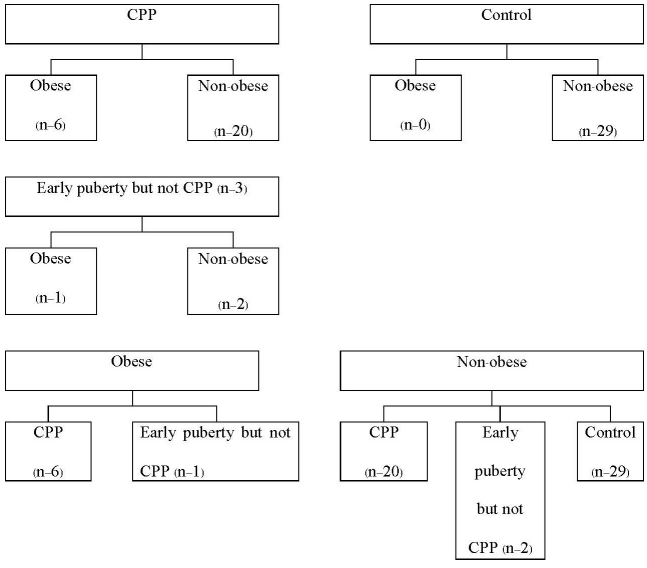


Fig 1. Subject allocation

CPP compared to non-obese control girls, as well as in obese CPP compared to non-obese control girls (all p<0.01). Basal FSH was significantly higher (p<0.01) while DBP percentile was significantly lower (p<0.05) in non-obese CPP compared to non-obese control girls. Maternal age of menarche (p=0.067) and midparental height (p=0.052) tended to be lower in non-obese CPP compared to non-obese control girls. Non-obese control girls tended to have lower average caloric intake compared to non-obese CPP girls (p=0.074) and had significantly lower average caloric intake compared to obese CPP girls (p<0.05). Percent fat and resting metabolic rate were significantly higher in obese CPP girls compared to non-obese CPP and control girls (all p<0.01). Bone age differences were significantly higher in obese CPP girls compared to non-obese CPP girls (p<0.05). Insulin, the HOMA-IR, the QUICKI, fasting glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride levels were not different between all groups.

Correlations of insulin, the HOMA-IR, and the QUICKI with clinical and biochemical parameters in CPP and control girls.

Correlations of insulin, the HOMA-IR, and the QUICKI with clinical and biochemical parameters in CPP and control girls are shown in Table 3.

Insulin and HOMA-IR had positive correlations with height and BW percentiles in both control (R≈0.6-0.7) and CPP girls (R≈0.4-0.5) and had positive correlation with BMI percentile, percent fat, fat mass, fat-free mass, SBP and/or DBP percentiles only in control girls (R≈0.4-0.8) (all p<0.05). Insulin had positive correlations with triglycerides, basal LH, and basal FSH (R≈0.4-0.7), but was negatively correlated with HDL-cholesterol only in CPP girls (R≈-0.4) (all p<0.05).

The QUICKI had negative correlations with height percentile and fat free mass in control (R≈-0.6-0.7) and CPP girls (R≈-0.4-0.5) and had negative correlations with BW and BMI percentiles, percent fat, fat mass, and SBP percentile only in control girls (R≈-0.4-0.7) (all p<0.05). The QUICKI had negative correlations with basal LH and basal FSH (R≈0.4-0.5) (all p<0.05).

Multivariate analysis of insulin, the HOMA-IR, and the QUICKI in the CPP girls and controls.

Multivariate analysis of insulin, the HOMA-IR, and the QUICKI in the CPP girls and control girls are shown in Table 4.

Insulin, which was set as the dependent variable, and showed significant associations with FSH (model 1) or FSH and fat-free mass (model 2) or LH (model 3) in CPP girls, and with fat mass in controls (all p<0.01). The HOMA-IR showed significant interactions with LH (model 1) or FSH (model 2) or FSH and fat-free mass (model 3) in CPP girls, and with fat mass in controls (all p<0.01). The QUICKI showed significant interactions with fat-free mass (model 1) or FSH (model 2) or fat-free mass and FSH (model 3) or LH (model 4) or LH and fat-free mass (model 5) in CPP girls, and with fat-free mass in controls (all p<0.05).

DISCUSSION

This study examined comparisons between CPP and age-matched control girls with subgroup analysis of obese and non-obese girls.

In our study, BMI, BW, and height percentiles were significantly higher in non-obese CPP girls and in obese CPP girls compared with non-obese controls. However, percent fat, the lipid profile, and glucose levels were not significantly different among non-obese CPP and non-obese controls. The higher BMI, BW, and height percentiles in CPP girls could be explained by increased growth velocity during pubertal progression. Average daily caloric intake was increased significantly in obese CPP girls, and also tended to increase in non-obese CPP girls, supporting that nutritional status could be associated with early onset of puberty. Maternal age of menarche and mid-parental height tended to be lower in non-obese CPP girls compared with non-obese controls, suggesting that genetic determination was associated with CPP, especially via a maternal relation. However, these factors were not different between obese CPP and non-obese controls, which might be due to the small number of subjects (n=6).

TABLE 3. Correlations of insulin, the HOMA-IR, and the QUICKI with clinical and biochemical parameters in CPP and control girls.

Variables	Controls (n=29) R	CPP (n=26) R
Insulin - height percentile	0.650***	0.476*
Insulin - BW percentile	0.702***	0.488*
Insulin - BMI percentile	0.627***	0.286
Insulin - percent fat	0.636***	0.089
Insulin - fat mass	0.799***	0.165
Insulin - fat-free mass	0.762***	0.352
Insulin - SBP percentile	0.476**	-0.166
Insulin - DBP percentile	0.368*	0.010
Insulin - triglycerides	0.111	0.401*
Insulin - HDL-cholesterol	-0.201	-0.400*
Insulin - basal LH	0.224	0.621**
Insulin - basal FSH	0.128	0.708***
HOMA-IR - height percentile	0.630***	0.485*
HOMA-IR - BW percentile	0.671***	0.504**
HOMA-IR - BMI percentile	0.597**	0.291
HOMA-IR - percent fat	0.615**	0.099
HOMA-IR - fat mass	0.783***	0.178
HOMA-IR - fat-free mass	0.736***	0.376
HOMA-IR - SBP percentile	0.449*	-0.154
HOMA-IR - triglyceride	0.087	0.399*
HOMA-IR - HDL-cholesterol	-0.197	-0.412*
HOMA-IR - basal LH	0.211	0.661***
HOMA-IR - basal FSH	0.114	0.732***
QUICKI - height percentile	-0.632***	-0.519*
QUICKI - BW percentile	-0.739***	-0.338
QUICKI - BMI percentile	-0.716***	-0.268
QUICKI - percent fat	-0.614**	-0.104
QUICKI - fat mass	-0.660***	-0.222
QUICKI - fat-free mass	-0.724***	-0.475*
QUICKI - SBP percentile	-0.469*	0.254
QUICKI - basal LH	-0.269	-0.578**
QUICKI - basal FSH	-0.128	-0.491*

*p<0.05, **p<0.01, ***p<0.001

TABLE 4. Multivariate analysis of insulin, the HOMA-IR, and the QUICKI in the CPP girls and controls.

Dependent variable	R	Model R2	p value		Coefficient	Standard error	t value	p value
Insulin of CPP	0.708	0.501	<0.001***	Constant	5.901	2.040	2.893	0.009
model 1				FSH	1.049	0.234	4.481	<0.001***
Insulin of CPP	0.759	0.576	<0.001***	Constant	-8.064	7.668	-1.052	0.306
model 2				FSH	0.997	0.218	4.582	<0.001***
				Fat-free mass	0.559	0.298	1.873	0.076
Insulin of CPP	0.621	0.386	0.001**	Constant	7.641	1.801	4.243	<0.001***
model 3				LH	2.193	0.565	3.884	0.001**
Insulin of Controls	0.799	0.638	<0.001***	Constant	1.105	1.983	0.557	0.583
				Fat mass	1.779	0.274	6.499	<0.001***
HOMA-IR of CPP	0.661	0.437	<0.001***	Constant	1.490	0.344	4.337	<0.001***
model 1				LH	0.465	0.108	4.316	<0.001***
HOMA-IR of CPP	0.732	0.535	<0.001***	Constant	1.151	0.390	2.948	0.008**
model 2				FSH	0.215	0.045	4.799	<0.001***
HOMA-IR of CPP	0.789	0.623	<0.001***	Constant	-1.856	1.473	-1.261	0.223
model 3				FSH	0.203	0.042	4.867	<0.001***
				Fat-free mass	0.121	0.058	2.107	0.049*
HOMA-IR of Controls	0.783	0.614	<0.001***	Constant	0.053	0.467	0.114	0.910
				Fat mass	0.399	0.065	6.176	<0.001***
QUICKI of CPP	0.475	0.226	0.014*	Constant	0.415	0.027	15.256	<0.001**
model 1				Fat-free mass	-0.003	0.001	-2.645	0.014*
QUICKI of CPP	0.491	0.241	0.017*	Constant	0.357	0.007	52.054	<0.001***
model 2				FSH	-0.002	0.001	-2.582	0.017*
QUICKI of CPP	0.660	0.436	0.003*	Constant	0.421	0.025	16.773	<0.001***
model 3				Fat-free mass	-0.003	0.001	-2.627	0.016*
				FSH	-0.002	0.001	-2.559	0.019*
QUICKI of CPP	0.578	0.334	0.002**	Constant	0.356	0.005	65.456	<0.001***
model 4				LH	-0.006	0.002	-3.466	0.002**
QUICKI of CPP	0.678	0.460	0.001**	Constant	0.409	0.023	17.560	<0.001***
model 5				LH	-0.005	0.002	-3.162	0.004**
				Fat-free mass	-0.002	0.001	-2.323	0.029*
QUICKI of Controls	0.724	0.524	<0.001***	Constant	0.552	0.039	14.000	<0.001***
				Fat-free mass	-0.009	0.002	-5.144	<0.001***

*p<0.05, **p<0.01, ***p<0.001.

Percent fat and resting metabolic rate were significantly higher in obese CPP girls compared to non-obese CPP and control girls, so these factors were specifically associated with obesity status. Furthermore, bone age difference from chronological age was significantly increased in the obese CPP girls compared to non-obese CPP girls. This finding could be explained by the effect of obesity on early onset of puberty.

Insulin, the HOMA-IR, and the QUICKI were not significantly different between girls with CPP and controls. However, insulin and the HOMA-IR were positively correlated with anthropometric parameters including height, BW, and BMI percentiles, percent fat, fat mass, and fat-free mass mainly in the control group. Insulin, the QUICKI, and the HOMA-IR were correlated with basal LH and FSH levels in the CPP group, but not in the control group. Multivariate regression analysis showed that insulin and the HOMA-IR were associated with fat-free mass and reproductive indices, including LH and FSH levels in girls with CPP, and with fat mass in controls. The QUICKI, which is an index of insulin sensitivity, was associated with fat-free mass in girls with CPP and controls, as well as with reproductive indices, including LH and FSH levels, in girls with CPP. These results suggested that insulin sensitivity and insulin resistance were related to anthropometric parameters in the prepubertal stage, but were more strongly associated with FSH and LH during pubertal advancement. Furthermore, it could be interpreted that insulin action in girls with CPP might be more related to reproductive regulation rather than its anabolic action. One possible underlying mechanism of these results could be that insulin has a central effect to stimulate reproduction in the pubertal stage.[23,24]

In the control group, not in the CPP group, insulin and the HOMA-IR were positively, but the QUICKI was negatively correlated with SBP and/or DBP percentiles. The positive correlation between insulin and blood pressure in control girls could be explained by the fact that insulin could increase renal sodium reabsorption leading to hypertension.[25] However, this association was not observed in the CPP girls, suggesting that the physiologic action of insulin might be changed during pubertal development.

In CPP girls, but not in control girls, insulin levels and the HOMA-IR showed positive correlations with triglyceride levels and negative correlations with HDL-cholesterol levels. These findings suggested that protective effects on the cardiovascular system and metabolism were decreased, while insulin levels and the HOMA-IR were elevated in girls with CPP. Another interpretation of our findings is that during initiation of pubertal development, glucose, insulin, adiposity, and blood pressure are not directly associated with their main peripheral function of metabolic and blood pressure regulations, but might be related to reproductive regulation. Insulin resistance could also be predisposed by genetic factors with the presence of a family history of type 2 DM. However, in our study, the family history of type 2 DM was not obtained from each subject.

The strengths of this study were as follows. 1) This study compared girls with CPP and controls. We also dissected out the effect of obesity associated with early puberty by subgroup analysis of comparisons between non-obese control, non-obese CPP, and obese CPP girls. 2) This study examined correlations and multivariate analyses of insulin, the HOMA-IR, and the QUCKI with clinical and biochemical parameters with subgroup analysis of CPP and control girls. Using this method, the role of factors associated with each pubertal status could be differentiated. However, there are some limitations of the study as follows. 1) The number of recruited obese subjects was low in the CPP group (n=6) and absent in the control group, so this might have led to flawed analysis. 2) Some parameters such as daily caloric intake calculated from food records, maternal age of menarche, and parental heights were reported from recall memory of the parents, so there could be some limitations of the data accuracy.

CONCLUSION

Girls with CPP had higher BW, BMI, and height percentiles, with a trend of lower maternal age of menarche and mid-parental height compared to controls. Insulin levels and the HOMA-IR were associated with anthropometric data in controls, but were more strongly associated with reproductive parameters in girls with CPP. Many correlations and multivariate associations between insulin, the HOMA-IR, and the QUICKI with anthropometric, metabolic, BP, and reproductive parameters in CPP girls were different from controls, indicating that these factors changed during pubertal development. During initiation of pubertal development, glucose, insulin, and adiposity might not be directly associated with their main peripheral function of metabolic regulation, but could be related to other functions involved in reproductive regulation.

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Improving International Standards in Surgical and Procedural Training through Comparative Operative Log Growth Charts

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ABSTRACT

Objective: To use these growth charts to critically assess sufficiency of cases and parity of cases between residents, and to compare these growth charts to available international standards for minimum case numbers.

Methods: Operative Log Growth Charts were developed for key indicator procedures for graduating otolaryngology residents in 2012-2014 at a large teaching hospital in the capital city of a newly industrialized country. Comparisons were made between years of training and required minimum case numbers published by the ACGME RRC for Otolaryngology.

Results: Data was available to create 7 key indicator operative log growth charts to include all available data from 2012-2014 residents. These growth charts were used to assess growth in operative procedures for residents in the program compared to historical norms in the program. Graduating residents surpassed ACGME minimum case numbers in Bronchoscopy only and were below the minimum numbers for the other key indicators tested.

Conclusion: There is significant heterogeneity in the standards for otolaryngology training between countries. It is possible to develop program-specific and country-specific operative log growth charts.

Keywords: Minimum case numbers; key indicator cases; surgical education (Siriraj Med J 2018;70: 123-126)

INTRODUCTION

Anecdotally, there is significant variance in case exposure between training programs on an international level. However, little data exists to quantify such differences in otolaryngology training. While case numbers are not a proxy for quality of education, a minimum exposure to certain key procedures may serve as a necessary baseline target. In recent years, the ability to obtain the Accreditation Council for Graduate Medical Education (ACGME)[1] international standardization offers a step towards the development of a minimum international standard for residency training.

Programs interested in improving case exposure must meticulously document and analyze resident operative logs. We offer one method for documenting case exposure over time, similar to a pediatric growth chart. Comparison can be made to the minimum case numbers for otolaryngology residency training published by the ACGME.

This study addresses the comparative standard minimum operative case numbers from the ACGME to the operative log growth charts for general otolaryngology training at Siriraj Hospital Mahidol University for 2012-2014 graduating residents.

MATERIALS AND METHODS

The sample of this study included the data from the educational division of the department of

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Otorhinolaryngology. This study included the operative log book of the residents who attended Otorhinolaryngology specialty residency training program in Siriraj Hospital, Thailand and graduated between 2012 and 2014 academic years. The permission to use the data was granted by the Department of Otorhinolaryngology, Faculty of Medicine Siriraj Hospital, Mahidol University. The data was collected regarding the total operative cases of residents in each year and divided by the number of residents per year to obtain an average case number for all available key indicator procedures by post-graduate year of training.

The Operative Log growth Charts[2] were graphed for each key indicator procedure using a curve of best fit and compared to the published minimum case numbers published by the ACGME for each procedure.

Key indicator data was available for Parotidectomy, Neck Dissection, Thyroidectomy, Mastoidectomy, Tympanoplasty, Endoscopic Sinus Surgery (Ethmoidectomy), and Bronchoscopy. Thus seven operative log growth chart graphs were developed.

In order to describe conditions between Thailand and US surgical education, the number of operative cases during residency training was used as an indicator of surgical case exposure. Seven graphs have been created to compare the operative cases between Thailand and the US program of residency training in seven main categories.

Statistical analysis

Analysis of data in the study involved a variety of descriptive and inferential statistics.

RESULTS

The seven graphs were made based on the data that was obtained in Siriraj Hospital, Thailand from 2012 to 2014 compared with the required minimum numbers of operative cases in the US 2014 retrieved from committee for Otolaryngologist by Operative Log Growth Charts.

On each Operative Log Growth Chart[3], the vertical axis represents the number of operative cases as primary surgeon or supervising surgeon, and the horizontal axis represents the post-graduate year of residency training. The average numbers of operative cases in each year of Siriraj Hospital residents were plotted for each of the three graduate classes (2012-2014). In comparison with the minimum cases for graduating residents in the US program, deficiencies in several key indicator procedures was readily noted (Figs 1-7). There was

TABLE 1. The required minimum cases of all Key Indicator Procedures for graduating residents US program, 2014.[1]

Category	Procedure	Min #
KEY INDICATOR: Head & Neck	Parotidectomy (all types)	15
	Neck Dissection (all types)	27
	Oral Cavity Resection	10
	Thyroid/Parathyroidectomy	22
KEY INDICATOR: Otology/Audiology	Tympanoplasty (all types)	17
	Mastoidectomy (all types)	15
	Stapedectomy/Ossiculoplasty	10
KEY INDICATOR: FPRS	Rhinoplasty (all types)	8
	Mandible/Midface Fractures	12
	Flaps and Grafts	20
KEY INDICATOR: General/Peds	Airway – Pediatric and Adult	20
	Congenital Neck Masses	7
	Ethmoidectomy	40
	Bronchoscopy	22

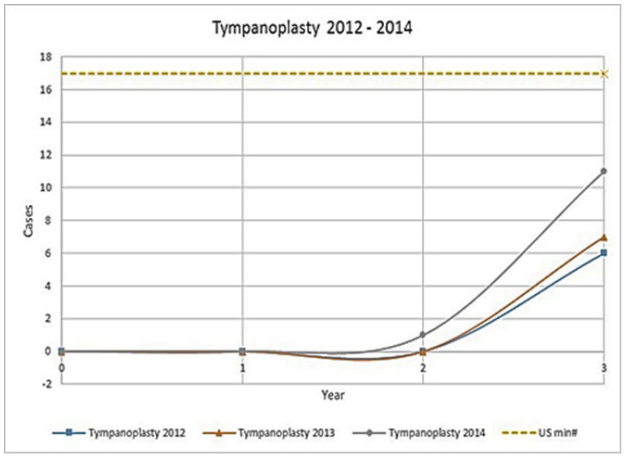


Fig 1.

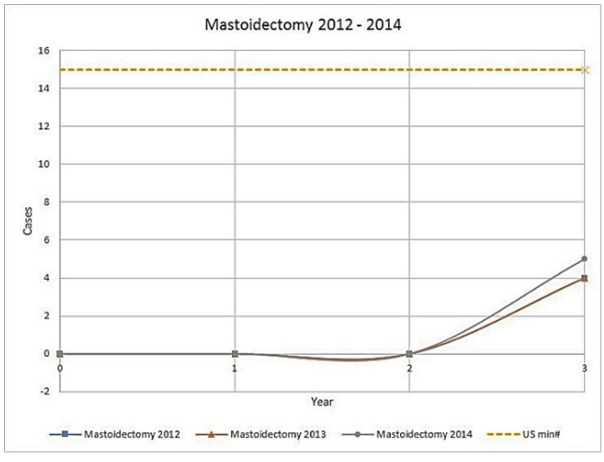


Fig 2.

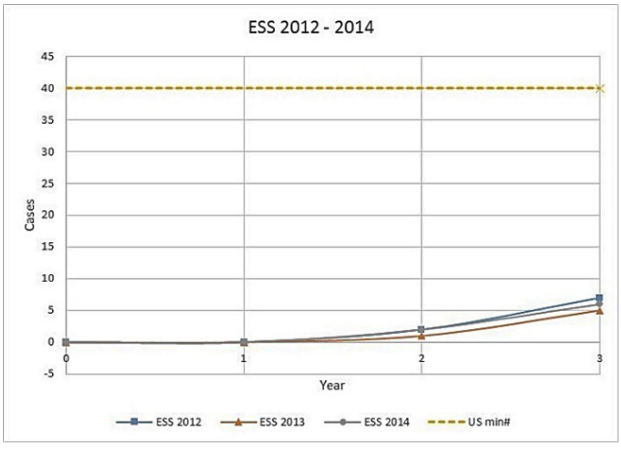


Fig 3.

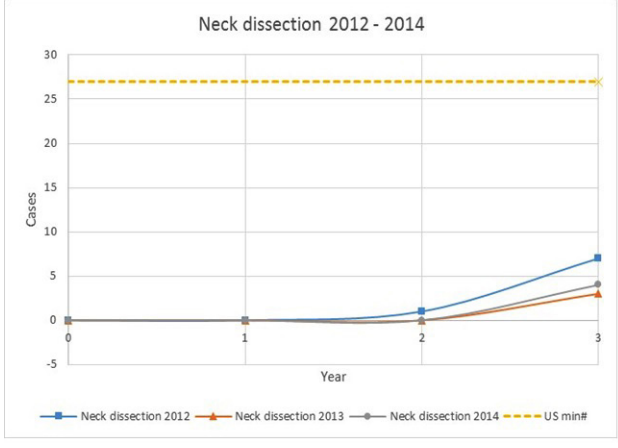


Fig 4.

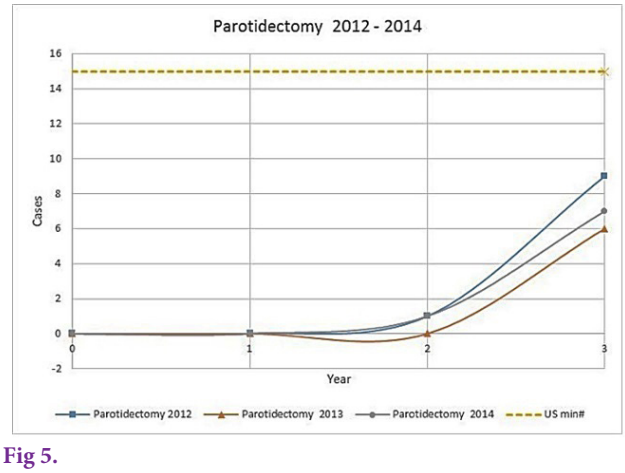


Fig 5.

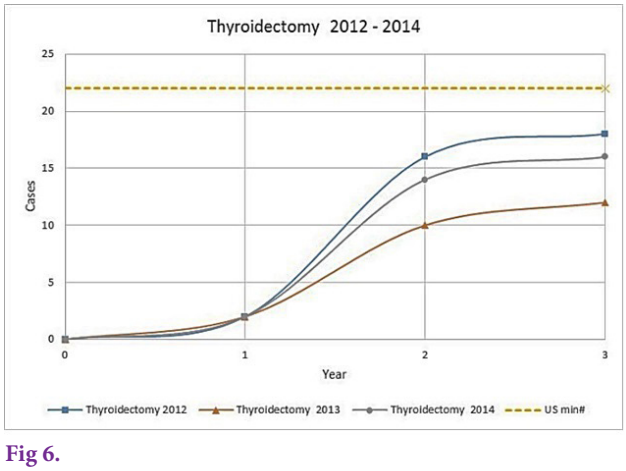


Fig 6.

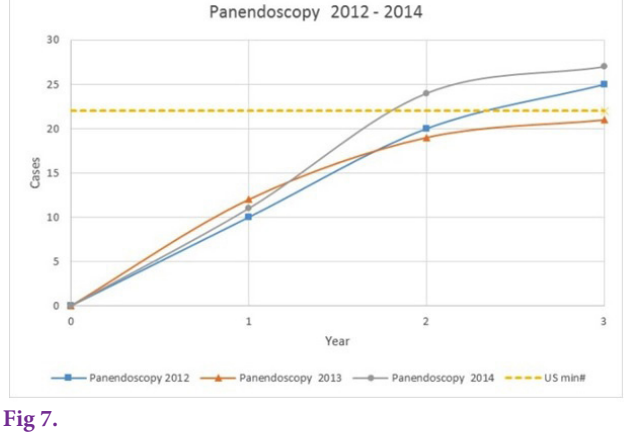


Fig 7.

Figs 1-7. The seven graphs were made based on the data that obtained in Siriraj Hospital, Thailand from 2012-2014 comparing with the required minimum numbers of operative cases in the US 2014 retrieving from committee for Otolaryngologist by Operative Log Growth Charts.

a lack of 6 cases in Tympanoplasty, 10 in Mastoidectomy, 34 in ESS, 20 in Neck Dissection, 6 in Parotidectomy, 4 in Thyroidectomy, and no difference in Bronchoscopy.

There were statistically significant differences between the operative cases of Siriraj Hospital medical school and the US program minimum in all key indicator cases except Bronchoscopy.

Examination of the mean and a one-sample t-test ($\alpha = .05$) indicated important differences between the operative cases of Siriraj Hospital medical school and the US program except for Panendoscopy (Bronchoscopy) category.

DISCSUSSION

Quantitative comparison of key indicator cases between international programs provides an essential starting point for improving international standards in otolaryngology training. Such a comparison provides an opportunity to consider methods for increasing case numbers, such as expanding the residency program to include other clinical sites or decreasing resident complement.

Developing program specific Operative Log Growth Charts may allow real-time monitoring of such changes. There is significant heterogeneity in the standards for otolaryngology training between countries. This project demonstrates that it is possible to develop program-specific and country-specific operative log growth charts.

While case exposure is not the equivalent of educational quality, a minimum exposure is essential for training. These growth charts may be used within a program to assess individual resident progress, to assess the effect of program changes such as training length and resident complement in real-time, and to compare case exposure to standards in other countries.

Partnerships between established programs in industrialized countries and those in newly industrialized countries may serve a valuable resource for both partners.

In addition to the monitoring of program changes, Operative Log Growth Charts may be used within a program to assess resident progress, parity of cases, and sufficiency of cases.

Similar growth chart comparisons may be made for other surgical specialties or medical specialties with minimum procedure numbers published by the ACGME.

CONCLUSION

Development of operative log growth charts allows for simple visualization of surgical case exposure over time. Comparisons can be made to historical experience within a program or to other national and international standards. We hope that this type of comparison may serve as a model for making program improvements on an international level.

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Identification of Uncommon Pathogenic Bacteria by MALDI-TOF Mass Spectrometry Using a Custom Library of Siriraj Hospital

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ABSTRACT

Objective: Matrix Assisted Laser Desorption Ionization Time-Of-Flight mass spectrometry (MALDI-TOF MS) is a rapid and cost-effective technique that serves as an alternative method for bacterial identification in microbiology laboratories. This study aimed to determine the performance of MALDI-TOF MS for identifying uncommon and/or fastidious bacteria comparing with automated identification method.

Methods: We observed the performances of original and extended databases after adding of in-house MALDI-TOF MS database for a total of 54 isolates belonging to 16 genera of uncommon and/or fastidious bacteria.

Results: The extended database improved identification of these bacteria significantly except for *Listeria monocytogenes* and *Elizabethkingia meningoseptica*.

Conclusion: This study provides a possibility for identification of uncommon and/or fastidious bacteria by MALDI-TOF MS method.

Keywords: Mass spectrometry; MALDI-TOF; fastidious bacteria; identification (Siriraj Med J 2018;70: 127-130)

INTRODUCTION

Rapid and accurate identification of bacteria can be challenging in a routine laboratory. Conventional methods provide standard results, but they are time consuming and require complicated procedures.[1] The automated Vitek 2 (bioMérieux. Marcy l'Etoile, France) provides identification result faster than conventional method, which is approximately 4-8 hours. However, misidentification may still occur.[2] Thus, molecular techniques, particularly 16S rDNA gene sequencing, have been developed as standard methods for bacteria identification.[3] Recently, MALDI-TOF MS has been developed as a rapid bacterial identification method, taking only 6-8.5 minutes for direct transfer method[4] and only 45 minutes for formic acid extraction method.[5] Several studies have utilized MALDI-TOF MS for identifying Gram-positive and Gram-negative bacteria[6] including, difficult-to-identify or uncommon bacteria.[7,8] Previous studies reported that, after using a combined in-house and original database, the identification rates have been improved.[9,10] Thus, the development of in-house database provided a useful tool for bacterial identification. The aim of this study was to evaluate the ability of a combined Bruker's MALDI-TOF MS database (Bruker Daltonics, Helios, Singapore) and our in-house database (extended database) for species differentiation among uncommon and/or fastidious bacteria.

MATERIALS AND METHODS

Generation of a combined MALDI-TOF MS database

To improve the MALDI-TOF database, uncommon

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and/or fastidious bacterial species to be included in the MALDI-TOF MS database were selected from uncommon and/or fastidious bacterial species isolated at the Faculty of Medicine Siriraj Hospital, Mahidol University (Bangkok, Thailand). According to their availability, between one and three isolates of each species were used. This study was approved by the Siriraj Institutional Ethical Review Board (Si 147/2013). A total of 54 bacterial isolates belonging to 20 species within 16 genera were added to the library including both Gram-positive and Gram-negative bacteria, namely: *Abiotrophia defective* (n=3), *Aggregatibacter actinomycetemcomitans* (n=2), *Campylobacter fetus* (n=3), *Chryseobacterium gleum* (n=3), *Corynebacterium jeikeium* (n=3), *Eikenella corrodens* (n=2), *Elizabethkingia meningoseptica* (n=3), *Enterococcus casseliflavus* (n=3), *Enterococcus gallinarum* (n=3), *Gemella haemolysans* (n=3), *Granulicatella adiacens* (n=3), *Lactococcus formosensis* (n=3), *Leuconostoc lactis* (n=3), *Listeria monocytogenes* (n=3), *Providencia rettgeri* (n=3), *Providencia stuartii* (n=3), *Roseomonas mucosa* (n=1), *Vibrio fluvialis* (n=2), *Vibrio parahaemolyticus* (n=3), and *Vibrio vulnificus* (n=2). Identification of each isolate was confirmed by 16S rDNA sequencing according to previous publications.[11,12] MALDI-TOF MS was performed on a Microflex LT instrument following formic acid extraction according to the manufacturer's instructions (Bruker Daltonics, Bremen, Germany). In-house databases of the 55 isolates of uncommon and/or fastidious bacteria were constructed as previously described.[13] For each isolate, a minimum of 20 spectra were collected using Flexanalysis software version 3.0 (Bruker Daltonics) and used to construct a reference main spectrum profile for adding into an in-house database using the automated function of the BioTyper software (Bruker Daltonics). The in-house database was combined with the Bruker's database (5,627 database entries according to Bruker Daltonics version 3.0) and this combined database was used for subsequent analysis.

Comparisons of the identification efficacy between automated methods and MADI-TOF MS

MALDI Biotyper RTC (real-time classification) software was used for bacterial identification. The parameters were 40 shots/steps of laser beam and collection of 240 shots were employed. In this study, peaks with *m/z* ratios in the range of 4,000–15,000 were used for MALDI-TOF MS identification. As shown in a previous study, the performance of MALDI-TOF MS was increased when this peak range was used.[14] Bacterial protein spectra were analyzed using the combined database. An identification score of ≥ 2.000 and a score within the range 1.700 to 1.999 indicated identification of the species and genus levels, respectively. For automated identification, bacterial isolates were identified by the Vitek 2 system (bioMérieux) according to the manufacturer's instructions. Gram-positive (GP), Gram-negative (GN), anaerobes and *Corynebacterium* (ANC) and *Neisseria-Haemophilus* (NH) identification cards were used in this study. Results were accepted at the species level if scores of more than 93% probability were obtained.[15] Genus-level identification was accepted when results were less than or equal to 93% probability.

RESULTS

The extended database improved bacterial identification by MALDI-TOF MS in comparison to the original database. The extended database identified 15 more strains correctly to the species level (Table 1). This suggested the need for addition of an in-house database as the original (company) database is typically not optimized for endemic microbes of each region. A comparison between the automated method and extended database of MALDI-TOF MS showed that 10 species were identified correctly to the species level only by the MALDI-TOF MS. Eight species were identified correctly to the species level by both methods. *E. meningoseptica* and *L. monocytogenes* were identified to the species level only by automated method. Interestingly, *A. actinomycetemcomitans, C. jeikeium, E. casseliflavus, E. gallinarum* and *G. haemolysans* were specifically identified by MALDI-TOF MS, while automated identification method had limitations for these organisms. Automated method had low discriminatory power for identifications of *E. casseliflavus, E. gallinarum* and *G. haemolysans*. However, yellow pigment production and satellite test in combination with acid production enabled discrimination of *E. casseliflavus/E. gallinarum* and *G. haemolysans/G. sanguinis/G. adiacens*, respectively.[16] Thus, phenotypic test and biochemical testing should be considered to support species identification of automated method.

In contrast, *L. monocytogenes* was more appropriately identified using automated method than MALDI-TOF MS because MALDI-TOF MS technique was insufficient for identifying *Listeria* species. A previous study reported that *Listeria* species including *L. innocua, L. ivanovii, L. monocytogenes, L. seeligeri* and *L. welshimeri* were difficult to be differentiated by MALDI-TOF MS because they were closely related based on their spectra.[17] In addition, MALDI-TOF MS also showed difficulty in discriminating *E. meningoseptica* from other species within the same genus. However, this issue has been proposed to be solved by 10% differential rule. This rule

TABLE 1. Identification of 54 isolates by original and extended databases of MALDI-TOF MS.

Bacterial species	Level of identification (No. of isolates with species/genus/misidentification)		
	Automated method	MALDI-TOF MS original database	MALDI-TOF MS extended database
A. actinomycetemcomitans	0/2/0	1/1/0	**2/0/0
A. defectiva	3/0/0	1/2/0	3/0/0
C. fetus	3/0/0	1/2/0	3/0/0
C. gleum	0/3/0	2/1/0	**3/0/0
C. jeikeium	0/3/0	3/0/0	**3/0/0
E. casseliflavus	0/3/0	3/0/0	**3/0/0
E. corrodens	2/0/0	2/0/0	2/0/0
E. gallinarum	0/3/0	3/0/0	**3/0/0
E. meningoseptica	*3/0/0	0/1/2[a]	1/2/0
G. adiacens	3/0/0	3/0/0	3/0/0
G. haemolysans	0/3/0	3/0/0	**3/0/0
L. formosensis	0/3/0	0/0/3[b]	**3/0/0
L. lactis	0/3/0	0/3/0	**3/0/0
L. monocytogenes	*3/0/0	1/2/0	1/2/0
P. rettgeri	3/0/0	3/0/0	3/0/0
P. stuartii	3/0/0	3/0/0	3/0/0
R. mucosa	0/1/0	1/0/0	**1/0/0
V. fluvialis	0/2/0	0/1/1[c]	**2/0/0
V. parahaemolyticus	3/0/0	3/0/0	3/0/0
V. vulnificus	2/0/0	2/0/0	2/0/0
Total 54 isolates	28/26/0	35/13/6	50/4/0

Notes: MALDI-TOF MS (original database) misidentification results: a) *E. meningoseptica* as *E. miricola*, b) *L. formosensis* as *L. garvieae* and c) *V. fluvialis* as *V. furnissii*; * Species identified better with the automated method; **Species identified better with the extended database.

will exclude any genus or species that show difference values between the top best match and next closest scores of higher than 10%.[8,18] In this study, this rule was not able to be used for the discrimination of *Elizabethkingia* and *Listeria* species because they provided difference values less than 10%. Thus, the resolution of MALDI–TOF MS for those species should be of concern.

DISCUSSION

In summary, our study indicated that MALDI-TOF MS is a potential choice for identification of uncommon and/or fastidious bacteria. However, misidentification or low discrimination results were found for some species when identified by using MALDI-TOF MS. Thus, identification results should be carefully interpreted. Furthermore, some uncommon and/or fastidious bacteria including *Abiotrophia, Aggregatibacter, Gemella, Granulicatella* and *Leuconostoc* grew as tiny colonies. Therefore, in these cases formic acid extraction would be recommended for creating high quality of spectra. In contrast, the identification of the genera *Campylobacter, Chryseobacterium, Corynebacterium, Elizabethkingia, Enterococcus, Lactococcus, Listeria, Providencia, Roseomonas* and *Vibrio*, were reliable when identified by MALDI-TOF using direct transfer method which has been demonstrated (data not shown).

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One-Year Anatomical and Functional Outcomes of 302 Elevate Posterior and Apical Mesh Repairs

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ABSTRACT

Objective: POP occurs as a consequence of fibromuscular and endofascial weakness. Women with prolapse often have global support defects causing difficulty in identification and repair. Fascial plication and levatorplasty in rectocele repair can cause over-narrowing of vagina and introitus leading to dyspareunia. Mesh reinforcement is used to correct multiple defects while avoiding vaginal narrowing. Although literatures have reported encouraging results of vaginal mesh repair, the use of mesh in posterior compartment is still controversial. Elevate system® has been developed as a minimally invasive method for mesh augmentation. Our objective is to evaluate outcomes of Elevate Posterior and Apical mesh in the posterior and apical prolapse repair.

Methods: We conducted a prospective study of 302 women undergoing Elevate Posterior mesh reconstruction between 2009 and 2015. Demographic and peri-operative information were recorded. Pre- and post-operative evaluation included symptom, quality of life and POP-Q assessment. Differences in quality-of-life and POP-Q scores were demonstrated using paired t test. Objective cure rates, symptom resolution, quality-of-life measures and peri-operative morbidities were evaluated.

Results: Three-hundred women had posterior vaginal prolapse while 131 had apical descent. Mean operative time was 87±21 minutes. Bleeding complication occurred in 9 while mesh extrusion occurred in 3 women. Significant symptom and quality-of-life improvement were demonstrated at one year. Objective cure rates of Elevate Posterior mesh in the treatment of posterior vaginal and apical prolapse were 93.7% and 92.4% respectively.

Conclusion: Elevate Posterior and Apical mesh reconstruction has delivered impressive clinical outcomes for the treatment of posterior vaginal and apical prolapse.

Keywords: Complications; Elevate Posterior and Apical mesh; objective cure; pelvic organ prolapse; recurrence rates; success rate; surgical outcomes (Siriraj Med J 2018;70: 131-138)

INTRODUCTION

Pelvic organ prolapse (POP), defined as the descent of one or more of the anterior vaginal wall, the posterior vaginal wall, the uterus (cervix) or the vaginal vault or cuff after hysterectomy, often occurs as a consequence of multifactorial etiology which contributes to the weakness of the fibromuscular pelvic supports, endopelvic fascial defects and pelvic floor muscle denervation.[1] As a result, women with prolapse often have global pelvic support defects which may make clear identification of each individual defect and comprehensive defect-specific repair difficult to achieve.

In the posterior vaginal compartment, damage to the rectovaginal septum, which extends from the posterior aspect of the cervix and the cardinal-uterosacral ligaments to its attachment on the perineal body, can result in the formation of both rectocele and enterocele. Conventional surgical techniques aiming to support the vaginal vault

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or uterus, plicate the rectovaginal fascia, and re-attach the fascia to the vaginal apex have reported variable success rates.[2] Although defect-specific rectocele repair has demonstrated favorable anatomical results with improvement in dyspareunia[3], it is not always easy to identify and repair all defects in the rectovaginal fascia. Furthermore, traditional rectocele repair which includes midline rectovaginal fascial plication and levatorplasty, has been shown to cause over-narrowing of vaginal lumen and introitus leading to dyspareunia, rectal and sexual dysfunction.[4]

Although there are diametrically opposing views and inconclusive data to support the use of synthetic graft material in the posterior compartment repairs[5], a number of prospective series have reported encouraging outcomes of the mesh kit procedures in the treatment of posterior vaginal and apical prolapse.[6,7] The premise of mesh reinforcement of the rectovaginal septum is to overcome global pelvic floor defects without excision of vaginal wall, thus avoiding vaginal narrowing and reducing the risk of recurrence. One of the currently available mesh kit, Elevate Posterior and Apical System (American Medical Systems, Minnetonka, MN, USA), has been developed as a transvaginal, minimally invasive method for correction of posterior compartment defects (rectocoele/ enterocoele) and providing vault support. This is done through a single incision using a soft type I macroporous polypropylene mesh.

The objective of this study is to evaluate the clinical outcomes of the Elevate Posterior and Apical mesh system in the treatment of posterior vaginal and/or apical prolapse.

MATERIALS AND METHODS

After obtaining ethical approval from the Institutional Review Board of the University of Sydney, we conducted a single-center, prospective study of women undergoing Elevate Posterior and Apical mesh reconstruction at our institute, the Centre for Advanced Reproductive Endosurgery, between December 2009 and December 2015. Three hundred and two women who presented with symptomatic posterior vaginal and/or apical prolapse of at least stage II were enrolled. At initial visit, women were asked to complete the Prolapse Quality of Life (P-QOL) questionnaire[8] to assess the severity of urogenital symptoms and how much their symptoms were affecting their quality of life. A four-point scoring system ranging from 0 ("none/not at all"), through 1 ("slightly/a little") and 2 ("moderately") to 3 ("a lot") was used to rate each quality of life domain including prolapse impact, role, physical and social limitations, personal relationships, emotional problems, sleep/energy disturbance and measurement of symptom severity. A higher total score indicated a greater impairment of quality of life, whereas a lower total score determined a better quality of life.

Following symptom and quality of life assessment, the stage and location of the prolapse were then defined according to the Pelvic Organ Prolapse Quantification (POP-Q) system.[9] Preoperative urodynamic studies were carried out when indicated. All women were provided with information regarding details of the surgical procedures, general surgical risks, risks associated with general anesthesia and transvaginal mesh complications, including the 2008 and 2011 FDA Safety Communications[10], before giving informed consent. Placement of the Elevate Posterior and Apical mesh and other concomitant procedures were performed by or under the supervision of the senior surgeon. Prophylactic intravenous antibiotic was administered at the induction of anesthesia. Operative time, intra-operative blood loss, early- and late-onset peri-operative complications as well as postoperative adverse events were recorded. Postoperative follow-up was scheduled at 6 weeks, 1 year and 5 years, or any adhoc time should there be any unexpected concerns. Each follow-up visit evaluation included reassessment of symptom, quality of life, POP-Q measurements and mesh-related complications.

The Elevate Posterior and Apical mesh system is composed of a central graft made of a soft Type I macroporous polypropylene mesh and two apical arms, each containing a small self-fixating tip which is anchored into the sacrospinous ligaments. Placement of the Elevate Posterior and Apical mesh was achieved using the standardized techniques outlined by the manufacturer. Pre-diluted local anesthetic solution, 0.5% Marcaine with Adrenaline 1:200,000, was infiltrated into the posterior vaginal wall. After making a vertical midline full thickness vaginal incision, lateral dissection was performed to free the rectum towards the ischial spines to reach the sacrospinous ligaments. Tissue overlying the sacrospinous ligaments was swept medially. Using the applicator device, each apical mesh arm was fixed to the ipsilateral sacrospinous ligament about 1.5-2.5 cm medial to the ischial spine. The mesh arms were then threaded through the central mesh lateral eyelets. Delayed absorbable suture was used to secure the mesh without tension to the vaginal apex or posterior cervix proximally, and to the lateral vaginal fornices and anorectal junction distally. After proper positioning of the mesh without tension, the self-locking button was slid down each mesh arm until desired tautness was obtained. Both mesh arms were then trimmed at 1-2 cm distal to the locking buttons. The excess portion of the

mesh was removed to suit the vaginal length. Per rectal examination was done to confirm rectal wall integrity and the absence of mesh through rectal wall. Closure of the vaginal incision was completed using delayed absorbable suture without trimming of redundant vaginal skin. Additional reconstructive and anti-incontinence procedures were carried out as indicated, either prior or after mesh placement. An indwelling catheter and vaginal pack were left in place for up to 24 hours.

Statistical analysis

All statistical data were analyzed using the Statistical Packages for the Social Sciences Version 18 for Windows (PASW statistic18). Continuous variables were expressed as means and standard deviations whereas categorical inputs were presented as numbers and percentages. Differences in P-QOL scores and POP-Q measurements were demonstrated using paired Student's t test. The P-value of less than 0.05 indicated statistical significance. The objective cure, which was defined according to the NICHD Pelvic Floor Disorders Network recommendations[11] as points Ap, Bp and C less than or equal to 0 cm, was considered as our primary outcome. Secondary outcomes were composed of (i) subjective resolution of prolapse symptoms, (ii) changes in bladder, bowel and sexual functions, as well as changes in P-QOL scores, and (iii) peri- and post-operative morbidities. Treatment success was defined as: 1) the posterior vaginal and/or apical descent at or above hymenal level 2) absence of bulge symptoms and 3) absence of re-treatment.

RESULTS

A total of 302 women had Elevate Posterior and Apical mesh implantation between December 2009 and December 2015. Information regarding baseline characteristics was displayed in Table 1. Most women (82.5%) had reached menopause. The mean age was 60.7 ± 11.3 years, the mean body mass index (BMI) was 26.6 ± 6.0 kg/m^2 and the average parity was 2.7 + 1.1. One-third (32.1%) was post-hysterectomy patients. Previous reconstructive procedures were categorized as native tissue repair (19.5%), mesh repair (2.6%) and anti-incontinence surgery (9.3%). More than half of women (54.6%) had previously tried pelvic floor muscle exercise without success. Vaginal estrogen was prescribed for preoperative use in 47% of postmenopausal women whereas 62% were given postoperatively. Of 169 women undergoing preoperative urodynamic studies, almost half (45%) were diagnosed with urodynamic stress incontinence.

Other clinical manifestations included overactive bladder symptoms, stress incontinence, voiding difficulty,

TABLE 1. Baseline characteristics (N = 302).

Variables	Mean ± SD or N (%)
Age (years)	60.7 ± 11.3
BMI (kg/m^2)	26.6 ± 6.0
Parity	2.7 ± 1.1
Menopause	249 (82.5)
Previous hysterectomy	97 (32.1)
Abdominal	52 (17.2)
Vaginal	31 (10.3)
Laparoscopic	14 (4.6)
Past native tissue repair	59 (19.5)
Anterior repair	44 (14.6)
Posterior repair	41 (13.6)
Vaginal vault suspension	5 (1.7)
Manchester operation	2 (0.7)
Laparoscopic pelvic floor repair	7 (2.3)
Past mesh repair	8 (2.6)
Anterior vaginal mesh	6 (2.0)
Posterior vaginal mesh	2 (0.7)
Sacrocolpo/hysteropexy	4 (1.3)
Past anti-incontinence surgery	28 (9.3)
Mid-urethral sling	5 (1.7)
Colposuspension	18 (6.0)
Others	5 (1.7)
Pessary use	30 (9.9)
Vaginal estrogen	189 (62.6)
Pre-operative use	35 (11.6)
Post-operative use	71 (23.5)
Pre- and post-operative use	83 (27.5)
Preoperative PFMT	165 (54.6)
Urodynamic diagnoses	169 (56.0)
USI	76 (25.2)
DO	36 (11.9)
Voiding dysfunction	52 (17.2)

Abbreviations: BMI= body mass index; PFMT = pelvic floor muscle training; USI = urodynamic stress incontinence; DO = detrusor overactivity

Data presented as mean ± standard deviation (SD) or number (%)

constipation, difficult bowel evacuation, need for vaginal digitation and fecal incontinence (Table 2). Half of those (56.8%) who were still sexually active also complained of difficulty having sex. Three hundred out of 302 women (99.3%) were found to have posterior vaginal prolapse ≥ stage II and 131 women (43.4%) with apical descent ≥ stage II (Table 5).

The average operative time for all carried out procedures was 87 ± 21 minutes (range 30-180 minutes) and the mean blood loss was 135 ± 105 ml (range 10-500 ml) (Table 3). Hysterectomy was performed at the time of mesh placement in 13 cases (4.3%). Other concomitant procedures comprised anterior fascial colporrhaphy (36.8%), anterior vaginal mesh augmentation (51.7%), laparoscopic sacrocolpo/hysteropexy (1%), mid-urethral sling (26.2%) and cervicoplasty (5.3%). While intra-operative bleeding ≥ 500 ml occurred in 9 out of 302 cases (3%), none required blood transfusion. Bladder perforation was encountered in 3 women who had undergone concurrent Elevate Anterior and Apical mesh implantation for anterior vaginal prolapse. All were effectively treated with intra-operative watertight repair and extended catheterization.

Voiding difficulty was the most common early postoperative adverse event found in 15 women, 7 of whom had concurrent sling procedures. De novo stress incontinence was reported by 36 out of 302 women (11.9 %), 9 of whom required surgical treatment with mid-urethral sling, the remainders were managed conservatively. Other postoperative complications included de novo urge incontinence (5 %), deep vaginal or buttock pain (4.6 %), dyspareunia (2.3 %), groin pain (1.7 %) and deep venous thrombosis (0.3 %). Three cases (1 %) of vaginal mesh extrusion were discovered, one on the posterior upper and two on the posterior lower vaginal wall with a median onset of 151 days (range 130-190 days). Following conservative treatment, surgical mesh excision was eventually performed as day-procedure in 2 women.

TABLE 2. Clinical presentations for pelvic organ prolapse (N = 302).

Presenting symptoms	N (%)
Prolapse-related	301 (99.7)
Bulging	296 (98.0)
Dragging	181 (59.9)
Backache	76 (25.2)
Urinary	271 (89.7)
Stress incontinence	165 (54.6)
Overactive bladder	233 (77.2)
Voiding difficulty	194 (64.2)
Defecatory	227 (75.2)
Difficult evacuation	180 (59.6)
Constipation/straining	177 (58.6)
Vaginal digitation	63 (20.9)
Fecal urgency/incontinence	53 (17.5)
Sexual difficulty	121 (40.1)

Data presented as number (%)

TABLE 3. Surgical outcomes and peri-operative complications (N = 302).

Variables	Mean ± SD or n (%)
Estimated blood loss (ml)	135 ± 105
Operative time (minutes)	87 ± 21
Concomitant procedures	
Hysterectomy	13 (4.3)
Anterior vaginal repair	111 (36.8)
Anterior vaginal mesh	156 (51.7)
Sacrocolpo/hysteropexy	3 (1.0)
Mid-urethral sling	79 (26.2)
Cervicoplasty	16 (5.3)
Intra-operative complication	
Bleeding (≥ 500 ml)	9 (3.0)
Bladder injury	3 (1.0)
Post-operative complications	
Bleeding/hematoma	9 (3.0)
Voiding difficulty	15 (5.0)
Fever	3 (1.0)
Urinary tract infection	12 (4.0)
Wound infection	1 (0.3)
de novo SUI	36 (11.9)
de novo urge	15 (5.0)
Deep vaginal/buttock pain	14 (4.6)
Groin pain	5 (1.7)
Dyspareunia	7 (2.3)
DVT/PE	1 (0.3)
Mesh extrusion	3 (1.0)

Abbreviations: SUI = stress urinary incontinence; DVT = deep venous thrombosis; PE = pulmonary embolism
Data are presented as mean ± standard deviation or number (%)

The mean follow-up time was 28 months, ranging from 1 month up to 5.9 years. A total of 292 women (96.7 %) returned for 6-week postoperative visit. Those who missed the early postoperative follow-up ultimately returned at one year. Sixteen women who came back for the 6-week follow-up failed to return at 12 months. Among these, two withdrew consent, two died from underlying medical problems, two moved to nursing home, one relocated overseas and the rest lived far away and were unable to travel due to long distance. Of the 286 out of 302 women (94.7 %) who returned for 12-month follow-up, improvement was noted in terms of prolapse-related symptoms (99 %), urinary (89 %), bowel (91 %) and sexual functions (74 %). Five out of eighty-nine women (5.6 %) who were not sexually active at baseline were able to resume sexual activity at 1-year follow-up. Postoperative evaluation of prolapse quality of life (P-QOL) scores revealed excellent results in all aspects (Table 4). All POP-Q measurements except total vaginal length (TVL) were significantly improved (Table 5). Using the hymenal level as the cut-off point to define prolapse recurrence (Ap, Bp and C > 0), three women were found to have recurrent posterior compartment defect whereas seven women were discovered with recurrent apical prolapse. If those who missed the 12-month postoperative follow-up were assumed as 'failures', the objective cure rates of the Elevate Posterior and Apical mesh system in the treatment of posterior vaginal and apical prolapse were 93.7 % (283/302) and 92.4 % (279/302) respectively (Table 6). Among three women with recurrent posterior vaginal prolapse, two later underwent posterior fascial colporrhaphy while one had laparoscopic sacrocolpopexy due to defect combination. For seven women with recurrent apical prolapse, six required laparoscopic sacrocolpo/hysteropexy while one underwent Elevate Anterior and Apical mesh repair to correct both anterior and apical defects. Postoperative anterior vaginal prolapse was discovered in 12 women, 10 of whom had already undergone either concomitant anterior fascial repair or Elevate Anterior mesh repair.

DISCUSSION

The use of mesh remains controversial as evidenced by statements from several international societies such as ACOG, IUGA and AUGS. To date, there is no evidence to support its use in the posterior compartment from the 2013 Cochrane review, based on the lack of difference in prolapse awareness between the native tissue repair group and the transvaginal mesh group.[5] However, there are several reasons why alternatives to traditional fascial repair should be considered as an option for patients. Posterior colporrhaphy has been associated with a high incidence of dyspareunia, sexual dysfunction and bowel complications post-operatively, such as incomplete bowel emptying and fecal incontinence.[4,12] In addition, the current evidence lacks high quality studies, particularly in the surgical treatment of posterior prolapse. From the findings in this current series, our results do support the Posterior Elevate mesh technique as feasible, safe and anatomically sound.

From the safety perspective, one of the foremost concerning complications with transvaginal mesh repair is mesh erosion. To the best of our knowledge, with 3 out of 302 (incidence of 1 %) women experiencing mesh exposure after 1 year, this is the lowest extrusion rate reported to date compared to incidence of 2% to 6.5% among other Elevate Posterior and Apical mesh series.[6,13-14] Overall, the mesh extrusion rate of Posterior

TABLE 4. Pre- and post-operative P-QOL questionnaire.

	Baseline scores	12-month scores	*P* value
Overall P-QOL score	2.0 ± 0.9	0.9 ± 1.0	< 0.001
Role limitations	1.1 ± 1.1	0.4 ± 0.7	< 0.001
Physical limitations	1.3 ± 1.0	0.5 ± 0.8	< 0.001
Social limitations	0.6 ± 0.9	0.3 ± 0.6	0.018
Personal relationship	0.9 ± 1.0	0.3 ± 0.8	< 0.001
Emotion	1.2 ± 0.9	0.4 ± 0.8	< 0.001
Sleep/energy	1.1 ± 0.9	0.6 ± 0.9	< 0.001

Data are presented as mean ± standard deviation

TABLE 5. Comparison between pre- and post-operative POP location and stage.

POP variables	Pre-operative	Post-operative	*P*-value
Aa	+0.8 ± 1.7	-2.3 ± 1.0	< 0.001
Ba	+1.1 ± 1.8	-2.2 ± 1.1	< 0.001
C	-2.0 ± 3.3	-6.7 ± 2.0	< 0.001
Ap	+1.4 ± 1.3	-2.8 ± 0.5	< 0.001
Bp	+1.6 ± 1.4	-2.8 ± 0.5	< 0.001
gh	5.8 ± 1.1	4.3 ± 0.6	< 0.001
pb	1.3 ± 0.7	2.2 ± 0.5	< 0.001
tvl	8.7 ± 0.9	8.7 ± 0.8	0.570
Posterior			
Stage 0 or I	2 (0.7)	275 (91.1)	
Stage II	113 (37.4)	27 (8.9)	
Stage III	185 (61.2)	-	
Stage IV	2 (0.7)	-	
Total	302 (100)	302 (100)	
Apical			
Stage 0 or I	171 (56.6)	273 (90.4)	
Stage II	80 (26.5)	25 (8.3)	
Stage III	48 (15.9)	3 (1.0)	
Stage IV	3 (1.0)	1 (0.3)	
Total	302 (100)	302 (100)	

Data are presented as mean ± standard deviation or number (%)

TABLE 6. Clinical outcomes at 1-year follow-up.

Follow-up	6-week	1-year	Cumulative
Return for follow-up	292 (96.7)	286 (94.7)	286 (94.7)
Recurrence			
Posterior	0 (0.0)	3 (1.0)	3 (1.0)
			19 (6.3)
Apical	1 (0.3)	6 (2.1)	7 (2.4)
			23 (7.6)
Objective cure			
Posterior	100 %	99 %	99.0 %
			93.7 %
Apical	99.7 %	97.9 %	97.6 %
			92.4 %

Data are presented as number (%)

Elevate is much lower than the published extrusion rates of other mesh kits, such as Prolift (11%) and Apogee (11%).[15] It is therefore reassuring to note that in our series, a 1% mesh erosion rate is lower than the erosion rate of sacrocolpopexy (4%).[16]

The second major concern with the use of a polypropylene mesh in the vagina is related to the risk of pain and dyspareunia postoperatively. In our series, 2.3% and 4.6% of women reported dyspaneuria and deep vaginal or buttock pain respectively, similar to those described by Lukban et al (2.9%) and Su, Tsung-Hsien et al (4 %).[6,15] However, these symptoms tend to be short-lasting, with no major impact on sexual activity and quality of life reported among the patients who experienced dyspareunia, based on the P-QOL questionnaire. Remarkably, three-fourths of our patients (74 %) reported noticeable improvement in their sexual function after the Elevate Posterior and Apical mesh placement. This sharply contrasts with studies which have looked specifically at sexual function after trocar-guided mesh placement. Su et al reported worsening sexual function (73 %) at 6-month follow-up after Prolift mesh repair based on Pelvic Organ Prolapse/Urinary Incontinence Sexual Questionnaire (PISQ-12).[17] Deterioration in sexual function (baseline score 15.5 VS postoperative score 11.7; $p < 0.001$) was also demonstrated in a prospective multi-center study by Altman et al, using the short form PISQ-12 before and one year after trocar-guided transvaginal mesh surgery.[18]

With regard to intra-operative morbidities, while transvaginal mesh application has been associated with greater blood loss and organ injury risk, in our series, we encountered no rectal or visceral injury and low incidence of bleeding complication. Nine women experienced intraoperative blood loss over 500 ml, four was from pararectal space dissection and five from paravesical space dissection during concomitant Anterior Elevate mesh placement. All bleeding was adequately controlled and no blood transfusion was required. Possible explanations for the low incidence of mesh erosion, postoperative pain and surgical morbidity in our series include softer and lighter mesh property, single-incision trocarless technique, appropriate tensioning during placement, full thickness vaginal dissection and surgeon's experience.

In relation to bladder function, voiding difficulty was observed amongst 5 % of our patients, all of which spontaneously resolved within six weeks. This is notably less prevalent when compared to the 31% rate from a prospective cohort study on trocar-guided mesh repair by Zhang et al.[19] De novo urge incontinence was also reported in 5% of women. This was conservatively managed with lifestyle modification, bladder re-training and vaginal estrogen, with only one patient subsequently confirmed to have detrusor overactivity by urodynamic testing requiring anti-cholinergic medication. At 12-month follow-up, de novo stress incontinence was identified in 36 out of 302 women (11.9 %). Our result is more comparable to the 7.7 % incidence determined by Jundt et al[20] than the exceptionally higher incidence of 43 % demonstrated by the OPUS trial.[21] Most women were not concerned of their stress urinary leakage and were able to perform Kegel exercise to help relieve the symptom. Only 9 out of 36 required subsequent anti-incontinence surgery.

From the anatomical support point of view, at one-year analysis, the Elevate Posterior and Apical mesh system performed at our center has demonstrated high objective cure rates at 93.7% and 92.4% for the treatment of posterior vaginal and apical prolapse. Our results are consistent with those obtained from both single-incision trocarless and trocar-based mesh studies. Published data on the Elevate Posterior and Apical mesh regarding its support in the posterior and apical compartment show 92.5% and 89.2% success at one year in a prospective study by Lukban et al[6], 99% and 94% after two years in a retrospective study by Huang et al[13], and 100% and 99% in a cohort study by Su et al.[14] Similarly, Prolift demonstrated 95.9% and 93.7% success rates in the posterior and apical compartments in a study by Withagen et al[22], and Apogee's objective cure rate approached 96% in a long term trial by Karmakar et al.[23] Anatomic cure after transvaginal free graft placement displayed almost identical figures. At a median follow-up of 22.7 months, de Tayrac et al identified an objective cure rate of 92.3 % after rectocele repair with bilateral sacrospinous ligament anchored polypropylene mesh.[24] Better anatomical and functional outcomes were also described in a retrospective cohort study by Cao et al comparing modified pelvic floor reconstructive surgery using precut polypropylene mesh and traditional anterior posterior colporrhaphy (88.1 % VS 64.9 %).[25]

Limitations in this study include the absence of randomization, the possibility of assessment bias and the short-term follow-up. However, our research has the advantages of having a large study population from a single center where one surgeon performs or supervises all the repairs. Patient selection is therefore consistent, and surgical technique standardized and systematic in every patient, especially pertaining to proper vaginal dissection and placement and tensioning of the mesh anchor points.

In conclusion, at one-year follow-up, this Elevate

Posterior and Apical mesh series has delivered impressive clinical outcomes including high success rates, anatomic durability, improved quality of life, low recurrence rates and low mesh-related complications for the treatment of both posterior vaginal and apical prolapse. Therefore, it is reasonable to advocate continuing research and refinement of the role of mesh in prolapse management in the hope of offering women surgical options which can deliver safe and improved anatomical and functional outcomes.

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Prevalence of *Helicobacter pylori* Infection in Patients with Perforated Peptic Ulcer in a Tertiary Hospital in Thailand: A Single Tertiary Hospital Study

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ABSTRACT

Objective: Because of limited resources, the approach in Thailand is to eradicate *Helicobacter pylori (H. Pylori)* infection in every patient with peptic ulcer perforation, but without infectious confirmation because of suspected high infection rates. Only one study reported the incidence of infection in perforated disease in Thailand with limited number of patents. Our aims were to study the prevalence of *H. pylori* infection in patients with perforation and to explore associated factors.

Methods: In a total 136 patients, infection was confirmed by rapid urease testing during post-operative esophagogastroduodenoscopy. The statistical analysis were Fisher's exact test and multiple logistic regression analysis.

Results: The infection rate was 69.6%. Age ≥ 60 years and male were correlated with infection in multivariable analysis (odds ratio: 3.4 and 4.3, p=0.01 and 0.03, respectively). Only age ≥ 60 years was associated with infection in the univariable analysis (odds ratio: 2.7, p=0.02) with an infection rate of 81.5%. Three upper gastrointestinal cancers were also seen.

Conclusion: The prevalence of *Helicobacter pylori* infection in perforated peptic ulcer patients in our study was high. Male patients ≥ 60 years of age appeared to be more prone to infection. The non-documented infection eradication approach may be more justified in older male patients, especially those with multiple comorbidities.

Keywords: *H. pylori* infection; perforated peptic ulcer; prevalence; Thailand (Siriraj Med J 2018;70: 139-144)

INTRODUCTION

Helicobacter pylori (H. Pylori) infection is the established etiology of peptic ulcer disease[1], and perforated peptic ulcer is a significant complication. Most affected patients require surgery, which can increase morbidity and may result in death. Several complicated surgical procedures have been designed to reduce acid secretion to prevent disease recurrence. However, with improved understanding of the pathophysiology and more effective medications, complicated surgery has been replaced with simple closure of the perforation site and/or reinforcing with an omental patch.[2-4]

Studies confirm that *H. pylori* eradication decreases recurrent perforation,[5-8] and the standard recommendation is to confirm and document infection status in all perforated ulcer patients.[5,6] However, in developing countries with limited resources, the recommendation is to eradicate infection in all patients with perforated ulcers regardless of infection documentation, because high infection rates are suspected. This practice has been adopted by most surgeons in Thailand, although there is a lack of supporting documentation and published studies. Surgeons in other countries also take this approach.[3] The infection rate in Thailand has been reported at approximately 27%-61.4%.[9-12] This wide variation may be a result of regional population differences and/or

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sampling differences based on whether patients are seen by gastroenterologists or non-specialists. However, to our knowledge, there was only one study which reported the incidence of infection in perforated disease in Thailand with limited number of patents.[13] The incidence in other countries varies greatly (26%-92%).[14-17] Our aim in this single-center study was to evaluate the prevalence of *H. pylori* infection in perforated peptic ulcer patients concurrently with other relevant etiologies and to explore correlated factors that may predict infection.

MATERIALS AND METHODS

Patients

We included all patients with perforated peptic ulcer from January 2014 to December 2016. Following surgery, postoperative rehabilitation, and discharge, patients were subsequently scheduled to return for esophagogastroduodenoscopy (EGD) after obtaining informed consent. EGD was performed in an outpatient setting.

The study was conducted in Sawanpracharak Hospital, a 653-bed public tertiary hospital in Nakhon Sawan province, located in the lower northern part of Thailand. This study was approved by the Sawanpracharak Hospital Medical Ethics Committee (Certification number: 41/2557).

Data collection and diagnostic method to confirm *H. pylori* infection

The following data was collected: sex, age, ulcer location, type of operation, smoking status (previous, on-going, or never), recurrent ulcer status, and nonsteroidal anti-inflammatory drug (NSAIDs) use in the two weeks prior to perforation. NSAID and smoking data were obtained from a questionnaire on the endoscopic examination day. The remaining variables were acquired from patients' medical records. On-going smoking and NSAID use less than two weeks before perforation events, only were included.

H. pylori infection was diagnosed using a rapid urease test (HelicotecUT^{R} Plus, Strong Biotech Corp., Taipei, Taiwan). EDG and gastric mucosal sampling are required for this test. Gastric mucosa was collected from both the antrum and body to decrease false negative results from more proximal distribution of the infection in patients who were taking proton-pump inhibitors (prescribed postoperatively in all patients).[18,19] Interpretation of the test's color was evaluated approximately 24 hours after the test commenced.

EGD was also used to evaluate whether healed or active ulcers and/or malignancy were present.

Statistical analysis

Variables considered to predict infection (age, sex, ulcer location, smoking, not consuming NSAIDs, and active ulcer) were individually examined for correlation using Fisher's exact test. Multiple logistic regression analysis was also used and incorporated the same variables. Data were expressed as mean ± standard deviation or percentage with 95% confidence intervals (95% CI). Significance was set at $p < 0.05$.

RESULTS

We studied a total of 136 patients. Patients' characteristics and associated variables are summarized in Table 1. Patients' ages ranged from 16-88 years. The average time between operation and EDG was 30 ± 10.8 days, most patients were male (86.0%), and ulcer locations were: gastric (9.6%), duodenal (47.1%), and pyloric (43.4%). Most surgeries involved simple closure of the perforated ulcer (95.6%).

Including all etiological factors, in a total of 135 patients, *H. pylori* prevalence was 69.6%. Infection status could not be documented in one patient because of obstructed esophageal cancer. Most patients had a history of smoking (62.5%), and the NSAID consumption rate was 66.9%. Fifteen (11.0%, 95% CI: 6.3-17.5) patients did not have *H. pylori* infection and had not consumed NSAIDs.

The current episode was recurrent perforation in five (3.7%) patients with four (80%) having infection. Previous infectious eradication information was not available in these patients' medical records. Ulcers were still active based on EGD in 41 (30.2%) patients with 28 (68.3%, 95% CI: 51.9-81.9) patients infected. Cancer was detected in three (2.2%) patients and confirmed by histology including one gastric cancer (adenocarcinoma) and two upper esophageal cancers (squamous cell carcinoma). All cancer patients were male. In the gastric cancer case, the surgeon was not aware of tumor while performing the ulcer repair operation. Documentation of infection could not be obtained in one esophageal cancer patient, although infection was confirmed in the two other cancer patients.

All relevant factors were examined in both univariable and multivariable analysis, and the results are summarized in Table 2. Age was the only factor that predicted infection in both analyses. However, after adjusting for other factors, male gender also reached statistical significance. Patients ≥ 60 years of age had an 81.5% (95% CI: 68.6-90.7) infection rate compared with 61.7% (95% CI: 50.372.3) for those < 60 years of age. Male patients had a 71.8% (95% CI: 62.7-79.7) infection rate compared with 55.6%

TABLE 1. Patients' characteristics, type of operation, and factors associated with the peptic ulcers. Data from 136 patients were analyzed.

Variables	Numbers	Percentage	95% Confidence interval
Age (Years) (Mean ± SD)	56.7	(± 13.3)	54.5 – 59.0
< 60	81	59.6	50.8 – 67.9
≥ 60	55	40.4	32.1 – 49.2
Sex			
Male	117	86.0	79.0 – 91.4
Female	19	14.0	8.6 – 21.0
Surgical procedure			
Simple closure	130	95.6	90.6 – 98.4
Acid reducing procedure	6	4.4	1.6 – 9.4
Ulcer type			
Gastric ulcer	13	9.6	5.2 – 15.8
Duodenal ulcer	64	47.1	38.4 – 55.8
Pyloric ulcer	59	43.4	34.9 – 52.1
NSAIDs consumption	91	66.9	58.3 – 74.7
Smoking	85	62.5	53.8 – 70.4
H pylori infection*	94	69.6	60.6 – 76.8
Active ulcer on EGD	41	30.2	22.6 – 38.6
Recurrence	5	3.7	1.2 – 8.4
Cancer	3	2.2	0.5 – 6.3

*A total of 135 patients for the *H. pylori* infection variable.

Abbreviations: SD = Standard deviation, NSAIDs = Nonsteroidal anti-inflammatory drugs, *H. pylori* = *Helicobacter pylori*, EGD = Esophagogastroduodenoscopy.

TABLE 2. Characteristic variables associated with *H. pylori* infection. Univariable and multivariable analyses in 135 patients.

Variables	Unadjusted odds ratio	95% Confidence interval	P- value	Adjusted odds ratio	95% Confidence interval	P- value
≥ 60 yrs	2.7	1.1 – 6.9	0.02	3.4	1.4 – 8.6	0.01
Male	2.0	0.6 – 6.3	0.18	4.3	1.1 – 16.2	0.03
Smoking	0.8	0.3 – 1.8	0.70	0.7	0.2 – 1.8	0.44
No- NSAIDs taking	0.8	0.3 – 1.8	0.55	0.7	0.3 – 1.5	0.33
Ulcer type						
Gastric	1.3	0.3 – 8.1	1.00	1.0	0.2 – 4.6	0.99
Duodenal	0.8	0.4 – 1.8	0.58	0.7	0.3 – 1.7	0.48
Pyloric	1.1	0.5 – 2.6	0.85	1.0	0.2 – 4.7	0.99
Active ulcer	0.9	0.4 – 2.2	0.84	0.8	0.4 – 2.1	0.81

Abbreviation: NSAIDs = Nonsteroidal anti-inflammatory drugs.

(95% CI: 30.8–78.5) for female patients. The infection rate was 88.1% (95% CI: 74.4-96.0) in male patients ≥ 60 years of age.

DISCUSSION

H. pylori and NSAIDs are two major factors in peptic ulcer pathogenesis. Our study was limited regarding patients' history of NSAID use. Obtaining NSAID history in Thailand is limited because of patient confusion in terminology between NSAIDs and antibiotics, availability of illegal pain-relief medication containing either NSAIDs or steroid, and lack of education. In the past, simple closure of perforated ulcers was considered inadequate because of high ulcer recurrence.[20] However, with newer more effective acid-reducing medications and a greater understanding of peptic ulcer pathogenesis, simple closer is now more widely used.[3,5-7,14,16] Considering the high proportion of patients with both main etiological factors in our study (89.0.%), simple closure also appeared to be adequate in our center. However, both etiological factors must be controlled postoperatively either by confirming and eradicating the infection or avoiding NSAIDs or using them with acid-reducing medications. Countries with limited resources follow a policy of eradicating the infection in every patient with perforated ulcers regardless of infection documentation, based on a suspected high prevalence of infection. Our results showed an infection prevalence in our hospital of 69.6%. Compared with the prevalence in other countries (26%-92%),[14-17] our rate is considered high.

Two large nationwide community-based studies of the prevalence of *H. pylori* infection in dyspeptic patients have been performed in Thailand. The prevalence was 48.2 %[12] in one study and 45.9 %[9] in the other, and both studies reported significant geographic variation.[9,12] The northern part (our region) was classified as having medium to high prevalence with the highest in the northeast region and the lowest in the southern region.[9] The reported prevalence in Nakhon Sawan province, specifically was 40.3 %.[9] Our infection rate was 69.6 %. This difference (69.6% vs 40.3%) can be indirectly interpreted that the incidence in perforated disease is higher than in community-based dyspeptic disease. However, compared to previous study in perforated disease[13], our incidence was lower (69.6% vs 86.3%). This can be explained by differences in geography, timing of examination (after recovery vs intra-operatively) and method of infectious confirmation (rapid ureas test vs rapid urease test and histologic examination).

Age, lower socioeconomic status, lower education level, and some foods are other factors reported to be associated with infection.[21-24] Differences in socioeconomic status and types of food consumed can explain the geographic variation in infection rates in Thailand.[9,12] However, in settings like ours (government hospital), most patients have low income and education level, so these factors cannot be used to classify factors associated with infection and may not have practical benefit. Regarding age, our results were similar to certain epidemiological studies,[21-24] but contrast with large studies in Thailand in which age was either not related[9] or inversely related.[11,12] Different *H. pylori* genotypes in different pathologies is a possible explanation[9,25] for this difference, but more evidence is required. Although ulcer location is reportedly related to infection,[11,13] in our study, perforated ulcer location failed to predict infection. Age was the only variable in our study significantly associated with infection in both uni- and multi-variable analyses. When adjusted for other factors, male gender was also significantly related to infection, and combining these two factors, the infection rate reached as high as 88.1%. This result may be valuable in decision-making, although at a 69.6% infection rate, proceeding with non-documented infection eradication may be a difficult decision. In patients ≥ 60 years of age, the incidence of infection was 81.5%, so the non-documented infection eradication approach appears more reasonable in these patients. Also, in older, frail patients with multiple comorbidities, intensive investigation may be impractical. Some patients may avoid follow-up sessions, which could result in these patients not receiving eradicative treatment.

We also detected a high (2.2 % in 3 years) prevalence of upper gastrointestinal cancer in our study, which was much higher than Thailand's specific cancer incidence for esophageal cancer of 1.6 per 100,000 men and 0.3 per 100,000 women and for gastric cancer of 1.5 per 100,000 men and 1.2 per 100,000 women (approximate rates).[26] Perforated gastric lesions can harbor malignancy, as seen in one of our patients.[20] The upper esophageal squamous cell carcinoma in the other two patients might be pathophysiologically unrelated to the perforated ulcer. The high prevalence of upper gastrointestinal cancer in our study might be explained by smoking as a common risk factor,[20,27] which was present in 62.5 % of our patients. Another relevant explanation might be reflux disease leading to esophageal cancer caused by gastric acid abnormalities, which also occurs in peptic ulcer disease. However, the expected histology of esophageal cancer is distal adenocarcinoma rather than proximal squamous cell cancer.[27] Given the high detection rate of upper gastrointestinal cancer, confirming *H. pylori* infection with EGD is an even more attractive

option. Other noninvasive confirmation methods cannot detect upper gastrointestinal cancer.[28,29] This high cancer detection rate also questions the suitability of the non-documented infection eradication approach because diagnosis of malignancy in some patients (albeit quite a low proportion) could be missed or delayed.

There were certain limitations in our study. First, even within a single country, there are geographic variations of infection prevalence.[9,12] In the southern part of Thailand, the prevalence of infection can be as low as 14.4%[9] (in dyspeptic patients). Second, as a single-hospital study, our results cannot be applied to the national population. Third, the number of subjects in our study was small, and this was reflected in the instability of the odds ratio (wide 95% CI). Fourth, information on certain variables was missing including alcohol consumption, family history, type of foods consumed, socioeconomic variables, preoperative use of acid-regulating medications, and previous *H. pylori* eradication status in recurrent patients. Fifth, because we relied on the only single test, inaccurate estimation was possible. Lastly, regarding the high incidence of cancer, because of the small sample size, this finding could have occurred by chance because the 95% CI was as low as 0.5%. Further large nationwide studies in specific groups of patients and possibly cost-effective analyses are needed.

CONCLUSION

The rate of *H. pylori* infection in perforated peptic ulcer patients in our study was high. Age and male gender identified high-risk patients, and older male patients were more likely to be infected. When resources are limited, the non-documented infection eradication approach is useful, especially in male patients ≥ 60 years of age. However, if feasible, documenting infection in every case is highly recommended. EGD with rapid urease testing appears to be the most attractive investigation because of the indirect benefit of malignancy detection. Finally, because of our small sample size, and the single-hospital design, our results should be interpreted with caution. Further larger nationwide studies are needed.

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Ultrasound-Guided Thoracic Paravertebral Block for Controlling Pain in Percutaneous Radiofrequency Ablation of Hepatic Tumors: A Prospective Case Series

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ABSTRACT

Objective: Patients with hepatic tumors undergo percutaneous radiofrequency ablation (PRFA) performed under local anesthesia and intravenous sedation can experienced severe post procedural pain. This prospective case series evaluated the feasibility, efficacy and safety of ultrasound assisted thoracic paravertebral blocks (TPVB) at three levels (T5-6, T7-8 and T9-10) for post procedural pain control.

Methods: 35 patients with hepatic tumors received 3 levels ultrasound guided right thoracic paravertebral blocks (TPVB) at T5-6, T7-8 and T9-10. Patients were administered with propofol bolus with continuous infusion for sedation. Pain score at post anesthesia care unit and maximum pain score during 24 hours were recorded.

Results: All patients except one reported minimal pain after PRFA. Mean NRS on arrival in PACU was 0.51 (SD: 1.50; range: 0-7) at rest and 1.20 (SD: 1.95; range: 0-10) on movement. The maximum pain score during 24 hour post-procedure period was 1.31 (SD: 1.93; range: 0-10). One case reported severe pain at shoulder due to the tumor adjacent to the diaphragm. Patients can tolerate TPVB well and there were no complications associated with the blocks.

Conclusion: 3 levels ultrasound guided TPVB can produce the effective post procedural pain control. The technique is promising and may also be appropriate for other interventional radiological procedures such as trans-arterial chemoembolization (TACE) of hepatic tumors and percutaneous trans-hepatic biliary drainage (PTBD).

Keywords: Percutaneous radiofrequency ablation of hepatic tumors; ultrasound-guided thoracic paravertebral block (Siriraj Med J 2018;70: 145-151)

INTRODUCTION

Percutaneous radiofrequency ablation (PRFA) is widely used in the management of small primary, inoperable primary, recurrent, or metastatic hepatic tumors.[1-4] PRFA is a localized thermal treatment technique designed to produce tumor destruction by heating tumor tissue to a temperature that approximates 50-60°C via a needle electrode. Anesthetic management for PRFA of hepatic tumors can be performed using mild sedation, deep sedation, general anesthesia, or epidural analgesia.[5-7] At our institution, local anesthesia with intravenous sedation is usually applied. However, early post procedural pain is reported by the majority of patients undergoing this procedure.[8-12] These results are similar to our pilot study, in which 11 out of 15 patients (73%) endured PRFA of hepatic tumors under usual anesthetic technique (local anesthesia with intravenous sedation) reported moderate to severe pain at recovery room (pain score 4-10 out of 10) and required the intravenous opioids such as morphine or pethidine.

Thoracic paravertebral block (TPVB) is a technique that involves injecting local anesthetic alongside the thoracic vertebral body, close to where the spinal nerves emerge from the intervertebral foramen. This procedure

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provides ipsilateral, segmental, somatic, and sympathetic nerve blockade in multiple contiguous dermatomes.[13] Paravertebral block is an analgesic technique used for pain relief after chest and abdominal surgery.[14, 15] There are several different techniques for performing TPVB, including loss-of-resistance, nerve stimulator-guided, and ultrasound-guided techniques. The ultrasound provides several advantages such as preventing pneumothorax which is the serious complication of TPVB.[16] From previous studies, multiple paravertebral injections resulted in more reliable radiographic and clinical distribution compared with a single-injection technique.[17,18] The objective of this study was to evaluate the pain control effectiveness of 3 levels ultrasound-guided right TPVB in patients undergoing PRFA of hepatic tumor. Effectiveness was defined as more than 90 percent of all patients having mild pain (less than 4 out of 10) and not requiring any rescue analgesic drugs in the post-anesthesia care unit.

MATERIALS AND METHODS

The protocol for this study was approved by the Siriraj Institutional Review Board (Si 070/2014), and written informed consent was obtained from all study participants. This study was registered on the ClinicalTrials.gov website (ID NCT02271646). During the March 2014 to December 2014 study period, 35 patients undergoing percutaneous radiofrequency ablation of hepatic tumors at Siriraj Hospital were included. Study subjects ranged in age from 47 to 84 years, each with ASA physical status of either 2 or 3. The number of patients was based on the hypothesis that more than 90 percent of all patients receiving paravertebral blocks will have mild pain. Using a two-sided test with 90% power and an alpha level of 0.05, the calculated sample size was 35 patients. Patients were excluded if they had a history of psychiatric illness, chronic pain, regular analgesic usage, or contraindications to TPVB, to include chest wall deformity, severe coagulopathy (PT > 16 sec, aPTT > 32 sec, and platelet < 45,000/mm^2), local or systemic infection, and/or allergy to local anesthetic drugs.

The study design was a prospective case series. All patients were instructed in the use of the Numeric Rating Scale for Pain (NRS: 0, no pain to 10, worst imaginable pain) for pain assessment during the pre-operative visit. Patients were also instructed regarding pain assessment at rest and when they cough (involving movement). Patients fasted for at least 6 hours before surgery and no premedication was given. Intravenous access and standard monitoring, including electrocardiography (ECG), pulse oximetry, and non-invasive blood pressure monitoring (cycle every 3 minutes), were established before TPVB was performed. Patients were positioned in the left lateral decubitus position with the neck slightly flexed and the right arm resting on a pillow to properly position the scapula. All patients received oxygen (2 L/min) via nasal cannula. Midazolam (1-2 mg) and fentanyl (25-50 mcg) were administered intravenously, as needed, for analgesia when performing the block. A preview scan was performed using a low frequency (2-5 MHz) curved ultrasound transducer, with the objective of obtaining a transverse view of the thoracic paravertebral space. The thoracic paravertebral levels were counted, starting from the seventh cervical vertebra which is not attached to a rib. The thoracic paravertebral spaces for T5-6, T7-8, and T9-10 were identified and marked with a marker pen. All patients were blocked by the principal investigator who experienced ultrasound-guided TPVB for more than 100 injections.

Using aseptic precautions, skin and subcutaneous tissue at the marked levels were anesthetized with 1-2 ml of 1% lidocaine. Starting at the T5-T6 level, a 10 cm 22 g block needle was advanced from a lateral to medial direction under ultrasound visualization, using the in-plane approach. When possible, needles were inserted at the articular process level, because the area of acoustic shadow is significantly smaller than the level of the transverse process (Fig 1). The advantages are this window offers less bony obstruction when advancing the block needle and the needle tip is not in the acoustic shadow of the transverse process. If not possible, needles were compromised and inserted at the transverse process level (Fig 2). Bupivacaine 0.5% with adrenaline 1:200,000 7 ml, 7 ml, and 6 ml was injected at T5-6, T7-8, and T9-10, respectively. After injection was completed, patients were returned to the supine position with any procedure-related complications (e.g., pneumothorax, hypotension

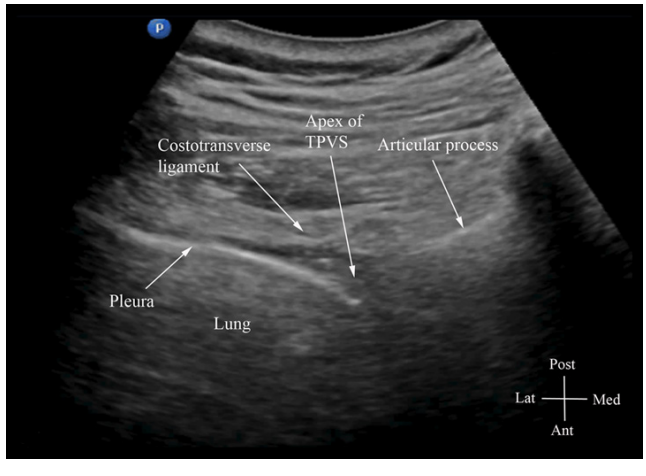


Fig 1. Transverse sonogram of the thoracic paravertebral space at the articular process window.
Abbreviation: TPVS = thoracic paravertebral space

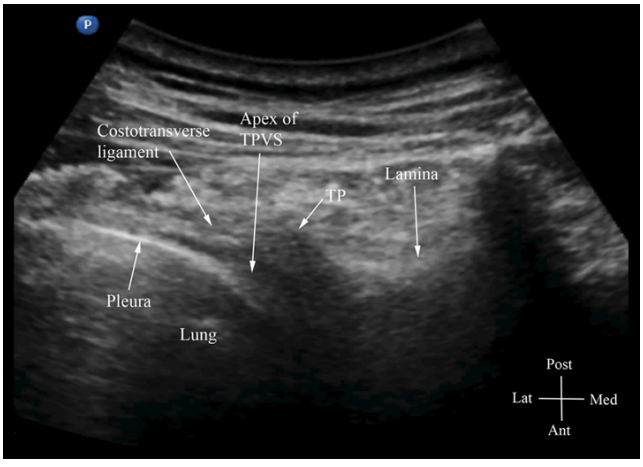


Fig 2. Transverse sonogram of the thoracic paravertebral space at the transverse process window.
Abbreviations: TPVS = thoracic paravertebral space; TP = transverse process

and local anesthetic systemic toxicity) being observed and appropriately treated. Sensation was tested by alcohol soaked cotton at 5, 10, 20, and 30 minutes after injection. Patients were then transferred to the intervention room which is equipped with CT fluoroscopy for difficult cases of localizing hepatic tumor.

After all standard monitoring devices were placed and activated, patients were positioned either supine with right arm resting above patient head or left lateral decubitus, according to the discretion of the interventional radiologist. The patient's right upper abdominal quadrant was then cleaned and draped with sterile sheath. Patients were asked to hold their breath to allow the interventionist to clearly identify and define the location of the tumor before needle electrode tip insertion. Before inserting the needle electrode, the interventionist tested patient skin sensation with forceps. Patients who experienced pain received xylocaine 1% 5-10 ml via skin and subcutaneous injection. After the needle electrode was positioned in the tumor, but before starting thermal burn (radiofrequency ablation), patients received propofol bolus with continuous infusion for sedation. The following information was taken and recorded: patient characteristics; number, size, and location of hepatic tumor(s); block performance time; patient discomfort score while performing paravertebral block; total PRFA procedure time and total burning time. Complications of TPVB (e.g., pneumothorax, hypotension and local anesthetic systemic toxicity), PRFA (e.g., pneumothorax), and anesthesia (e.g., bradycardia and hypotension) were also recorded. Bradycardia was defined as patients' heart rate less than 40 bpm or heart rate between 40 to 60 bpm with hypotension. Hypotension was defined as a 25% decrease in systolic blood pressure from baseline. Atropine, ephedrine or norepinephrine were appropriately administered by anesthesiologists in charge. At the end of PRFA procedure, the interventional radiologist's satisfaction with the anesthetic technique was assessed and recorded (satisfaction score: 0, totally dissatisfied to 10, very satisfied). After completion of PRFA, sedation was discontinued and patient was transferred to the post-anesthetic care unit (PACU) where vital signs were monitored every 5 min for 30 min. A recovery room nurse not involved with this research evaluated patient pain score on arrival and before discharge from the PACU as normal. For patients with NRS ≥ 5 while in the PACU, morphine (2 mg intravenous bolus for patient age < 70 year and 1 mg for patient age ≥ 70 year) for analgesia was given, with dose being repeated, as needed, every 5 min for a maximum of 3 doses. Upon return to the surgical ward, patients were allowed to eat and drink as tolerated. All patients received paracetamol 500 mg oral every 6 hours and either paracetamol 500 mg oral when NRS < 5 or morphine 1-2 mg intravenous when NRS ≥ 5 for breakthrough pain. A blinded nurse anesthetist visited patients 24 h after PRFA and recorded maximum pain score during previous 24 hour period, patient satisfaction with anesthesia technique, and any complications.

Statistical analysis

Data were processed using SPSS for Windows v.14.0 (SPSS, Inc., Chicago, IL, USA). Shapiro-Wilk test was used to evaluate the distribution of continuous data. Data that were normally distributed were presented as mean (SD) (range) and data that were not normally distributed were presented as median (range).

RESULTS

Patients' demographic data were demonstrated in Table 1. None of the enrolled patients had any exclusion criteria. Mean diameter of the first tumor nodule (35 nodules) was 1.94 cm (SD: 1.05; range: 0.50-4.70), with the mean diameter of the second nodule (14 nodules) being 1.50 cm (SD: 0.88; range: 0.40-3.20). Tumor nodule was located in right lobe of the liver in 22 patients, left lobe in 10 patients, and in both lobes in 3 patients. Regarding coagulation values, 13 patients (37%) had platelet less than 100,000/mm^3 (11 patients had platelet count between 50,000-100,000 and 2 patients had platelet less than 50,000) and all patients had PT ≤ 16 sec and aPTT ≤ 32 sec.

Mean duration of 3 levels thoracic paravertebral block was 14.6 min (SD: 5.07; range: 5-25) and mean maximum NRS pain score experienced by patients

TABLE 1. Demographic information of all patients (n=35).

Age (years)	61.51 (8.72)(47-83)
Sex (n/%)	
Male	26 (65.2%)
Female	9 (34.8%)
Weight (kg)	65.94 (11.59)(48-100)
BMI (kg/m^2)	24.77 (4.49)(17.71-34.60)
ASA classification (n/%)	
II	24 (68.6%)
III	11 (31.4%)

Continuous data were shown as mean (SD) (min-max) and categorical data were presented as count (percentage).
Abbreviations: BMI = body mass index; ASA = American Society of Anesthesiologists

during block was 2.91 (SD: 1.4; range: 0-6). Onset of sensory loss after injection as determined by cold test (defined as decrease in sensation from 100% to 20%) was 20 min in 14 patients and 30 min in 11 patients. At 30 min block produced ipsilateral sensory blockade in over 5 dermatomes (range: 1-9), and in most cases the dermatome extent of anesthesia was T5 to T10. There was no bilateral blockade in any patients. Twenty percent of patients (7 of 35) required local anesthesia before inserting needle electrode. All 7 of those patients had left lobe hepatic tumor, which required puncture point at left side of the abdomen.

The entire radiofrequency ablation procedure lasted an average of 82.06 min (SD: 50.32; range: 25-260), with mean RFA burn time lasting 30.69 min (SD: 24.59; range: 2.61-100.45). One case of pneumothorax from PRFA procedure developed, which was treated with supplemental oxygen therapy. Two patients developed bradycardia, which was treated with atropine injection.

All patients except one reported minimal pain after PRFA. Mean NRS on arrival in PACU was 0.51 (SD: 1.50; range: 0-7) at rest and 1.20 (SD: 1.95; range: 0-10) on movement. While pain score on discharge from PACU was 0.23 (SD: 0.64; range: 0-2) at rest and 0.88 (SD: 1.02; range 0-3) on movement. The maximum pain score during 24 hour post-procedure period was 1.31 (SD: 1.93; range: 0-10) (Table 2). One patient reported severe pain in her shoulder in the PACU (pain score 10). This patient had tumor at segment 8, which was adjacent to capsule and diaphragm. After giving morphine 1 mg, 3 times, the patient reported a satisfactory decrease in pain. NRS score reduced from 10 to 3 and patient was uneventfully discharged from PACU. Regarding overall satisfaction with the anesthetic technique, mean patient satisfaction score was 9.83 (SD: 0.71; range: 7-10) and mean interventional radiologist satisfaction score was 9.63 (SD: 0.64; range: 7-10). There were no complications from TPVB in all patients.

TABLE 2. The numeric rating scale (NRS) reported by patients (n=35) at different times.

	Numeric Rating Scale (NRS)	
	At rest	**At movement**
On arrival in PACU	0.51 (SD 1.50; range 0-7)	1.20 (SD 1.95; range 0-10)
On discharge from PACU	0.23 (SD 0.64; range: 0-2)	0.88 (SD 1.02; range 0-3)
Maximum pain score within 24 hr	1.31 (SD: 1.93; range: 0-10)	

The data was shown as mean (SD; range)
Abbreviation: PACU = post-anesthetic care unit

DISCUSSION

In this prospective case series, we successfully demonstrated the effectiveness of 3 levels ultrasound-assisted right TPVB for postoperative pain control in patients undergoing PRFA of hepatic tumors. All patients except one had mild pain (less than 4 out of 10) and did not require any rescue analgesic drug in the post-anesthesia care unit. This study focused on patients' pain score in PACU according to a pilot study showing the period of maximum pain in most patients was in PACU. Moreover in the literature review, early post procedural pain was reported by the majority of patients undergoing this procedure.[8-12] Results from this study were consistent with those of Cheung, *et al.*, who used 5 level thoracic paravertebral blocks (T6-T10) with loss-of-resistance with glass syringe technique. Their patients (20 patients) reported minimal pain after PRFA, with mean (SD) NRS on arrival in PACU 2.0 (2.5), at discharge from PACU 1.7 (2.1), and at 24 h ward visit 1.3 (1.6). The spreading pattern of paravertebral block is unpredictable. Injected solution may remain localized in the space where it was injected[19] or it may spread to the contiguous spaces above and/or below[20], the intercostal space laterally[13], the epidural space medially[21-23], or a combination of the above outcomes.[13] A recent magnetic resonance imaging study (MRI) showed the spread of local anesthetic solution after ultrasound-guided thoracic paravertebral block.[24] Using 20 ml at T6 level, they detected variability in the spread of local anesthetic between two and six levels. Moreover, the extent of sensory block was highly variable and significantly larger, when compared with the spread of local anesthetic. The ultrasound-assisted technique may facilitate more accurate placement and more controlled spread of local anesthetic. For this study, we decided to evaluate the efficacy of 3 levels, as compared to 5 levels in the Cheung, *et al.*, study.[25] Patients would suffer less puncture-related discomfort and be at lower risk with less injection punctures.

Picconi, *et al.*,[26] used 2 level thoracic paravertebral block with nerve stimulator guidance as the sole anesthetic for percutaneous radiofrequency ablation of hepatic tumor. Their result delivered adequate pain relief during RFA, with median VNS (verbal numeric scale) of 0.5 (range: 0-6). No patients requested sedatives or opioids during the procedures in the Picconi, *et al.*, study. Using only ultrasound guidance, the patients in our study were not able to tolerate procedure-related pain and required sedative medication. For the last 10 cases, we decided to combine with nerve stimulator, but we were not able to elicit intercostal muscle contraction at low current (0.5 mA). We successfully elicited muscle contraction at 0.5 mA for all 3 levels of injection in only 3 patients, with all 3 patients able to tolerate the burning segment without any sedation requirement. Because patients were awake, the radiologists were very satisfied with the patients' cooperation (breath holding) during the procedure especially when they inserted the needle at the second nodule. However, there were disadvantages of not giving sedation which included patient fatigue and malaise due to an extended period in the supine position and a pronounced unpleasant feeling from heat created by the RFA procedure. Furthermore, theoretically TPVB alone may not provide adequate anesthetic for the PRFA procedure, because the liver and its capsule are innervated by sympathetic and parasympathetic nerves via the hepatic plexus. Sympathetic fibers extend from the thoracic sympathetic chain from T5 (or T6) to T11 and reach the liver via the greater and lesser splanchnic nerves and the coeliac plexus. Thus, using only thoracic paravertebral block as the sole anesthetic is not yet clearly explained.

Two out of 3 patients that were able to tolerate the burning segment without any sedation requirement developed severe bradycardia during the PRFA procedure which was not seen in patients receiving sedation. The Valsalva maneuver or repeating the Valsalva maneuver may have been responsible for this bradycardia.[27] This maneuver involves compressing the liver to prevent it from shifting or moving during placement of the RFA needle. The Valsalva maneuver causes increased vagal tone and decreased venous blood return, resulting in bradycardia and low cardiac output. In this situation, vagal crisis can be treated with atropine administration.

From this study, one patient with tumor adjacent to diaphragm that had severe shoulder pain may have benefited from superficial cervical plexus block, because the phrenic nerve receives innervation from both the cervical plexus and the brachial plexus.

Onset of paravertebral block in our study took longer, as compared with Cheung, *et al.*, and Picconi, *et al.*, Only 71.43% of our patients (25 of 35 patients) had onset within 30 minutes, while Cheung, *et al.*, reported onset within 10 minutes and Picconi, *et al.*, reported onset within 15-20 minutes. This may be explained by differences in technique and/or number of injections. There was no contralateral spreading in our study. This result was similar to Picconi, *et al.*, but remains in contrast with Cheung, *et al.* Similar to onset, this may be explained by differences in technique.

Thoracic paravertebral block may prove to be an excellent post-procedure analgesia for other radiology procedures, such as transarterial chemoembolization

(TACE). Most patients scheduled for TACE procedure have platelet and coagulopathy complications, for which paravertebral block can safely be used. TPVB is a technique that involves injecting local anesthetic solution alongside vertebra; unlike epidural block, which may cause epidural hematoma. In this study, paravertebral block could be performed in 11 patients who had platelet count between 50,000 and 100,000 and 2 patients with platelet count lower than 50,000/mm^3 without any following complications. However the future study with larger sample size should be investigated before drawing this conclusion. In addition, the continuous presence of an ultrasound machine in the radiology department is an added advantage to using the ultrasound-guided technique.

In this study we were unable to compare TPVB with an alternative anesthetic technique for overall analgesia efficacy and complication rates. A double-blind randomized controlled trial would be a better study design. However, this involves performing a sham TPVB in patients, which is ethically less acceptable for a pilot study investigating the safety and efficacy of ultrasound guided TPVB. Nevertheless, 3 levels ultrasound-guided TPVB demonstrated effectiveness in postoperative pain control as shown in Table 2. Interventionists expressed satisfaction that patients were able to cooperate with them while experiencing only mild pain. In patients that have more than one tumor, we recommend selecting the most difficult one first, because onset of sedation for burning procedure makes it difficult for the patient to cooperate with the inserting needle electrode on the next nodule. While a distinct advantage of ultrasound-guided TPVB is low risk of pneumothorax, a combination of nerve stimulator-guided and ultrasound-guided TPVB may be the safest and most efficacious method. We may be able to use only thoracic paravertebral block as the sole anesthetic for PRFA of hepatic tumors.

CONCLUSION

A 3 levels ultrasound guided right TPVB is an effective technique for providing postoperative pain control for PRFA of hepatic tumors. This blocking technique was well-tolerated by patients and was well-received by interventional radiologists.

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Conflict of interest declaration: The authors hereby declare no personal or professional conflicts of interest regarding any aspect of this paper.

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The Association between Mammographic and Ultrasound Features and Histologic Grade in Invasive Ductal Carcinoma of the Breast

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ABSTRACT

Objective: To evaluate the relationships between mammographic and ultrasound features and histological grades of invasive ductal carcinoma using the terminology of the fifth edition of Breast Imaging Reporting and Data System (BI-RADS).

Methods: Mammographic and ultrasound features of invasive breast carcinomas diagnosed in 104 consecutive women between January 2011 and August 2014 were retrospectively reviewed, described according to the BI-RADS lexicon, and correlated with histological tumor grade according to the Nottingham histologic scoring system.

Results: 104 invasive ductal carcinomas were graded histologically as follows: grade 1, 12.5%; grade 2, 50%; and grade 3, 37.5%. In multivariate analyses, mammographically identified oval or round shape of masses and indistinct margin were significantly associated with grade 3 tumors ($p < 0.05$). In addition, ultrasound-identified microlobulated margin was significantly associated ($p = 0.013$) with grade 3 tumors. In contrast, mammographically identified spiculated margin and ultrasound-identified posterior shadowing occurred significantly more frequently in grade 1 tumors ($p < 0.05$).

Conclusion: Mammographic and ultrasound features are effective predictors of histologic tumor grade of breast cancer.

Keywords: Mammography; invasive ductal carcinoma; ultrasonography (Siriraj Med J 2018;70:152-158)

INTRODUCTION

Mammography has been proven as an effective screening tool and as highly accurate and sensitive means of diagnosing and early detecting breast cancer, although the sensitivity for breast cancer detection is lower in patients with dense breasts.[1] Additionally, ultrasound is a useful breast cancer screening tool and adjunct to mammography for detecting breast cancer, especially in dense breasts.[2,3] Most previous studies have focused on differentiating malignant from benign lesions. It has been clearly shown that the most specific evidence of malignancy is a spiculated and irregular mass.[4]

Breast cancer is a heterogeneous disease with a variety of tumor cell types and histologic grades. Histologic grade is an important prognostic factor for predicting tumor aggressiveness, treatment response, and long-term survival rate. Nonetheless, few previous studies have focused on the relationship between conventional breast imaging and histologic grading. Moreover, published findings have been inconclusive, and some studies reported an association between imaging findings and histologic grade.[5,6] However, Watermann et al.,[7] found no correlation between ultrasound features and histologic grade.

The Breast Imaging Reporting and Data System

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(BI-RADS) has been developed by the American College of Radiology, which incorporates mammographic and ultrasound findings, and is currently used to assess the likelihood of malignancy.[8] To our knowledge, no previous studies have used the fifth edition of BI-RADS to predict histologic grading.

Objective

The main purpose of our study was to evaluate whether the mammographic and ultrasound findings used in the fifth edition of BI-RADS can predict histologic grade. We studied only invasive ductal carcinoma, which is the commonest type of invasive breast cancer, to eliminate some potentially confounding imaging features of other tumor types, such as mucinous cancer, which commonly has a well-defined margin.

MATERIALS AND METHODS

Patients

This study was approved by the Institutional Review Board for human investigation (Si 668/2014). A retrospective review of data of 104 consecutive women who had been diagnosed as having invasive ductal carcinoma and underwent breast imaging, including mammography and ultrasound, at our institution between January 2011 and August 2014 was performed. The indications for breast imaging included both screening and diagnosis. The patients underwent wide excision, modified radical mastectomy, or total mastectomy.

Mammography

Standard bilateral mediolateral (MLO) and craniocaudal (CC) views had been obtained by mammography using the LORAD Selenia or Selenia Dimensions mammography system (Hologic, Marlborough, MA, USA). For the present study, all images were reviewed by two radiologists who had 5 and 15 years' experience in breast imaging and were blinded to histologic tumor grade until consensus was reached. Mammographic features were described according to terminology used in the fifth edition of BI-RADS.[9] Breast composition was classified as almost entirely fatty, scattered areas of fibroglandular density, heterogeneously dense, and extremely dense.

Ultrasound

Bilateral whole breast ultrasound scanning was performed with high frequency linear-array 10-14 MHz transducers on LOGIQ 7 or LOGIQ E9 machines (General Electric Healthcare, Milwaukee, WI, USA). Ultrasound images were interpreted by two breast radiologists until consensus was reached using the descriptors in the fifth edition of BI-RADS lexicon.[10] The size of the tumors was determined by the longest dimension. Finally, in accordance with BI-RADS, a combination of the mammographic and ultrasound features that were most suspicious of malignancy was used to allocate lesions to BI-RADS categories.

Pathology

Histologic grade was allocated by retrospective review of the study patients' charts. All included lesions were defined as invasive ductal carcinoma. Histologic grading of invasive ductal carcinoma was based on the Nottingham histologic score system and allocated as follows: grade 1 (low-grade, well differentiated), grade 2 (intermediate-grade, moderately differentiated), and grade 3 (high-grade, poorly differentiated).

Statistics and Data Analysis

General patients' characteristics were presented using descriptive statistics. Pearson's chi^2- test or Fisher's exact test were used to compare mammographic and sonographic features among the three histologic grades. To identify associations between mammographic or ultrasound findings and high grade breast cancer (grade 3), simple and multiple logistic regression were employed. Results were presented as odds ratios (ORs) and 95% confidence intervals (CIs). All statistical analyses were performed using PASW 18.0 (IBM SPSS Inc., Chicago, IL). *P* values < 0.05 were considered statistically significant.

RESULTS

Patients' Characteristics

Our study cohort comprised 104 consecutive women (mean age, 55.7 ± 9.8 years [SD]; age range 29–81 years; median 55 years) all of whom had single tumor masses. Therefore, 104 invasive ductal carcinomas classified into three histologic grades, grade 1, 2, and 3 carcinomas comprising 12.5% (13/104), 50% (52/104), and 37.5% (39/104), respectively. Table 1 shows associations between patients' characteristics and tumor grades. When we divided participants into two age groups (≤ 50 years and > 50 years); we found that women aged ≤ 50 years had a significantly greater proportion of higher grade tumors (grade 2 and 3) than of low-grade tumors (grade 1) (p = 0.017). Additionally, patients who performed diagnostic mammography had higher tumor grading than in screening women (p = 0.008). However, menstruation status (pre- vs. post-menopausal) did not predict histologic tumor grade.

The mean size of tumors in our study was 2.1 cm

TABLE 1. Descriptive statistics and distribution of patients' characteristics and imaging features according to histologic tumor grading.

Features	Histologic grade: Number (%) Grade 1	Grade 2	Grade 3	*p*-value
Patients' characteristics (*n* = 104)				
Age groups				0.017
0-50 years	0	15 (50)	15 (50)	
>50 years	13 (17.6)	37 (50)	24 (32.4)	
Menstrual status				1
Pre-menopausal	4 (11.4)	18 (51.4)	13 (37.1)	
Post-menopausal	9 (13)	34 (49.3)	26 (37.7)	
Indication for investigation				0.008
Screening	6 (31.6)	10 (52.6)	3 (15.8)	
Diagnosis	7 (8.2)	42 (49.4)	36 (42.4)	
Mammographic features (*n* = 67)				
Shape				0.298
Oval	3 (37.5)	10 (34.5)	18 (60)	
Round	0 (0)	2 (6.9)	1 (3.3)	
Irregular	5 (62.5)	17 (58.6)	11 (36.7)	
Margin				< 0.001
Circumscribed	0	1 (3.4)	0	
Obscured	0	1 (3.4)	0	
Microlobulated	1 (12.5)	2 (6.9)	7 (23.3)	
Indistinct	0 (0)	13 (44.8)	20 (66.7)	
Spiculated	7 (87.5)	12 (41.4)	3 (10)	
Density				0.046
Low	0	0	0	
Equal	6 (75)	13 (44.8)	8 (26.7)	
High	2 (25)	16 (55.2)	22 (73.3)	
Calcification				0.038
No	8 (100)	20 (69)	16 (53.3)	
Yes	0	9 (31)	14 (46.7)	
Associated features				0.111
No	13 (100)	46 (88.5)	34 (87.2)	
Yes	0	6 (11.5)	5 (12.8)	
Ultrasound features (*n* = 102)				
Shape				0.0242
Oval	6 (46.2)	15 (30)	24 (61.5)	
Round	0	2 (4)	0	
Irregular	7 (53.8)	33 (66)	15 (38.5)	
Margin				0.001
Circumscribed	0	3 (6)	1 (2.6)	
Indistinct	4 (30.8)	12 (24)	8 (20.5)	
Angular	2 (15.4)	9 (18)	1 (2.6)	
Microlobulated	3 (23.1)	16 (32)	28 (71.8)	
Spiculated	4 (30.8)	10 (20)	1 (2.6)	
Echo pattern				0.510
Hypoechoic	13 (100)	50 (100)	38 (97.4)	
Heterogeneous	0	0	1 (2.6)	
Calcification in mass				0.075
No	13 (100)	38 (76)	28 (71.8)	
Yes	0	12 (24)	11 (28.2)	
Posterior features				<0.001
None	3 (23.1)	11 (22)	6 (15.4)	
Enhancement	1 (7.7)	14 (28)	25 (64.1)	
Shadowing	8 (61.5)	12 (24)	3 (7.7)	
Combination	1 (7.7)	13 (26)	5 (12.8)	
Orientation				0.112
Parallel	8 (61.5)	37 (74)	34 (87.2)	
Not parallel	5 (38.5)	13 (26)	5 (12.8)	

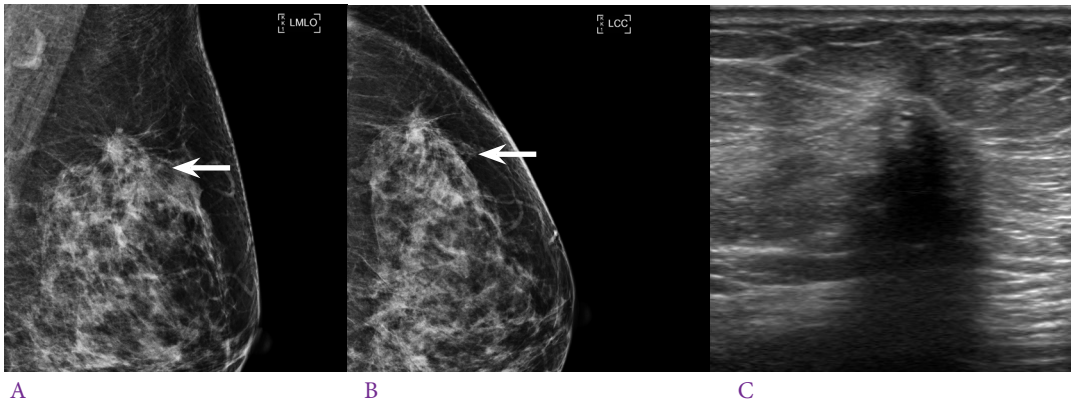
(SD 1.3 cm). We found significant correlations between size of lesions and histologic grade: grade 1 median size was 1.4 cm. (range 0.6–2.7), grade 2 median size was 1.7 cm. (range 0.4–5.7) and grade 3 median size was 2.2 cm. (range 0.8–7.0) (p = 0.0064). Thus, the larger tumors have tendency to be higher tumor grading.

Mammographic Features

The mammographic presentations of our patients' invasive ductal carcinomas were as follows: masses (67 cases, 64.4%), asymmetries (19 cases, 18.3%), calcifications (three cases, 2.9%), architectural distortion (one case, 1%), and no detectable abnormality (14 cases, 13.5%). As to major breast composition in our study, 68.3% of patients had dense breasts (including heterogeneously dense and extremely dense), whereas almost entirely fat and scattered areas of fibroglandular density were found in only 9.6% and 22.1%, respectively.

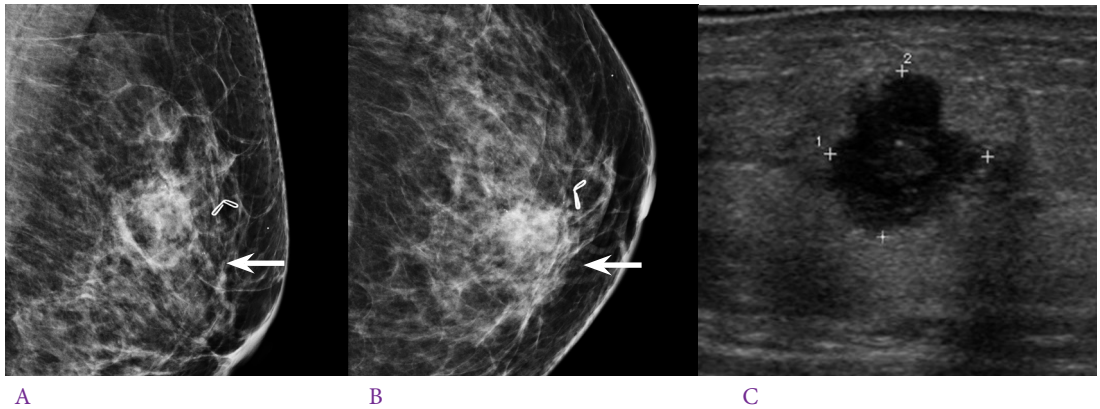
As shown in Table 1, we mainly focused on predicting histologic grading by mammographic features of masses (67 cases) and found that shape and associated findings (findings denoting locally advanced breast cancer) were not correlated with histologic grade. However, the nature of the tumor margins was a strong predictor of histologic grade ($p < 0.001$). As shown in Fig 1, spiculated margin was associated with grade 1 tumors (87.5% of Grade 1 tumors). In contrast, indistinct margin was associated with grade 3 tumors (66.7% of Grade 3 tumors). Density and calcification also moderately correlated with histologic grade ($p < 0.05$); higher grade tumors (grade 2 and 3) were significantly more likely to have high density or calcifications.

Associations between mammographic mass features and grade 3 tumors according to multivariate analyses are shown in Table 2. In multivariate analyses, we found that not only oval or round shapes were significantly associated with grade 3 tumors (odds ratio [OR], 3.40, 95% CI, 1.04-11.15, p = 0.044), but also indistinct margins (OR, 4.80, 95% CI, 1.45-15.92, p = 0.010), as shown in Fig 2. However, high density and calcification tended to be associated with grade 3 tumors without statistical significance.



A B C

Fig 1. Mammographic images of grade 1 invasive ductal carcinoma in a 54-year-old woman demonstrating a spiculated isodensity mass (*arrow*) on mediolateral oblique (A) and craniocaudal views (B). Ultrasound image (C) showing an angular irregular hypoechoic mass with posterior shadowing.



A B C

Fig 2. Mammographic images of grade 3 invasive ductal carcinoma in a 58-year-old woman, who presented with a palpable mass. An indistinct high density mass (*arrow*) is visible in the area of the palpable mass on mediolateral oblique (A) and craniocaudal views (B). Ultrasound image (C) showing a hypoechoic mass with microlobulated margins and posterior enhancement.

TABLE 2. Univariate and multivariate analyses of predictors of grade 3 tumors with mammographic and ultrasound features.

Features	Histologic grade: Number (%)		Univariate analysis		Multivariate analysis	
	Grades 1 and 2	Grade 3	*p*-value	OR (95% CI)	*p*-value	adjusted OR (95% CI)
Mammographic features (*n* = 67)						
Shape						
Irregular	22 (59.5)	11 (36.7)		Reference		Reference
Oval, round	15 (40.5)	19 (63.3)	0.064	2.53 (0.94-6.83)	0.044	3.40 (1.04-11.15)
Margin						
Other margins	24 (64.9)	10 (33.3)		Reference		Reference
Indistinct	13 (35.1)	20 (66.7)	0.010	3.69 (1.34-10.20)	0.010	4.80 (1.45-15.92)
Density						
Low, isodensity	19 (51.4)	8 (26.7)		Reference		Reference
High density	18 (48.6)	22 (73.3)	0.041	2.90 (1.03-8.17)	0.053	3.42 (0.98-11.90)
Calcification						
No	47 (72.3)	20 (51.3)		Reference		Reference
Yes	18 (27.7)	19 (48.7)	0.030	2.48 (1.08-5.69)	0.134	2.56 (0.75-8.77)
Associated features						
No	59 (90.8)	34 (87.2)		Reference		
Yes	6 (9.2)	5 (12.8)	0.564	1.45 (0.41-5.10)	NA	NA
Ultrasound features (*n* = 102)						
Shape						
Oval, round	40 (63.5)	15 (38.5)		Reference		Reference
Irregular	23 (36.5)	24 (61.5)	0.014	2.78 (1.22-6.34)	0.278	1.70 (0.65-4.41)
Margin						
Others	44 (69.8)	11 (28.2)		Reference		Reference
Microlobulated	19 (30.2)	28 (71.8)	<0.001	5.90 (2.44-14.22)	0.013	3.63 (1.31-10.02)
Calcification						
No	53 (81.5)	28 (71.8)		Reference		
Yes	12 (18.5)	11 (28.2)	0.246	1.74 (0.68-4.43)	NA	NA
Posterior features						
Others	48 (76.2)	14 (35.9)		Reference		Reference
Enhancement	15 (23.8)	25 (64.1)	<0.001	5.71 (2.38-13.70)	0.076	2.55 (0.91-7.17)
Orientation						
Not parallel	18 (28.6)	5 (12.8)		Reference		
Parallel	45 (71.4)	34 (87.2)	0.064	2.72 (0.92-8.06)	NA	NA

Abbreviations: OR = odds ratio, 95% CI = 95% confidence interval, NA= not applicable

Ultrasound Features

Ultrasound detected 98% of the 104 invasive ductal carcinomas as masses, 1% by finding focal duct dilatation and 1% by finding calcifications. We studied only the features of masses (102 cases) to predict histologic grade (Table 1) and found that shape, margin, and posterior features were significantly correlated with histologic grade ($p < 0.05$). Oval shape, microlobulated margin, and posterior enhancement were commonly associated with grade 3 tumors, whereas posterior shadowing was more likely to be associated with grade 1 tumors (Fig 1). The echogenicity of invasive ductal carcinomas was not

related to histologic grade, and almost all lesions were hypoechoic (101 lesions, 99%), while the remaining lesion was heterogeneous echo (one lesion, 1%).

Results of multivariate analyses of associations between ultrasound features of masses and grade 3 tumors are shown in Table 2. In multivariate analyses, microlobulated margin was significantly associated with grade 3 tumors (OR, 3.63, 95% CI, 1.31-10.02, p = 0.013) (Fig 2). Oval shape and posterior enhancement were more likely to be associated with grade 3 tumors, although these associations were not statistically significant.

The overall assessment of BI-RADS 4 or 5 categories derived by combining mammography and ultrasound features did not correlate with histologic grade (p = 0.391).

DISCUSSION

It is currently widely accepted that mammography and ultrasound are valuable in differentiating malignant from benign breast lesions. However, previous studies that evaluated imaging features have rarely addressed prediction of histologic grade. In this study, we used the terminology of the fifth edition of BI-RADS and focused mainly on the ability of mammographic and ultrasound features to predict histologic grade of the invasive ductal carcinomas.

Spiculated margin has hitherto been the most commonly recognized as highly specific mammographic finding associated with invasive breast cancer and often results in BI-RADS Category 5.[8,11] However, in our series we found that spiculated margin was mostly found in grade 1 tumors, whereas it found only in 10% of grade 3 tumors. Lamb et al.,[5] and Evan et al.[12] have reported results similar to our study that spiculated margin is a good predictor of low grade tumor. Furthermore, we found that posterior features in ultrasound findings were predictors of histologic grade. Posterior shadowing was found in about 61.8% of grade 1 tumors versus 7.7% of grade 3 tumors. This result was consistent with previous studies that reported correlations between the presence of posterior shadowing and low grade tumors.[5,13-15] In low grade tumor, several researchers have proposed a theoretical explanation for the impact of histopathologic pattern on radiologic findings in which low grade tumors are usually slow growing and have low mitotic rates, inducing marked desmoplastic reactions.[5,14] Desmoplastic reactions cause spiculated margin on mammography and posterior shadowing on ultrasound. According to our results, spiculated margin on mammography and posterior shadowing on ultrasound effectively predict grade 1 tumors and also correlate with histological features.

Interestingly, we found that some mammographic and ultrasound features are reliable predictors of grade 3 tumors. Mammographically indistinct margin was strongly associated with grade 3 tumors (p = 0.010), which was consistent with the finding of Lamb et al,.[5] However, there are some differences in description of the margin between our study and other studies due to new BI-RADS lexicon. Furthermore, mammographically oval and round shape tumor masses, which have been considered benign features, were moderately correlated with grade 3 tumors (p = 0.044) in our study. According to our ultrasound findings, we found that microlobulated margins strongly predicted grade 3 tumors (p = 0.013) consistent with other studies.[5,13,17] It is a fact that higher grade tumors grow rapidly and there is no desmoplastic reaction causing "pushing margins" which would result in relatively well-defined tumors and can explain our results in imaging features of grade 3 tumors.[16] Moreover, a few previous studies have reported that calcification in masses is significantly correlated with high-grade tumors and poor prognosis.[6,18] However, our study did not identify significant correlations between this feature and tumor grade.

Our study has some limitations. First, it was a retrospective study. To minimize the resultant bias, two radiologists who were blinded to the clinical context and pathological tumor grade interpreted the imaging findings until they achieved consensus. Second, the radiologists knew that all lesions in this study were malignancy when they interpreted the imaging features. Third, our sample size was small and comprised only consecutive women from only a single institution. Fourth, the lesions had multiple features and we chose only one feature to describe in accordance with the BI-RADS lexicon. Fifth, we did not assess interobserver variability in this study because moderate to substantial interobserver agreement has been demonstrated in previous studies.[19,20]

In conclusion, our study showed that certain mammographic and ultrasound features can be the predictors of histologic grade of breast cancer. In particular, oval and round shape, and indistinct margins on mammography are associated with grade 3 tumors. The microlobulated margin in ultrasound feature is also associated with grade 3 tumors. Conversely, mammographic spiculated margin and sonographic posterior shadowing occur more commonly in Grade 1 than in higher grade tumors. Thus, radiologists should be cautious when interpreting apparently benign tumors because high-grade tumors can appear similarly as a result of dense cellularity and lack of desmoplastic reaction.

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Chemical Profiling of an Antipyretic Drug, Thai Herbal Harak Formula, by Liquid Chromatography Coupled with Quadrupole Time-of-Flight Mass Spectrometry

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ABSTRACT

Objective: To isolate and identify chemical compounds in Harak, a Thai herbal formula widely used as an antipyretic drug in Thailand.

Methods: Methanol extraction of the Harak formula and its five herbal components were separated by Ultra high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. This method has been developed for "untargeted" profiling of this formula and its components. An online database was used to identify chemical compounds by comparing its empirical molecular formula, isotope pattern, and fragmentation pattern.

Results: Nineteen chemical constituents were found from positive and negative electrospray ionization (ESI) modes. These compounds included flavonoids, hesperitine, and iso-corydine, which are known to possess antioxidant and anticancer activity. Moreover, data from the principle component analysis (PCA) score plot of positive and negative ESI modes showed that the chemical constituents of Thai herbal Harak formula were similar to those found in *Ficus racemosa* Linn. and *Capparis micracantha* DC.

Conclusion: Under this optimization method, nineteen chemical constituents including phenolic and flavonoids were characterized in both positive and negative ESI mode.

Keywords: Chemical profiling; Thai herbal Harak formula; Thai traditional medicine; time-of-flight mass spectrometry; ultra high performance liquid chromatography (Siriraj Med J 2018;70: 159-168)

INTRODUCTION

A Thai herbal Harak formula (HRF) or Ha-Rak recipe has been widely used in Thailand as an antipyretic drug. The formula has been included in the essential drug list of herbal medicinal products since 2006 by National Drug Committee of Thailand.[1] HRF is prepared by the combination of five dried roots powder in the equal parts by weight; *Ficus racemosa* Linn. (FR), *Capparis micracantha* DC. (CP), *Harrisonia perforata* (Blanco) Merr. (HP), *Tiliacora triandra* (Colebr.) Diels. (TT) and *Clerodendrum petasites* (Lour.) S. Moore (CP). Pharmacological activities of HRF have been previously studied such as antipyretic[2], antioxidant[3] and anti-inflammatory.[4] Methanol and aqueous extracts of Ben-Cha-Lo-Ka-Wi-Chian remedy; another name for HRF, had antipyretic and antinociceptive properties in animal models[2]. Ethanol extracts of HRF and its herbal components suppressed UVA-induced matrix metalloproteinase-1 (MMP-1) activity in keratinocyte HaCaT cells. Moreover, they regulated the endogenous antioxidants including

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glutathione (GSH), catalase, and glutathione peroxidase (GPx).[3] Previous study also showed non-direct mutagenic activity of this formula.[4] Many scientific studies[5-17] have suggested that the main chemical constituents of each component of HRF, excluding CM, were phenolic acid and flavonoids which are known as pharmacological substances of antioxidant, anti-inflammatory and anticancer actions. However, scientific data that support the chemical constituents in a polyherbal formula HRF was rare. Until now, there was only one work that studied chemical components from HRF remedy. Singharachai C, et al. 2011[4] had found twelve components (Cappine B, Yanangine, Tiliacosine, Tiliacorinine, Tiliasine, Nevedencin 7-rutinoside, Hispidulin 7-methyglucoside, Hispidulin, Heteropeucenin 7-Me-ether, Perforatin G, Racemosa and Isowingtione) from HRF remedy using 3D-HPLC followed with UV spectrum patterns data comparison.

In the past decades, many reports had been developed the best way for isolation and identification of chemical constituents from herbal medicines (HMs). However, HMs comprise hundreds of different constituents that belong to numerous compound classes.[18] Moreover, the challenging task of HMs is some HMs contain two or more than two herbal components. These HMs are more complex, therefore, analysis of HMs are more difficult. The analytical selectivity and sensitivity of liquid chromatography coupled with mass spectroscopy (LC-MS) together make the most selective technique for rapid screening and characterization of known and unknown constituents from the HMs. Nowadays, Liquid Chromatography Quadrupole Time-of-flight Mass Spectrometry (LC-Q-TOF) is one of the powerful tools to isolate and identify chemical constituents in HMs. LC-Q-TOF provides accurate mass measurements (possibility to yield mass accuracy <5 ppm with an adequate calibration range)[19, 20] and high resolutions. The accurate mass measurement from LC-Q-TOF gives elemental composition of both parent and fragment ions.[19, 21-23] Thus, it is appropriate for identification of unknown constituents. There are many applications of LC-Q-TOF for HMs analysis such as qualitative, quantitative of HMs or study of metabolites. HMs include known and unknown chemical compounds. An interesting function of LC-Q-TOF for HMs qualitative analysis is identification of complex chemical constituents which involves the confirmation of targeted compounds and elucidation of non-targeted compounds. To screen non-targeted compounds from HMs using LC-Q-TOF, the exact elemental compositions of parent ions are generated from several steps which are applied from Chen XF, et al. 2011.[20] LC-Q-TOF software, MarkerLynx™ XS (Waters Corp., MA, USA), generated the possible molecular composition according to accurate mass measurements after low fragmentation voltages. Then, the most probable molecular composition was selected and searched against an on-line chemical library. Finally, the library match was recorded for each chemical formula. Because of the complexity of HMs, only parent ions identification may not be enough for confirmation. Therefore, fragment ions of parent ion are generated using high fragmentation voltages and the software of Q-TOF, MassFragment™ software (Waters Corp., MA, USA). This provides valuable structural information by producing various characteristic fragment ions together with their elemental compositions.

The present study, developed the first chemical profiling of a Thai herbal Harak formula and its five herbal components. LC-Q-TOF was used as a tool for isolation and identification of chemical profiling. Due to the complexity and lacking of chemical constituents data in HRF, chemical compositions of HRF were generated by screening method. Moreover, we focussed on the most possible chemical targets, therefore mass spectrometry detection was performed in both positive and negative electrospray ionization mode (ESI). The most complete datasets were identified by matching and comparison of empirical molecular formula with online database. This work provided the rapid and reliable method for isolation and identification of the chemical profiling in HRF and the other HMs.

MATERIALS AND METHODS

Chemicals solvents and herbal materials

LC-MS grade acetonitrile, methanol, formic acid and analytical grade ethanol were purchased from Scharlau (Spain). Purified water was prepared by a Milli-Q water system (Millipore, France).

HRF and its five herbal components (FR, CM, HP, TT and CP) were prepared by the good manufacturing practice (GMP) certified Manufacturing Unit of Herbal Medicines and Products, Center of Applied Thai Traditional Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. All of them were authenticated by experienced Thai traditional practitioners. Then, the powders were stored and kept at room temperature in air-dried condition. The five herbal components of HRF were shown in Fig 1.

Preparation of HRF and its five herbal components

For identification of chemical constituents, the HRF, FR, CM, HP, TT and CP powders were accurately weighed (100 mg) and ultrasonically extracted with methanol 1 mL for 60 min. Each extracted solution was centrifuged



Fig 1. Five dried roots of HRF's herbal components: (A) *Ficus racemosa* Linn., (B) *Capparis micracantha* DC., (C) *Harrisonia perforata* (Blanco) Merr., (D) *Tiliacora triandra* (Colebr.) Diels. and (E) *Clerodendrum petasites* (Lour.) S. Moore.

(Eppendorf, Germany) at 12,000 rpm for 10 min at 4°C and the supernatant was filtered through 0.2 µm polyvinylidenedifluoride (PVDF) syringe filter (Vertical Chromatography, Thailand). The final concentrations of all sample solutions with 10 mg/mL and 4 µL were injected into UHPLC-Q-TOF.

Chromatographic conditions

Chromatographic analysis was performed with a Waters ACQUITY UPLC® system (Waters Corp., MA, USA) equipped with a binary solvent delivery system, an online degasser, an auto sampler and a thermostatically controlled column system. The separation was performed on a Waters ACQUITY HSS PFP (100 mm × 2.1 mm, 1.8 µm, Waters, Ireland) column at 30°C. The auto sampler was maintained at 10°C. The mobile phase consisted of (A) 0.1% formic acid in purified water and (B) 0.1% formic acid in acetonitrile using an optimization linear gradient elution of 2-99%B over 0-9.0 min, kept at 99%B for 1 min, 99-2%B over 10.0-12.0 and kept at 2%B for 3 min. The sample volume injected was 4 µL and the flow rate was 400 µL/min.

Mass spectrometry detection was performed on Waters® Xevo™ Q-TOF Mass spectrometer (Waters Corp., MA, USA) equipped with ESI source. The analysis carried out by positive ion and negative ion mode of MS^E analysis mode, involving low collision energy of 4 V for the precursor analysis and for the fragment ions the analysis was performed using high collision energy of 20-40 V. Mass range was set as m/z 100-1,000. The source parameters were electrospray capillary voltage 3 kV, source temperature at 120°C and desolvation temperature at 600°C. The cone voltage was set at 20 V. Nitrogen and argon were used as cone and collision gases, respectively. The cone and desolvation gas flow were set at 50 L/hr and 900 L/hr respectively.

To ensure the accuracy and reproducibility of all analysis, a reference standard lock spray interference, leucine encephalin (Waters Corp., MA, USA) was used. The lock mass of leucine encephalin for positive mode and negative mode were 556.2771 and 554.2615 respectively, at a concentration 200 pg/mL, with the infusion flow rate of 20 µL/min and scan injection every 30 sec.

Data processing and statistical analysis

The MassLynx™ V4.1 software (Waters Corp., Milford, USA) were used to collecting all data. The EZinfo software (Umetrics UK Ltd, UK) was used for multivariate statistical analysis; PCA (principal component analysis). The possible markers were extracted from S-plot of the OPLS-DA (an orthogonal partial least-squares-discriminant analysis). To avoid the chemical noise, pareto scaling was used in all the models. The MarkerLynx™ XS (Waters Corp., Milford, USA) and MassFragment™ software (Waters Corp., Milford, USA) were used for identifying chemical markers which were confirmed by mass spectrum and chromatographic retention times following databases, such as ChemSpider, Natural Remedies, KEGG and Pubmed.

RESULTS

Chromatographic conditions and Q-TOF method development

Preliminarily, these chromatographic conditions were tested on four columns. The ACQUITY UPLC® HSS PFP (100 mm × 2.1 mm, 1.8 µm, Waters, Ireland) had a good separation and gave a large number of compounds thus it was chosen for this study. The other columns were Waters ACQUITY BEH Shield RP18 (100 mm × 2.1 mm, 1.8 µm), Waters ACQUITY CSH C18 (100 mm × 2.1 mm, 1.8 µm) and Waters ACQUITY HSS T3 (50 mm × 2.1 mm, 1.8 µm). A solvent system consisted of 0.1% formic acid in purified water and acetonitrile provided the superior separation and baseline stability. Gradient elution was used for the best separation in 10 min at flow rate 400 µL/min.

Mass spectrometry detection was performed on a Waters® Xevo™ Q-TOF mass spectrometer equipped with ESI source. Untargeted analysis was applied for identify abundant chemical constituents in HRF and its components. Since there was no target analysis, samples should be separated as much as possible thus the separations were in both positive and negative ionization mode. Analyses of the other parameters were conducted to get more peak and fragmentation information for further identification of the chemical constituents.

Chemical profiling of HRF and its herbal components

This work developed the first chemical profiling of HRF and its five herbal components; FR, CM, HP, TT and CP, by LC-Q-TOF. HRF and all herbal components were separated by UHPLC-Q-TOF, which in this case the ionizations were done in both positive and negative mode. Chemical profiling of HRF (a.), FR (b.), CM (c.), HP (d.), TT (e.) and CP (f.) in positive and negative ionization mode were exhibited in Fig 2A and 2B respectively. All of the chemical profiling showed in base peak intensity (BPI) chromatograms. Both positive and negative ESI mode demonstrated the complexity of chemical constituents in HRF and all herbal components. The UHPLC-Q-TOF system over the m/z 100-1,000 within 12 min could detect many peaks.

Peak assignment from HRF and its herbal components

Currently, chemical constituent data of HRF were less and remain unclear. Most of HRF's herbal components were found to be phenolic acid and flavonoids. For this study, we would like to find some chemical constituents from HRF and its components. Therefore, "Untargeted" screening method was applied to generate chemical constituents from HRF and each herbal component. In order to obtain the chemical data, principal component analysis (PCA) was utilized to depict the different chemical constituents between the HRF and its five herbal components. PCA Score plot of HRF and its five herbal components in positive and negative ESI mode were shown in Fig 3A and 3B respectively. As shown in the PCA Score plot of positive and negative ESI modes, each sample was represented as a point. The HRF, FR, CM, HP, TT and CP were clearly separated as the principal components in both positive and negative ESI mode. The red circles in PCA exhibited that chemical constituents of HRF were similar to those found in FR and CM by the principal component 3 (PC3) and 4 (PC4). In addition, the key chemical constituents were generated

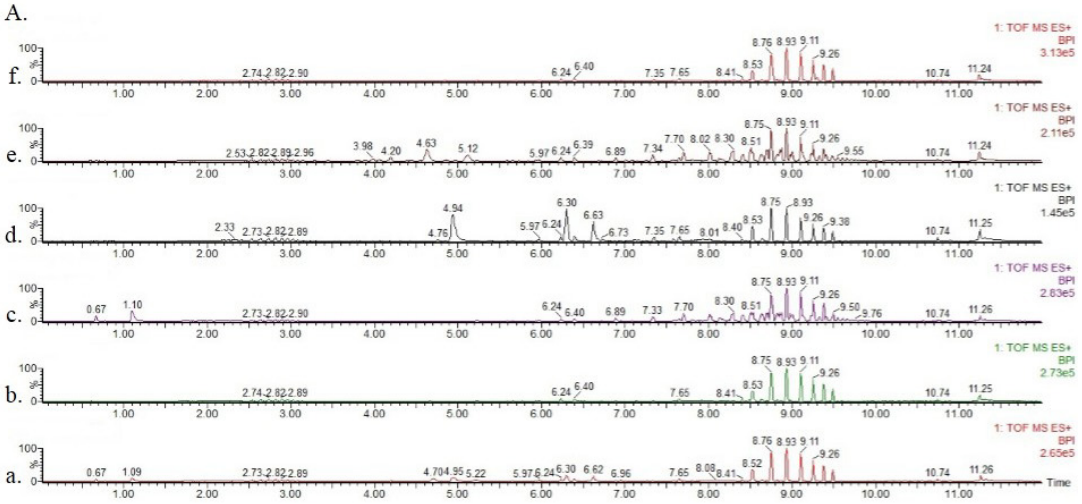


Fig 2A.

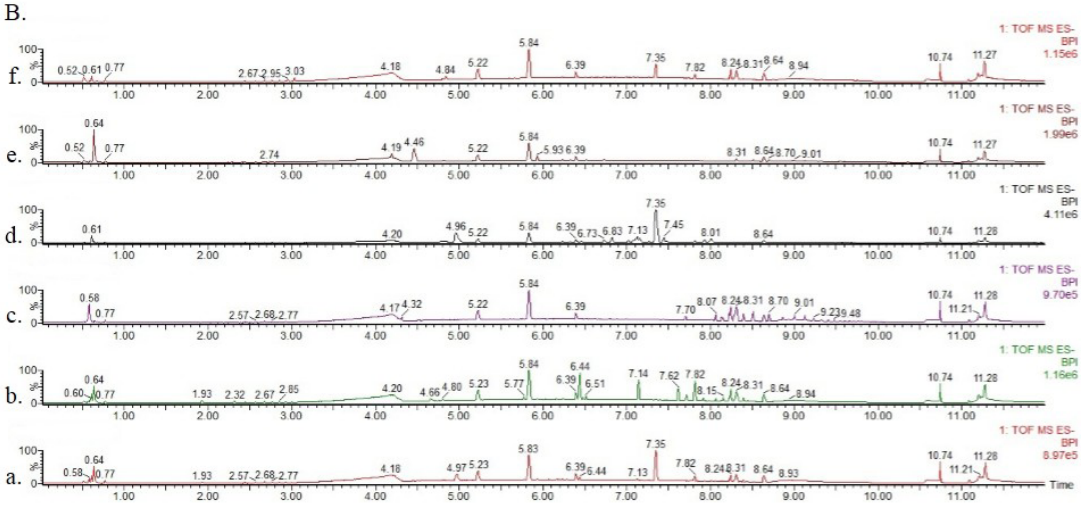


Fig 2B.

Fig. 2 Base-peak ion (BPI) chromatograms of HRF and its five herbal components from LC-QTOF analysis with (A) positive ESI mode and (B) negative ESI mode: a) HRF, b) *Ficus racemosa* Linn., c) *Capparis micracantha* DC., d) *Harrisonia perforata* (Blanco) Merr., e) *Tiliacora triandra* (Colebr.) Diels. and f) *Clerodendrum petasites* (Lour.) S. Moore.

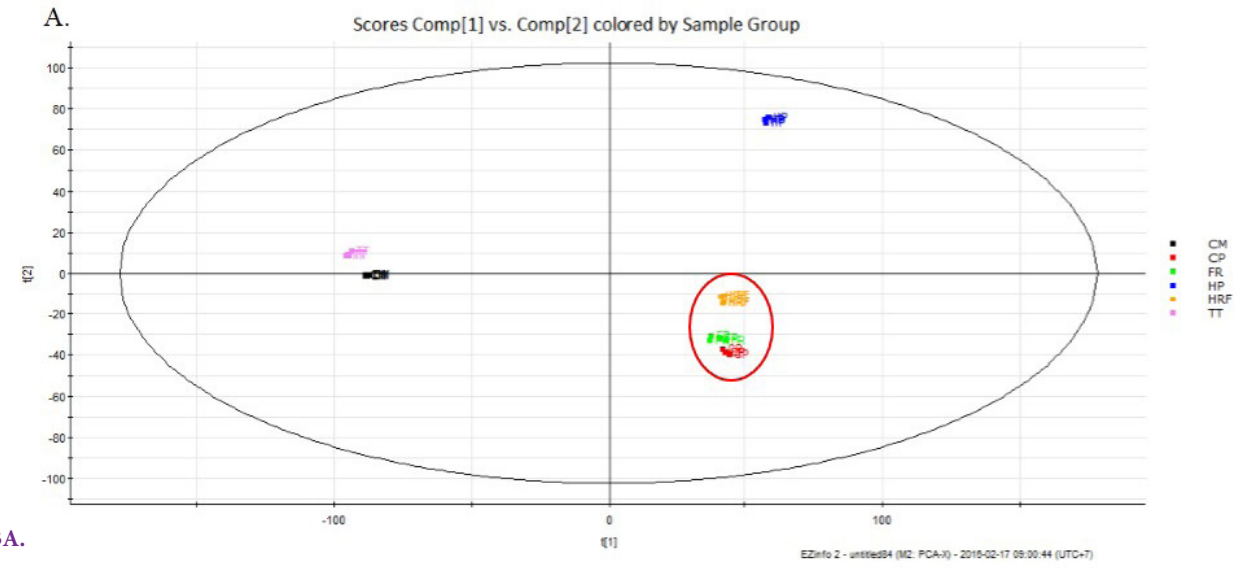


Fig 3A.

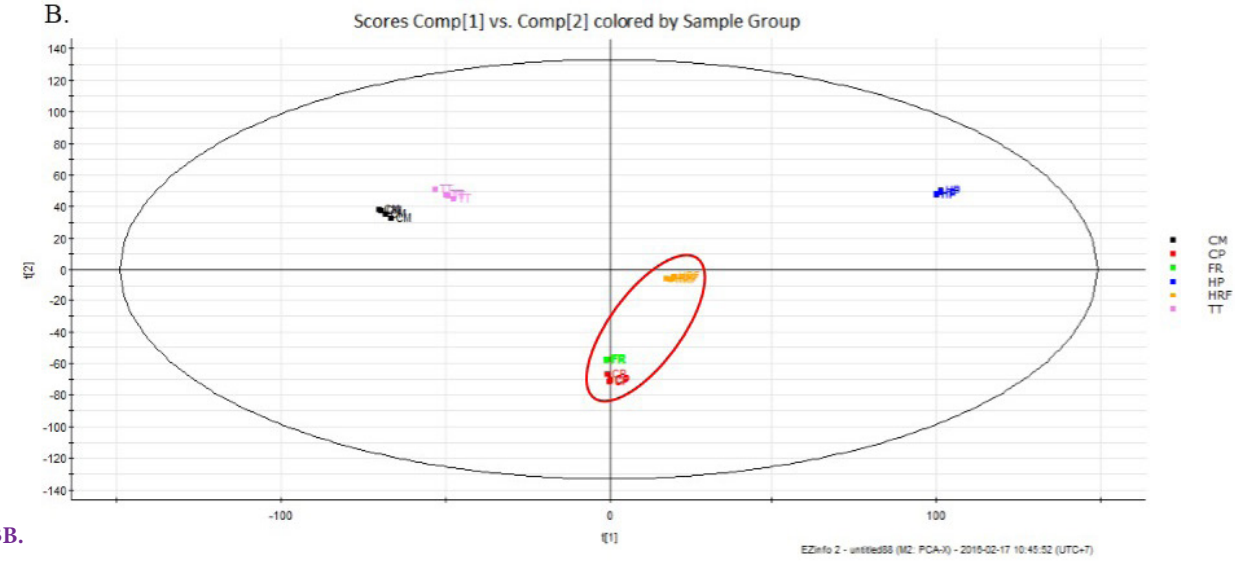


Fig 3B.

Fig. 3 PCA Score plot of HRF and its five components in (A) positive ESI mode and (B) negative ESI mode; Thai herbal Harak formula (HRF), *Ficus racemosa* Linn. (FR), *Clerodendrum petasites* (Lour.) S. Moore (CM), *Harrisonia perforata* (Blanco) Merr. (HP), *Tiliacora triandra* (Colebr.) Diels. (TT) and *Capparis micracantha* DC. (CP).

by OPLS-DA S-plot (Fig 4 and Fig 5 for positive and negative ESI mode respectively). Identification of the differences between sample groups was elucidated by MarkerLynx™ XS data processing which then generated the key chemical targets. All chromatograms in each positive and negative ESI mode were aligned. Possible key chemical markers were created by correlation and coefficient value of OPLS-DA S-plot. Each sample was compared together with the rest samples. In Fig 4 and Fig 5, marker ions had correlation value nearly -1 and coefficient value far from the center of OPLS-DA S-plot, suggesting that was the more important compound. These candidate markers were tentatively identified by isotope pattern matching and comparison of empirical molecular formula with online database (Chembank, ChemDB, KEGG, Natural Remedies, Nature Chemical Biology, Nature Chemistry and Pubmed). Moreover, for structural elucidation conformation, fragmentation pattern matching was done by MassFragment™ software.

Nineteen chemical constituents including phenolic and flavonoids which are known as pharmacological substances of anti-fungal and anticancer activities,[24,25] were found from positive and negative ESI mode. The data showed only compounds which matched with online database. Table 1 showed eight candidate compounds found in both HRF and its herbal components in positive ESI mode. In negative ESI mode, twelve candidate compounds were identified from both HRF and its herbal components (Table 2). Surprisingly, 5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4H-chromen-4-one or hesperitine was found in both positive and negative ESI modes and expressed in all HRF's five components.

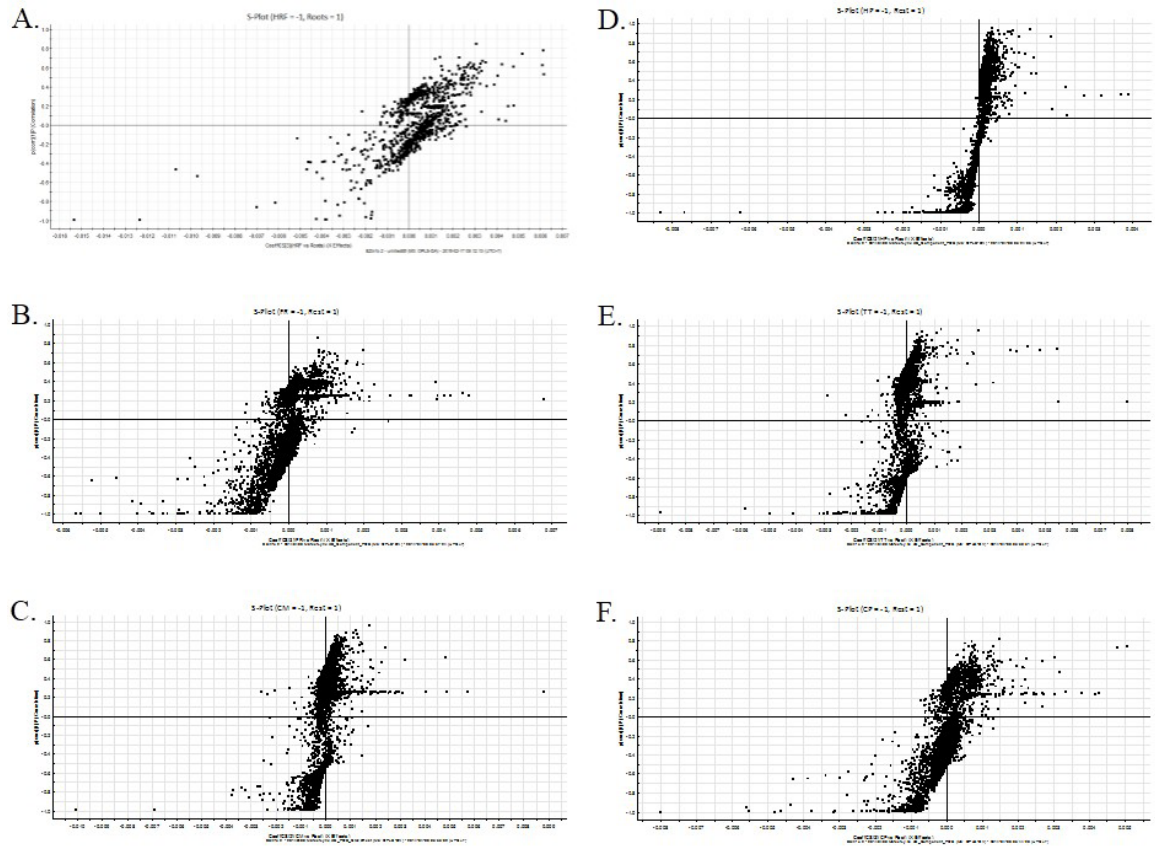


Fig 4. OPLS-DA S-plot of HRF and its five herbal components in positive ESI; HRF (A), *Ficus racemosa* Linn. (B), *Clerodendrum petasites* (Lour.) S. Moore (C), *Harrisonia perforata* (Blanco) Merr. (D), *Tiliacora triandra* (Colebr.) Diels. (E) and *Capparis micracantha* DC. (F).

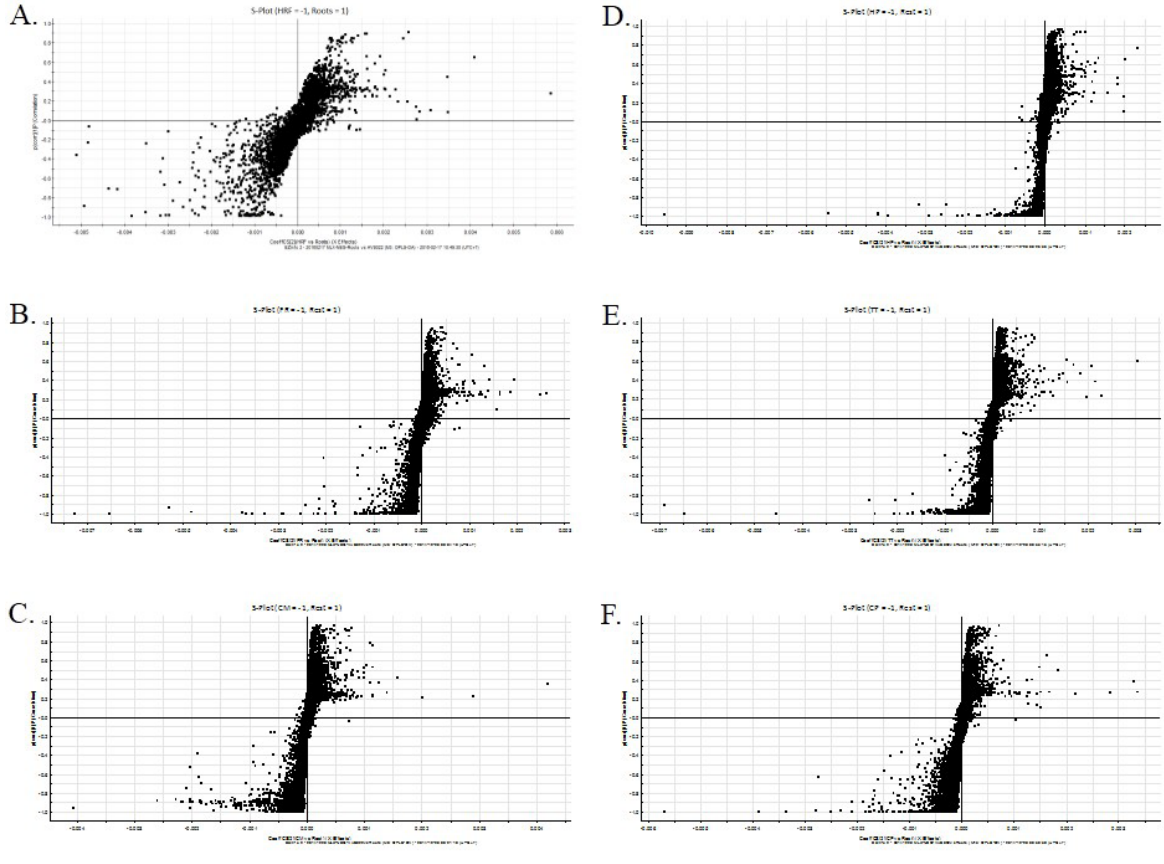


Fig 5. OPLS-DA S-plot of HRF and its five herbal components in negative ESI; HRF (A), *Ficus racemosa* Linn. (B), *Clerodendrum petasites* (Lour.) S. Moore (C), *Harrisonia perforata* (Blanco) Merr. (D), *Tiliacora triandra* (Colebr.) Diels. (E) and *Capparis micracantha* DC. (F).

TABLE 1. Tentatively identified of compounds from HRF and its five components using LC-QTOF in positive ESI mode.

RT(min)	Parent ions (m/z)	Actual mass	Tentative identification	Elemental composition	HRF	FR	CM	HP	TT	CP
0.6684	160.0972 $[M+H]^+$	159.0894	Methyl 2-[(isopropylideneamino)oxy] propanoate	$C_7H_{13}NO_3$	/		/			
1.1061	144.1023 $[M+H]^+$	143.0945	1-(Hydroxymethyl)-2-azepanone	$C_7H_{13}NO_2$	/		/			
2.5310	388.2544 $[M+H]^+$	387.2465	N-{[(2-Methyl-2-propanyl)oxy]carbonylleucyl-N~5~-(diaminomethylene) ornithine	$C_{17}H_{33}N_5O_5$	/	/	/	/	/	/
4.2009	314.1396 $[M+H]^+$	313.1318	Ethyl 11-oxo-2,3,6,7-tetrahydro-1H,5H,11H-pyrano[2,3-f]pyrido[3,2,1-ij] quinoline-10-carboxylate or Coumarin 314	$C_{18}H_{19}NO_4$	/				/	
4.8512	301.0714 $[M+H]^+$	300.2629	5,7-Dihydroxy-2-(4-hydroxyphenyl)-6-methoxy-4H-chromen-4-one or Hispidulin	$C_{16}H_{12}O_6$	/					/
4.7225	342.1710 $[M+H]^+$	341.1631	(6aS)-1,2,10-Trimethoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-11-ol or iso-Corydine	$C_{20}H_{23}NO_4$	/					
4.9435	303.0876 $[M+H]^+$	302.0798	5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4H-chromen-4-one or Hesperitine	$C_{16}H_{14}O_6$	/			/		
6.2992	273.1133 $[M+H]^+$	272.1055	4,4'-(1,2-Hydrazinediylidene)bis (4,5,6,7-tetrahydro-2,1,3-benzoxadiazole)	$C_{12}H_{12}N_6O_2$	/			/		

Abbraviations: FR: *Ficus racemosa* Linn., CM: *Clerodendrum petasites* (Lour.) S. Moore, HP: *Harrisonia perforata* (Blanco) Merr., TT: *Tiliacora triandra* (Colebr.) Diels. and CP: *Capparis micracantha* DC.

TABLE 2. Tentatively identified of compounds from HRF and its five components using LC-QTOF in negative ESI mode.

RT(min)	Parent ions (m/z)	Actual mass	Tentative identification	Elemental composition	HRF	FR	CM	HP	TT	CP
0.5892	332.0107 $[M-H]^-$	333.0185	1-S-[N-(Sulfooxy) ethanimidoyl]-1-thiohexopyranose	$C_8H_{15}NO_9S_2$	/		/			
0.6462	191.0556	192.0634	1-Oxaspiro[2.5]octane-4,5,6,7,8-pentol	$C_7H_{12}O_6$	/			/	/	
4.1972	312.1235 $[M-H]^-$	313.1313	1,3,7-Trimethyl-8-[2-(2-pyridinylmethylene)hydrazino] -3,7-dihydro-1H-purine-2,6-dione	$C_{14}H_{15}N_7O_2$	/				/	
4.6585	269.0449 $[M-H]^-$	270.0527	1-[(4-Methylphenyl)sulfonyl]-2-(methylsulfanyl)-4,5-dihydro-1H-imidazole	$C_{11}H_{14}N_2O_2S_2$	/	/				
4.9827	301.0704 $[M-H]^-$	302.0782	5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4H-chromen-4-one or Hesperitine	$C_{16}H_{14}O_6$	/	/	/	/	/	/
5.7725	337.1072 $[M-H]^-$	338.1150	3-(1-Methyl-1H-pyrrol-2-yl)-N'-(3-nitrobenzylidene)-1H-pyrazole-5-carbohydrazide	$C_{16}H_{14}N_6O_3$	/	/				
5.8380	277.1808 $[M-H]^-$	278.1886	[2,6-Bis(dimethylamino)-4-pyrimidinyl](1-piperazinyl) methanone	$C_{13}H_{22}N_6O$	/	/				/
5.9324	295.2273 $[M-H]^-$	296.2352	1-Ethyl-4-{1-[1-(2-methoxyethyl)-1H-tetrazol-5-yl]-2-methylpropyl} piperazine	$C_{14}H_{28}N_6O$	/	/			/	/
6.5171	403.1543 $[M-H]^-$	404.1621	2-(2,3-Dimethoxyphenyl) cycloheptyl 4-methylbenzenesulfonate	$C_{22}H_{28}O_5S$	/	/				
6.5236	349.1077 $[M-H]^-$	350.1155	2-Oxo-3-(3-oxo-1-phenylbutyl)-2H-chromen-4-yl acetate	$C_{21}H_{18}O_5$	/	/				
6.4405	405.1699 $[M-H]^-$	406.1777	2-{[2-(1H-Indol-3-yl)ethyl] amino}-4',6'-dimethyl-6-oxo-5,6-dihydro-4H-1,2'-bipyrimidine-4-carboxylic acid	$C_{21}H_{22}N_6O_3$	/	/				
6.4733	351.1231 $[M-H]^-$	352.1309	Diethyl 2,2'-(2'-hydroxy-4',5-dioxo-2,4',5,5'-tetrahydro-1H,1'H-3,3'-bipyrrole-1,1'-diyl) diacetate	$C_{16}H_{20}N_2O_7$	/	/				

Abbraviations: FR: *Ficus racemosa* Linn., CM: *Clerodendrum petasites* (Lour.) S. Moore, HP: *Harrisonia perforata* (Blanco) Merr., TT: *Tiliacora triandra* (Colebr.) Diels. and CP: *Capparis micracantha* DC.

DISCUSSION

The present study, UHPLC-Q-TOF was used as a tool for isolation and identification of chemical constituents from HRF and its five herbal components. Untargeted screening method has been developed for identification of the possible chemical constituents of HRF and its five herbal components which is applicable for another complex herbal formula. The chemical compounds were identified by comparison of empirical molecular formula with online database, isotope pattern matching, and fragmentation pattern matching. According to the results, some peak intensities which were found in HRF are the same as those found in all or some herbal components too, which represented these peaks of HRF which were from its herbal components. On the other hand, some peak intensity was only established in HRF. It is possibly that the new compounds were produced after these herbal components were mixed. Moreover, some chemical constituents of the five herbal components were presented in a tiny concentration. When these herbal components were mixed in equal weight, the concentrations of chemical constituents were diluted. There was a possibility that some chemical constituents which were found in some or all five herbal components could not be found in HRF. Moreover, the chemical change of the constituents in HRF could be caused by chemical interaction of each herb during extraction processes.[26] This method was developed for rapid screening of the possible chemical constituents. However, the classification method which compares with reference standard should be done after screening.

CONCLUSION

This study has demonstrated a convenient, rapid screening, high-throughput and reliable UHPLC-Q-TOF method for analysis of chemical profiles. Under this optimization method, nineteen chemical constituents including phenolic and flavonoids were characterized in both positive and negative ESI mode. In addition, as shown in the PCA Score plot of positive and negative ESI modes, the chemical constituents of HRF were similar to those found in *Ficus racemosa* Linn. and *Capparis micracantha* DC. Hence, this work generated the new scientific data for HRF and its five herbal components to support the use of HRF and further study on identified compounds in each herbal component.

ACKNOWLEDGMENTS

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Conflict of Interest: The authors declare no conflict of interest.

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Impact of ^{18}F-FDG PET/CT Scan on Management of Non-small Cell Lung Cancer Patients in Contexts of University-Based Hospital in Thailand

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ABSTRACT

Objective: To determine the impact of Fluorine-18 fluorodeoxy glucose (^{18}F-FDG) positron emission tomography with computed tomography (PET/CT scan) on management of non-small cell lung cancer (NSCLC) in contexts of university-based hospital in Thailand where most patients were in advanced disease and conventional imaging (CI) had previously been done.

Methods: Between December 2006 to December 2014, PET/CT scan and CI - e.g. chest and upper abdominal CT scan and bone scintigraphy of 120 pathologically proven NSCLC patients were independently reviewed. Stagings based on CI and PET/CT scan were evaluated. Proposed treatment plan based on CI and given treatment based on PET/CT scan were compared in patients whose medical records were available. Impact of PET/CT scan on management was categorized into inter-modality and intra-modality treatment change – i.e. change in radiation fields.

Results: Of 120 NSCLC patients, PET/CT scans were performed for initial staging (INS) in 60 patients, post-treatment evaluation (PTE) in 41 patients and restaging at time of recurrence or progression (RR) in 19 patients. PET/CT scan changed the disease status in 38 patients (31.7%). Changes to more advanced and less advanced disease were found in 26 patients (11 INS, 8 PTE and 7 RR patients) and 12 patients (3 INS, 6 PTE and 3 RR patients), respectively. Among 83 patients with available proposed treatment plan, inter-modality treatment changes were observed in 19 patients (22.9%). Changes were more frequent in setting of PTE and RR (14/19 patients; 73.7%). The most common changes were toward palliative chemotherapy (15/19 patients, 78.9%) owing to unexpected distant metastasis. Among 43 patients who received radiation therapy, changes in radiation approach were found in 18 patients (41.9%), mostly due to positive or negative lymph node (LN) metastasis.

Conclusion: PET/CT scan has considerable impact on staging and both inter-modality and intra-modality treatment change in NSCLC patients in all settings – INS, PTE and RR even with the presence of CI. Therefore, PET/CT scan should be considered for staging and guiding the multidisciplinary treatment.

Keywords: PET/CT; non-small cell lung cancer; NSCLC; staging; management; treatment (Siriraj Med J 2018;70: 169-174)

INTRODUCTION

In non-small cell lung cancer (NSCLC), conventional imaging (CI) – e.g. computed tomography (CT), magnetic resonance imaging (MRI) and bone scintigraphy generally provides adequate information for staging and subsequent management. However, patients have to undergo multiple imaging modalities causing delayed treatment. Additionally, CI may occasionally have limitation in differentiating between benign and malignant lesions. With these reasons, whole body functional imaging, ^{18}F-FDG PET/CT scan has been developed to overcome these problems. For decades, ^{18}F-FDG PET or PET/CT scan has been proved by many studies in developed countries to provide benefits in almost every aspect of

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management of NSCLC from staging to detection of recurrence.[1-14] Nevertheless, lack of effective screening program and unawareness of malignancy have resulted in late presentation of many lung cancer patients in Thailand. Moreover, to be eligible for reimbursement, CT scan of chest including upper abdomen and bone scintigraphy must be done prior to PET/CT scan. We hypothesized that benefit of PET/CT scan may be altered in patients with advanced disease and who previously received the CI. Therefore, this study was elicited to evaluate the impact of PET/CT scan on NSCLC treatment in the contexts of our university-based hospital in Thailand.

MATERIALS AND METHODS

During December 2006 to December 2014, 120 pathologically proven NSCLC patients who underwent PET/CT scan for 1) initial staging or evaluation of disease extension at time of diagnosis (INS), 2) post-treatment evaluation (PTE), 3) evaluation of suspected residual or recurrent disease or restaging at time of recurrent disease (RR) were included into the study. Patients with other co-existing malignancy were excluded. Medical records and all diagnostic imaging studies were retrospectively reviewed to collect patient, tumor and treatment characteristics following approval from the Institute Ethical Committee (Si 190/2015).

Conventional imaging in this study included 1) CT scan performed as part of PET/CT scan, 2) CT scan of chest with or without upper abdomen, 3)^{99m}Tc-MDP whole body bone scintigraphy and 4) contrast-enhanced CT scan or MRI of brain. Apart from CT scans which were simultaneously performed as part of PET/CT scans, other imaging must be performed within 2 months prior to PET/CT scan.

The PET/CT scan required fasting for at least 6 hours with blood sugar less than 200 mg/dL. ^{18}F-FDG of 5.2-7.4 MBq/kg was intravenously injected and followed by uptake period of 60 minutes. Standard whole body imaging - base of skull to mid-thigh or further extension as indicated was performed. CT acquisition parameter was 120 kV, auto mA (30-300 mA) and slice thickness of 3.75 mm. Contrast media was used in all cases, if patient had no contraindication. PET/CT scan and CI of each patient were separately reviewed with at least 1 week apart by consensus of two reviewers.

Primary purpose of the study was to evaluate the impact of PET/CT scan on inter-modality treatment changes - i.e. a surgical candidate received chemotherapy (CMT) instead due to presence of unexpected distant metastasis on PET/CT scan. Proposed treatment plans were based on clinical data including available CI and derived from opinion of referring physicians stated in medical records at time of requesting for PET/CT scans. Proposed treatment was compared to given treatment after performing PET/CT scan in all patients whose medical records were available.

Secondary purpose was whether PET/CT scan triggered intra-modality change - i.e. change in target delineation or radiation (RT) field due to detection of additional metastatic mediastinal LN. Contouring based on CI was compared to contouring based on PET/CT scan in order to assess the change in target coverage.

Other secondary purpose was if PET/CT scan led to upstage and downstage in setting of INS and changed disease status to more advanced (equivalent to upstage) or less advanced (equivalent to downstage) in PTE and RR. The 7th edition AJCC staging system[15] was applied for staging in all settings – INS, PTE and RR. Therefore, patients without detectable disease after treatment were categorized as stage 0. Staging based on findings on all available CI was compared to that on PET/CT scan and reported in term of upstage, unchange or downstage.

RESULTS

Of 120 patients, 73 patients were male (60.8%). Age range was 35-92 years with mean age of 64 years. Major histopathology was adenocarcinoma in 95 patients (79.2%). CT scan of chest with or without upper abdomen was available in all patients. Bone scintigraphy was done in 49 patients (40.8%) and CT scan or MRI of brain was done in 3 patients (2.5%). Most common indication was INS in 60 patients (50%), followed by PTE in 41 patients (34.2%). (Table 1)

TABLE 1. Patients and tumors' characteristics.

Characteristics		Number (%)
Mean age (years)		64
Gender	Male	73 (60.8)
	Female	47 (39.2)
Pathology	Adenocarcinoma	95 (79.2)
	Squamous cell carcinoma	9 (7.5)
	Adenosquamous carcinoma	3 (2.5)
	Not specified subtype	13 (10.8)
Indication	Initial staging	60 (50)
	Post-treatment evaluation	41 (34.2)
	Recurrence/restaging	19 (15.8)

PET/CT scan led to staging change

In comparison to CI, PET/CT scan led to more advanced disease in 26 patients and less advanced disease in 12 patients. Among these 38 out of 120 patients (31.7%), there were 14 INS patients, 14 PTE patients and 10 RR patients.

PET/CT scan changed initial staging in 14 of 60 patients (23.3%) - upstage in 11 patients and downstage in 3 patients (Table 2). T-staging was upstage in 2 patients and none was downstage. N-staging was changed in 12 patients – upstage in 7 patients and downstage in 5 patients. M-staging was more advanced in 6 patients and none was downstage. Most common additional findings detected on PET/CT scan, which affected staging were mediastinal LN in 8 patients (13.3%) and bone in 3 patients (5%).

Regarding PTE setting, PET/CT scans were resulted in staging change in 14 patients (34.1%) – upstage in 8 patients and downstage in 6 patients (Table 3). T-staging was upstage in none and downstage in 1 patient. N-staging was upstage in 4 patients and downstage in 5 patients. M-staging was upstage in 7 patients and downstage in 1 patient. Most common additional findings detected on PET/CT scan which affected staging were bone in 6 patients and mediastinal LN in 5 patients.

PET/CT scan led to staging change in 10 out of 19 RR patients (52.6%) – upstage in 7 patients and downstage in 3 patients (Table 4). T-staging was upstage in none and downstage in 3 patients. N-staging was upstage in 3 patients and downstage in 1 patient. M-staging was upstage in 4 patients and downstage in none. Most common additional findings detected on PET/CT scan

TABLE 2. Impact of PET/CT on change of initial staging.

		Conventional imaging staging							
		IA	IB	IIA	IIB	IIIA	IIIB	IV	Total
PET/CT Staging	IA	4	0	0	0	0	0	0	4
	IB	0	4	0	0	1^{N}	0	0	5
	IIA	0	1^{N}	2	0	0	1^{N}	0	4
	IIB	0	0	1^{PL}	2	0	0	0	3
	IIIA	0	0	1^{N}	0	8	1^{N}	0	10
	IIIB	0	0	0	0	3^{N}	13	0	16
	IV	0	0	1^{B}	1^{IP}	1^{IP}	2^{B}	13	18
	Total	4	5	5	3	13	17	13	60

Staging on PET/CT scan was different from CI due to visceral pleural involvement (PL), intrapulmonary metastasis (IP), bone metastasis (B) and mediastinal LN (N)

TABLE 3. Impact of PET/CT on change of staging in post-treatment evaluation.

		Conventional imaging staging								
		0	IA	IB	IIA	IIB	IIIA	IIIB	IV	Total
PET/CT Staging	0	3	1^{T}	0	0	0	2^{N}	0	1^{PL}	7
	IA	0	0	0	0	0	0	0	0	0
	IB	0	0	0	0	0	0	0	0	0
	IIA	0	0	0	2	0	0	0	0	2
	IIB	0	0	0	0	0	0	1^{N}	0	1
	IIIA	0	0	0	0	0	3	1^{N}	0	4
	IIIB	0	0	0	0	0	1^{N}	7	0	8
	IV	$2^{*PL,B}$	0	0	0	0	3^{B}	2^{B}	12	19
	Total	5	1	0	2	0	9	11	13	41

Staging on PET/CT scan was different from CI due to no uptake at tumor bed (T), pleural metastasis (PL), bone metastasis (B) and mediastinal LN (N). *Staging change due to pleural metastasis in one patient and bone metastasis in other.

TABLE 4. Impact of PET/CT on change of staging in residual or recurrent disease.

		Conventional imaging staging								
		0	IA	IB	IIA	IIB	IIIA	IIIB	IV	Total
PET/CT	0	1	2[T]	0	1[T]	0	0	0	0	4
Staging	IA	0	3	0	0	0	0	0	0	3
	IB	0	0	0	0	0	0	0	0	0
	IIA	0	0	0	0	0	0	0	0	0
	IIB	0	0	0	0	0	0	0	0	0
	IIIA	0	0	0	0	0	0	0	0	0
	IIIB	0	1[N]	0	0	0	1[N]	1	0	3
	IV	2*[B,CN]	0	1[IP]	0	0	0	1[B]	5	9
	Total	3	6	1	1	0	1	3	4	19

Staging on PET/CT scan was different from CI due to primary tumor or tumor bed (T), intrapulmonary metastasis (IP), bone metastasis (B), mediastinal LN (N) and cervical LN (CN), *Staging change due to bone metastasis in one patient and both cervical LN and bone metastasis in other.

which affected staging were tumor bed or primary tumor in 3 patients and bone in 3 patients.

Of 49 patients who underwent bone scintigraphy, change of M-staging due to newly diagnosed bone metastasis on PET/CT scan were seen in 4 patients (8.16%) - 3 INS patients and 1 PTE patient. As for 71 patients without bone scintigraphy, first diagnosis of bone metastasis on PET/CT scan were observed in 8 patients (11.3%) – 5 PTE patients and 3 RR patients.

Impact on management

Consequent management resulted from PET/CT scan were analyzed in 2 aspects as follows;

1) PET/CT scan triggered inter-modality treatment change; Out of 83 patients with available proposed treatment plan prior to PET/CT scan (38 INS patients, 33 PTE patients, 12 RR patients), given treatment were inter-modality change in 19 patients (22.9%). Treatment modality changed from surgery or RT to CMT due to distant metastasis in 15 patients (18.1%) and from surgery or radiation to observation due to negative PET/CT scan in 4 patients (4.8%) (Table 5).

2) PET/CT scan triggered intra-modality treatment change; Out of 43 patients who received RT as a locoregional treatment for intra-thoracic disease, findings of PET/CT scan, particularly FDG avid subcentimeter intrathoracic LN, led to changes in radiation approach i.e. target delineation in 18 patients (41.9%).

TABLE 5. Inter-modality treatment change according to indication and given treatment.

	Sx/RT to CMT	Sx/RT to OBS	Total
INS (n=38)	5	0	5
PTE (n=33)	8	3	11
RR (n=12)	2	1	3
Total	15	4	19

Treatment modality; surgery (Sx), radiation (RT), chemotherapy (CMT) and observe (OBS)

DISCUSSION

According to previous studies conducted in developed countries, PET and PET/CT scan has been proven to provide higher diagnostic performance than CI and consequently affected the management.[1-14,16-19] However, benefit of PET/CT scan may be questionable in different contexts – i.e. more advanced disease and presence of other CI prior to PET/CT scan. Consequently, this study was aimed to assess the impact of PET/CT scan on management of NSCLC in the context of a university-based hospital in Thailand.

While most studies evaluated the effect of PET/CT scan on stage migration in INS, in this study we evaluated the effect in all settings - INS, PTE and RR. We found that PET/CT scans benefit in all settings as 31.7% of patients were changed to more advanced/upstage or less advanced/downstage after performing PET/CT scan.

Regarding PET/CT scan for INS, stage changing in current study was 23.3% corresponding to previous studies which reported changes ranging from 22-42%.[4,6-8] These figures varied among the studies due to several reasons.

First, settings were different – i.e. evaluation of solitary pulmonary nodule, staging of newly diagnosed NSCLC and staging in surgical candidate. Our study included patients in all aforementioned settings and we found that change in staging was consistent to other studies. Second, CI in each study included different imaging modalities – e.g. the study integrating bone scintigraphy as part of CI may detect more bone metastatic lesions. Therefore, upstage to stage IV disease following PET/CT scan might be less frequent in a study which included bone scintigraphy as part of CI. Third, interpretation of equivocal lesions on CI had a considerable impact on stage shifting. For example, an adrenal nodule on CT scan could be considered as either benign adenoma or metastasis. FDG avid adrenal nodule on subsequent PET/CT scan would lead to upstage or unchanged, respectively. Lastly, time interval between CI and PET/CT scan may lead to disease progression giving the false impression of upstaging by PET/CT scan. With this reason, we compared findings on PET/CT scan and CI which included CT imaging of PET/CT scans themselves to diminish the upstage from tumor progression during waiting time. Therefore, additional benefit of PET/CT scan was more accurately assessed.

Among TNM-staging of INS, change of M-staging was the most common one.[6-7] However, changes of M-staging in our study was less frequent than N-staging. The possible explanation was 41% of patients underwent bone scintigraphy prior to PET/CT scan. CT scan from base of skull to mid-thigh as part of PET/CT scan was also incorporated as part of CI. Therefore, M-staging change due to unexpected bony metastasis was decreased. Additionally, over 70% of patients in our study were in advanced stage. These patients carried high disease burden and high potential of overt distant metastasis which might be easily detected even on CI. As a result, unexpected distant metastasis discovered on PET/CT scan was less frequent.

Change of M-staging was more common in PTE and RR. We postulated that after receiving treatment these patients carried lower disease burden and risk of distant metastasis. Consequently, occult or small metastasis might not be detected easily on CI. Moreover, some CI as a metastatic screening might be omitted by referring physician. As a result, detection of unexpected distant metastasis was increased on PET/CT scan.

Noticeably, most of inter-modality changes in our study were toward CMT owing to presence of distant metastasis (15 of 19 patients; 78.9%). Moreover, majority of changes were observed in PTE and RR (11 of 33 patients; 33.3% and 3 of 12 patients; 25%, respectively). Regarding inter-modality treatment change in INS patients, it was only 13.2% (5 of 38 patients) and all of them were given CMT. This result was quite different from that of 19.2-39% in the previous studies.[4-6] We hypothesized that it might relate to advanced stage of disease and practices of our institute. Although, stage III patients had several treatment options – i.e. preoperative CMT, preoperative CMT and RT and definitive CCRT, definitive CCRT tended be chosen in our institute. Therefore, PET/CT scan seemed to have only modest impact on treatment.

PET/CT scan has been proven to provide benefit in evaluation of post-treatment residual disease[20] and prediction of treatment outcome by monitoring change of metabolic activity parameters.[1,21] Even so PET/CT scan in our institute was infrequently used to differentiate between fibrosis and residual disease. Majority of PET/CT scans in PTE setting were to guide further adjuvant treatment. Remarkably, among 12 PTE patients who received curative surgery prior to PET/CT scan, in 5 of them unexpected metastasis were found on PET/CT scan. In much the same way, there were 2 of 12 RR patients whose PET/CT scan showed unexpected metastasis leading to exclusion of futile surgery/radiation. Consequently, performing of PET/CT scan before starting treatment might assist to prevent futile definitive treatment.

In the study containing early stage NSCLC patients, intra-modality treatment change following PET scan was observed in 15% of patients, and change in surgical approach was the most common one (7.3%).[5] However, there were too few patients who underwent surgery in our study. Our study had high proportion of N2 and N3 staging on CI staging (63%). Main treatment modalities were radiation and change of radiation approach resulting from exclusion or detection of metastatic LN which was 42%.

We conducted this retrospective study in a university-based hospital where treatment of any malignancy is based on a multidisciplinary team and patients accrued into the study can be representative of patients in Thailand. We believed that applicability to clinical practices is the strength of this study. Nevertheless, incomplete data is unavoidable in a retrospective study. Our hospital is one of the largest tertiary care units. Accordingly, many patients were referred for investigation – i.e. PET/CT scan, bone scintigraphy and returned to their primary or secondary care unit for further treatment. Treatment plans of these patients were unavailable and treatment outcome related to information from PET/CT scan could not be achieved. This group included almost one third of patients (37/120 patients; 31%) and absolutely affected the result of the study more or less. Another

limitation was lack of pathological confirmation and follow-up imaging which prevented us from evaluation of accuracy of PET/CT scan.

In conclusion, PET/CT scan had considerable impact on TNM-staging and management of NSCLC in all settings – INS, PTE and RR even with the presence of previous CI. However, It seems like the benefits of PET/CT were more obvious in M-staging for patients with low disease burden or in early stage as detection of distant metastasis might help to avoid futile definitive treatment. In contrast, patients with high disease burden or in advanced stage might gain more benefit in N-staging reflecting in change in RT treatment planning.

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Retrieval Analysis of Polyethylene in Total Knee Arthroplasty in Thailand: A Case Report

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ABSTRACT

Objective: Retrieval analysis is one of the effective methods that can provide the information on in-vivo characteristics of wear implants. The clinicians can correlate the damage pattern from this method with other findings in a reproducible manner. Nevertheless, there has been no previous retrieval analyses of PE wear in total knee arthroplasty (TKA) in Thailand.

Case Presentation: A 80-year-old female underwent left TKA due to osteoarthritic knee in 1994. She was 135 cm in height and weighed 42.7 kg. The cemented posterior stabilized press-fit condylar (PFC) (Depuy, Warsaw, IN, US) total knee system was used. Patellar resurfacing was performed with a cemented all-polyethylene implant. The femoral component was cobalt-chrome, while the tibial component was titanium alloy. A 12.5-mm thickness of stabilized plus tibial insert was used.

After surgery, her knee had functioned well for 20 years. One year ago, she had intermittent swelling and effusion of the knee, together with occasional pain. The symptoms had gradually progressed with increasing episodes of giving way. She was diagnosed with aseptic loosening of TKA and consequently underwent revision TKA in 2014. All components were removed and retrieval analysis of PE tibial insert was performed at the National Metal and Materials Technology Center (MTEC), Thailand.

Wear assessment of the articulating and non-articulating surfaces was performed in 16 zones. We found that PE wear was present in 11 zones without any backsides wear. The stereomicroscopy was performed, in which the scratches and delamination of the PE surface were demonstrated. The specimen was cut and analyzed using a scanning electron microscopy (SEM) to observe the insides of PE. Delamination, subsurface cracking and damage were identified in the damaged zones. In the metal part of PE, the surface damage and metal wear debris were also displayed using SEM.

Conclusion: This retrieval analysis could demonstrate various modes of wear on UHMWPE. However, we propose that future studies, which are based on the association between wear of UHMWPE and possible precipitating factors, are mandatory.

Keywords: Retrieval analysis; mode of failure; fail total knee arthroplasty; wear assessment (Siriraj Med J 2018;70: 175-177)

INTRODUCTION

Total knee arthroplasty (TKA) is one of the most effective orthopaedic procedures for treating end-staged osteoarthritic knee. However, this artificial joint has limited longevity. The common modes of failure after TKA include aseptic loosening, polyethylene (PE) wear and infection.[1] In TKA, PE wear and debris are the major concerning problems, which can cause osteolysis and lead to aseptic loosening. There are several factors that are related to the PE wear such as PE processing, implant

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design, patient factors and poor surgical technique.[2]

The delamination, pitting and fatigue failure of PE have been reported in previous literature.[3,4] Third-body wear can also occur from bone cement or bone fragments. Retrieval analysis is one of the effective methods that can provide the information on in-vivo characteristics of wear implants. The clinicians can correlate the damage pattern from this method with other findings in a reproducible manner. Nevertheless, there has been no previous retrieval analyses of PE wear in TKA in Thailand. This is the first report that can guide further retrieval analyses of artificial joint replacements.

CASE PRESENTATION

An 80-year-old female underwent left TKA due to osteoarthritic knee in 1994. She was 135 cm in height and weighed 42.7 kg. The cemented posterior stabilized press-fit condylar (PFC) (Depuy, Warsaw, IN, US) total knee system was used. Patellar resurfacing was performed with a cemented all-polyethylene implant. The femoral component was cobalt-chrome, while the tibial component was titanium alloy. A 12.5-mm thickness of stabilized plus tibial insert was used.

After surgery, her knee had been functioned well for 20 years. One year ago, she had intermittent swelling and effusion of the knee, together with occasional pain. The symptoms had gradually progressed with increasing episodes of giving way. Finally, she felt instability of the knee and decided to come to the hospital. The radiographs showed asymmetrical PE wear with the evidence of loosening of the tibial component (Fig 1). She was diagnosed with aseptic loosening of TKA and consequently underwent revision TKA in 2014. The intraoperative findings included an effusion, hypertrophic synovial tissue and loose fragments of PE. The visible wear was observed at both tibial insert and patellar component. The tibial component was grossly loosened, whereas the femoral and patellar components were well-fixed. Osteolysis was also found in both distal part of the femur and proximal part of the tibia. All components were removed and retrieval analysis of PE tibial insert was performed at the National Metal and Materials Technology Center (MTEC), Thailand.

Retrieval Analysis Results

Wear assessment of the articulating and non-articulating surfaces was performed in 16 zones.[5] The authors found that PE wear was present in 11 zones without wear on the backsides (Fig 1). In order to detect the changes of color and texture in the damaged areas[6], the stereomicroscopy was performed, in which the scratches (Fig 2) and delamination of the PE surface were demonstrated. The specimen was cut and analyzed using a scanning electron microscope (SEM) to observe the insides of PE[7]. Delamination, subsurface cracking and damage were identified in the damaged zones (Fig 3). Furthermore, in the metal part of PE, the surface damage and metal wear debris were also displayed using SEM (Fig 4).

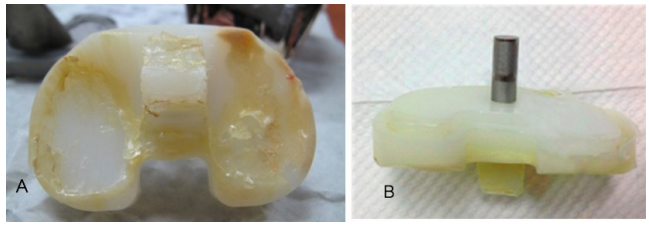


Fig 1. A) Articulating and B) Non-articulating surfaces of retrieved polyethylene tibial insert.

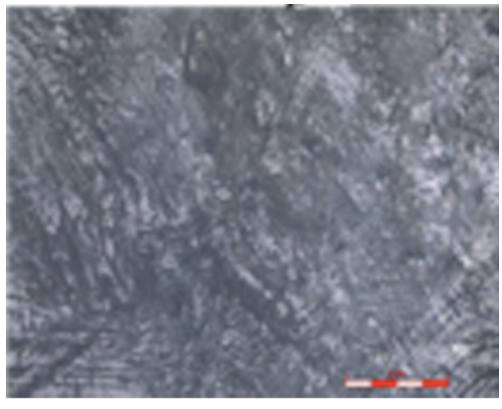


Fig 2. Stereomicroscopy photograph shows scratch and surface damage of polyethylene tibial insert.

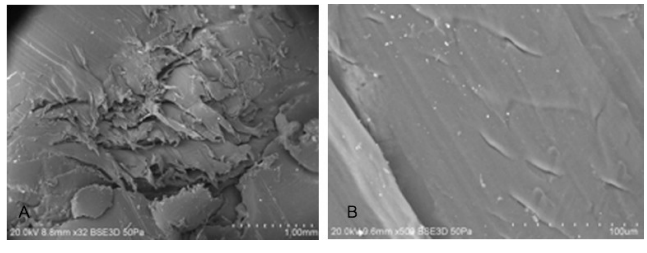


Fig 3. Scanning electron microscopy photographs show delamination (A) and subsurface crack (B) of polyethylene tibial insert.

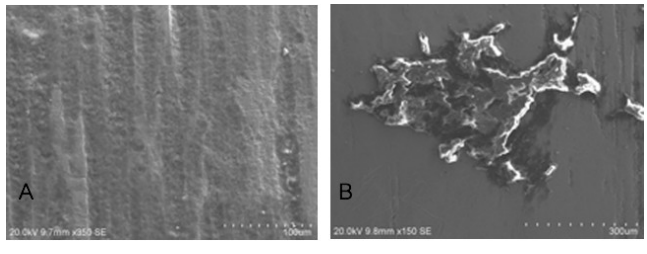


Fig 4. Scanning electron microscopy photographs show surface damage (A) and metal wear debris (B) on metal part of polyethylene tibial.

DISCUSSION

This is the first case report in Thailand regarding the retrieval analysis of ultra-high molecular weight polyethylene (UHMWPE) cemented PFC (Depuy, Warsaw, IN, US) in a definite aseptic loosened case after undergoing TKA for 20 years. Our purpose was to find out the mode of failure of PE implant that contributed to wearing of the UHMWPE, which will eventually lead to aseptic loosening after TKA.

From visual inspection, we found that the damages of the UHMWPE component were very severe. Delamination, pitting, burnishing and abrasion, as was shown in figure 1A, were located in all the wear areas consisting of medial articulation, lateral articulation and the post of the UHMWPE.

The UHMWPE tibial components was roughly analyzed by stereomicroscopy to identify the damaged area. We found scratches and surface damage of PE tibial insert, implying that fatigue wear had occurred (Fig 2). Then we examined damaged area using scanning electron microscope (SEM) in order to get the exact details of failure mode. Surface cracks were observed in a wide area and also in various directions. Furthermore, SEM was employed to scan through both articular part (Fig 1A) and non-articular part (Fig 1B) of the UHMWPE. Results showed delamination (Fig 3A) of the medial and lateral articular contact surface and subsurface crack of UHMWPE (Fig 3B), suggesting that adhesive and fatigue wear had occurred. The wear debris was also found on the surface.

The SEM exam of the metal part of PE showed surface crack (Fig 4A) and metal wear debris (Fig 4B) at the metal bar of tibial component on the side of metal part of the UHMWPE. The findings were evidently caused by fretting and abrasive wear mode. This might have happened because of motion between bar of UHMWPE component and the metal component of tibia.

The demonstrated modes of UHMWPE wear, such as abrasive, adhesive, fatigue, fretting and corrosive wear, were similar to many previous reports. Many factors have been reported to have influence on the wear of UHMWPE, for example joint loading, position, motion, degree of bearing conformity and contact stresses through range of motion. Further studies are necessary to evaluate the association between wear mode and these factors.

Summary

This retrieval analysis could demonstrate various modes of wear on UHMWPE. However, we propose that future studies based on the association between wear of UHMWPE and possible precipitating factors are mandatory.

The retrieval analysis is a very important method for the study of details of failed TKA. Since the number of TKA cases are gradually increasing, preventing its failure is therefore of great importance. The retrieval analysis is a way to prevent such consequences for it could help to improve prosthesis or bearing design and select appropriate material and surgical technique. This primary research could be a great start towards better longevity of TKA and improved quality of life of the patients.

What is already known on this topic?

The retrieval analysis is a very important method for the study of details of failed TKA.

What this study adds?

We propose that future studies based on the association between wear of UHMWPE and possible precipitating factors are mandatory.

What are the implications for public health practice?

Retrieval analysis is now feasible and achievable in Thailand. This method could facilitate further trials to investigate the correlation between mode of failure and other risk factors.

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Perforated Duodenal Ulcer after Laparoscopic Roux-en-Y Gastric Bypass for Morbid Obesity

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ABSTRACT

Objective: Duodenal ulcer perforation after laparoscopic Roux-ex-Y gastric bypass (LRYGB) for morbidly obese patient is uncommon. However, the morbidity and mortality rate will be increased if the diagnosis is delayed. We reported clinical presentations, surgical approach as well as outcome of first perforated duodenal ulcer after LRYGB in E-Da hospital.

Case presentation: A 34 years old morbidly obese female who underwent successful LRYGB in 2006. Five years later, she developed severe epigastric pain and marked tenderness at this area. No pneumoperitoneum was demonstrated on abdominal CT scan. First diagnostic laparoscopy was done and showed unexplained hemoperitoneum at subhepatic area without definite cause of abdominal pain. Unfortunately, she had to undergo re-diagnostic laparoscopy on postoperative day 2 because bile content was present in abdominal drain. Luckily, the perforated duodenal ulcer was detected at 1st part of duodenum and closed properly by simple suture techniques. Postoperatively, there was no complication and she was discharged home uneventfully. Helicobacter pylori and life-long proton pump inhibitor were prescribed. Upon 3-month follow-up, she had no abdominal pain or other complications.

Conclusion: The diagnosis of perforated duodenal ulcer after LRYGB for morbidly obese patients is challenging. Although laparoscopic simple suture is safe and feasible in acute perforated scenario, this might carry high recurrent rate due to the remaining parietal cells in gastric remnant. Nevertheless, the role and timing of definitive acid-reducing surgery need to be addressed by having well-designed studies in future.

Keywords: Perforated duodenal ulcer after laparoscopic Roux-en-Y gastric bypass; laparoscopic simple suture with omental patch (Siriraj Med J 2018;70: 178-181)

INTRODUCTION

Laparoscopic Roux-en-Y Gastric Bypass (LRYGB) which combines restriction and malabsorption mechanism, has been known as one of the most popular bariatric surgeries with significant excess weight loss.[1-3] With an increasing of bariatric surgeries performed nowadays, we should be aware of the changes in gastrointestinal pathophysiology. It is inevitable that we must face these uncommon complications after bariatric surgery in the future. Diagnosis of peptic ulcer disease (duodenal and gastric ulcer) in the bypassed segment is usually more complicated and results in delayed treatment. The common complications of peptic ulcer are bleeding and perforation. Although incidence of perforated peptic ulcer in bypassed segment is rarely reported, it is usually a fatal complication when happened.[4] We reported first perforated duodenal ulcer after LRYGB in our hospital (E-Da hospital, Taiwan).

CASE PRESENTATION

This is a 34-year-old female with a body mass index (BMI) of 35.4 kg/m^2 and Type 2 diabetes mellitus who underwent successful antecolic and antegastric LRYGB in 2006. Preoperative endoscopic finding was unremarkable

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and no H. Pylori was detected. The operation went well without any perioperative complications and she was discharged home on postoperative day 3. Unfortunately, 5 years later, she came to our hospital with severe epigastric pain which was not ameliorated by medications. Her BMI was 26.5 kg/m^2 and blood pressure as well as pulse rate were normal. Abdominal exam showed marked tenderness without definite peritonitis at epigastric area. Significant Laboratory revealed hematocrit was 30% and white blood cell was 15,000 cell/ml. An abdominal computed tomography scan (abdominal CT scan) was performed and showed only generalized dilated small bowel loop and pneumoperitoneum was not detected. However, her clinical was not improved after we admitted her in the hospital. Few hours after admission, diagnostic laparoscopy was performed in order to identify cause of severe abdominal pain.

Upon diagnostic laparoscopy, we found unexplained hemoperitoneum and blood clot with at hepatic hilum and around gallbladder area. No source of bleeding could be identified at that moment. Therefore, all the hemoperitoneum and blood clot were removed and a 10 mm. Jackson-Pratt (JP) drain was placed at this subhepatic area. Unfortunately, she still developed persistent epigastric pain and 100 ml of bile content was present in JP drain on postoperative day 2. Therefore, re-diagnostic laparoscopy was scheduled and demonstrated bile contamination around subhepatic area as well as perforated acute ulcer at first part of duodenum. The perforated site was closed by laparoscopic simple suture with 2-0 Monocryl interrupted stitches. Also, a 10 mm. JP drain was placed at subhepatic area. Postoperatively, her clinical was improved and she was able to resume full liquid diet on postoperative day 4 (started sipping water on postoperative day 2 then we advanced to full liquid diet on postoperative day 4). The JP drain showed no more bile content and it was removed on postoperative day 4. She was discharged home uneventfully on postoperative day 5. Helicobacter pylori (*H. pylori*) eradication and life-long proton pump inhibitor were prescribed. On the 3-month follow-up, she was doing well without any abdominal pain or other complications.

DISCUSSION

Perforated peptic ulcer in bypassed segment are unusual complication after gastric bypass. Some publications have been reported in the past (Table 1). Macgregor et al. reported 11 cases (0.25%) out of 4,300 cases of perforated peptic ulcer after open gastric bypass. Among them, 9 patients had duodenal ulcer perforation, 1 patient had gastric ulcer perforation and 1 patient had both. This was the largest series of perforated peptic ulcer after gastric bypass.[5]

The pathophysiology of this perforation is not clear. The bypassed segment has proven to have acidity with a mean pH of 2-3.[6,7] Remnant stomach is able to react to normal stimuli from vagal nerves. Bjorkman et al. claimed that duodenal ulcer formation might cause by unbuffered acid in bypassed segment. This could occur because there was no contact between acid production in remnant stomach and ingested food.[4] Beside that, pancreas' bicarbonate secretion in response to food stimuli is absent after gastric bypass. Another possibility, bile reflux in bypassed segment caused mucosal disintegration in the remnant stomach.[4,8]

This lady was the first case of perforated duodenal ulcer after LRYGB in our bariatric center. Her preoperative upper endoscopy for LRYGB in 2006 showed unremarkable finding and *H. pylori* was not detected. Also, she denied any risk factor of peptic ulcer disease such as smoking, alcohol, NSAIDs, other ulcerogenic usage and physical-psychological stress. In 2011, she presented with sudden onset of epigastric pain without definite sign of peritonitis 2 days before admission. Abdominal CT scan was done in order to identify cause of abdominal pain. However, we could see any free air and no definite cause of abdominal

TABLE 1. Some previous publications about perforation in bypassed segment after RYGB.

No.	Journal	Total Cases	Perforated	Duodenal	Gastric	Both
1	Bjorkman et al[4] (1989)	NA	1	1	-	-
2	Macgregor et al[5] (1999)	4300	11	9	1	1
3	Papasavas et al[20] (2003)	NA	1	-	1	-
4	Mittermair et al[21] (2007)	NA	1	1	-	-
5	Snyder[22] (2007)	477(360)	4	3	1	-
6	Gypen et al[9] (2008)	NA	1	1	-	-

pain could be detected on the CT scan. Basically, abdominal CT scan carries high sensitivity in pneumoperitoneum detection but it might not be identified in perforated peptic ulcer after RYGB because air in remnant stomach is usually absorbed after RYGB. We must note that radiological sign of perforation in RYGB is different from normal patients.[9]

Fundamentally, management of perforated duodenal ulcers including closure of the defect, wide drainage, proton pump inhibitors, broad spectrum antibiotics, and eradication of *H. pylori*. However, few studies stated that there was high recurrent peptic ulcer rate which required re-operation after primary closure. Macgregor et al. reported nine from eleven cases of perforation require subsequent surgery.[5] However, our patient was eventually successful managed by only laparoscopic simple suture without any perioperative complications. Life-time proton pump inhibitor use was advised to reduce possibility of recurrent perforated duodenal ulcer in the future.

Definitive surgery for perforation in bypassed segment after LRYGB are resection of bypassed segment as well as concomitant truncal vagotomy. This procedure is purposed to eliminate the source of acidic environment.[4,5,9] The ideal time for definitive surgery remains inconclusive. Definitive surgery during perforation may cause duodenal stump leak since there are a lot of edema and inflammation process in the perforation area. On the other hand, definitive surgery at later time needs another next operation and there will be adhesion and scar tissue in this area.[9] Further well-designed studies are required in order to clarify role and best timing for definitive surgery in these perforated peptic ulcer patients.

Regarding postoperative surveillance for the perforated peptic ulcer, it is very challenging to reach peptic ulcer in bypassed segment by conventional upper endoscopy due to new anatomical reconstruction after RYGB. Fobi et al. described utilization of a temporary gastrostomy tube with radio-opaque marker in the remnant stomach during gastric bypass to facilitate access for feeding, decompression and endoscopy access when needed.[10] Percutaneous access to gastric remnant using CT or ultrasound guidance also has been studied. This technique allows endoscope could be introduced through gastric remnant and peptic ulcer easily. However, it requires a good endoscopist and radiologist because of difficulty in technique.[11,12] Moreover, some authors claimed success rate of 68% cases using long pediatric enteroscope to reach bypassed segment. Another noninvasive diagnostic examination is a CT or MRI virtual gastroduodenoscopy but the limitation of this procedure is lack of tissue sampling for histological examination.[13] Furthermore, there is a new approach to the remnant stomach and duodenum by double balloon enteroscopy which is recently described.[14]

Helicobacter pylori infection has been recognized as one of the significant causes of peptic ulcer disease.[15] Many modalities have been described to identify *H. pylori* including noninvasive and invasive procedures. Urea breath test is the most common noninvasive procedure. Unluckily, the result is usually negative because urea swallowed by patient could not reach the bypassed segment.[16] Stool antigen detection has been proven as the best non-invasive test for confirming the presence of *H. pylori*.[17] Measurement of serum anti-*H. pylori* antibody titers can also be used for detection of *H. pylori* infection. However, it needs multiple sampling for longer than 12 months since resolution from infection takes time after eradication therapy.[18] The most reliable method is a tissue biopsy by endoscopy for a rapid urease test, histological examination, and microbial culture[19-21]. Nevertheless, it is technically difficult to obtain the tissue by endoscopic approach due to anatomical problems as we described previously. Therefore, we decided to eradicate *H. pylori* by classical triple therapy in our patient without any tissue confirmation. Upon 3-month follow-up, she denied any abdominal pain and other complication.

CONCLUSION

The perforated duodenal ulcer after LRYGB is rare but the way how to diagnose this condition is very challenging. The delay diagnosis and treatment could result in high morbidity and mortality rate. Although laparoscopic simple suture is safe and feasible for this patient in acute perforated setting, it might carry high re-operative rate. The role and ideal timing of definitive acid-reducing procedure are still inconclusive. More well-designed studies are required to clarify its benefit.

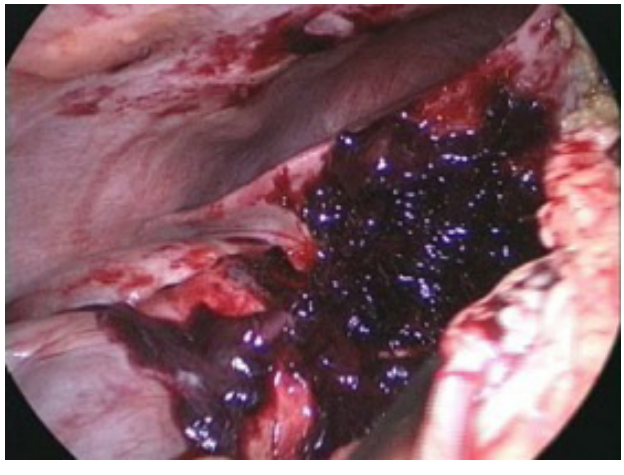


Fig 1. Blood clots were noticed at subhepatic and around gallbladder area

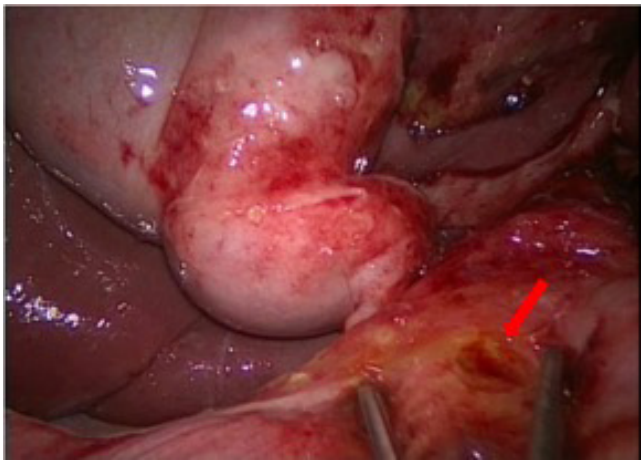


Fig 2. After all the blood clot was removed, acute perforated ulcer at first part of duodenum was found (arrow)

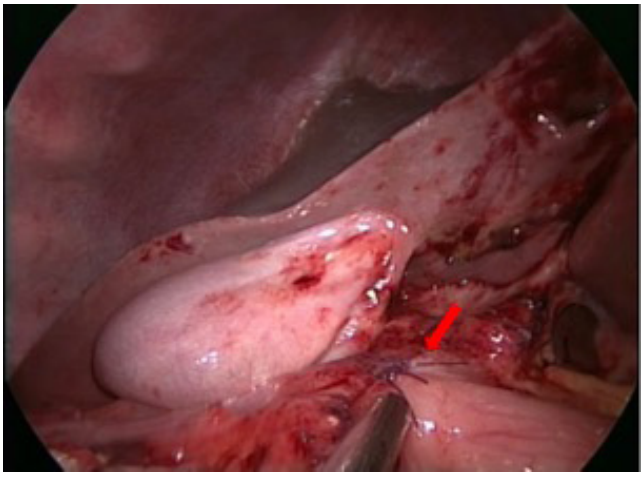


Fig 3. Simple suture with full thickness interrupted sutures over the perforated site laparoscopically (arrow)

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Posaconazole Induced Diffuse Lentigines: A Case Report

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ABSTRACT

Objective: To report a case of posaconazole induced diffuse lentigines.

Case presentation: A 57-year-old Thai male with acute promyelocytic leukemia who received chemotherapy and antifungal drug prophylaxis then appeared abruptly generalized multiple discrete well-defined brownish macules on face, lips, trunk, back, both arms, forearms, both thighs, legs, both palms and soles about eight days after received posaconazole.

Conclusion: Multiple lentigines could be found in patients who received immunosuppression or immunomodulation, and antifungal drugs such as voriconazole.

Keywords: Drugs induces multiple lentigines; eruptive lentiginosis (Siriraj Med J 2018;70: 182-183)

INTRODUCTION

Lentigo is a skin lesion which is a well circumscribed, light to dark-brown macule. The pathogenesis is included melanocytic hyperplasias which comprise of intraepidermal melanocytic hyperplasia and increased melanin formation. Lentigo simplex is associated with multiple clinical manifestations such as LEOPARD syndrome (lentigines, electrocardiographic (ECG) conduction defects, ocular hypertelorism, pulmonary stenosis, abnormalities of genitals, growth retardation, and sensorineural deafness), and LAMB syndrome (lentigines, atrial myxomas, mucocutaneous myxomas, and blue naevi), and Peutz–Jeghers syndrome.[1,2] LEOPARD syndrome is determined by an autosomal dominant with a mutation in PTPN11 which results in excessive signaling through the RAS/MAP kinase pathway, which can lead to the development of junctional naevi.[2] In the syndromic lentiginosis, the lentigines are usually present at birth or early life and increase in number by age.

Shortly, development of multiple lentigines should raise more concern about medication induced lentigines. The immunosuppressive or immunomodulatory drugs can cause eruptive naevi or eruptive lentiginosis.[2] Furthermore antifungal drugs have been shown to cause generalized lentiginosis.[3,4,5]

CASE PRESENTATION

A 57-year-old Thai male presented with dizziness and dyspnea and was diagnosed with acute myeloid leukemia from bone marrow biopsy in July 2016. Initially, he was admitted for chemotherapy as 3+7 regimen which included cytarabine and idarubicin for 3 days. After flow cytometry result shown as acute promyelocytic leukemia, chemotherapy regimen was changed to all-trans retinoic acid (ATRA) and posaconazole was prescribed for prophylaxis of fungal infection.

After 8 days of posaconazole and ATRA treatment, he abruptly developed generalized multiple discrete well-defined brownish macules on the face, upper lip (Fig 1), trunk, back, both arms, forearms, both thighs, legs, both palms, and soles. The skin lesions were diagnosed as lentigines by dermatologists. He was discharged with posaconazole treatment for prophylaxis of fungal infection was prescribed, because the number of lentigines was continually increasing. Photoprotection and observation for skin cancer were advised.

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Fig 1. Multiple discrete well-defined brownish macules on face and upper lip representing multiple lentigines in the reported patient.

At follow-up period, posaconazole was withdrawn due to the improvement of leukemic disease. Lentigines faded to light brown macules and no new lesion was detected after 2 months of posaconazole discontinuity.

He had underlying diseases which included hypertension, dyslipidemia, impaired fasting glucose. His current medications were quinapril, atenolol, manidipine, simvastatin, and gemfibrozil which he had received for many years before this current illness.

DISCUSSION

Multiple lentigines could be induced from immunosuppression or immunomodulation, which have been reported in cyclophosphamide, doxorubicin, vincristine, oral prednisolone,[2] etanercept[6] and antifungal drugs such as voriconazole. Voriconazole was frequently reported as a drug which caused diffuse lentigines and photosensitivity.[3,4,5]

Voriconazole is a broad-spectrum triazole antifungal agent which is associated with acute photosensitivity and carcinogenesis of skin. The mechanism of the photosensitivity is unknown, while an indirect retinol effect secondary to the antifungal's impact on CYP450 enzymes has been proposed to contribute to the underlying mechanism. Voriconazole inhibits P450 enzymes, including CYP3A4 and CYP2CP which metabolize tretinoin, which leads to increased retinol levels which might exacerbate the acute photosensitivity.[3] Affected patients may present with photo distributed erythema, cheilitis or erosions (usually of the lower lip which is a sun-exposed area), accelerated photoaging, or pseudoporphyria cutanea tarda.

Chronic phototoxic effects and lentigo formation from voriconazole frequently affects a patient with Fitzpatrick type VI skin. The development of lentiginous pigmentation can be found in both children and adults who received voriconazole.[4] After discontinuation of voriconazole, the skin lesions of sun-exposed areas may leave solar elastotic changes, multiple lentigines, and ephelides.[5]

Posaconazole is the second-generation triazole antifungal agent like voriconazole and is currently approved only for use against invasive fungal infections as prophylaxis in immunocompromised patients.[7] From our literature review, there was no recently reported posaconazole induced photosensitivity. However, in this case, the patient received chemotherapy which included cytarabine, idarubicin, and all-trans retinoic acid (ATRA) which had no reports about inducing lentigines and photosensitivity. Regarding timeline of his medication, the start of posaconazole was associated with the development of skin lesions and improvement of lentigines was demonstrated after stopping posaconazole. Although there was no report that it induced multiple lentigines, this may be due to its recent wide usage. On the other hand, there were many studies which revealed voriconazole-induced multiple lentigines. Posaconazole was the same generation triazole antifungal agent as voricosanazole, therefore posaconazole might induce diffuse lentigines. The more extensive study would be required to determine association and risk factors of posaconazole and multiple lentigines or photosensitivity.

Concerning treatment, most reports of voriconazole-associated squamous cell carcinoma have occurred in immunocompromised patients with elevated risk of skin cancer. Hence, the treatment recommended was photoprotection and surveillance for skin cancer.[4]

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