ISSN 2629-995X
Volume 70, Number 5, September-October 2018

SMJ
Siriraj Medical Journal



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A Prospective Randomized Controlled Trial Comparing Infection Rates of the Daily Dressing and Scheduled Dressing of Sutured Traumatic Wounds

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ABSTRACT

Objective: To evaluate outcome differences between DD and SD in 1) wound infection rates, 2) patients' satisfaction according to the wound care and cost of treatment.

Methods: A single-center, prospective randomized controlled trial of 350 traumatic-wound patients was conducted. Only adult traumatic wounds without a fracture, tendon injury and neurovascular injury were included. The patients were randomized into two groups: daily dressing (DD) and scheduled dressing (SD) groups. DD group patients received daily wound dressing changes, while those in SD group typically had dressing changed on days 3, 7 and one other scheduled day. Analyzed data included patients' demographics, clinical parameters, patient's satisfaction according to wound treatment and cost.

Results: Total wound infection rate was 1.2%. Although the incidence of wound infection in the SD group was lower than that of the DD group (0% vs 2.4%), statistically it was not significant (p=0.06). Patients' satisfaction level was also not significantly different between the two groups.

Conclusion: The study showed clinical non-inferiority of SD compared to DD while offering saving benefits both in time and cost. Therefore, SD can be substituted for DD for traumatic wound care after primary repair.

Keywords: Traumatic wound infection; scheduled dressing; daily dressing (Siriraj Med J 2018;70: 377-381)

INTRODUCTION

Traumatic wounds are defined as cuts, lacerations or puncture wounds that inflict damage to the skin and underlying tissues.[1] All traumatic wounds are considered to be contaminated upon presentation at an emergency department. They are generally sutured after wound irrigation and debridement. The risk of wound infection depends on the type and location of the wound, and the patient's clinical conditions.[2] Traditionally, a patient with sutured wound is required to come for wound dressing change every day until the stitches are removed. This daily dressing is commonly assumed to be better than the alternative non-daily dressing change because, firstly, the physicians or nurses can more regularly evaluate the wound for infection and, secondly, daily dressing change is believed to keep the wound cleaner. However, this wound care strategy not only leads to higher expenses, but also costs both patient's and medical personnel's time. Our study was designed to test the hypothesis that the scheduled non-daily dressing change is not inferior to daily dressing change scheme.

MATERIALS AND METHODS

We conducted a single-center, prospective, randomized controlled study between February 2009 and June 2016. All adult patients (≥20 years old) with traumatic wounds, defined by wound depth as encompassing the skin and subcutaneous layer, were included. Sites of the wound

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Received 15 March 2018 Revised 30 May 2018 Accepted 4 June 2018
doi:10.14456/smj.2018.60

were face, head, neck, body and extremities. Excluded were patients who required surgery or admission, or those with bone fracture, tendon injury or neurovascular injury. Wound contamination was classified as 1) 'minor' if arrival time (T) to the hospital was less than 6 hours and happened in a clean environment or by a clean instrument, 2) 'moderate' – if T > 6 h, happened in a dirty environment or by a dirty instrument, or 3) 'heavy' – if T> 12 h and/or grossly contaminated and/or with foreign body present. Written informed consents were obtained from all participants.

A total of 350 patients were enrolled in the study. Block randomization, with randomly-selected block sizes of 2, 4 and 8, was generated by computer, and the patient assignments were delivered in sealed, opaque, sequentially-numbered envelopes. The patients were allocated equally (175 patients each) into 2: daily dressings (DD) and scheduled dressings (SD) groups. All wounds were routinely irrigated with normal saline solution, sutured under local anesthesia and sterilely dressed on the first day. In addition, all patients in both study groups were prescribed prophylactic oral antibiotics, usually cephalosporin. For cephalosporin-allergic patients, clindamycin was given instead.

Patients in DD group had their wound dressings changed every day by nurses while those in SD group only on the third and seventh days. However, if the stitches were to be removed before the seventh day, the third day dressing change would then be postponed to the day of suture removal. On the other hand, if the stitches were scheduled to be removed after the seventh day, then an additional dressing would be scheduled on that suture removal day (which could be either the tenth or the fourteenth day). This strategy reduced the outpatient visits for patients in the SD group to only 2, 3 or 4 times, depending on the site or condition of the wound, and the day of suture removal.

The protocol for this study was approved by the Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (Si 277/2009).

Objectives of the study

Primary endpoint – wound infection rate

Definition of infected wound

An "infected wound" was defined as a suture wound with inflammatory changes. These changes were further divided into major and minor categories. The major category consisted of 3 signs: fever (a temperature over 38 degrees Celsius), abscess, and lymphangitis, while the minor consisted of 5 signs: erythema, tenderness, swelling, purulent discharge, and leukocytosis. The diagnosis of infected wound was made when there were at least 1 out of 3 major signs or 4 out of 5 minor signs present.[3,4] A physician in the trauma outpatient unit assessed all wounds and made a determination. If an infected wound was detected, the stitches would be removed and a wet dressing applied daily until the infection cleared. Associated risk factors were recorded.

Secondary endpoints – patient's satisfaction level with the treatment cost and outcomes

After completion of the treatment, all patients were given a rating scale questionnaire, which comprised four questions: Was the cost of traveling appropriate? Was the charge for the wound dressing reasonable? Were you satisfied with the results of the wound dressing? and what was your impression of the overall treatment? Patients answered by marking one of the following simple responses: very satisfied, satisfied, not satisfied.

Sample size calculation and statistical analysis

Given that the purpose of the study was an equivalence trial, we performed a sample size calculation for non-inferiority. Based on an estimated incidence of wound infections of 7%, an equivalence limit of 5%, an alpha of 0.05 and 85% power, the sample size was determined to be 175 per group.

Descriptive statistics for the baseline demographic and clinical variables were reported. Data were analyzed according to the intention-to-treat principle. Differences in the infection rates and satisfaction levels were analyzed using a Chi-square test. All statistical analyses were performed using SPSS Statistics for Windows, version 18.0 (SPSS Inc., Chicago, Ill., USA).

RESULTS

Eighteen patients out of 350 were lost to follow-up, leaving 332 for analysis (Fig 1).

Patients' demographics and other clinical parameters are as shown in Table 1. Their ages ranged from 20 to 84 (Mean 39 years). The average wound size was 2.5 cm (2.53 ± 1.76). Distribution of wounds by body site between DD and SD group was similar (p=0.455), with face and upper extremities being the two most common sites. All patients were prescribed prophylactic antibiotics and had wound sutured under local anesthesia. The median arrival time to the hospital from injury was 30 minutes. The majority (>90%) of wounds in both groups were classified as "moderately" contaminated wounds.

There were 4 infected wounds in this study and all were (Table 2) female and 3 of those sustained injury

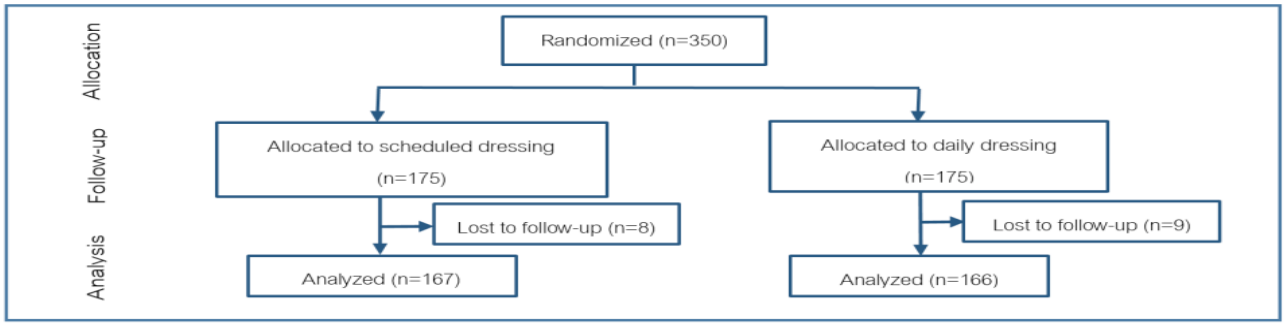


Fig 1. The flowchart showing the process of randomization.

TABLE 1. Patients' demographics and clinical parameters.

	Daily dressing (n=166)	Scheduled dressing (n=167)
Age (mean±SD)	38.26 ± 13.1	39.01 ± 16.3
Sex (male, %)	55.6%	50.9%
Antibiotics	100%	100%
Diabetes mellitus (%)	3.2%	3.4%
Mechanism of injury		
Penetrating wound	41.5%	34.1%
Crushed/blunt	58.5%	65.9%
Site of wound (n, %)		
Head	30 (18.1%)	36 (21.6%)
Face	57 (34.3%)	68 (40.7%)
Chest/abdomen	1 (0.6%)	0
Upper extremities	39 (23.5%)	36 (21.6%)
Lower extremities	39 (13.5%)	27 (16.2%)
Size of wound (cm, mean, SD)	2.78 ± 2.04	2.29 ± 1.39
Wound contamination		
Minor		
Moderate	1%	0%
Heavy	94.7%	95.5%
Debridement (n)	4.3%	4.5%
Local anesthesia	2	1
	100%	100%

to the foot. Two patients had a large-sized wound. All 4 infections occurred on or after post-repair day 2, with the latest one found on post-repair day 10.

Regarding risk factors, only wound size and location in the lower extremities were found to be significant factors associated with wound infection (Table 3).

Results of endpoints analysis are summarized in Table 4. There were 4 infected wounds out of 333 (1.2%). All 4 were found in wounds size larger than 2 cm in the lower extremities of patients in the DD group. In terms of the patients' perceptions, the majority of patients were "satisfied" to "very satisfied" both in the costs and the outcomes of the treatment. There was no significant difference between the groups.

TABLE 2. Characteristic of the patients who had wound infection.

Patient	Age (years)	Sex	Wound size (cm)	Wound site	Occupation	Underlying disease	Mechanism of injury	Day of infection
1	49	Female	15	Leg	Laborer	Obesity	Toilet seat injury	10
2	78	Female	12	Foot	Laborer	Allergic rhinitis	Fall	7
3	55	Female	3	Foot	Housewife	Bipolar disease	Fall	5
4	29	Female	3	Foot	Laborer	Thalassemia	Motorcycle accident	2

TABLE 3. Risk factors of wound infection.

	Non infection group	Infection group	P-value
Arrival time to hospital (min)	30	30	0.76
Wound size (cm)	2	7.5	0.012
Diabetes mellitus (%)	3.6%	0%	1.00
Heavy wound contamination	4.7%	0%	1.00
Crush/blunt injury (%)	61.5%	66.7%	1.00
Wound location: lower extremities (%)	19%	100%	0.015

TABLE 4. Summary of primary and secondary endpoints analysis.

	Daily dressing (n=166)	Scheduled dressing (n=167)	P-value
Wound infection (n, %)	4 (2.4%)	0	0.06
Wound infection in lower extremities wound (n, %)	4 (10.5%)	0	0.13
Wound infection in wound size > 2 cm (n, %)	4 (5.4%)	0	0.13
Wound infection in wound size > 2 cm and in lower extremities (n, %)	4 (23.5%)	0	0.13
Patient's perception (%)			
Traveling cost			
Very satisfied	73.4%	73.3%	0.31
Satisfied	24.7%	26.1%	
Not satisfied	1.9%	0.6%	
Dressing cost			
Very satisfied	9.4%	13.1%	0.24
Satisfied	89.3%	86.9%	
Not satisfied	1.3%	0%	
Wound care			
Very satisfied	91.2%	94.4%	0.29
Satisfied	8.8%	5.6%	
Not satisfied	0%	0%	
Overall Impression			
Very satisfied	92.5%	93.8%	0.66
Satisfied	7.5%	6.2%	
Not satisfied	0%	0%	

DISCUSSION

Traumatic wounds are classified as contaminated wounds that are associated with high risk of wound infection. Although the risk can be reduced by adequate wound cleansing and debridement, it is still significant in some groups of patients with certain risk factors. Quinn et al., reported a high 2.6% infection rate in their series of patients with traumatic lacerations. They identified diabetes, wound contamination, wound length greater than 5 cm and a location on the lower extremities as high risk factors.[2,5] For patients with such risk factors, prophylactic antibiotics are often recommended, especially for those with dirty wounds or wounds with signs of infection.[6,7] Taylor et al., routinely administered prophylactic antibiotics for patients with traumatic wounds undergoing suture repair and found it to be cost effective.[8]

Conventionally, patients are advised to keep their wound clean and dry by using a protective dressing for at least 24 hours after repair.[9] However, there has not been to date any evidence-based data to suggest the optimal frequency of the wound dressing change after suturing. Based on the incubation period, wound infection would generally be detected on day 3 post-repair.[10] Therefore, some physicians would schedule wound-dressing change on the third day and subsequent 2 to 3 days interval thereafter until stitches are eventually removed. However, most physicians in Thailand would still err on the cautious side and perform a wound-dressing change daily. To date, the frequency of wound dressing change remains a contentious and unsettled issue.

We conducted our study in an attempt to provide evidence, if there is any, of the difference in the infection rate between the two approaches to wound-dressing changes: DD vs SD. Although all 4-infection cases were found in the DD group and none form the SD group, there was no significant clinical-superiority of the SD statistically in lowering the infection rate. Regarding the patients' satisfaction with the wound treatment and the associated costs, there were no differences between the two study groups: patients in each group were satisfied with the wound dressing quality, and each perceived the costs as reasonable.

As for risk factors, we found wound size (>2 cm) and location of wounds (lower extremities) to be the only significant factors associated with wound infection. The lower-than-expected infection rates could be attributed to small-average wound size and the higher proportion of wounds on the face and upper extremities, which carry a lower risk of infection.

Although our study did not show significant superiority of SD compared to DD in lower wound infection rate, SD offers obvious economic advantages both in reducing treatment costs and time.

CONCLUSION

Our study demonstrated a lower incidence of wound infections among SD patients compared to those in DD group. Although it was not statistically significant, given that SD showed clinical non-inferiority to DD in preventing wound infection while being more cost-effective and time-efficient, it could be considered as a standard treatment for traumatic wounds.

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Efficacy of 0.0125% Capsaicin Patch at Acupuncture Point for Pain Relief in Knee Osteoarthritis: A Randomized Controlled Trial

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ABSTRACT

Objective: To study the efficacy of 0.0125% capsaicin patch at acupuncture point for pain relief in knee osteoarthritis (OA).

Methods: This randomized, double-blind, placebo-controlled clinical trial was conducted during September 2014 to March 2015. Sixty-two ambulatory patients over 50 years of age with a diagnosis of knee OA were included. All enrolled patients had a pain score of 4-7 out of 10. Participants were randomized into either the treatment (capsaicin, n=31) or control (placebo, n=31) group.

Interventions: Capsaicin vs. placebo patch at ST34 (1), SP10 (2), ST35 (3), EX-LE4 (Neixiyan) (4), ST36 (5), and SP9 (6) acupuncture points for 4 weeks. Main outcome measure: Pain subscale of modified Thai version of Western Ontario and McMaster (WOMAC) Osteoarthritis Index was assessed at baseline, 2, 4, 6, and 8 weeks after treatment.

Results: Pain subscale of modified Thai version of WOMAC showed no significant difference between groups at baseline, 2, 4, 6, and 8 weeks after treatment. After 2 weeks of treatment, the pain subscale score in the treatment group was significantly decreased from baseline ($p<0.001$). After 4 weeks of treatment, the pain subscale score in the placebo group was significantly decreased from baseline ($p<0.006$).

Conclusion: Capsaicin patch at acupuncture point for pain relief in knee OA yielded no significant difference between groups. However, significant pain relief from baseline was observed in the treatment group after 2 weeks of treatment and in the placebo group after 4 weeks of treatment. Further study with higher capsaicin concentration and/or larger size patch should be considered.

Keywords: Capsaicin patch; acupuncture point; knee osteoarthritis (Siriraj Med J 2018;70: 382-390)

INTRODUCTION

Osteoarthritis (OA) is a major cause of pain and locomotor disability in the older population.[1] Knee OA is one of the most common forms of osteoarthritis and the most frequent chronic condition leading to functional limitation in older adults.[2] Prevalence of knee OA in an older Thai population with a mean age of 67.8 years was 34.5-45.6 percent.[3] Current treatment guidelines recommend that optimal management of knee OA requires a combination of non-pharmacological and pharmacological treatment modalities. Surgical intervention is normally used in severe cases as the final treatment option.[4,5] However, oral pharmacologic management is often accompanied by gastrointestinal, cardiovascular, and/or renal side effects. Thus, the demand for safe and noninvasive therapies to treat the symptoms of OA is increasing.

Topical capsaicin is increasingly used in OA, because it reversibly desensitizes nociceptive C fibers by acting on VR-1 vanilloid receptors. Sound evidence has been

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Received 5 October 2017 Revised 21 November 2017 Accepted 8 December 2017
doi:10.14456/smj.2018.61

reported regarding the efficacy of capsaicin in knee OA[6] and no systemic side effects were reported.[5] Multiple preparations of topical capsaicin (0.0125%, 0.025%, and 0.075%) were used in a 4-week, double-blind, randomized, placebo-controlled trial for treatment of painful joints from rheumatoid arthritis (RA) and osteoarthritis (OA).[6-8] In these studies, a local burning sensation was the only side effect noted, although none of the patients withdrew from any of these studies because of this side effect. These findings suggest that topical capsaicin is a safe and potentially useful drug for the treatment of painful OA.

Capsaicin patch was developed for ease of application and accuracy of drug dosage release. Several indications for capsaicin patch have been reported, including chronic lower back pain, reducing postoperative analgesic requirement after orthognathic surgery, prevention and reduction of postoperative nausea and vomiting after surgery, and treatment of notalgia paresthetica and post-herpetic neuralgia.[9-15] Previous studies applied capsaicin patch at acupuncture points to reduce postoperative analgesic requirement and to prevent postoperative nausea and vomiting after surgery.[10,11,13,15] However, the efficacy and point of application of capsaicin patch for pain relief in knee OA has not been reported. The objective of this study was to evaluate the efficacy of 0.0125% capsaicin patch at acupuncture points for pain relief in knee OA.

MATERIALS AND METHODS

Preparation of 0.0125% capsaicin patch

Capsaicin patches (0.0125% concentration) were produced by the Herbal Medicine and Products Manufacturing Unit at the Center of Applied Thai Traditional Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, with manufacturing quality controlled according to standards of Good Manufacturing Practice and British Pharmacopoeia 2008. The patch has been made from polyacrylate-based products which allow the active substances in patch form (sustained release) to treat during normal activity of the human body. A single 0.0125% capsaicin patch contained 1 μg or 0.25 μg/cm^2 of capsaicin. Patches were stored at room temperature in a well-closed container and kept dry and clean.

Study design

This study was conducted at the outpatient rehabilitation clinic of Siriraj Hospital – Thailand's largest university-based tertiary referral center (Bangkok, Thailand). We equally allocated 62 patients between 2 study groups in a double-blind, randomized, placebo-controlled trial designed to compare the efficacy of 0.125% capsaicin patch vs. placebo patch for relief of pain in knee OA. The protocol for this study was approved by the Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University (Si 204/2556). This trial was registered with the Thai Clinical Trials Registry (TCTR) (TCTR20141111001).

Participants

Eligible patients were recruited during the September 2014 to March 2015 study period from the Outpatient Unit, Department of Rehabilitation Medicine, Faculty of Medicine Siriraj Hospital using the following inclusion criteria:

1. Outpatients who attended the outpatient rehabilitation clinic that were diagnosed with knee OA according to American College of Rheumatology (ACR) clinical criteria for classification of idiopathic (primary) OA[16]; and
2. Ambulatory patients over 50 years of age; and
3. Pain score according to numerical rating scale (pain on weight bearing) of 4-7 out of 10; and
4. Willing to participate in this study.

Patients with one or more of the following were excluded:

1. History of hypersensitivity to capsaicin or related compounds, paracetamol, naproxen or NSAIDs;
2. Skin lesion at the affected (treated) knee;
3. History of acupuncture for knee pain relief within 1 month prior to screening;
4. History of lower extremity surgery within 6 months prior to screening;
5. Other rheumatological diseases potentially affecting knee joint, such as rheumatoid arthritis or gouty arthritis;
6. Disease of spine or other lower extremity joints that could affect walking ability;
7. Treatment with other drugs potentially affecting bone or cartilage metabolism, such as:
   - Chronic systemic corticosteroids;
   - Glucosamine or chondroitin sulphate treatment within the last 15 days;
   - Hyaluronan injection into the affected knee within the previous 6 months;
   - Diacerin treatment within the last 12 months.

Interventions

Sixty-two participants were randomly assigned to receive capsaicin or placebo patch. The first participant was randomized in September 2014 and the last participant assessment took place in March 2015.

Using the www.randomization.com website,

randomization was performed into blocks of 8 8 8 8 8 6 8 8 in a 1:1 ratio to receive capsaicin or placebo patch. Staff of the Center of Applied Thai Traditional Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University provided capsaicin or placebo patches in opaque packages according to the generated randomization list. Study investigators, assessors, and participants were blinded to treatment assignment.

Participants in both groups received identical looking patches (2x2 centimeters in size) and instructions to apply 2 patches at 2 acupuncture points (one each) on the affected knee for at least 6 hours twice daily during the first week and once daily during the second to the fourth week.

A combination of two of the following six acupuncture points for knee OA were selected: ST34 (1), SP10 (2), ST35 (3), EX-LE4 (Neixiyan) (4), ST36 (5), and SP9 (6). The acupuncture point combinations were: 1 & 4, 2 & 3, 4 & 5, and 3 & 6, respectively – as shown in each patient's treatment diary (Fig 1).

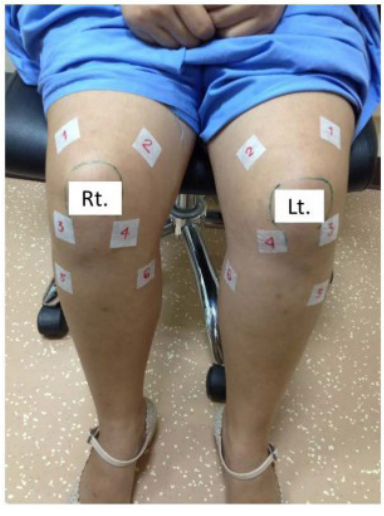


Fig 1. Locations of six acupuncture points in each knee.

All patients in both groups received basic education on knee OA and quadriceps exercise technique. Paracetamol 500 mg (Tylenol®; Johnson & Johnson, Ayutthaya, Thailand) every 4-6 hours or naproxen 250 mg (Berlin Pharmaceutical Industry Co., Ltd., Bangkok, Thailand) every 12 hours were allowed as a rescue medication, if necessary. Patients attended to their normal daily routines and were instructed not to seek, participate in, or take any other treatments (e.g., physical therapy, other pain relief medicines). Patients maintained a treatment diary that included the times patches were applied and removed, type and amount of medications used, and side effects, such as skin irritation and burning sensation.

Participants were seen in the clinic at baseline, 4 weeks, and 8 weeks after treatment for primary and secondary outcome evaluation, and interviewed via telephone at 2 weeks and 6 weeks after treatment for primary outcome evaluation.

Outcomes

The primary outcome was the pain subscale of modified Thai version of WOMAC osteoarthritis index for knee OA (measured on a 0- to 50-point scale, with higher scores indicating greater level of pain) at baseline, 2, 4, 6, and 8 weeks after treatment.[17,18]

Predefined secondary outcomes were modified Thai version of WOMAC osteoarthritis index for knee OA stiffness subscale (0-20), functional subscale (0-150), and total WOMAC score (0-220); visual analogue scale (VAS) for pain (0-10); Timed Up and Go test (seconds); participants' global assessment of improvement by asking participants to rate how much their knee pain had changed since using the patches (measured on a Likert scale from 1 [very much better] to 7 [very much worse]); participants' satisfaction of treatment (measured on a Likert scale from 1 [very satisfied] to 5 [very unsatisfied]); VAS for burning sensation (0-10); and, side effects from treatment.

Sample size

For the primary outcome of pain subscale of modified Thai version of WOMAC score, we initially estimated that 26 participants would be needed in each group to detect a difference of 3.42 between groups with a standard deviation (SD) of 4.3 (from previous study)[7], with a two-tailed α of 0.05 and a statistical power of 80% for comparison of 2 independent means. Based on a potential 20% withdrawal rate assumption, the final calculated sample size was 31 participants per group or a total sample of 62 participants.

Statistical Analysis

Data were reported using mean ± standard deviation (SD), median (min, max), or number. To compare quantitative data between 2 groups, independent t-test was used for normally distributed data and Mann-Whitney U test was employed to analyze for non-normally distributed data. Analysis of covariance (ANCOVA) was used to compare pain subscale of modified Thai version of WOMAC score between capsaicin and placebo groups. To compare quantitative data within group, repeated measures ANOVA was used with Bonferroni corrections for multiple comparisons. Chi-square test or Fisher's exact test was used to compare qualitative data between groups. For intention-to-treat (ITT) analysis, the last available score was carried forward. All statistical

analyses were performed using SSPS Statistics version 18.0 (SPSS, Inc., Chicago, IL, USA). A p-value<0.05 was considered to be statistically significant.

RESULTS

A total of 80 patients were screened for eligibility. Sixty-two patients met the eligibility criteria and were randomized between September 2014 and March 2015 (Fig 2). Baseline demographic and clinical characteristics were similar between groups (Table 1). Pain subscale of modified Thai version of WOMAC score between capsaicin and placebo groups at baseline and at each visit showed no significant difference between groups (Table 2). Pain subscale of modified Thai version of WOMAC score within group (pre-post treatment) at baseline and at each visit (weeks 2, 4, 6, and 8) in the capsaicin group showed pain subscale of modified Thai version of WOMAC was significantly decreased from baseline after 2 weeks of treatment (p<0.001 for all). In the placebo group, modified Thai version of WOMAC pain subscale was significantly decreased from baseline after 4 weeks of treatment (p=0.108, p=0.004, p=0.005, and p=0.004, respectively) (Table 3). Trend of pain subscale of modified Thai WOMAC score improvement from baseline to each follow-up visit in the capsaicin and placebo groups is shown in Fig 3.

Stiffness subscale, functional subscale, and total modified Thai version of WOMAC score at baseline and at each visit showed no significant differences between groups (Table 2). VAS for pain, Timed Up and Go test (seconds), participants' global assessment of improvement, and participant satisfaction with treatment at baseline and at each visit showed no significant differences between groups (Tables 4, 5).

A significant difference between groups was observed for burning sensation VAS at week 4 (p=0.029) (Table 5). Only one subject in the placebo group mention about burning sensation. This was probably because of the uncomfortably feeling from patch was miss-interpreted to burning sensation in the questionnaire in that particular patient.

Five out of 31 patients in each group described feeling uncomfortable wearing patches. Reported side effects included allergy, rash, itching, and ankle swelling (Table 6). None of the patients in either group withdrew as a result of any of these side effects.

The amounts of medication used to control knee pain over the 8-week course of patch treatment for paracetamol and naproxen was not significantly different between groups (p=0.227 and p=0.789 respectively) (Table 5).

Patient treatment compliance was recorded in each patient's treatment diary. Twenty-seven patients (87.1%) in the treatment group and 29 patients (93.5%) in the placebo group had good compliance (over 80% compliance), with no significant difference between groups (p=0.671).

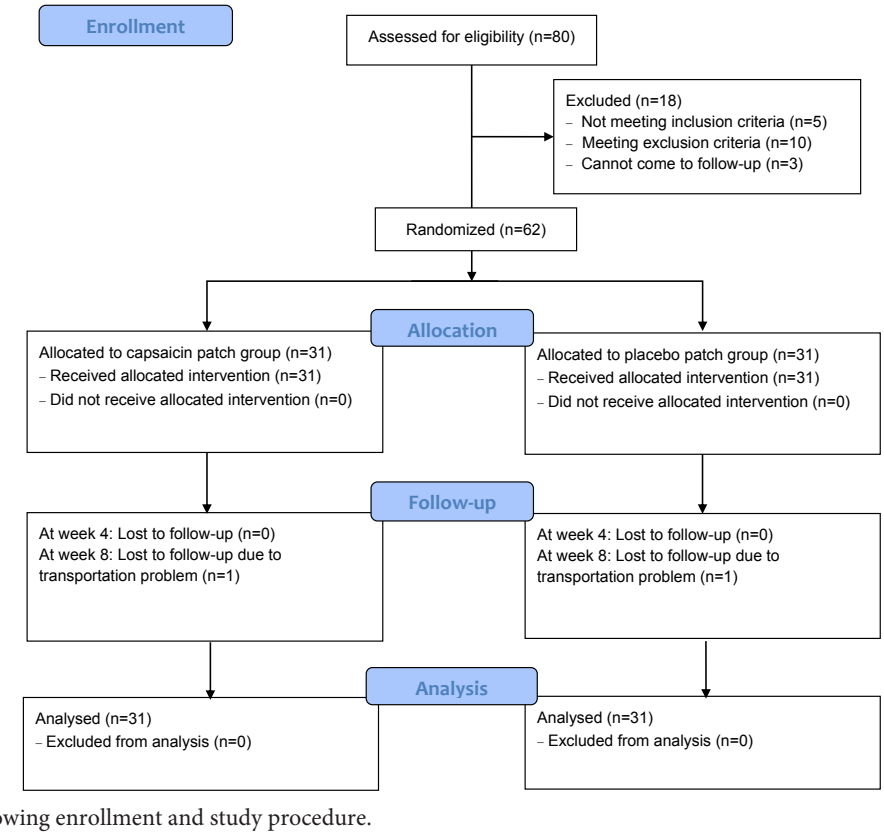


Fig 2. Flow diagram showing enrollment and study procedure.

TABLE 1. Clinical characteristics of study population at baseline.

	Treatment (n=31)	Placebo (n=31)	*p*-value
Age	63.3±8.9	62.8±6.3	0.767
Gender (female:male)	27:4	30:1	0.354
Body Mass Index	26.3±4.8	25.9±5.2	0.792
Affected knee (right:left)	18:13	14:17	0.309
Duration of pain (years)	3 (0.2, 10)	2 (0.2, 20)	0.876
WOMAC pain	22.0±9.7	20.7±7.9	0.556
WOMAC stiffness	8.5±5.2	8.9±4.2	0.727
WOMAC function	58.5±33.7	57.7±27.0	0.927
WOMAC total	89.0±46.5	87.4±36.4	0.880
VAS for pain	5.38±1.84	5.20±1.61	0.685
Timed Up and Go test	14.18±4.22	12.64±3.31	0.117

Data presented as mean±SD, median (min, max), or number

Abbreviations: WOMAC = Western Ontario and McMaster Osteoarthritis Index, VAS = visual analogue scale

TABLE 2. Comparison of WOMAC subscale and total scores between treatment and placebo groups at baseline and at each follow-up visit.

	Week	Treatment (n=31)	Placebo (n=31)	*p*-value
Pain subscale	0	22.0±9.7	20.7±7.9	0.556
	2	17.5±9.8	17.0±7.2	0.745
	4	15.0±10.8	14.9±7.2	0.630
	6	13.2±10.4	14.7±7.3	0.225
	8	13.5±11.1	14.4±8.5	0.432
Stiffness subscale	0	8.5±5.2	8.9±4.2	0.727
	4	5.2±4.6	5.6±3.7	0.691
	8	5.2±4.8	4.5±3.5	0.490
Functional subscale	0	58.5±33.7	57.7±27.0	0.927
	4	45.6±32.6	44.3±23.1	0.858
	8	43.2±35.2	44.9±28.5	0.837
Total WOMAC score	0	89.0±46.5	87.4±36.4	0.880
	4	65.7±46.0	64.8±30.5	0.923
	8	62.0±50.0	63.8±37.5	0.873

Abbreviation: WOMAC = Western Ontario and McMaster Osteoarthritis Index

TABLE 3. Comparison of pain subscale of modified Thai version of WOMAC score between baseline and each follow-up visit within group (pre-post treatment) for the capsaicin and placebo groups (repeated measures ANOVA).

Week	Treatment (n=31)	Placebo (n=31)
0 *vs.* 2	*p*<0.001	*p*=0.108
0 *vs.* 4	*p*<0.001	*p*=0.004
0 *vs.* 6	*p*<0.001	*p*=0.005
0 *vs.* 8	*p*<0.001	*p*=0.004

p-value <0.05 indicates statistical significance

TABLE 4. Comparison of pain VAS and Timed Up and Go test at baseline and at each follow-up visit between capsaicin and placebo groups.

	Week	Treatment (n=31)	Placebo (n=31)	*p*-value
Pain VAS	0	5.38±1.84	5.20±1.61	0.685
	4	3.57±2.09	3.9±2.05	0.526
	8	3.45±2.56	3.93±2.74	0.477
Timed Up and Go test	0	14.18±4.22	12.64±3.31	0.116
(seconds)	4	12.36±3.57	11.67±2.53	0.381
	8	12.56±4.28	10.93±2.00	0.062

Abbreviation: VAS = visual analogue scale

TABLE 5. Comparison of global change after treatment, patient satisfaction, burning sensation VAS and number of medication used (tab) between capsaicin and placebo groups.

		Treatment (n=31)	Placebo (n=31)	*p*-value
Global change after treatment	Week 4	2 (1, 5)	3 (1, 6)	0.246
	Week 8	2 (1, 4)	2 (1, 7)	0.341
Patient satisfaction	Week 4	2 (1, 3)	2 (1, 4)	0.994
	Week 8	2 (1, 4)	2 (1, 3)	0.943
Burning sensation VAS	Week 4	0 (0, 9.46)	0 (0, 6.88)	0.029
	Week 8	0 (0, 9.46)	0 (0, 0)	N/A
Amount of medications used	Paracetamol	0 (0, 62)	0 (0, 25)	0.227
	Naproxen	0 (0, 48)	0 (0, 15)	0.789

Data presented as median (min, max), *p*-value <0.05 indicates statistical significance

Abbreviations: N/A = not applicable, VAS = visual analogue scale

TABLE 6. Comparison of side effects between capsaicin and placebo groups (patients can have more than 1 side effect).

Side effect	Treatment (n=5)	Placebo (n=5)	*p*-value
Allergy	1	1	1.000
Rash	3	3	1.000
Itching	4	4	1.000
Other	0	1*	1.000

*Ankle swelling

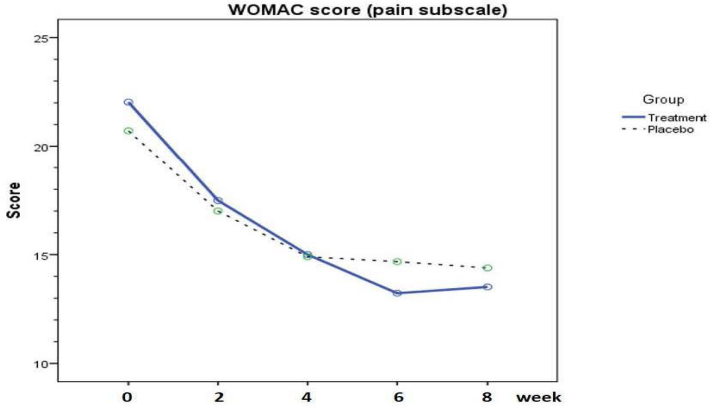


Fig 3. Pain subscale of modified Thai version of WOMAC score at baseline and at each visit in the capsaicin and placebo groups.

DISCUSSION

In this study and relative to the pain subscale of modified Thai version of WOMAC score, the treatment group showed early significant improvement after 2 weeks of treatment and the placebo group demonstrated significant improvement after 4 weeks of treatment. However and overall after 8 weeks of treatment, there was no significant difference in reduction of pain subscale of modified Thai version of WOMAC score between groups.

Possible explanations why there was no statistical difference in overall pain subscale of modified Thai version of WOMAC score between groups include: (1) insufficient concentration and volume of capsaicin; (2) acupuncture point effect; (3) effect of education and/or exercise; and, (4) placebo effect.

Based on our review of the literature specific to preparations of topical capsaicin and acupuncture point in knee OA, we decided upon using the lowest concentration of capsaicin preparation, which is 0.125%.[7] In addition, only 2 of 6 acupuncture points were used at each patch treatment (for a total of 4 different acupuncture point combinations) in order to avoid local side effect, as two local points may be sufficient to treat knee OA.[19] The concentration and volume of capsaicin used in this study may have been insufficient. As such, additional studies using a higher concentration of capsaicin and/or a larger size of the patch should be conducted.

Stimulation of acupuncture point with capsaicin or placebo patch may have acupuncture effect, which can reduce pain via gate control theory.[20] Additionally, placebo patch may replicate sham acupuncture, which may also have some therapeutic effects.[21] Education and exercise might also affect outcome of treatment. From the results, pain subscales were decreased in both groups, partially because quadriceps strengthening exercises were advised for every subject in this study. There is evidence that a 5-week isometric quadriceps exercise program showed beneficial effects on quadriceps muscle strength, reduction in pain intensity, and improvement in function in patients with osteoarthritis of the knee.[22] However, in this study, the decrement of pain subscale between both groups showed no statistically significant difference probably because the effect size of quadriceps exercise is over and above the effect of capsaicin patch alone.

From a systematic review of the effects of alternative placebo types on pain outcomes in knee OA, Bannuru, *et al.*

reported a 0.20 (95% CI: 0.02-0.38) effect size for topical placebo.[23] Administration of a placebo with higher efficacy will decrease the apparent effect size of the active treatment in a between-group comparison. Thus, the magnitude of the placebo effect can influence the results of a randomized controlled trial. Accordingly, there might have been a placebo effect relative to pain reduction in this study.

Study limitations

This study accomplished its research objective, but not without some mentionable limitations. First, the follow-up period was relatively short, although longer-term follow-up was not available. Second, rescue drugs for pain relief were authorized in all patients in both groups since all initial pain scores ranged from 4 to 7 out of 10 on visual analog scale, which we considered to be moderate pain. Third, although all patients were prescribed the same behavior modification and exercise regimens as co-interventions, data relating to frequency and intensity of exercise were not recorded by our patients. Finally, only patients with moderate knee pain were recruited for this study. Therefore, the findings of this study cannot be considered generalizable to patients with mild or severe knee pain.

CONCLUSION

Capsaicin patches (0.0125% concentration) at acupuncture points for pain relief in moderately painful OA knees yielded no significant difference in pain relief between groups. However, significant pain relief from baseline was observed in the treatment group after 2 weeks of treatment and in the placebo group after 4 weeks of treatment. Further study with higher capsaicin concentration and/or larger size patch should be considered.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Mr. Suthipol Udompunturak for assistance with statistical analysis.

This trial was supported by a grant from the Routine to Research Unit, Faculty of Medicine Siriraj Hospital, Mahidol University (Trial #12RE00014/024/12). The funder had no role in the design, preparation, or outcomes of this study.

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

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Optimizing Fractional Intensity Threshold for FSL-Brain Extraction Tool (BET) and Comparing with FreeSurfer on 3D T1W MR Images

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ABSTRACT

Objective: To find out the optimal Brain Extraction Tool (BET) parameter (fractional intensity threshold) for measuring the brain volume compared with the standard manual method in our institute and to compare with those of automated FreeSurfer software

Methods: This retrospective study was performed in 10 healthy adult subjects with data of 3D-T1W on 3T MR machine. The manual gold standard brain volume measurements were done by two independent readers. The automated segmentations using BET with varied parameters and FreeSurfer software were also performed. Then, the two automated methods were compared with the manual tracing to make the optimal parameter by seeking for the highest intraclass correlation coefficient (ICC) using SPSS software.

Results: The fractional intensity threshold for whole brain volume measurement of 0.1- 0.6 showed high ICC with the manual gold standard (ranging from 0.639 to 0.748). The best value was 0.1, showing highest ICC of 0.748 ($p < 0.006$) with confidence interval of 95% equal to (0.242; 0.932). There were no optimal parameters for right and left hippocampus volume measurement by BET due to very low ICC between BET and the reader (ICC ranging from 0.017 to 0.139 and from 0.012 to 0.110, for the right and left hippocampus volume, respectively). The ICC values of the automated FreeSurfer method with the manual tracing were also very low (0.063, 0.068, and 0.063 for right, left and bilateral hippocampi respectively).

Conclusion: The optimal BET parameter (fractional intensity threshold) for automated brain volume measurement in our institute is 0.1 similar to the suggested value by prior study with high agreement (ICC=0.748) to the manual method.

Keywords: Fractional intensity threshold; MRI; BET; FreeSurfer (Siriraj Med J 2018;70: 391-396)

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with disruption of neuronal function and gradual deterioration in cognition, function and behavior.[1] It is the most common cause of dementia in the elderly[1,2] and characterized by deposition of neurofibrillary tangles and amyloid plaques.[2,3] However, it is clinically diagnosed by progressive memory loss affecting activities of daily living.[4] The presence of medial temporal lobe atrophy is considered to associate with Alzheimer's disease. As a result, the measurement of medial temporal lobe or hippocampus has been accepted to be useful in detection of the disease.[5]

A manual segmentation is generally accepted as the gold standard measurement of brain volume for atrophic changes. However, it is not practical due to being

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Received 25 September 2017 Revised 12 July 2018 Accepted 16 February 2018
doi:10.14456/smj.2018.62

time-consuming. Fortunately, automated techniques have been developed to replace the conventional method. FreeSurfer is a set of powerful automated software tools for reconstruction of the brain's cortical surface from structural MRI data, including volumetric segmentation of visible brain structures, especially the hippocampus.[6] The white and grey matter is segmented by integrating information of image intensity, location and spatial relationships between subcortical structures.[7] The automated Brain Extraction Tool (BET) refers to the separation of brain and non-brain tissue technique, and is provided as part of the FSL software. The program is fast and easy to use.[8,9,10] The automated BET measurement values vary depending on the parameter called "fractional intensity threshold", ranging from 0 to 1. There was a prior study about the optimal value showing that the BET parameter of 0.1 was the best for all acquisition protocols after removing the neck slices.[11] However, due to different settings in our institute, the best value may be not the same. Therefore, the purpose of this study was to find out the optimal fractional intensity threshold used to measure the Thai brain volumes compared with the standard manual method and to compare between the two automated methods.

MATERIALS AND METHODS

Subjects

The study was part of the Siriraj Thai language paradigm for functional imaging which is approved by the Siriraj Institutional Review Board (Si 423/2011). The data for this retrospective study were retrieved from the database of the project with official permission. The subjects were excluded if they have gross brain abnormalities. Finally, a total of 10 Thai healthy volunteers (5 men and 5 women) were enrolled in this study. The mean age for all subjects was 25.5 years (ranging from 22 to 36 years). The mean ages for male and female subjects were 25 years (ranging from 23 to 29 years) and 26 years (ranging from 22 to 36 years), respectively.

Image acquisition

The examinations were performed by 3.0 T MRI scanner (Achieva, Philips Medical Systems, Best, NL) in whole brain T1-weighted axial 3D Turbo fast field echo (3D TFE) (voxel size = 1x1x1 mm , repetition time (TR) = 7.7 msec, echo time (TE) = 3.6 msec, flip angles = 8°, TFE factor = 144, FOV = 230x290 mm, matrix = 232x288, slice thickness = 1 mm, NSA = 1).

Manual segmentations

The manual segmentations were performed by one reader, a resident in diagnostic radiology, using AW VolumeShare 2 software. The whole brain tracing was made by drawing along the outer surface of the dura using the lowest point of the medulla oblongata as the most inferior point (outlined by border of the foramen magnum). The signal threshold was visually adjusted to exclude low signal of the CSF in the ventricle and subarachnoid space whereas preserving brain parenchyma was aware. The ventricular system, the cavernous sinus, the optic nerves, the optic tracts, the optic chiasm and the pituitary gland were excluded. Then the volume of whole brain was calculated.

The hippocampal tracing was made by drawing along the outer surface of the bilateral hippocampi in coronal plane. The anterior aspect of the hippocampus was limited by tracing from the body part and only amygdala was seen on the images. The posterior aspect was limited at the last image before the visualized crus of the fornix. Only the hippocampal proper was selected and separated from the parahippocampal gyrus by an imaginary line perpendicular to the border between subiculum and entorhinal cortex.

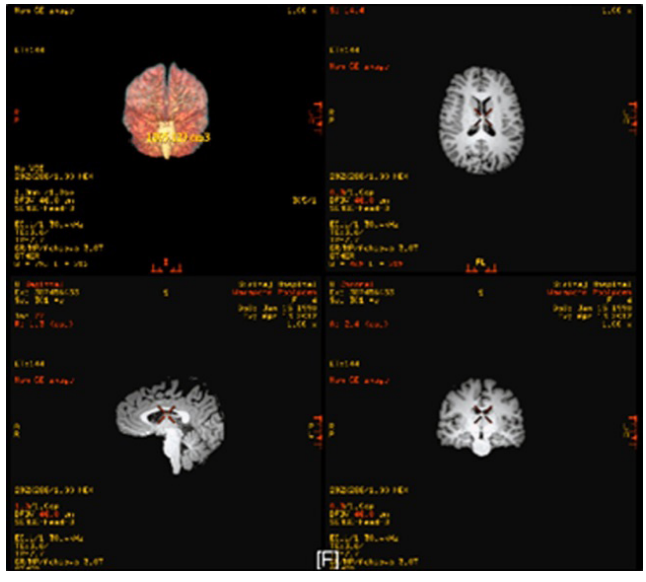


Fig 1. Manual segmentation for whole brain volume.

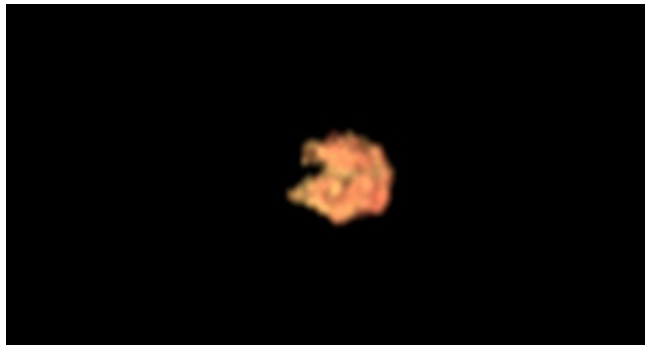


Fig 2. Manual segmentation of hippocampal volume.

Automated segmentations

The automated segmentations were performed using FSL-Brain Extraction Tool (BET) and FreeSurfer 5.0 software. The BET parameter (fractional intensity threshold) was used in different values, ranging from 0 to 1. Then, the brain volumes measured by BET at various parameters and FreeSurfer were compared with those of the manual segmentations by the reader to seek for the highest intraclass correlation coefficient (ICC) computed by SPSS software (Mahidol University license).[12,13]

RESULTS

The measurement of whole brain volume was shown in Table 1. The whole brain volumes measured by the reader ranged from 1,071.53 to 1,370.37 cm^3 ($\bar{x}$ =1,191.45 cm^3, *SD* =100.73).

The whole brain volume measurement by BET with different fractional intensity threshold and ICC of brain volume measurement between BET and the reader's manual segmentations was shown in Table 2.

According to the results in Table 2, the fractional intensity threshold between 0.1 to 0.6 showed high agreement to the manual gold standard range of the reader with the value of ICC ranging from 0.639 to 0.748. The optimal value was 0.1, indicating the highest ICC of 0.748 ($p < 0.006$) with confidence interval of 95% equal to (0.242; 0.932).

The result of measurement of the right and left hippocampus volumes by BET with different fractional intensity threshold values; and the ICC values between BET and the reader were shown in Table 3 and Table 4, respectively.

For the right hippocampus volume measurement, the fractional intensity threshold between 0 to 1 showed the low agreement with the manual gold standard (ICC ranging from 0.017 to 0.139). Similar to the left hippocampus volume measurement, the fractional intensity threshold between 0 to 1 showed the low agreement with the manual gold standard (ICC ranging from 0.012 to 0.110).

The hippocampal volume measurement by FreeSurfer software and the ICC values between FreeSufer and the reader manual tracing were shown in Table 5.

The ICC values of hippocampus volume measured by FreeSurfer and the reader show very low agreement with the manual gold standard at ICC = 0.063 (Right hippocampus) and 0.068 (Left hippocampus), respectively.

TABLE 1. The manual measurement of whole brain volume

Subjects/ Whole brain volume (cm^3)	Reader
1	1,098.80
2	1,081.33
3	1,370.37
4	1,205.12
5	1,134.70
6	1,298.24
7	1,071.53
8	1,287.47
9	1,188.34
10	1,178.60
Mean	1,191.45
Standard Deviation	100.73

TABLE 2. Whole brain volume (cm^3) measured by BET at various parameters and ICC of measured volumes between BET and the reader.

Subjects/ **WB volumes	0*	0.1*	0.2*	0.3*	0.4*	0.5*	0.6*	0.7*	0.8*	0.9*	1*
1	3,169.31	1,080.51	1,070.05	1,059.11	1,053.04	1,042.87	1,030.80	1,023.06	771.29	470.00	261.57
2	2,973.92	1,013.21	1,000.55	986.77	972.50	968.01	942.88	905.77	638.17	382.56	184.13
3	3,050.44	1,312.47	1,288.89	1,270.90	1,266.69	1,262.18	1,239.85	1,166.60	715.22	467.63	277.24
4	3,297.36	1,156.56	1,135.46	1,127.00	1,108.60	1,106.54	1,111.22	1,059.41	805.77	482.54	257.42
5	1,163.12	1,169.06	1,145.61	1,134.41	1,125.75	1,122.19	1,102.81	1,036.94	752.65	418.99	230.81
6	3,061.31	1,342.73	1,315.32	1,301.61	1,292.64	1,276.98	1,275.95	1,229.25	886.09	518.02	263.08
7	3,773.39	1,260.64	1,229.79	1,193.35	1,177.77	1,165.84	1,143.98	1,117.45	869.67	559.56	322.86
8	2,916.10	1,269.01	1,220.82	1,210.47	1,197.34	1,195.34	1,175.30	1,138.99	847.91	507.36	282.39
9	2,617.09	1,175.65	1,160.65	1,153.97	1,143.20	1,141.14	1,142.32	1,083.18	765.53	446.45	237.20
10	1,298.75	1,145.57	1,099.89	1,084.09	1,079.84	1,066.68	1,049.33	1,030.00	740.76	430.36	226.83
***Reader	0.002	0.748	0.719	0.719	0.697	0.683	0.639	0.438	0.017	0.005	0.002

*BET Parameter (Fractional Intensity Threshold), **WB volumes: whole brain volumes in cm^3
***ICC of measured whole brain volumes using BET at various parameters and reader's measured volumes.

TABLE 3. Right hippocampus volume (cm^3) measured by BET at various parameters and ICC of measured volumes between BET and the reader.

Subjects/ ***RH volumes (cm^3)	0*	0.1*	0.2*	0.3*	0.4*	0.5*	0.6*	0.7*	0.8*	0.9*	1*
1	3.600	4.613	4.956	5.096	4.646	4.735	4.847	4.765	4.952	0.928	**N/A
2	1.620	4.067	3.802	4.073	4.007	3.976	0.129	3.997	0.361	**N/A	1.202
3	3.982	0.744	4.772	5.09	5.112	5.08	4.366	4.134	4.403	3.773	**N/A
4	1.125	4.901	4.967	4.95	4.844	4.943	4.956	4.784	4.598	4.567	0.793
5	**N/A	5.562	4.792	4.535	4.716	2.691	4.531	4.762	1.201	4.102	0.908
6	5.763	3.846	5.842	7.769	**N/A	5.723	5.684	10.291	5.65	5.444	5.484
7	9.609	5.416	5.558	1.248	5.43	5.383	5.396	5.359	5.466	5.408	0.308
8	7.375	5.437	1.138	1.199	0.633	**N/A	**N/A	1.131	5.42	0.575	0.937
9	4.938	4.987	4.979	5.017	4.94	5.019	5.023	4.935	3.911	4.867	4.935
10	2.916	1.197	4.842	4.751	6.543	4.761	4.693	4.987	3.133	1.197	2.934
****Reader	0.139	0.034	0.028	0.017	0.029	0.021	0.126	0.080	0.126	0.095	0.073

*BET Parameter (Fractional Intensity Threshold), **N/A: data not available, ***RH volumes: right hippocampus volumes,****ICC of measured right hippocampus volumes using BET at various parameters and reader's measured volumes.

TABLE 4. Left hippocampus volume (cm^3) measured by BET at various parameters and ICC of measured volumes between BET and the reader.

Subjects/ ***LH volumes (cm^3)	0*	0.1*	0.2*	0.3*	0.4*	0.5*	0.6*	0.7*	0.8*	0.9*	1*
1.	3.938	4.358	4.354	4.473	4.394	4.366	4.366	4.457	4.166	0.841	**N/A
2.	2.419	4.329	4.265	4.325	4.379	4.310	0.154	4.294	0.286	1.433	1.106
3.	1.663	0.735	5.556	5.396	5.348	5.456	5.316	5.345	5.230	4.640	**N/A
4.	0.955	4.835	4.830	4.877	4.877	4.872	4.852	4.910	4.792	4.607	0.416
5.	**N/A	2.478	4.098	4.027	4.034	1.029	3.857	4.026	1.105	3.151	0.589
6.	5.467	2.652	5.587	11.691	8.426	5.598	5.592	11.156	5.113	5.071	5.027
7.	8.945	4.734	4.774	1.240	4.894	4.906	4.747	4.876	4.697	4.633	0.190
8.	3.343	5.250	1.050	1.052	1.125	8.863	8.207	0.722	5.060	0.689	1.201
9.	4.837	4.982	4.957	4.956	4.973	4.971	4.949	4.932	2.490	4.883	4.768
10.	4.800	1.161	4.776	4.781	6.618	4.809	4.716	4.741	2.027	1.152	1.130
****Reader	0.110	0.080	0.014	0.029	0.022	0.012	0.087	0.035	0.105	0.019	0.089

*BET Parameter (Fractional Intensity Threshold), **N/A: data not available, ***LH volumes: left hippocampus volumes, ****ICC of measured left hippocampus volumes using BET at various parameters and reader's measured volumes.

TABLE 5. Hippocampal volumes (cm^3) measured by FreeSurfer and the reader with ICC values.

Subjects/ volume (cm^3)	FreeSurfer: Right hippocampus	Reader 2: Right hippocampus	FreeSurfer: Left hippocampus	Reader 2: Left hippocampus	FreeSurfer: Bilateral hippocampi	Reader 2: Bilateral hippocampi
1	4.040	2.529	3.866	2.795	7.906	5.324
2	3.991	2.101	3.754	2.171	7.745	4.272
3	4.697	2.698	4.492	2.690	9.189	5.388
4	4.092	2.263	3.453	2.259	7.545	4.522
5	4.719	2.960	4.084	2.874	8.803	5.834
6	5.029	3.311	4.723	3.064	9.752	6.375
7	4.286	2.929	4.052	2.820	8.338	5.749
8	5.185	2.803	4.832	2.855	10.017	5.658
9	4.498	2.306	4.397	2.585	8.895	4.891
10	4.119	2.404	4.056	2.713	8.175	5.117
*Reader	0.063		0.068		0.063	

*ICC of measured hippocampus volumes using FreeSurfer and reader's measured volumes.

DISCUSSION

This study was initiated to adjust the optimal BET parameter called "fractional intensity threshold" which affects the estimated brain volumes[8] and to compare the two automated methods. According to the previous study about the optimal BET parameter of automated brain volume measurement in patients with multiple sclerosis, the best value for all MRI sequences after removing the neck slices was 0.1.[14] However, the study included patients who performed both 1.5 T and 3.0 T MRI studies.[14] This variety might affect the study result.[15] This study was done in the healthy adults who performed the same MRI scanner (3.0 T MRI scanner). Thereby, such variation was eliminated.

The optimal fractional intensity threshold for whole brain volume measurement in this study was 0.1 which is similar to the suggested value in the previous study.[15] However, this study could not seek optimal values for automatic hippocampus volume extraction using BET and Freesurfer since the agreements with the manual goal standard were very low. FreeSurfer was superior to BET in terms of no need to adjust parameters, although the result from this study show limited use of both BET and FreeSurfer for measuring hippocampal volume.

This exploratory study implied that in different situations, research with automated segmentation needs to be adjusted and validated with an acceptable gold standard before clinical implementation. The study also showed low agreements of the measurement implying inappropriateness for clinical practice. Further study for the solution is needed with a larger sample size and more readers. This study was limited by the small sample size and the validity of the manual gold standard measurement which depended on operators' skills, experiences and knowledge. Furthermore, it was done in only healthy subjects. The application of the study to patients with brain abnormalities especially atrophy, such as Alzheimer's disease may be questionable.

CONCLUSION

The optimal BET parameter (fractional intensity threshold) for automated whole brain volume measurement in our institute is 0.1 with high ICC value. Adjusted threshold with manual segmentation should be performed and absolute value from BET was not appropriate to use in clinical practice unless an appropriate solution is found.

ACKNOWLEDGMENTS

This study was supported by the Faculty of Medicine Siriraj Hospital, Mahidol University.

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Do Wristbands Used as an Adjunct to a Weight-Loss Program Affect the Outcomes in the Treatment of Obesity?

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ABSTRACT

Objective: The purpose of the present study was to evaluate the efficacy of a wristband as a method for reminding patients of lifestyle modification in weight-loss program.

Methods: Researchers randomly assigned 64 obese patients in our outpatient department to receive either a wristband plus brief lifestyle modification program (wristband group) or a brief lifestyle modification program alone (control group). The primary outcome was the change in body weight at 6 months. The secondary outcomes included the change in body weight at 12 months; the change in waist circumference at 6 and 12 months; the percentage of participants who maintained a weight loss at 6 and 12 months; and the categorical weight loss of the participants at the 6- and 12-month follow-ups.

Results: At 6 months, participants in both groups had lost weight. The mean (±SE) weight change among the participants in the wristband group and the control group was -4.5±1.38 and -2.13±1.09 kg, respectively (p-value = 0.193). All of the secondary outcomes displayed a trend in favor of the wristband group.

Conclusion: The results of this trial of a weight-loss intervention provide potential support to use a wristband for habitual change as an adjunct to lifestyle modification in a weight-loss program.

Keywords: Wristband; obesity; lifestyle modification program; weight-loss program; body weight; waist circumference; habit (Siriraj Med J 2018;70: 397-405)

INTRODUCTION

Obesity is a growing problem. Compared to people with normal, or healthy, weight, people who are obese are at increased risk for many serious diseases.[1-3] Lifestyle modification is considered the first option to achieve healthy weight.[4-7] Several trials, in which primary care providers offered counseling about diet and physical activity, have seen weight losses of 4.6 kg or less over study periods ranging from 6 months to 2 years.[8-11]

Behavioral therapy is one of the key components of lifestyle modification. Behavioral therapy refers to a set of principles and techniques for helping obese individuals modify their eating, activity and thinking habits which are contributing to their excess weight.[6]

Ten Top Tip program (10TT) is a brief and effective weight control intervention that can be delivered without specialist skills. It is suitable for our healthcare environment which has limited resources to select comprehensive lifestyle modification program. It is based on habit-formation theory that had quantitative and qualitative evidences to help lose weight. According to a randomized control trial by RJ Beeken et al., 10TT delivered through primary care is effective in the short-term and a low-cost option over the longer term. Participants receiving

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Received 16 March 2018 Revised 29 June 2018 Accepted 9 July 2018
doi:10.14456/smj.2018.63

10TT lost significantly more weight over 3 months than those receiving usual care.[12] 10TT has shown qualitative evidences about the experiences of participants in the program. Participants reported positive experiences, both during and after the interventions. Participants particularly enjoyed the novelty of the interventions as they shifted focus from diet and exercise, to practical everyday habit changes. They also reported indirect health benefits such as increased energy levels, increased confidence and improved self-awareness. 10TT enhanced self-regulatory skills and changes in self-regulatory skills and automaticity mediated the effect of the intervention on weight loss.[13,14]

Researchers searched for tools that help as an adjunct to a weight-loss program and considered wristbands. These are made from a loop of material that is placed around the wrist, and they are used for many different reasons, but most typically, for identification purposes. Wristbands are an effective intervention for cuing process in habit loop which resulted in reducing drinking among designated drivers.[15] Despite their widespread use, their efficacy in weight loss program has not been evaluated before this study. This tool is based on neurobehavioral model and habit-formation theory that offers a unique perspective from which to derive an approach to weight management.[16-20] Habits are automatically triggered actions, learned through repetition of the action in a consistent context. Researchers postulated that wristbands might be the intervention that targets automatic actions in a consistent context. Wristbands might help participants to lose weight, given that they had the potential to enhance particular components of cognitive behavioral therapy by regularly alerting and reminding participants of their goal to lose weight, the need to strive for healthy habits, and the value of regular self-monitoring. Researchers conducted a 12-month, randomized controlled trial to evaluate the effectiveness of wristbands as an adjunct to a simple, weight-loss program.

MATERIALS AND METHODS

Study design

Researchers randomized patients in a controlled trial that compared the clinical consequences of a wristband plus brief lifestyle modification program (wristband group), versus a brief lifestyle modification program alone (control group).

The study, conducted between November 2013 and March 2016, was approved by the Research Ethics Board of the Panyananthapikkhu Chonprathan Medical Center (PCMC), Srinakharinwirot University.

Study participants

Participants were recruited and treated at the outpatient department at PCMC.

For inclusion in the study, participants had to be 18 years of age or older and have a body-mass index (BMI, the weight in kilograms divided by the square of the height in meters) of 30 or higher.

Exclusion criteria were chronic kidney disease with an estimated GFR below 30; pregnancy; lactation; a weight loss of 5 kg or more in the previous 3 months; an eating disorder; a thyroid disease; major surgery in the previous 3 months; severe comorbidity; and receiving a medication that affected the body weight in the previous 3 months (e.g., diuretics, glucocorticoids, insulin or thiazolidinedione).

Interventions

All participants were prescribed a brief lifestyle-modification program encompassing three principal components: diet, physical activity, and behavioral therapy. By permission of J Wardle, the behavioral treatment was based on the Ten Top Tips program.[21]

Wristband group

Researchers prescribed a brief lifestyle-modification program encompassing three, principal components: diet, physical activity, and behavioral therapy. The behavioral treatment was based on the Ten Top Tip program. The model consists of a list of ten behaviors. Seven are simple behaviors associated with a negative energy balance that is estimated to create a daily deficit of 800-900 kcal for a person changing from doing none of the behaviors to doing all of them. Two additional behaviors are designed to improve awareness of food intake, while the tenth behavior promotes routines.[21] Health professionals were provided with a script to assist them to deliver the intervention in a standardized way. Immediately after randomization, patients received the Ten Top Tips leaflet, a simple logbook for self-monitoring of target behaviors and weight issued by Weight Concern and Cancer Research UK[22] together with a wristband made from silicone, which was labelled "Healthy habits". Researchers encouraged participants to wear the wristband all the time, if possible, to help in reminding them about both their commitment to lose weight and the need for regular, healthy habits formation. A 15-minute session was provided to take participants through the leaflet and logbook using a flip chart and defined script. These leaflets outline weight-loss tips and suggest how to develop healthy lifestyle-habits.

At 2 months, researchers reviewed the participants' logbooks and emphasized the importance of having a healthy diet. In addition, researchers gave instructions on how to use a food diary provided to them during the session, so that they could begin to record their food and calorie intakes. At 4 months, researchers reviewed their logbooks and the food-and-calorie-intake records in their food diaries. As well, researchers discussed the benefits of doing exercise and physical activities. At 6 months, researchers emphasized the need for habit formation for weight maintenance. The researchers made no further contact with the participants until the 12 months follow-up, although the participants still attended the hospital as needed to receive ongoing follow-up for their various and individual medical conditions.

Control group

Participants randomized to control group, except for the wristband, were provided the same simple, self-guided, lifestyle-modification program, including leaflet, logbook and sessions, as the wristband group.

Outcomes and assessments

The primary outcome was the change in body weight at 6 months for members of each group.

The secondary outcomes included the change in body weight at 12 months; the change in waist circumference at the 6- and 12-month follow-ups; the percentage of participants who maintained a weight loss at the 6- and 12-month follow-ups; and the categorical weight loss at the 6- and 12-month follow-ups (categorized into participants whose initial weight had decreased by less than 3%, 3% or more, 5% or more, 7% or more, and 10% or more).[23]

Screening, randomization and blinding

The participants were recruited by physician-referral for obesity management. After screening for the inclusion and exclusion criteria, participants gave their informed consent and were then assessed by means of standardized measures for weight, height, waist circumference, blood pressure and laboratory tests (namely, FPG, HbA1c, total cholesterol, triglyceride, HDL and LDL).

Participants were randomly assigned to either the control or the wristband group with the aid of computer-generated assignment with block randomization, using a block size of 4. The researchers and the participants were blinded to the group assignment status until the envelope was opened, from when neither the physicians conducting the investigations nor the participants were blinded, as specified by the protocol.

In addition, both the data collector and the statistician (responsible for administering the outcome assessments, managing the databases and analyzing the data) were blinded to the group assignments.

Statistical analysis

Changes in body weight in the intention-to-treat participants were compared with the use of repeated measure ANOVA. An independent t-test was used to analyze the differences in the changes in the waist circumferences. Either a chi-squared test or a Fisher's exact test was used to determine the differences in the percentage of participants who maintained a weight loss and in the categorical weight loss for each group.

This is the first study to assess efficacy of wristband for weight loss. The study had a power of 80% to detect a 2-kg difference in the body-weight change at the 6-month follow-up for each group. A p-value of 0.05 or less was considered to show statistical significance. Researchers set 2 kg difference in weight change because an analysis of Diabetes Prevention Program lifestyle intervention data showed that weight loss was the dominant determinant of the reduced risk of diabetes. For every kilogram lost, there was a 16% reduction in risk. This effect began with losses as low as 2% of body weight which was estimated to be 2 kg in this study. The intervention should have clinically meaningful benefit if it can achieve that target.[24]

RESULTS

Study population

From November 4, 2013 through September 31, 2015, a total of 72 participants were assessed for eligibility. A total of 64 participants met the eligibility criteria and were randomly assigned to receive either a wristband plus brief lifestyle modification program (wristband group with 32 participants), or a brief, lifestyle-modification program alone (control group, with 32 participants).

The main reasons that participants did not undergo randomization were not meeting the inclusion criteria (BMI less than or equal to 30 kg/m^2, n = 2, and taking drugs that affect body weight, n = 3).

Of the 64 initial participants, 47 completed the trial. In the wristband group, 1 participant died (from pneumonia) after randomization, and 6 participants were lost to follow-up. In the control group, 8 patients were lost to follow-up, and 2 patients became pregnant during the follow-up period (Fig 1).

The baseline demographic and clinical characteristics were similar for both groups (Table 1). The mean age of the participants was 51.4 years, the mean body weight at baseline was 100.2 kg, the mean BMI was 39.5 kg/m^2,

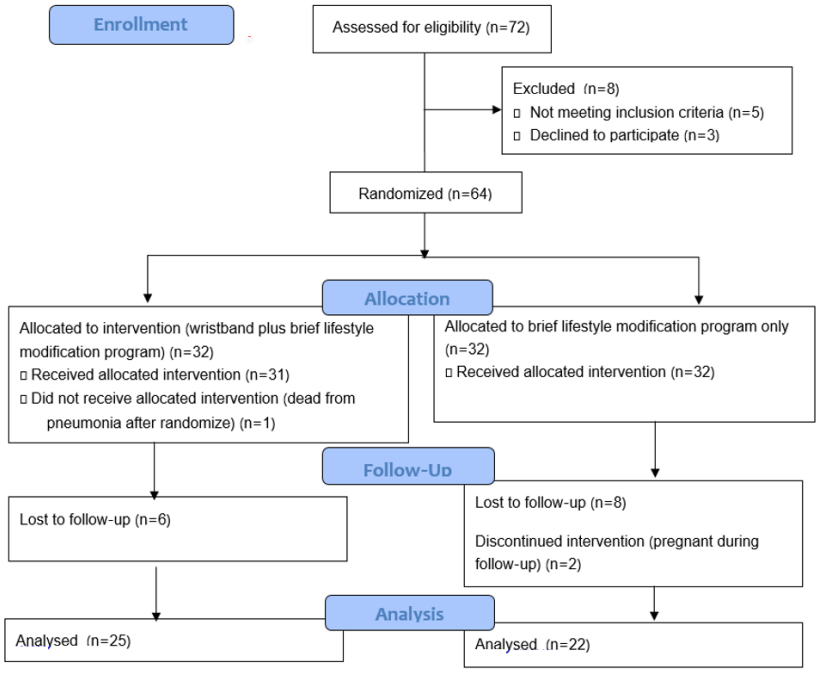


Fig 1. Flow diagram.

TABLE 1. Baseline characteristics of the participants.

Characteristic	Wristband (n = 25)	Control (n = 22)	p-value
Gender n (%)			
Male	5 (20%)	8 (36.4%)	0.211
Female	20 (80%)	14 (63.6%)	
Age, mean (SD), years	51.64 ± 14.8	51.18 ± 12.61	0.91
Education, n (%)			
Less than high school	2 (8%)	1 (4.5%)	0.757
High school	14 (56%)	11 (50%)	
College or above	9 (36%)	10 (45.5%)	
Medical conditions n (%)			
Diabetes mellitus	15 (60%)	10 (45.5%)	0.319
Hypertension	23 (92%)	21 (95.5%)	1
Hypercholesterolemia	21 (84%)	20 (90.9%)	0.67
Coronary artery disease	4 (16%)	3 (13.6%)	1
Sleep disorder	4 (16%)	4 (18.2%)	1
Weight (kg)	101.29 ± 26.8	99.11 ± 19.03	0.753
Height (cm)	156.88 ± 9.78	160.64 ± 9.07	0.181
Body-mass index (kg m^{-2})	40.72 ± 7.44	38.31 ± 6.39	0.243
Waist circumference (cm)	114.76 ± 19.06	115.55 ± 14.37	0.875
Blood pressure (mmHg)			
Systolic	136.32 ± 18.58	134.66 ± 18.87	0.763
Diastolic	80.48 ± 12.38	81.18 ± 11.92	0.844
Fasting glucose (mg/dL)	158.79 ± 68.79	139.27 ± 50.08	0.438
HbA1c	7.4 ± 1.87	7.23 ± 1.97	0.855
Cholesterol (mg/dL)	182.23 ± 49.12	168.5 ± 26.53	0.478
Triglyceride (mg/dL)	187 ± 166.46	100 ± 19.89	0.19
High-density lipoprotein	48.38 ± 9.98	59.86 ± 15.23	0.056
Low-density lipoprotein	96.25 ± 36.61	99.08 ± 25.97	0.83

and the mean waist circumference was 115.1 centimeters. A total of 72.3% of the patients were women, 53.2% had diabetes mellitus, 93.6% had hypertension, 87.2% had hyperlipidemia, 14.9% had coronary artery disease, and 12.7% reported a history of osteoarthritis of the lower extremities.

Outcomes

Weight loss

During the initial 6-month period, participants in the wristband group tended to lose more body weight than those participants who were in the control group. However, this did not reach statistical significance (Fig 2).

By the 6-month follow-up, participants in both groups had lost weight. The mean (±SE) weight change for the participants in the wristband group was -4.5±1.38 kg, whereas those who were in the control group was -2.13±1.09 kg. Nevertheless, the difference between the two groups was not statistically significant (p-value = 0.193).

Twelve months after the commencement of the study, 56% (14/25) of the participants in the wristband group and 54.5% (12/22) of the patients in the control group were followed up. The mean (±SE) weight changes for the wristband group and the control group were -4.25±1.26 and -1.83±1.13 kg (p-value = 0.169), respectively (Table 2). The percentage reduction from the initial body weight and the changes in the participants' BMI are also in Table 2.

Waist circumference

By the 6-month follow-up, participants in both groups had decreased their waist circumference, with the mean (±SE) waist-circumference change among participants in the wristband group and the control group being -2.84±0.99 and -1.3±0.92 cm (p-value = 0.269), respectively.

At the 12-month follow-up, the mean waist-circumference loss for the wristband group was still numerically larger than for the control group, with losses of -2.27±1.18 and -0.57±1.15 cm (p-value = 0.314), respectively.

Categorical weight loss

Fig 3 shows that the percentage of participants who maintained their weight losses at both the 6-month and 12-month follow-ups were greater for the wristband group than for the control group, although the effect was not statistically significant.

Fig 4 shows that participants in the wristband group tended to lose more weight than participants in the control group.

Adverse effect

There were few adverse events. One participant in the wristband group died from pneumonia after randomization, but before commencing the intervention, while 2 participants in the control group became pregnant during the six-month follow-up period.

The study physician deemed that none of the adverse events were related to the intervention.

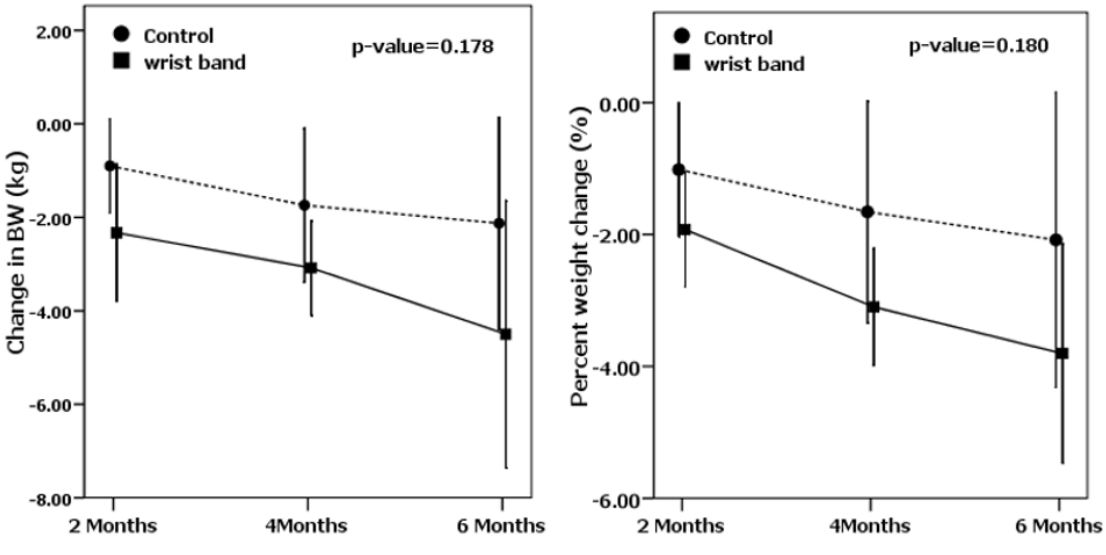


Fig 2. Changes in body weight (kg) and percentage of weight change (%) over 6-month period. p-value corresponds to repeated measure ANOVA.

TABLE 2. Estimated mean weight loss, percentage reduction in body weight, change in Body-Mass Index, and change in waist circumference over a 12-month period. Values presented as mean ± SE. p-value corresponds to the independent t-test.

	Wristband		Control		
	n	Mean ± SE.	n	Mean ± SE.	p-value
Change in BW (kg)					
At month 2	23	-2.33 ± 0.71	22	-0.9 ± 0.48	0.106
At month 4	22	-3.08 ± 0.49	22	-1.74 ± 0.79	0.156
At month 6	25	-4.5 ± 1.38	22	-2.13 ± 1.09	0.193
At month 12	14	-4.25 ± 1.26	12	-1.83 ± 1.13	0.169
Change in BW (%)					
At month 2	23	-1.93 ± 0.42	22	-1.01 ± 0.49	0.160
At month 4	22	-3.1 ± 0.43	22	-1.65 ± 0.81	0.125
At month 6	25	-3.8 ± 0.81	22	-2.08 ± 1.08	0.201
At month 12	14	-3.84 ± 1.04	12	-1.73 ± 1.07	0.171
Change in BMI (kg m^{-2})					
At month 2	23	-0.85 ± 0.22	22	-0.35 ± 0.18	0.089
At month 4	22	-1.23 ± 0.18	22	-0.64 ± 0.29	0.091
At month 6	25	-1.67 ± 0.43	22	-0.74 ± 0.41	0.127
At month 12	14	-1.7 ± 0.51	12	-0.69 ± 0.42	0.147
Change in Waist circumference (cm)					
At month 6	25	-2.84 ± 0.99	20	-1.3 ± 0.92	0.269
At month 12	15	-2.27 ± 1.18	14	-0.57 ± 1.15	0.314

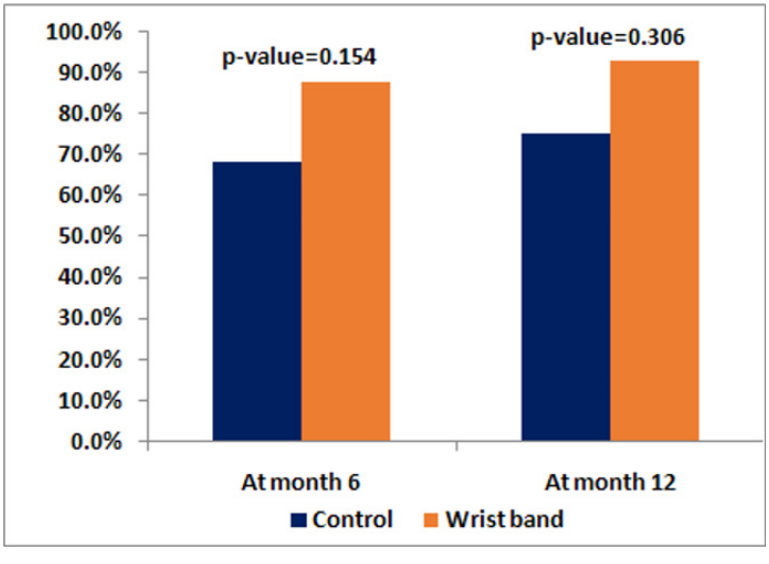


Fig 3. Percentage of participants in each group who maintained their weight losses at the 6- and 12-month follow-ups.

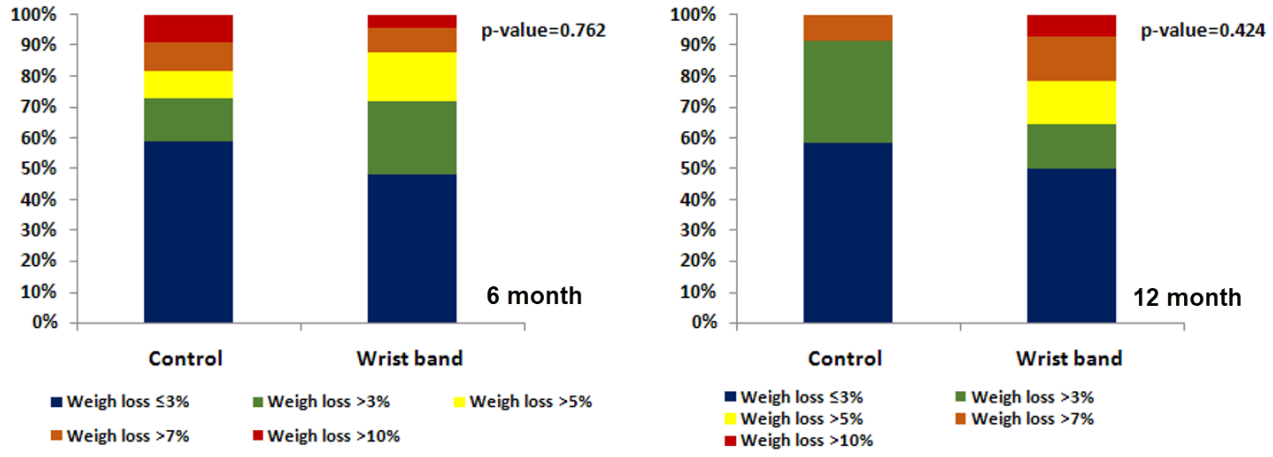


Fig 4. Categorical weight losses at the 6 and 12-month follow-ups.

DISCUSSION

Participants in both groups in this study had lost weight by the 6- and 12-month follow-ups, compared to their baseline measurements. The extent of the body weight loss tended to be greater among participants in the wristband group, although the differences in the weight loss between the two groups did not reach statistical significance. Similar trends were observed for the changes in waist circumference, BMI, and percentage weight-loss.

The reasons for the results not reaching statistical significance were mostly due to a high attrition rate and substantial variations in the degree of weight loss. Researchers aimed to randomize 64 participants in this trial and collect data from at least 50 participants, but due to underestimating the extent of participant loss at follow-up, researchers were only able to collect data from 47 participants.

The difference in point estimate between the 2 groups was 2.37 kg, which researchers had estimated would be > 2 kg, but the confidence interval was large, which shows that there were substantial variations in the degrees of individual weight losses. A larger study may provide more reliable outcomes.

In this study, by the 6-month follow-up, participants in the control group had lost a mean weight of 2.13 kg compared to a mean weight loss of 4.5 kg in the wristband group. The magnitude of those reductions in body weight was comparable with losses reported in several other trials in which the primary care providers also offered brief counseling sessions.[8-11]

By the 12-month follow-up, during weight maintenance phase, participants in both groups maintained their reduced body weight, although they regained 0.25 to 0.3 kg from 6-month. This degree of regained weight has been consistently observed in several comprehensive lifestyle modification program trials.[25,26]

Researchers advised the participants in intervention group to use the wristband as a cue, a trigger that told participants to go into automatic mode and which habit to use to control weight and create habitual health behaviors. The body-weight and waist-circumference reductions in the wristband group were likely to result from the wristbands helping participants by regularly reminding them of their goal to lose weight, the need to strive for healthy habits, and the value of regular self-monitoring.

All participants in this study had chronic medical conditions requiring them to regularly attend the OPD to follow up those conditions. However, the results of this study may be applied to a healthy, but overweight population, in which case they may have greater potential due to the higher exercise capacity and physical activity of a healthy population.[27-29]

There is important limitation to this study. According to the study protocol, both participants and physicians were not blinded to the group assignment, so this may have affected the subsequent, differential cointerventions, or biased the assessments of the outcomes. Researchers tried to reduce this bias by standardizing the protocol and blinding the data collector and the statistician to the group assignments.

To date, there have been no other studies evaluating wristbands or other similar, wearable tools (such as a smartwatch or a wearable fitness tracker) which could be used to compare with this study. However, there is an ongoing trial to evaluate the effectiveness of an

experimental intervention that is based on standard diet recommendations plus a free smartphone application and a wearable device for weight loss.[30]

The use of a smartphone with weight loss applications (but without a wearable device) has been shown to be effective for weight loss.[31-36] Unfortunately, many smartphone applications are sophisticated, which can make their use challenging for some people, and there is an associated cost for the smartphone, which can be a financial barrier to uptake. In contrast, this study has shown that the use of only a simple wristband may help with weight loss.

Although this study lacked the power to detect statistically-significant differences between the control and intervention groups in terms of the differences in weight changes, the trends supported our hypothesis. Therefore, researchers encourage the use of a wristband to help with weight loss, particularly given this tool's zero adverse events and very low cost.

CONCLUSION

The utilization of a wristband as an adjunct to a weight-loss program showed a trend toward a decreased body weight and waist circumference at the 6- and 12-month follow-ups. Because this tool is risk-free, low-cost and potentially beneficial, it is worth using with obese patients.

ACKNOWLEDGMENTS

The study was funded by the Panyananthapikkhu Chonprathan Medical Center, Srinakharinwirot University. Ms. Dollapas Punpanich was involved with the data analyses and interpretation. Also, thanks go to Mrs. Piyawun for help with the data collection and entry.

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Health-Related Quality of Life as Measured by EQ-5D and TFLIC-2 in Liver Cancer Patients

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ABSTRACT

Objective: To assess the relation and estimation function between utility weight (UW) from EQ-5D and T-FLIC 2 score (TFS). To compare quality of life (QOL) between global burden of disease (GBD) and TNM staging.

Methods: All liver cancer patients which presented in selected hospitals in 2009, were asked their QOL by EQ-5D and T-FLIC 2 questionnaires. The relation between UW and TFS was analyzed by using Pearson's correlation and the linear regression. The means of UW and TFS in specific conditions were compared with independent t-test and ANOVA.

Results: There were 53 cases. The majority (73.6 %) were men, mean age was 57.2 years, lived in suburban area (67.9%), worked in agriculture (47.2%) and graduated secondary level or lower (83.0%). These 73.6% of all samples had household monthly income not greater than 5,000 THB and used universal coverage scheme (64.2%). Seventeen cases had not been identified TNM staging because of the condition of patients. However, those cases fulfilled to be diagnosed terminal stage. The multiple comparisons between means of UW and TFS showed no statistical significance e.g. pre-terminal and terminal (0.5 vs 0.4, p=0.3 and 38.8 vs 34.0, p=0.2 respectively), TNM-stage 4 vs TNM-stage 1-3 (0.3 vs 0.5, p=0.3 and 38.9, 30.5, p=0.1), and supportive only vs. other treatment (0.5 vs 0.5, p=0.9, and 32.8 vs 36.2, p=0.5). The correlation between UW and TFS was significantly related (p<0.001) with linear pattern (p<0.001). The equation for estimating UW from TFS and specific Q1-Q22 questions was formulated.

Conclusion: This study revealed the significant correlation between UW and TFS with linear pattern. A major advantage of using regression analysis was predicting UW from TFS for economic evaluation in Thai liver cancer patients. The treatment might not alter QOL significantly. Given cancer stage and treatment choice and their QOL in this study, our findings should not be over-interpreted.

Keywords: Liver cancer; quality of life; EQ-5D; T-FLIC 2 (Siriraj Med J 2018;70: 406-412)

INTRODUCTION

Liver cancer is an abnormal and malignant growth of liver cells. The most common type of liver cancer is called hepatocellular carcinoma (HCC).[1] Liver cancer is a tumor with high lethality. The 5-year relative survival rates were about 6.5% to 8.3%.[2]

From global overall years of life lost (YLL) by cancer, liver cancer ranked the third in year 1990 and stepped up to the second rank in 2013.[3]

In Thailand, liver cancer contributed to the burden of diseases by number 5 in men and number 8 in women.[4] Moreover, liver cancer ranked the second top cancer incidence in Thailand.[5] The incidence of liver cancer in males and females ranged between 6.4 to 87.5 in males and 1.4 to 37.2 per 100,000 in females.[6]

To estimate and validate disability-adjusted life years lost from cancer, the disease model categorized liver cancer patients into two stages; terminal and

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Received 20/11/2017 Revised 21/5/2018 Accepted 20/6/2018
doi:10.14456/smj.2018.64

pre-terminal stages.[7] World Health Organization-Global Burden of Disease (GBD) was used in this two phases model in calculation. Terminal stage was defined by the patient-survival time less than 1 month.[8] The terminal and Pre-terminal stage definition were totally different from TNM staging[9] which has been widely used by clinicians. This difference has led to a renewed interest in comparison for quality of life (QOL) in TNM staging and GBD definition. Additionally, the QOL measurement tools could be one of these disease specific or generic tools. The generic tools e.g. EQ-5D, SF-36 etcetera were most likely to report score and then converted to utility weight (UW) by its algorithm. The EQ-5D, the tool which has been used for measuring patient preference-based quality of life, consists of 5 questions about mobility, self-care, main activity, social relationships, pain, and mood.[10] The EQ-5D for adults has 2 versions; 5L and 3L. Both versions are valid and it is now generally accepted that the generic health status questionnaires were easy to use and able to compare other diseases in case of economic evaluation, and were also valid. Whilst, quality of life in cancer patients can be measured by various tools, none of that can be converted to UW directly. The Functional Living Index Cancer (FLIC) was one of the famous questionnaires which was was first reported for using in clinical trials in 1984[11], The FLIC is a 22-item questionnaire which measures QOL in multidimensions of global, role, social, emotional, pain, and nausea scales. The latest FLIC version 2 was translated and validated into Thai language in 2005.[12] The TFLIC 2 was a reliable and valid measurement of the quality of life in clinical trials, studies of outcome and research in oncology.[12]

In spite of recent studies[13] which have explored the similarities between disease specific questionnaires and generic health status questionnaire, relatively little is known about the relationship between T-FLIC 2 and generic health status questionnaire. To avoid redundant measurement and bridging the clinical trial QOL and UW for cost-effectiveness analysis, we needed a tool to convert TFLIC-2 to UW.

MATERIALS AND METHODS

A multicenter, cross-sectional observational survey was conducted in three provinces of Thailand during 2009-2010. This study was ethical approved by The Ethical Review Committee for Research in Human Subjects. Ministry of Public Health, Thailand (Document No 98/2009).

Patients

All liver cancer patients who presented at the hospitals in the selected period [June 2009 to December 2009] and fulfilled the inclusion criteria below were enrolled to this study.

1. Samples must have been diagnosed liver cancer as a primary source.
2. Samples must be able to read and write, co-operative and willing to complete questionnaire or interviewing.
3. Samples must agree to participate in our study.

Exclusion:

1. Samples have other underlying diseases not related to liver cancer.
2. Samples who have other congenital anomaly or co-morbidity.
3. Samples who have acute condition of sickness or injury.
4. Samples who were negative to participate in our study.

Sample size was calculated based on correlation coefficient between UW and TFS with α=0.05, β=0.2, expected correlation coefficient (r)=0.5. The equation[14] was shown below.

$N = [(Z_{\alpha}+Z_{\beta})/C]^2 + 3$ where $C = 0.5 * \ln[(1+r)/(1-r)]$

Thus the calculated sample size was 29. Then, we ensured the sample number by multiplying it by 2, so the expected number was around 60 cases. The recruitment proportion was shown in Fig 1.

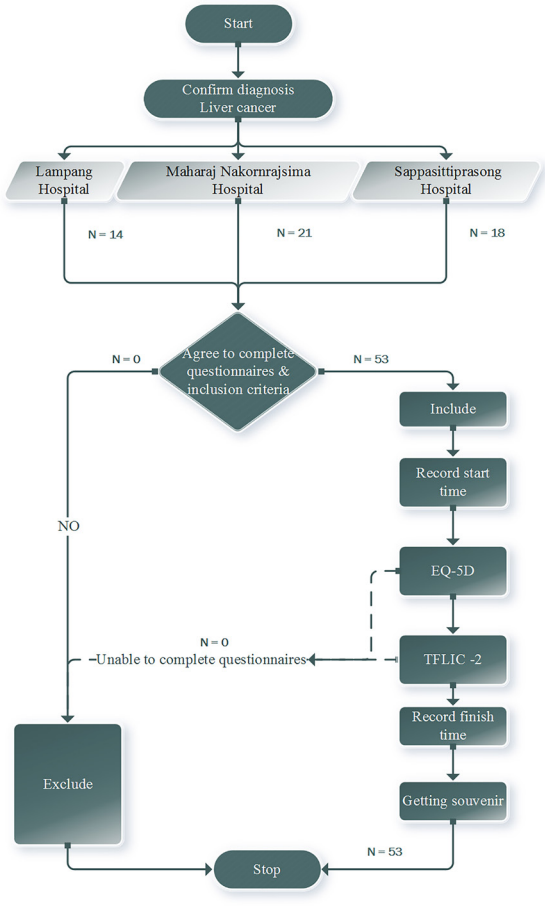


Fig 1. Sample recruitment process..

Definition

The Global Burden of Disease (GBD) group divided the liver cancer patients into two groups: pre-terminal and terminal which had definition[7] as shown below.

Pre-terminal stage of patient who lives with liver cancer not described as terminal stage.

Terminal stage means the final last month period of living of the patients.

Tools

The tools for measuring QOL were EQ-5D and T-FLIC 2 score (TFS). The EQ-5D was created by EURO-QOL® to measure QOL in all cases in term of utility weight (UW). The UW reflected the QOL by continuous score which ranged from 0 to 1. The meaning of UW 1.0 was perfect health and 0 was the worst imaginary health which roughly equaled to dead. Sometimes the UW was less than 0 which means dead would be better than that condition. The EQ-5D score was transformed to UW with specific Thai Algorithm.[15]

In addition, this study focused on the relationship of the quality of life which was measured by T-FLIC 2 and EQ-5D. T-FLIC -2 was a disease specific QOL measurement tool which was validated to use in Thai cancer patients.[12] The interpretation for both UW and TFS were likely to be the same, so higher is better.

The data collection protocol was established and standardized with resulted in a standard of practice (SOP) manual. All data collection staffs in all sites were trained for data collecting and interviewing process. Data verification and validation process were done, following the SOP manual.

Statistical analysis

Sample demographics were reported as number and percent. The relation between UW and T-FLIC 2 Score (TFS) was analyzed by using Pearson's correlation and the linear regression was performed to derive the UW from TFS and TFLIC-2 specific questions. The mean difference between UW and TFS in each groups of patients were compared with independent sample t test and ANOVA with LSD. All calculations were done by SPSS version 24.0.

RESULTS

Table 1 presented the general characteristics of 53 samples. The percentage of male was 73.6. The age was varied between 17 and 89 and the average age was 57.2 years old. The cases were collected mainly from Northeastern region based on their epidemiologic pattern. The 67.9 percent of samples lived in suburban area, and mainly worked in agriculture (47.2 percent). The main education level was secondary level or lower. The percentages of health insurance scheme sorted from maximum to minimum were universal coverage, civil servant, and social security scheme respectively. The 73.6% of all samples had household monthly income not greater than 5,000 THB. Specifically, the characteristics of our samples mainly pointed to low socioeconomic status based on income and education level.

As shown in Table 2, seventeen cases from a total of 53 cases had not been identified TNM staging because

TABLE 1. Characteristic of subjects.

Demographic characteristics	n (%)
Gender	
Male	39(73.6%)
Female	14(26.4%)
Age	
Mean±SD	57.2±15.8
Range	17-89
Data collection sites	
Lampang Hospital	14(26.4%)
Sappasittiprasong Hospital	21(39.6%)
Maharaj Nakornrajsima Hospital	18(34.0%)
Health insurance scheme	
Universal coverage scheme	34(64.2%)
Social security scheme	4(7.5%)
Civil servant medical benefit scheme	15(28.3%)
Address	
Urban	17(32.1%)
Sub-urban	36(67.9%)
Education level	
Uneducated	3(5.7%)
Secondary level or lower	44(83.0%)
Upper than secondary level	6(11.3%)
Occupation	
Unemployed	7(13.2%)
Agriculture	25(47.2%)
Non-agriculture	21(39.6%)
Monthly income	
0-5,000 THB	39(73.6%)
5,001-15,000 THB	8(15.1%)
15,001+ THB	6(11.3%)

of the condition of patients. In addition, those seventeen cases were also diagnosed in terminal stage. Table 2 presented, that the terminal stage didn't exactly equal to stage 4 of TNM staging because there was one case of stage 1-3 which was classified to terminal stage. However, all liver cancer cases of TNM stage 4 were categorized to terminal stage.

Table 3 showed the lower values of QOL which were measured by EQ-5D and T-FLIC 2 in case of terminal stage, but there was no statistical significant. The UW and TFS in pre-terminal and terminal were 0.54, 38.78 and 0.41, 34.03 respectively.

A positive correlation between UW and TFS was obtained with correlation 0.67 and $p<0.001$.

To estimate UW from TFS and specific Q1-Q22 T-FLIC questions, we performed linear regression. Table 4 presented the significant ($p<0.001$) result of linear regression analysis between UW and TFS with R^2 0.428 and the equation for estimating UW was shown in Equation 1.

As shown in Fig 2, even the R^2 was not high, but there was no skewness of expected probability and observed probability in Probability-Probability Plot (P-P Plot). In order to improve the accuracy of UW prediction from

TABLE 2. Cross table of cases who have been diagnosed in TNM staging and GBD definition.

Disease stage		Unidentified	Stage 1-3	Stage 4	Total N (Col %)
Pre-terminal vs. terminal stage	Pre-terminal N (row %)	0	19(100%)	0	19(35.8%)
	Terminal N (row %)	17(50.0%)	1(1.9%)	16(48.1%)	34(64.2%)
Total N (row %)	17(32.1%)	20(37.7%)	16(30.2%)	53(100%)	

Abbreviations: TNM = , GBD = burden of disease

TABLE 3. UW and TFS in pre-terminal vs. terminal stage of liver cancer patient.

The QOL score	Pre-terminal stage (Mean±SD)	Terminal stage (Mean±SD)	p-value
UW	0.54±0.40	0.41±0.40	0.293
TFS	38.78±12.22	34.03±12.56	0.203

Abbreviations: UW = utility weight , TFS = T-FLIC 2 score

TABLE 4. Linear regression analysis between UW and TFS.

	B	Std. Error	Beta	t	Sig.
(Constant)	-0.3	0.136		-2.211	0.032
			0.655		
TFS	0.021	0.004		5.935	0

Abbreviation: TFS = T-FLIC 2 score

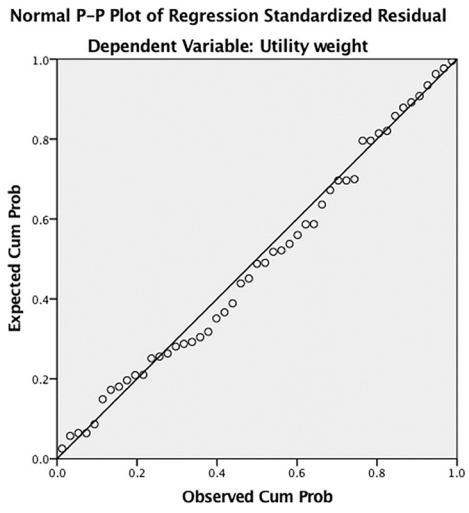


Fig 2. P-P Plot of observed UW and expected UW from TFS to UW conversion equation.

T-FLIC 2, we developed the linear equation based on each of the 22 items as seen on Table 5 with R^2 0.715.

Table 6 presented that the treatment receiving group had higher UW, but was not statistically significant. TFS seemed to have similar effect on only chemotherapy receiving group which had higher TFS than not receiving, although the difference showed no significance.

The samples who were diagnosed in terminal stage in the Table 7, had lower QOL in terms of UW and TFS, but this was not statistically significant. Similarly, the TNM stage 4 patients had the lowest QOL when compared to Stage 1-3 and unidentified group. Incidentally, the unidentified group in TNM staging were similarly equal UW and TFS to TNM stage1-3.

TABLE 5. Linear regression analysis between UW and individual Q1-Q22 TFLIC questions.

	B	Std. Error	Beta	t	Sig.
(Constant)	-0.08	0.25		-0.33	0.75
Q1: 1. Feel depressed?	0.04	0.11	0.076	0.37	0.71
Q2: 2. Cope well with stress?	0.00	0.12	-0.003	-0.01	0.99
Q3: 3. Think about illness?	0.18	0.16	0.241	1.16	0.26
Q4: 4. Maintain leisure activities?	0.11	0.08	0.273	1.41	0.17
Q5: 5. Nausea affecting daily functioning?	0.10	0.08	0.253	1.34	0.19
Q6: 6. Feel well?	-0.06	0.12	-0.127	-0.52	0.60
Q7: 7. Well enough for meals or repairs?	0.00	0.09	0.004	0.02	0.99
Q8: 8. Hardship on the closest?	-0.05	0.11	-0.114	-0.45	0.65
Q9: 9. Discouraged about life?	-0.08	0.11	-0.134	-0.71	0.49
Q10: 10. Satisfied with work?	-0.02	0.11	-0.042	-0.16	0.87
Q11: 11. Feel uncomfortable?	0.04	0.09	0.088	0.39	0.70
Q12: 12. Disruptive to the closest?	-0.03	0.13	-0.073	-0.25	0.81
Q13: 13. Pain/discomfort interfering activities?	-0.02	0.08	-0.052	-0.25	0.80
Q14: 14. Hardship on yourself?	0.20	0.11	0.505	1.79	0.09
Q15: 15. Able to complete housework?	0.08	0.06	0.211	1.21	0.24
Q16: 16. Willing to spend time with family?	-0.03	0.11	-0.056	-0.26	0.80
Q17: 17. How much nausea?	-0.10	0.08	-0.166	-1.21	0.24
Q18: 18. Frightened of future?	0.16	0.10	0.300	1.72	0.10
Q19: 19. Willing to spend time with friends?	-0.01	0.09	-0.031	-0.15	0.88
Q20: 20. Pain/discomfort related to cancer?	0.04	0.11	0.084	0.37	0.72
Q21: 21. Confident of treatment?	-0.08	0.07	-0.176	-1.14	0.27
Q22: 22. Appear well?	-0.09	0.13	-0.169	-0.66	0.52

TABLE 6. The QOL in Liver cancer patient categorized by their treatment choice.

Choices of treatment	n	UW			TFS		
		Yes	No	p-value	Yes	No	p-value
		Mean±SD	Mean±SD	(2-tails)	Mean±SD	Mean±SD	(2-tails)
Surgery	34	0.55±0.46	0.45±0.38	0.43	34.17±10.47	36.71±13.65	0.47
Chemotherapy	15	0.61±0.38	0.43±0.41	0.16	37.43±10.62	35.11±13.29	0.53
Surgery and chemotherapy	8	0.35±0.44	0.47±0.38	0.33	33.83±11.52	36.63±14.2	0.49
Supportive treatment only	27	0.50±0.48	0.48±0.40	0.93	32.83±9.07	36.19±12.96	0.45

TABLE 7. The mean UW and TFS in liver cancer patients defined stage by GBD definition and TNM staging.

	GBD Staging			TNM-Staging			
	Pre-terminal	Terminal	p-value†	Stage 1-3	Stage 4	Unidentified	p-value‡
	Mean± SD	Mean±SD		Mean±SD	Mean±SD	Mean±SD	
UW	0.54±0.41	0.41±0.41	0.29	0.53±0.40	0.32±0.40	0.51±0.42	0.29
TFS	38.78±12.51	34.03±12.56	0.20	38.89±11.88	30.53±12.54	37.07±12.42	0.14

NOTE: † p-value in 2-tailed test in case of equal variances not assumed in significant level .05
‡p-value in ANOVA test between groups in significant level .05

DISCUSSION

To interpret quality of life, the socioeconomic status (SES)[16], should also be considered with other aspects of patients. The lower SES in these samples might lead to the lower set of UW and TFS. As in previous studies[17], the results of this analysis confirmed that liver cancer was more predomint in males more than females, with the ratio about 3:1 and this ratio was in the same range from previous study (between 6.4 to 1.4 and 87.5 to 37.2 per 100,000 in females).[6] Thus, the sex difference of epidemic data urged researchers to investigate the role of sex hormone in liver tumors.[18] For instance, there was a study which suggested increasing prolactin level in men might help to reduce incidence of HCC.[19] Therefore, the level of prolactin level comparison between north-eastern, Thailand and other regions might be studied further.

There were many tools which could be used for measuring QOL in cancer patient apart from EQ-5D and T-FLIC 2 e.g. The European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30), Functional Assessment of Cancer Therapy-General (FACT-G), Functional Assessment of Cancer Therapy-Hepatobiliary (FACT-Hep), Functional Hepatobiliary Symptom Index-8 (FHSI-8) Questionnaire, Short Form-36 (SF-36) survey, WHOQOL-100 and WHOQOL-BREF, Spitzer QoL Index (SQLI), and SF-12.[20] etcetera. However, there were no tools to estimate UW from their specific disease questionnaires. By our synthesized equation, a researcher can estimate the UW from T-FLIC 2 score, although the value should not exceed -0.59 to 1.00.[21]

In contrast, the treatment showed no significant difference to alter QOL, which it might seem counter intuitive that QOL after operation gradually increased[22], so the effect of treatment and QOL varied from time to time. The other study which compared between EQ-5D and T-FLIC also mentioned EQ-5D changed significantly over time.[23]

Our UW findings were consistent with previous results showing the range of mean index-based scores

from 0.33 (SD 0.4) to 0.93 (SD 0.12).[24] However, there was restriction to compare our TFS with other study due to no prior data, but we found our TFS results were lower than stage IV lung cancer patients (46-55).[25] The liver cancer patients might present the lower TFS from the nature of this disease.

Accordingly, future studies will have to continue to explore for a new version of EQ-5D-5L.[26] To clarify the relationship between liver cancer stage and quality of life, more samples should be considered. Moreover, this study stated the low SES has been diagnosed in liver cancer and most likely to be in terminal stage, so the recommendation of liver cancer prevention and early detection in this group of people should be supported by government.

To conclude, this study synthesized the equation to converse TFS to UW and confirmed the linear relation between these scores. The TNM stage 4 was likely to be diagnosed terminal stage in GBD definition. Given cancer stage and treatment choice and their QOL in this study, our findings should not be over-interpreted.

ACKNOWLEDGMENTS

This study has been financially supported by Burden of Diseases Project, International Health Policy Program (IHPP). The researcher would like to fully thank to Dr. Jittrakul Lertsakulpanitch for good advice, Dr. Sirinart Tongsiri for EQ-5D algorithm and also Jiamjit Wongphan for a perfect physical and mental support. This study had a full contribution in data collection process from Lampang, Nakornratchasima and Sappasitiprasong Hospital.

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Prevalence of and Factors Associated with Undernutrition in a Geriatric Outpatient Setting: Results from a Multidimensional Nutritional Assessment

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ABSTRACT

Objective: To determine the prevalence of and factors associated with undernutrition in older patients in a geriatric outpatient setting.

Methods: A cross-sectional study in people aged 65 years or older was conducted at a geriatric outpatient clinic in Bangkok, Thailand. Nutritional status was assessed by Mini Nutritional Assessment (MNA) score. Demographic data, activities of daily living, food frequency questionnaire (FFQ), anthropometric measurements, complete blood count, and serum albumin were recorded in every subject. Body composition analysis was measured by bioelectrical analysis (BIA) at the same visit.

Results: Three hundred and twenty-four adults (63% women) with a mean age of 77.5 years (SD 6.8 years) were enrolled. Prevalence of undernutrition, at-risk of undernutrition, and well-nourished were 8.3%, 35.5%, and 56.2%, respectively. In univariate logistic regression analysis, factors associated with increased risk of undernutrition (defined by MNA <24) were dependency in carrying out instrumental activities of daily living (IADL) or basic activities of daily living (BADL), dementia, depression, low education, cerebrovascular disease, polypharmacy, female, multiple co-morbidities, and increasing age. In multivariate logistic regression analysis adjusted for age and gender, depression, cerebrovascular disease, and dementia were significantly associated with risk of undernutrition.

Conclusion: Although older patients in our study had higher socioeconomic status than general population, the prevalence of undernutrition in our geriatric clinic was high. Factors significantly associated with undernutrition in our study were depression, cerebrovascular disease, and dementia. Early recognition and intervention are essential for effective undernutrition prevention.

Keywords: Mini nutritional assessment; nutritional status; geriatric clinic; geriatric outpatients; undernutrition (Siriraj Med J 2018;70: 413-418)

INTRODUCTION

The increasing number of older people is one of society's most important and pressing health policy planning issues. The prevalence of undernutrition is increasing more among the elderly than in any other age group. Undernutrition is associated with mortality, morbidity, infection, hospitalization, and lower quality of life.[1,2] The prevalence of undernutrition in previous studies ranged between 5.3% and 53.1%,varying according to clinical setting and diagnostic criteria.[3-6] Prevalence of undernutrition may be higher in the hospital or institutional setting than in the community-dwelling setting.[3] Few studies have explored undernutrition in a geriatric outpatient setting[2,7,8], which is a specialized setting for

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Received 8 February 2017 Revised 26 September 2017 Accepted 3 October 2017
doi:10.14456/smj.2018.65

elderly patients who have multiple comorbid diseases or geriatric syndromes, such as cognitive impairment and mobility disorders. The prevalence of undernutrition in this patient population is likely to be high and factors associated with undernutrition should be identified, so that an undernutrition prevention strategy can be developed. Accordingly, the objective of this study was to determine the prevalence of and factors associated with undernutrition in older patients in a geriatric outpatient setting.

MATERIALS AND METHODS

Study design and population

This cross-sectional study recruited patients who visited the Geriatric Clinic, Faculty of Medicine Siriraj Hospital, Mahidol University (Bangkok, Thailand) during January 2014 to October 2015 study period. The study followed STROBE statement for observational study.[9] Inclusion criteria were patients aged 65 years or older who scored higher than 23 on the Thai Mental State Examination (TMSE) cognition test. Subject candidates with a TMSE score < 23 who had well-informed caregivers that were able to give reliable information were also included. Patients with a TMSE score < 23 who were unable to communicate or who were without a caregiver able to provide reliable information were excluded. Written informed consent was obtained from all patients or legal representatives. The protocol for this study was approved by the Siriraj Institutional Review Board (Si 016/2014).

Data collection

Patient demographic data, including age, education, and socioeconomic status were collected by face-to-face interview and/or medical record review. Comorbid diseases were scored by Charlson Comorbidity Index.[10] All drugs taken by patients in this study were reviewed for potentially inappropriate use using the American Geriatrics Society updated Beers Criteria.[11]

All patients underwent activities of daily living assessment using Barthel Index[12] and Lawton Index[13], respectively. Thai Mental State Examination (TMSE)[14] and Thai Geriatric Depression Scale (TGDS)[15] were used to evaluated cognitive function and mood respectively. A food frequency questionnaire which was validated by both geriatricians and nutritionists was used for the estimation food intake. All patients underwent physical examination and anthropometric measurement. Complete blood count and serum albumin level were obtained to determine nutritional status. Body composition analysis by bioelectrical analysis method using Inbody® 720 (InBody Co., Ltd, Cerritos, CA, USA) was performed in all patients not having pacemaker or metallic surgical implantation.

Patient nutritional status was assessed by Mini Nutritional Assessment (MNA).[16] MNA is a 30-point validated questionnaire which consists of questions that include anthropometric, general, dietary, and subjective assessment. Scores of <17, 17-23.5, and ≥24 defined undernutrition, at-risk of undernutrition, and well-nourished status, respectively.

Data analysis

Patient clinical and demographic data were reported as percentage, mean and standard deviation (SD), or median and inter quartile range (IQR). Comparisons among 3 nutritional status groups were performed using analysis of variance (ANOVA) or Kruskall-Wallis test depend on distribution of variables. Chi-square test was used for categorical variables.

Univariate and multivariate logistic regression analyses adjusted for age and gender were performed to assess and identify factors associated with MNA <24. These results were presented as odds ratio (OR) and 95% confidence interval (95% CI). Data analysis was performed using SPSS Statistics version 18 for Windows (SPSS, Inc., Chicago, IL, USA).

RESULTS

Three hundred and twenty-four patients were enrolled in this study. Mean (SD) age was 77.5 (6.8) years and 63.3% were female. According to MNA classification, 8.3%were undernourished, 35.5% were at-risk for being undernourished, and 56.2% were well-nourished. Patient demographic and clinical data by nourishment classification are shown in Table 1. There were statistically significant differences among groups for age, gender, ambulation status, educational level, history of physical exercise, Charlson Comorbidity Index, BADL score, IADL score, TMSE score, number of medications used, diagnosis of each disease in dementia, depression, Parkinson disease, and stroke. We also found body mass index (BMI), fat mass, skeletal muscle mass, hemoglobin, total lymphocyte count, and serum albumin level to be significantly different among the 3 nutritional status groups.

Data from the 60-item semi-quantitative food frequency questionnaire did not demonstrate significant differences in food consumption among the 3 groups.

In univariate logistic regression analysis, factors associated with increasing risk of undernutrition (defined as MNA <24) included dependency in carrying out IADLs or BADLs, dementia, depression, education level of less than 4 years, cerebrovascular disease, drug usage > 5 items,

TABLE 1. Demographic and clinical characteristics of the study population (n= 324), classified by nutritional status.

Characteristics	Undernutrition (N=27)	At risk of undernutrition (N=115)	Well-nourished (N=182)	*p*-value
Age, (years)*	81.2 (7.3)	78.9 (6.5)	76.6 (6.6)	<0.001
Female, (%)	70.4	71.3	57.1	0.035
Walking independently, (%)	33.3	61.7	80.2	<0.001
Education < 4 years, (%)	55.5	63.5	42.8	0.03
Current alcohol use, (%)	0.0	0.9	6.0	0.119
Current exercise, (%)	14.8	33.0	41.8	0.016
Income (baht),†	12,000 (10,000.0-22,000)	17,500 (10,000.0-36,500.0)	20,000 (10,000.0-32,500.0)	0.749
Charlson Comorbidity Index,†	6.0 (5.0-7.0)	5.0 (4.0-6.0)	4.0 (3.0-5.0)	<0.001
Dementia, (%)	88.9	69.6	41.2	<0.001
Depression, (%)	85.0	53.9	21.0	<0.001
Parkinson disease, (%)	37.0	40.9	16.5	<0.001
Cerebrovascular disease, (%)	33.3	20.0	11.5	0.007
Hypertension, (%)	59.3	68.7	69.2	0.578
Diabetes, (%)	37.0	36.5	32.4	0.731
Malignancy, (%)	7.4	2.6	5.5	0.395
Barthel Index,†	80.0 (45.0-100.0)	100.0 (90.0-100.0)	100.0 (100.0-100.0)	<0.001
Lawton Index,†	8.0 (8.0-17.0)	15.0 (9.0-23.0)	23.0 (6.0-24.0)	<0.001
TMSE,†	20.0(16.0-24.0)	23.0 (19.0-26.0)	26.0 (23.3-28.0)	<0.001
Number of medications,†	7.0 (4.0-9.0)	7.0 (5.0-10.0)	6.0 (4.0-9.0)	0.022
Anticholinergic drug use, (%)	14.8	16.7	19.9	0.695
Metformin use (111 cases), (%)	30.0	42.9	49.2	0.497
Body mass index, (kg/m^2)*	19.2 (3.0)	22.7 (4.1)	25.9 (4.2)	<0.001
Fat mass, (kg)†‡	11.0 (8.1-17.3)	17.9 (11.5-23.4)	20.6 (15.9-27.0)	<0.001
Skeletal muscle mass, (kg)†‡	16.6 (12.3-20.0)	17.4 (15.9-20.6)	20.2 (17.5-25.1)	<0.001
Hemoglobin, (g/dl)*	11.3 (1.4)	12.0 (1.6)	12.8 (1.4)	<0.001
Total lymphocyte count, (cells/mm^3)†	1,630.2 (1,265.5-2,118.8)	1,725.2 (1,347.2-2,259.8)	2,010.6 (1,661.7-2,487.1)	0.002
Serum albumin, (gm/dl)†	3.7 (0.6)	4.0 (0.3)	4.1 (0.3)	<0.001

*Mean (SD); † Median (IQR); ‡231 cases

Abbreviation: TMSE = Thai Mental State Examination

female gender, multiple co-morbidity, and increasing age. Patients who reported that they currently consume alcohol and patients having high total lymphocyte count, hemoglobin, or serum albumin level were at decreased risk of being malnourished.

In age- and gender-adjusted multivariate logistic regression analysis, depression, cerebrovascular disease, and dementia remained associated with an increased risk of undernutrition, with OR's (95% CI) of 2.9 (1.6-5.2), 3.2 (1.6-6.6), and 2.3 (1.3-4.2), respectively (Table 3).

TABLE 2. Factors significantly associated factors with MNA score <24, according to univariate logistic regression analysis.

Factors	Odds ratio (95% CI)
IADL dependence	5.3 (3.1-9.2) †
BADL dependence	3.6 (2.2-6.0) †
Dementia	3.9 (2.4-6.3) †
Depression	3.4 (2.0-5.7) †
Education level (<4 years)	2.2 (1.4-3.4)*
Cerebrovascular disease	2.2(1.2-4.1)*
Medication (>5 items)	1.9 (1.1-3.1)*
Female	1.8 (1.2-2.9)*
Charlson Comorbidity Index (each score)	1.4(1.2-1.7) †
Age, (years)	1.1 (1.0-1.1) †
Total lymphocyte count, (per 100 cell/mm^3)	1.0 (0.9-1.0)*
Hemoglobin, (g/dL)	0.7 (0.6-0.8) †
Serum albumin, (gm/dL)	0.2 (0.1-0.4) †
Current alcohol use	0.1 (0.0-0.9)*

*p<0.05, †p<0.001

TABLE 3. Factors significantly associated with MNA score <24, according to multivariate logistic regression analysis (adjusted for age and gender).

Factors	Odds ratio (95% CI)
Depression	2.9 (1.6-5.2)†
Cerebrovascular disease	3.2 (1.6-6.6)*
Dementia	2.3 (1.3-4.2)*

*p<0.05, †p<0.001

DISCUSSION

Based on MNA, the prevalence of malnourished and at-risk of undernourished patients was 8.3% and 35.5%, respectively. Factors associated with undernutrition in multivariate analysis were depression, cerebrovascular disease, and dementia.

Compared to results from several studies with similar undernutrition diagnostic criteria and population settings[2,7,8], these prevalence figures are slightly lower than previous studies in geriatric outpatient clinics in Turkey and the Netherlands which reported prevalence of 13% and 17%, respectively. These differences may result

from differences in the study population, including age, comorbidity, and function. The baseline characteristics of subjects in the present study had more advanced age, more severe comorbidity (defined by higher score of Charlson Comorbidity Index), and more functional dependency.

Among all associated factors, we found that depression had the highest association with MNA score of less than 24 (OR 2.9; 95% CI 1.6-5.2), whereas, other studies demonstrated various degrees of association. Anorexia is one of the diagnostic criteria for depression and social isolation is a common feature in patients who are depressed. These clinical manifestations are likely to affect nutritional status. Furthermore, depressed patients who take some specific antidepressants (eg. flueoxetine) are at potential risk of undernutrition.[17,18]

Cerebrovascular disease (CVD) was identified as one of the significant determinants of undernutrition in this study (OR 3.2; 95% CI 1.6-6.6). This result was consistent with findings from a study by Saka.[2] Mobility problem is a common symptom in stroke patients that limits their access to food and their food preparation capacity. In addition, dysphagia and aspiration in stroke patients directly results in undernutrition. A systemic review that included 8 studies showed that the odds of being malnourished were higher among subjects with dysphagia, as compared to subjects without dysphagia (OR 2.425; 95% CI 1.264-4.649, $p<0.008$).[19] Also, stroke patients may be depressed, have altered level of consciousness, and be cognitively impaired, which could result in poor oral intake leading to undernutrition.[20,21]

Dementia was also identified as an essential determinant for undernutrition (OR 2.3; 95% CI 1.3-4.2). This association was shown in previous studies from Turkey. The pathophysiology of weight loss in dementia patients is a multifactorial process.[22-24] Some previous studies reported the effect of degenerative processes in particular brain areas, especially in the olfactory system and the brain area involved in appetite and eating habit.[23] Other studies found an increase in several inflammatory cytokines, such as tumor necrotic factor alpha (TNF-α) and interleukin -6 (IL-6), in the plasma of Alzheimer's disease and vascular dementia patients and this pro-inflammatory state in these patients may promote anorexia and weight loss.[24-25] Moreover, dementia patients with reduced self-care abilities, particularly ones without caregivers, would have limited access to food, thus leading to even poorer nutritional status.

Undernutrition is associated with several adverse clinical outcomes. However, this process is reversible. Early detection of undernutrition with appropriate screening tools is the key to prevention of undernutrition-related consequences, especially in elderly patients with depression, cerebrovascular disease, and/or dementia. The European Society for Clinical Nutrition and Metabolism (ESPEN) recommends screening for undernutrition in every person with dementia at the time dementia is diagnosed.[24] However, at the present time, no consensus has been reached regarding how often undernutrition screening should be performed. Although, previous studies have shown association between undernutrition and depression and stroke,[7,20] there are currently no recommendations regarding screening and assessment of undernutrition in patients with these conditions.

The strengths of this study include all 4 dimensions of nutritional evaluation, including food intake estimation, anthropometric measurement, laboratory data analysis, and body composition analysis. In clinical practice, we evaluated patient nutritional status using a combination of diagnostic tools. It can be logically explained that the more methods used to evaluate and diagnose, the more accurate the diagnosis. Moreover, all diagnostic tools used in this study were validated, well-recognized, widely-used, and easily and reliably reproducible.

This study had some mentionable limitations. Our geriatric clinic is a special clinic for complicated cases that is located in a highly populated urban metropolis (Bangkok, Thailand) and at the largest university-based tertiary referral center in Thailand. As such, the findings of this study may not be generalizable to all settings. In addition, subjects in this study had a generally higher socioeconomic status than the normal elderly population living in the same area. It is, therefore, possible that the prevalence of undernutrition maybe underreported. Further studies with larger study populations in general geriatric outpatient settings should be underestimated to confirm the results of this study before nutritional screenings' policy implementation.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Mrs. Angkana Jongsawadipatana and the staff of the Siriraj Hospital Geriatric Clinic for their assistance during this study.

Source of support: This study was supported by a grant from the Siriraj Research Development Fund (Managed by Routine to Research: R2R).

Conflict-of-Interest Notification Page: The authors hereby declare no personal or professional conflicts of interest regarding any aspect of this study.

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The Teratogenic Effects of Glyphosate Based Herbicide (GBH) on the Development of Chick Embryos

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ABSTRACT

Objective: This study was conducted to investigate the teratogenic effects of GBH by using chick embryo as an animal model.

Methods: The equal volume of 0.1 ml of 0.01%, 0.05%, 0.3%, and 0.5% w/v glyphosate solution were injected into yolk sacs of fertilized White Leghorn eggs at 21 h of incubation and repeated at the volume of 0.05 ml on the 3rd day of incubation. The embryos were observed for abnormalities on day 3, 6 and 10 of incubation.

Results: The results showed that the mortality percentages increased as the concentration of glyphosate increased. Day 3 chick embryos showed retardation of development and several abnormalities, for instance, the irregular shape of the brain vesicles with an opening of anterior neuropore, small eye primordia with the optic cup and lens vesicle retardation, looser of the heart looping with dilated lumen, lesser number of branchial arches, absent of limb bud or tail fold. Day 6 chick embryos showed severe retardation of several organs. Microphthalmia, anophthalmia, ectopia cordis and ectopic viscerae were observed in day 6 chick embryos. On day 10, most embryos died earlier and living embryos showed normal external features but delayed ossifications which were significantly different from the control *(P<0.05)*.

Conclusion: Glyphosate was toxic to chick embryos which directly caused mortality and was also a powerful teratogen which caused growth retardation and malformations on day 3 and day 6 and skeletal alteration on day. The effects were predicted to occur in other embryos including humans. Pregnant women should avoid contamination with GBH especially in the first trimester of pregnancy.

Keywords: Glyphosate; ROUNDUP; teratogen; chick embryo (Siriraj Med J 2018;70: 419-428)

INTRODUCTION

The broad spectrum herbicide glyphosate is widely used in agriculture worldwide particularly in association with genetically modified organisms (GMO), but has possible adverse effects on the environment and on human health. Reports of neural defects and craniofacial malformations from regions where glyphosate based herbicides (GBH) are used led us to undertake an embryological approach to explore the effects of low doses of glyphosate on development. Glyphosate has contaminated land, water, air and our food chain since its use with the genetically modified (GM) glyphosate tolerant crops. The generation of plants tolerant to glyphosate allows farmers to apply glyphosate while crops are growing, theoretically killing every plant, but the crop. The consequence is that crops now contain residual levels, directly exposing consumers. Not only that, glyphosate tolerant crops accumulate the herbicide and transport it to the roots, excreting it into the root zone of the soil, harming the next crop to be planted in the same field.

Glyphosate (N-(phosphonomethyl) glycine) is an organ ophosphorated, broad-spectrum, non-selective and post-emergence herbicide. It kills unwanted weeds by

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Received 22 June 2017 Revised 12 October 2017 Accepted 21 November 2017
doi:10.14456/smj.2018.66

inhibiting the synthesis of aromatic amino acids necessary for protein formation in plants.[1-4] Glyphosate in form of isopropylamine salt is an active ingredient of Roundup®, herbicide's trade name under Monsanto's company since the 1970s.[7] Glyphosate is believed to be less toxic than other pesticides and no potential to pose a health risk to humans,[8] with low toxicity and environmental friendliness. However, several studies showed that glyphosate may not be as safe as previously thought. Its has potential adverse health effects to humans as it may induce apoptosis and necrosis in human umbilical cord, embryonic, and placental cells.[9] Glyphosate acts as an endocrine disruptor by inhibiting activity of enzyme CYP19, an essential component of cytochrome P450 aromatase. CYP19 is responsible for the irreversible conversion of androgens into estrogens.[10] After studying the epididymal region of drakes, the animal model, showed the glyphosate-roundup affected the expression of the steroidogenic acute regulatory protein (StAR) protein, which mediated the initial step of steroid hormone synthesis.[11] Furthermore, several studies stated glyphosate acted as a teratogen.[12] The malformations were microcephaly, microphthalmia, cyclopia and craniofacial malformations,[13] of the fetal skeleton in Wistarrats.[13] Residual glyphosate was found in many organs of malformed offspring pigs.The piglets showed many abnormalities such as ear atrophy, leg atrophy, spinal and cranial malformations, elephant tongue, female piglet with testes, and piglets born alive having short legs and one eye. Residual glyphosate in GMO foods may effect to pig embryos via pregnant pigs which were fed with GMO foods.[14]

This experiment was conducted to study the teratogenic effects of glyphosate based herbicide (Roundup®) on development *in ovo* chick embryos. The serial sections of chick embryos at different stages and the development of bone and cartilage of chick embryos were studied by comparing to normal development. The results of this work may be consistent with the well-known evidences which have occurred to the babies born in the area using this herbicide and come to the conclusion that GBH is a teratogen, so its use should be limited.

MATERIALS AND METHODS

Ethics statement

According to German animal care guidelines, no IACUC approval was necessary to perform this experiment. According to the local guidelines, only experiment with chick embryos of E18 and more need IACUC approval. However, this study used earlier stages of chick embryos, day 3 - day 11.

ACUC Guideline

Use and Euthanasia Procedures of Chicken/Avian Embryo

Avian embryos are not considered live animals under PHS policy. However, there is a consensus in the scientific community that at a certain point in development, avian embryos can experience pain. Because that exact point is not known for chicken embryos, chicken use and euthanasia guidelines differ across institutions. Cal Poly Pomona has chosen to adopt a guideline with the belief that pain occurs on or after gestation day 13, in anticipation of reviewing protocols including them. However, this study used earlier stages.

Experiment protocol

Glyphosate based herbicide preparation

Roundup® herbicide, a commercial product of Monsanto Company, Malaysia, which consisted of 48% W/V SL Isopropylamine salt with unknown surfactants, was used to prepare the stock solution. 1 ml of Roundup® herbicide was diluted in 47 ml of normal saline. The working solutions of 0.5%, 0.3%, 0.05% and 0.01% w/v Roundup® were further diluted with normal saline solution.

Animal treatment

The 140 white leghorn hen *(Gallus domesticus)* eggs were obtained from Luang Suwan Vajokkasikij farm, Department of Animal Science, Faculty of Agriculture, Kasetsart University. The eggs were divided into 5 groups, one control and four experimental groups. At 21 hours after incubation, eggs were drilled with dental driller to get a hole at the blunt end and injected with normal saline solution (control group), 0.01%, 0.05%, 0.3% and 0.5% w/v glyphosate (experimental groups), at the equal volume of 0.1 ml into the yolk sac by sterile needle. The holes were sealed with paraffin and further incubated until day 3. Embryos from each group were collected. The remaining were repeated with corresponding dosages at the volume of 0.05 ml to yolk sac and further incubated until days 6 and 10. Day 3 embryos were fixed in dietrich's FAA fixative for about 4 hours, recorded viability and processed for total mount and serial sections. Day 6 embryos were fixed in dietrich's FAA fixative for one day and recorded viability, weight, morphology, and measured external features for instance, crown-rump length (CRL), limb length, and width of head, then they were processed for serial sections. Day 10 embryos were recorded viability, morphology, weight, and measured external features such as crown-rump length (CRL), beak, limb length and width of head then they were deskinned and eviscerated for alcian blue and alizarin red staining transparency to study the cartilage and bone abnormalities.

RESULTS

The survival and mortality ratesof day 3 chick embryo

The living embryos were indicated by heart beating and blood circulation. The survival rateof day 3 chick embryosof the control group was 100% while the highest dose of glyphosate injected to group, 0.5% w/v, was 0%. The 0.01%, 0.05% and 0.3% w/v groups showed that the survival rates were 92.86%, 85.71% and 33.33%, respectively, which indicated that the survival rate decreased as the concentration increased.

The mortality and survival rates day 6 chick embryo

The survival rates of the day 6 chick embryos were 100%, 87.50%, 85.71%, 41.67% and 14.29% in the control, 0.01% w/v, 0.05% w/v, 0.3% w/v and 0.5% w/v glyphosate, respectively. The living embryos were observed by the heart beating and blood circulation. The data indicated that the increasing glyphosate concentration affected to the viability as the survival percentage was decreased.

The survival and mortality rates of day 10 chick embryo

Table 3 showed the viability was 100% in the control group injected with NSS, and 42.86% and 50% in the experimental groups injected with 0.01% and 0.05% GBH, respectively. Both 0.3% and 0.5% glyphosate injected groups showed no survival. The surviving embryos were indicated by the blood circulation and heart beating.

The total mount of day 3 chick embryo

The normal day 3 chick embryo by total mount preparation

On day 3, the normal chick embryo developed to the stage 18 of Hamburger and Hamilton.[15] The control group showed normal embryonic development which comprised 36 pairs of somite. The brain developed to the secondary brain vesicles with 5 components, telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon. The total mount showed head fold rotation about 2/3 to the right side and illustrated the

TABLE 1. The survival and mortality rates of day 3 chick embryos after injected with 4 different concentrations of GBH and normal saline solution.

Group	% Survival	% Mortality
Control (n=8)	100	0
0.01% (n=14)	92.86	7.14
0.05% (n=14)	85.71	14.29
0.3% (n=12)	33.33	66.67
0.5% (n=8)	0	100
Total (n=56)		

TABLE 2. Percentages of survival and mortality of day 6 chick embryos after injected twice with 4 different concentrations of GBH and normal saline solution

Groups	% Survival	% Mortality
Control (n=5)	100	0
0.01% (n=8)	87.50	12.50
0.05% (n=14)	85.71	14.29
0.3% (n=12)	41.67	58.33
0.5% (n=7)	14.29	85.71
Total (n=46)		

TABLE 3. Percentages of survival and mortality of day 10 chick embryo after injected with 4 different concentrations of GBH and normal saline solution

Group	% Survival	% Mortality
Control (n=7)	100.0	0
0.01% (n=7)	42.86	57.14
0.05% (n=12)	50	50
0.3% (n=8)	0	100
0.5% (n=4)	0	100
Total (n=38)		

dextrodorsal view. There were 2 head flexures, one at the midbrain called "cephalic flexure" and at the point between myelencephalon and spinal cord called "cervical flexure". The otocyst situated at the level of myelencephalon. The eye primordia, optic cups and lens vesicle situated at the level of diencephalon. The optic cup appeared as horseshoe-shaped and lens vesicle located in the middle. About 3-4 branchial arches were visible. Heart tube was S-shaped loop. The neural tube appeared as two parallel dense lines which were parallel to each other to the caudal end along sides with the continuous blocks of somite which also exceeded the caudal end. The limb buds enlarged symmetrically, and the posterior pair were slightly larger than the anterior pair (Fig 1A).

The total mount of glyphosate injected groups

All treated groups showed more or less malformations and growth retardation. The 0.01% treated group (Fig 1B) showed severe looser of heart looping with minor growth retardation of brain, eye, branchial apparatus and limb buds. Retardation of formation of brain caused no cervical flexure. Moreover Fig1C showed abnormal body bending. Fig 1D showed severe abnormality of brain and spinal cord with opened anterior and posterior neuropores, abnormal heart looping, eye primordia was rudimentary and neural tube showed unparallel dense lines. There were retardations in somite formation and absence of branchial arch and limb bud. The 0.3% glyphosate group in Fig 1E showed retardation of head bending which

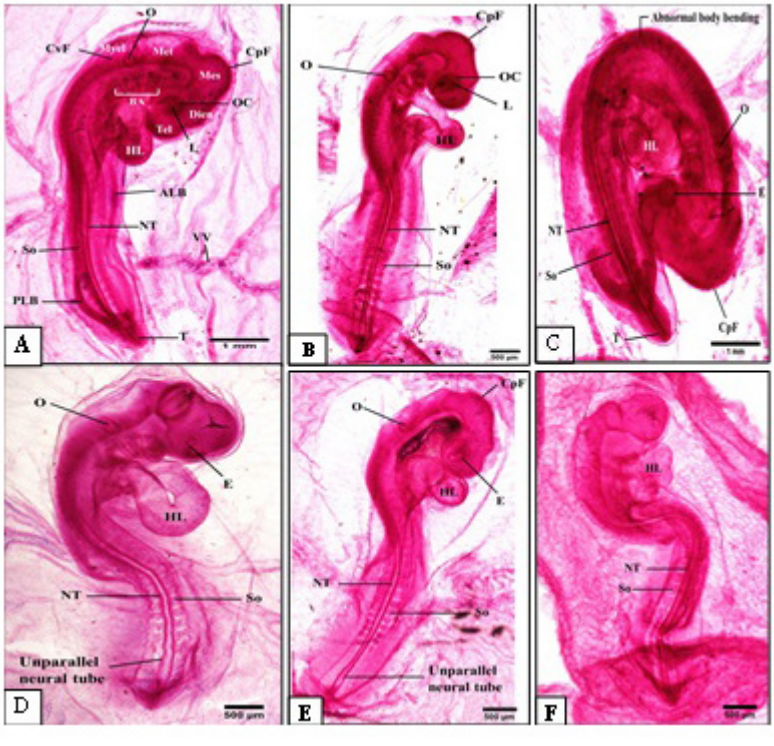


Fig 1. A Micrograph of the total mount of the 3rd day chick embryo of the control group showed normal development of Hamburger and Hamilton stage 18. Tel, Telencephalon; Dien, Diencephalon; Mes, Mesencephalon; Met, Metencephalon; Myel, Myelencephalon;CpF, cephalic flexure; CvF, cervical flexure; BA, branchial arch; O, otocyst; OC, optic cup; L, lens; HL, heart loop; So, somite; NT, neural tube; ALB, anterior limb bud; PLB, posterior limb bud; VV, vitelline vessel; T, tail fold.

B was the total mount of chick embryo of the 0.01%w/v treated group, C and D were the 0.05%w/v treated group. B showed severe loosening of heart looping with minor growth retardation of brain, eye and limb buds. C showed abnormal body bending. D showed severe abnormal of brain and spinal cord with opened anterior and posterior neuropores, abnormal heart looping, eye primordia was rudimentary and neural tube showed unparallel dense lines. O,otocyst, OC, optic cup, L, lens E, eye, NT , neural tube, So, somite. E and F were total mount of chick embryos of the 0.3% and 0.5% w/v group, D showed retardation of head bending which appeared only the cephalic flexure (CpF), abnormal heart looping(Ht), delay of somite formation(So) with unparallel dense lines of neural tube and absent of limb bud. E showed abnormal body bending, abnormal heart looping, anophthalmos and opened anterior neuropore,(HL, heart looping), NT, neural tube.

showed only the cephalic flexure (CpF), abnormal heart looping (Ht), and delay of somite formation(So) with unparallel dense lines of neural tube and absence of limb bud. The 0.5% glyphosate in Fig 1F showed abnormal body bending, abnormal heart looping, anophthalmiaand opened anterior neuropore, absence of branchial apparatus and limb bud.

External appearance of the day 6 chick embryo

The normal day 6 chick embryo developed to the Hamburger and Hamilton stage 29,[15] and the external appearance of the day 6 chick embryo showed the prominent mesencephalon and cephalic flexure. The eyes were blackprominent protruding organs of the head. The upper and lower limbs distinctly appeared. The wing bent at the elbow with the second digit distinctly longer than the other. The ends of the upper and lower limbs were paddle-shaped with groove between the 1st, 2nd and 3rd digits. The 2nd to the 4th toes were prominent as ridges separated by distinct grooves with the webs between them. Beak was prominent, but the egg-tooth was not visible. Mandibular processes lengthened and were broadly fused. The visceral organs were enclosed by the body wall except the area of umbilicus. The control showed normal development while the experimental groups showed smaller body, eye and head. The upper and lower limbs were shorter than the control by observation. An embryo treated with 0.3%w/v glyphosate indicated the unilateral microphthalmia and an embryo treated with 0.01%w/v glyphosate indicated bilateral anophthalmia with opening of anterior neuropore.

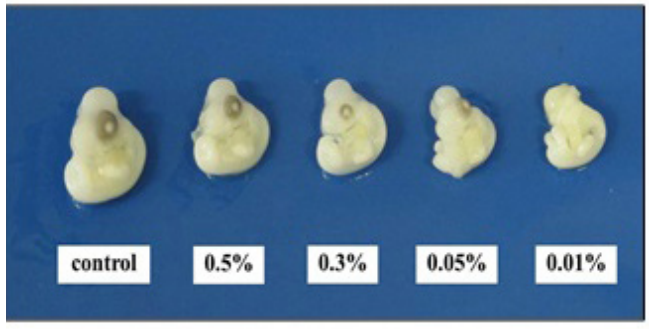


Fig 2. The photographs showed the comparison of external features between the experimental groups and the control group. All showed smaller than the control. The 0.01% w/v group was the smallest and showed anophthalmia and anencephaly.

Measurement and analysis of body parameters

The comparison of body parameters of day 6 chick embryos between the control group and the experimental groups of 0.01% w/v, 0.05% w/v, 0.3% w/v and 0.5% w/v GBH were shown in Table 4. The data analysis of body weight, crown rump length (CRL), width of head, width of eye, length of beak, length of upper limb and length of lower limb were tested statistically using Statistical

TABLE 4. The effect of GBH on body parameters of the 6th day chick embryo development showed the data by the mean ± standard error which compared with the control group.

Body parameter	Control (n=5)	0.01% (n=7)	0.05% (n=12)	0.3% (n=5)	0.5% (n=1)
Body weight (mg±SE)	0.34±0.02	0.25±0.02*	0.27±0.01*	0.19±0.04*	0.19±0.01*
CRL (mm±SE)	14.08±0.15	12.27±0.52*	12.20±0.33*	12.06±0.60*	11.28±0.0*
Width of eye (mm±SE)	3.43±0.04	2.90±0.48	2.98±0.12	2.34±0.20*	2.52±0.0*
Width of head (mm±SE)	7.53±0.22	6.35±0.56*	6.67±0.15	6.40±0.69	6.07±0.0*
Upper limb (mm±SE)	3.66±0.09	3.27±0.16*	3.37±0.09	3.48±0.11	3.16±0.0*
Lower limb (mm±SE)	4.18±0.06	3.72±0.18*	3.97±0.13	4.10±0.16	3.65±0.0*

Significant difference from control: *$P<0.05$. One-way ANOVAs was used for statistical analysis.

Table 4 indicated that the body weight and the CRL of the day 6 chick embryo of all experiment groups were significantly lesser than the control. Moreover all parameters of the 0.5%w/v glyphosate treated group were also significantly lesser than the control. The width ofhead, upper limb and the lower limb of the 0.01% w/v glyphosate treated group were significantly lesser than the control.

Package for the Social Science (SPSS) by one way analysis of Variance (ANOVA), and the data represented by mean ± SD with significant difference at $p<0.05$

The glyphosate affects to the eye development of day 6

The normal day 6 eyes comprised oval-shaped optic cup and lens. The optic cup comprised two layers, the outer pigment layer and inner nervous layer which were closely 4/5 attached and obliterated the intraretinal space. The posterior 4/5 of the optic cup which is called pars optica retinae comprised of single layer of outer pigment layer and thick inner nervous layer with numerous cell layers developed into retina. Lens comprised of anterior lens epithelium and posterior lens fiber. The anterior lens epithelium with the mitosis cells migrated to the equatorial zone and elongated to form the posterior lens fiber that developed to obliterate the lens cavity. Anterior to it were anterior chamber and cornea. The eye in the control group showed normal eye development following HH stage 29.

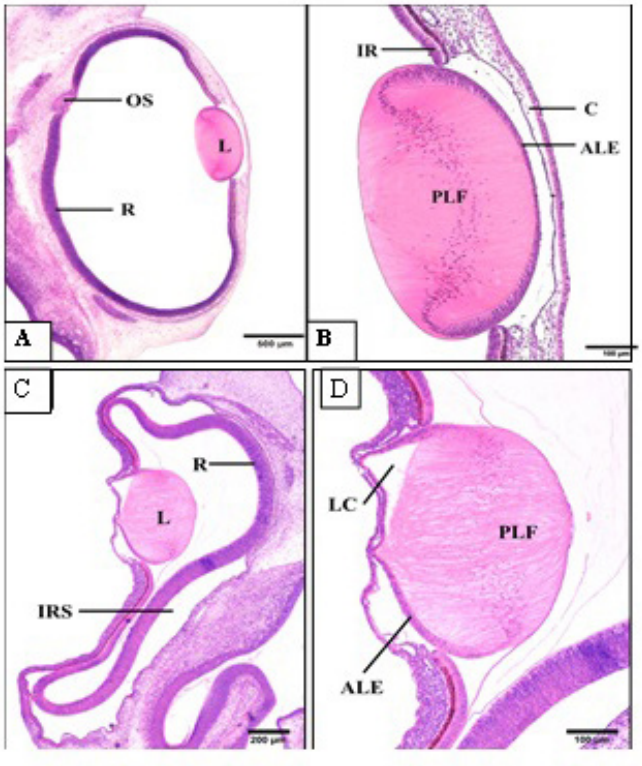


Fig 3. The micrographs showed eye development of the control A, B and in 0.05% w/v of glyphosate injected group C, D. Fig A exhibited normal optic cup which was oval-shaped without intraretinal space B showed lens without lens cavity, cornea (C) and smooth anterior chamber. Fig C and D showed irregular-shaped of optic cup and lens. R, retina; L, lens; IRS, intraretinal space; LC, lens cavity; ALE, anterior lens epithelium; PLF, posterior lens fiber. Other experiment groups showed the same appearances as in C and D.

The severe malformation of the eye was shown in an embryo that was injected with 0.01% w/v glyphosate. Both eyes were absent, called "anophthalmia". In addition, the other embryos in 0.01% glyphosate injected group and other experimental groups showed under development of the eye compared with the control. The embryo showed irregular-shape of the optic cup with wide intraretinal space. The lens protruded into the anterior chamber and contacted with the cornea which obstructed the anterior chamber. The lens cavity still persisted between anterior lens epithelium and posterior lens fiber.The embryos in 0.05% injected group (Fig 3) showed collapsed optic cup with persistence of the intraretinal space which as well as irregularity of the cornea and displacement of lens to obstruct the anterior chamber. Non homogenous lens could be observed at area of posterior lens fiber which might cause congenital cataract.

One of the day 6 chick embryos treated with 0.3% w/v of glyphosate exhibited unilateral microphthalmia (Fig 4), the right eye showed normal size with slightly retarded optic cup while the left eye exhibited much smaller optic cup with relatively large lens with persistence of hyaloids canal called the microphthalmic eye.

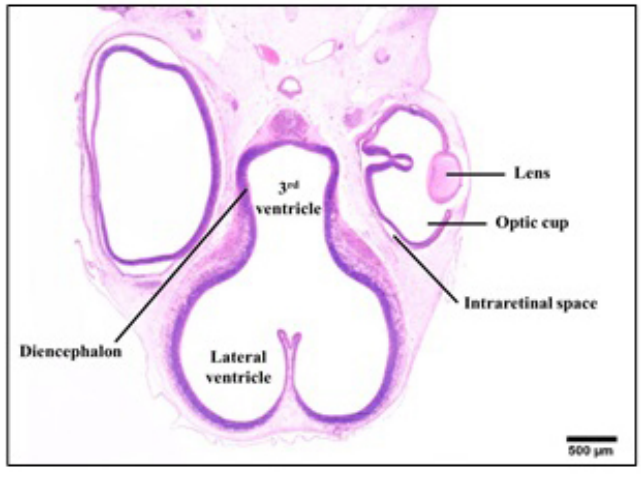


Fig 4. The micrograph showed unilateral microphthalmia in 6-day-old chick embryo after injected with 0.3% w/v of glyphosate.

Effect to the heart of day 6 chick embryo

The control group showed thin atrium lied dorsal to the thick ventricle. They were divided internally by fused endocardial cushion with two opening of left and right atrioventricular canals. The atrium was divided to two chambers, left and right atrium by septum primum that extended from the roof of atrium to the endocardial cushion. The left and right ventricles were divided by

muscular part of the interventricular septum. On this stage, the chamber of the ventricle was not completely divided and they were connected by the interventricular foramen. The results showed both abnormal and delayed developments of the heart. The ectopiacordis, the congenital displacement of the heart were observed in the embryos which were injected with 0.01% and 0.3% w/v of glyphosate. In addition in the 0.3% group, the embryo showed severe heart retardation when compared with the control. Some embryos in 0.05% and 0.3% injected group showed thin heart wall and the ventricle wall was thinner than the control. The embryo of the highest dose, 0.5% w/v of glyphosate showed that the heart size was smaller than the control without any malformation.

The day 6 embryo treated with 0.01 % w/v glyphosate (Fig 5A) showed the heart and liver (Li) situated outside the body cavity (ectopiaviscerae). The heart was severely retarded with the unfused endocardial cushion with the thinned-wall and underdeveloped atrium and ventricle. Figs 5B and C showed ectopiacordis with the concentration of 0.3% w/v of glyphosate-based herbicid. This heart composed of atrium and ventricle. The atrium situated inside the body cavity and showed more or less retardation of development. Ventricle was separated into two parts the proximal lied inside the body cavity while the distal part lied outside (Fig 5B and C) and the ventral body wall separated them as indicated in Fig 5C.

The day 10 embryo developed to stage 40 of Hamburger and Hamilton,[15] and the embryo showed an increasing length of beak and toe. The egg tooth which is used to break the eggshell for hatching developed. The longer feather-germs covered the body and wing. The four to five rows of feather-germs were at the edges of lower eyelids. The eyes showed the opening between lids. Both the control group and the experiment groups showed normal development of the living embryos by external feature observation. Only the 0.01% and 0.05% survived, and the other two groups totally died. The dead embryo of the 0.3% treated group showed anophthalmia, microphthalmia and claw digits (Fig 6).

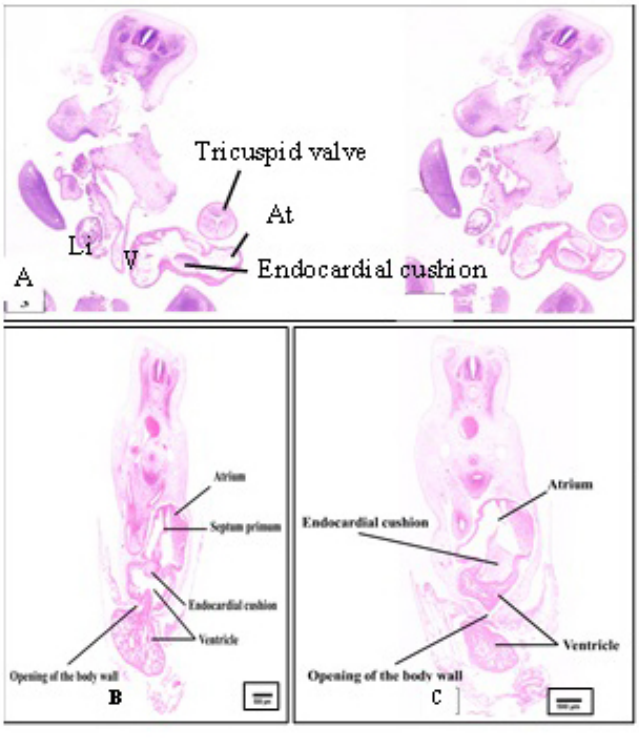


Fig 5. A The day 6 embryo treated with 0.01% w/v glyphosate showed the heart and liver(Li) situated outside the body cavity (ectopia viscerae). The heart showed severe retardation, the unfused endocardial cushion with the thinned-wall and underdeveloped atrium and ventricle. Fig B and C Micrographs showed the serial sections of 0.3% w/v of glyphosate injected group at the heart developing level. This embryo showed a part of the ventricle situated outside the body cavity. This occurred as a result of abnormal lateral body folds fused at the abnormal dorsal level. This was accompanied with small and retarded heart.

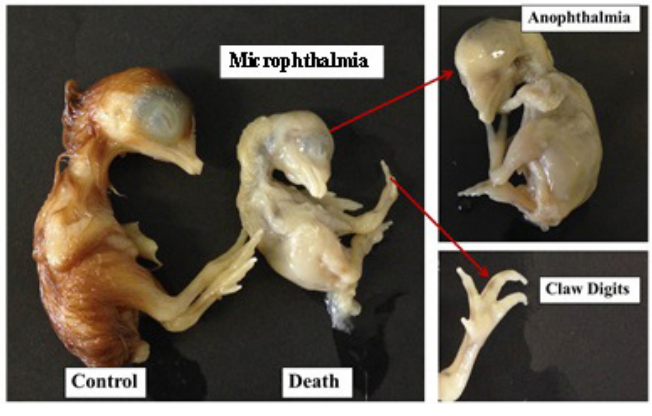


Fig 6. The photographs showed abnormal external appearance of dead chick embryo. The body size was smaller than the control. The anophthalmia, microphthalmia and claw digits were observed in the dead embryo.

Most embryos died before day 10 and the surviving embryos showed normal external appearance with no significantly difference of body parameters. The alcian blue and alizarin red staining showed lesser ossification of several bones. The ossification area of the skull, beak, mandible and the cervical vertebrae were stained red by the alizarin red while the cartilaginous parts stained blue by the alcian blue. Fig 7A was the control group which showed more ossification area of the skull, beak, mandible and the cervical vertebrae while Fig 7B of the 0.01% and Fig 7C of the 0.05% showed lesser ossification.

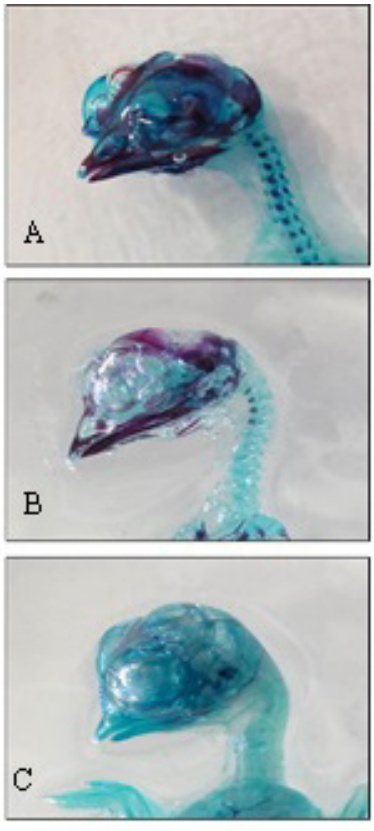


Fig 7. The photographs showed the head of day 11 chick fetus which were stained with alcian blue and alizarin red. The normal formation showed in the control group (Fig A) and the 0.01% glyphosate injected group (Fig B). The retardation of skull ossification showed in the 0.05% glyphosate injected group (Fig C).

DISCUSSION

Humans may expose to Roundup herbicide residues by agricultural practices[16] or when the residues enter the food chain.[17] Glyphosate residues in animal food, which is from GMO's products may be the cause of malformed piglets.[15]

The mortality rates were equal to the enhancing rate of glyphosate concentration. This result corresponded to Yusof *et al.*, 2014 who studied the effects of GBH on Java medaka (*Oryziasjavanicus*).[18] The survival and hatching percentage of fish embryos decreased as glyphosate concentration and time increased. In addition, the rats which were treated with high dose, 1000 mg/kg glyphosate-Roundup showed 50% mortality between days 7-14 of pregnancy.[14] This study also indicated that glyphosate-Roundup was highly toxic and lead to embryonic death.

In 2003, Lajmanovich *et al.*, found the external malformations in tadpole stage of frog embryos (*Scinaxnasicus*) such as craniofacial and mouth deformities, eye abnormalities and bent curved tails.[19] These abnormalities occurred in all tests and increased with time and glyphosate-Roundup concentrations. In 2014, Yusof *et al.*, found the effects of GBH in pre-hatching stage of the fish embryo that showed several developmental abnormalities such as shrunken yolk, abnormal body curvature, growth retardation and disproportion of head and body size. In post- hatching stage, birth defect was found as absence of cornea and pectoral fins, permanently bent tail, abdominal enlargement and cell disruption in fin head and abdomen.[18] In this study, day 3 embryos showed retardation of brain vesicles, delayed flexing of head, looser of heart looping with thin heart's wall, fewer branchial arches, retardation of optic cup and lens and absence of limb buds. The day 6 chick embryos showed several significantly smaller parameters ($P<0.05$, one way ANOVA) such as body weight, CRL, width of eye, width of head, length of upper limbs and length of lower limbs. The serial sections revealed growth retardation of optic cup, lens and heart. The delayed development corresponded to the study of toxicity of GBH to four North American frog species.[20] Glyphosate-Roundup inhibited transcription of hatching enzyme and correlated with a delay in hatching process of sea urchin embryos.[21]

The irregularly-shaped brain with opening of anterior neuropore presented on day 3 and day 6 chick embryos. The width of head of the embryos in 0.01% and 0.5% experimental groups were significantly smaller than the control ($P<0.05$, one way ANOVA) which revealed microcephaly. This agreed with the study of Pagamelli *et al.*, in 2010, in which brain microcephaly occurred in vertebrate embryos; amphibians and chickens at different developmental stages.[13]

Abnormalities were presented in eye development on day 3 and day 6 chick embryos. The range of abnormal eye development included irregular-shaped optic cup, lens displacement, congenital cataract, microphthalmia, and anophthalmia. GBH caused bilateral anophthalmia and unilateral microphthalmia that were distinctly presented in 0.01% and 0.3%, respectively on the day 6 chick embryos. This result was in agreement with a previous study reported in 2010 by Paganelli *et al.*, who found GBH and pure glyphosate produced microphthamia in amphibians (*Xenopuslaevis*) at neurula stage, tail bud stage and tadpole stage. Injecting of glyphosate into bilateral side on the frog embryo at 2-cell stage produced cyclopia. Similarity of glyphosate and glyphosate-based herbicide induced bilateral microphthalmia in chick embryos on Hamburger and Hamilton stage 8. During embryonic development, the *pax6*protein was thought to activate genes involved in the formation of the eyes, the brain and spinal cord. According to Paganelli *et al.*, the potential effects of GBH were by increasing retinoic acid signaling which inhibited or decreased sonic hedgehog

(Shh), otx2, sox9 and *pax6* genes which caused cyclopia, microchephaly, affected cranial neural crest as well as craniofacial malformations.[13]

Cardiac abnormalities have been reported in rabbit fetuses that mothers were exposed to glyphosate during pregnancy such as defects of aorticopulmonary (spiral) septum, ventricular septal defects, dilated heart, dilated ventricle, and cardiomegaly.[22] In this study, there was dilated heart tube with thin wall and looser of heart looping on day 3, and small heart size with thin wall of atrium and ventricle on day 6. The ectopiacordis showed severe abnormality which was found on day 6 chick embryos of 0.3% and 0.01% w/v of glyphosate concentration. There was no report that glyphosate herbicide exposure in chick embryos caused ectopic viscerae. This abnormality was found in chick embryos which were exposed to various concentrations of the insecticides.[23]

Most embryos died before day 10 and the surviving embryos showed normal external appearance with no significantly difference of body parameters. The alcian blue and alizarin red staining showed lesser ossification of several bones. This agreed with the previous study that revealed 21-day-old fetus skeletal development retardations consisted of incomplete skull ossification and enlarged fontanel in all test groups of tadpole stage of amphibians that were exposed to low concentrations of glyphosate and GBH (Roundup).[19]

The results of this study indicated that mortality rate increased with the concentration while degrees of the severity of abnormalities were not dependent on the increasing concentrations of the GBH. The malformations randomly occurred in all experimental groups which did not correspond with the previous studies that stated the malformation increased with dose and time of exposure.[14,18] The abnormalities were unpredictable and also the repeated exposures or single exposure of this herbicide on varios periods of development also produced uneven results. This issue needs to be emphasized in further study.

The result of this study indicates that glyphosate based herbicide is highly toxic to chick embryo which leads to several developmental abnormalities and embryonic death. The mortality rate increased with the concentration of glyphosate while degree of abnormalities did not depend on the increasing concentration. Glyphosate is therefore predicted to be harmful to humans.

CONCLUSION

Glyphosate-based herbicide (Roundup) caused lethality in every concentration on day 3, 6 and day 10 chick embryo which indicated that it was an embryocidal agent which may cause the same effect, miscarriage, to humans. Moreover glyphosate also caused several malformations and growth retardation. The living embryos of day10 showed normal external features but revealed delayed ossification. The dead fetus had unilateral anophthalmia and microphthalmia, and small body size with claw digits. This indicated that most of the abnormal embryos could not survive and died before day 10. The pregnant should be highly cautious when living in the area using this herbicide during pregnancy to avoid abortion or congenital malformations. The results of this work showed that the malformations produced by GBH in chick embryos were embryonic death, the abnormality of nervous system (anencephaly), eye (anophthalmia, microphthalmia), heart, ectopiacordis, ectopia viscerae, delayed ossification, which were consistent with the well-known whichevidences occurred to the babies born in the areas using such herbicides. Why is GBH still promoted as "SAFE" despite damning evidence of serious harm to health and environment? Thus, future studies should investigate the teratogenic effects of GBH in different routes such as oral route, inhalation to mimic contamination to this herbicide from the residue in soil, water, air, vegetable or fish and meat fed with the GMO products.

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Obesity Significantly Affects the Incidence of Hepatic Injury in Patients with Colorectal Liver Metastasis

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ABSTRACT

Objective: Surgical resection is the mainstay treatment for colorectal liver metastasis. In unresectable cases, chemotherapy is used to transform the tumor into resectable lesions, with related concerns about toxicity to non-tumoral liver parenchyma. Liver toxicity, including steatosis, steatohepatitis, and sinusoidal dilation, has been reported. However, these changes are difficult to histologically distinguish from non-alcoholic fatty liver disease, which is commonly found in populations and attributed mainly to metabolic syndrome. The aim of this study was to investigate the factors associated with liver injury in patients with colorectal liver metastasis.

Methods: This retrospective study included the patients who underwent hepatic resection for colorectal liver metastasis at Siriraj Hospital during the eight-year period (2006 to 2013). Patient demographic data, clinical characteristics, and histologic changes related to liver injury were collected and analyzed. Ten factors were evaluated for association with liver injury in selected patients.

Results: Ninety-two patients (50 men, 42 women) were included, with a mean age of 59.4 years (range: 48.5-70.3). Forty-four patients (47.8%) received preoperative chemotherapy (CMT). Incidence of liver injury was not significantly different between the CMT and non-CMT groups (65.9% vs. 62.5%; p=0.902). However, incidence of liver injury was significantly higher in obese patients than in non-obese patients (82.8% vs. 55.6%; p=0.022, odds ratio=3.95). Multivariate analysis showed that obesity (BMI >25 kg/m^2) was the only factor significantly associated with liver injury in patients with colorectal liver metastasis.

Conclusion: Of the ten factors evaluated, obesity was the only factor found to be significantly associated with liver injury in patients with colorectal liver metastasis.

Keywords: Obesity; hepatic injury; colorectal liver metastasis (Siriraj Med J 2018;70: 429-437)

INTRODUCTION

Colorectal carcinoma is the third most common cancer in men and the second in women worldwide.[1] According to the National Cancer Institute and a population-based cancer registry in Thailand, colorectal carcinoma is the third most common cancer among males after liver cancer and lung cancer, and the fifth after cancer of the breast, cervix, liver and lung among females.[2] A search of the database at our center (Siriraj Hospital) revealed colorectal carcinoma to be the third and second most common cancer in males and females, respectively.[3] The most common site of distant metastasis is the liver.

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Received 22 December 2017 Revised 9 July 2018 Accepted 31 July 2018
doi:10.14456/smj.2018.67

Current management of colorectal liver metastasis requires a multidisciplinary approach, with combination therapy consisting of complete surgical resection and systemic chemotherapy offering the best opportunity for prolonged survival. However, there are, only a small percentage of patients that are eligible for upfront resection. For patients with initially unresectable metastasis, preoperative chemotherapy may transform the tumor into a resectable lesion.[4] However, chemotherapy may adversely affect non-tumoral areas of the liver parenchyma. Different patterns of liver injury are attributed to different types of chemotherapy. By way of example, fluorouracil can cause steatosis, oxaliplatin can cause steatosis and sinusoidal dilation, and irinotecan can cause steatosis and steatohepatitis.[4-8] Many studies have reported that chemotherapy-induced liver injuries cause increased perioperative morbidities and mortality.[9-12] In contrast, some other studies reported that chemotherapy-induced liver injuries had no effect on clinical outcomes.[13-16] Conflicting results among studies were attributed to differences in duration and number of cycles, and interval time before surgery.[5-7]

Chemotherapy-induced liver injury cannot be distinguished histologically from nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). NAFLD and NASH are associated with obesity and metabolic syndrome, both of which are now very common in many, if not most, populations.[17] According to the Fourth National Health Examination Survey (2008-2009) conducted by the Thailand National Health Examination Survey Office (NHESO), the rate of obesity in Thailand (BMI ≥25 kg/m^2) is 28.3% among male adults and 40.7% among female adults.[18]

A finding of liver injury among patients with colorectal liver metastasis is relatively common in routine pathology practice. Accordingly, the aim of this study was to investigate the factors associated with liver injury in patients with colorectal liver metastasis.

MATERIALS AND METHODS

This retrospective study included patients with colorectal liver metastasis who underwent hepatic resection at Siriraj Hospital during the 2006 to 2013 study period that were identified from the electronic database of the Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University using the keywords *liver* and *adenocarcinoma*. Siriraj Hospital is located in Bangkok, and is Thailand's largest national tertiary referral center. The protocol for this study was approved by the Siriraj Institutional Review Board (Si 552/2014).

The following demographic and clinical data were collected: age; gender; underlying diseases, including diabetes mellitus (DM), hypertension (HT), dyslipidemia (DLP), ischemic heart disease (IHD), hepatitis B virus (HBV) infection, and hepatitis C virus (HCV) infection, history of preoperative chemotherapy (CMT), and, height and weight. Obesity in this study was defined as body mass index (BMI) >25 kg/m^2 (Asian adults).[19] Patients having one or more of the following were excluded: 1) greater than 1 year interval between the last cycle of chemotherapy and hepatic surgery; 2) change in chemotherapy regimen during treatment; 3) incomplete chemotherapy treatment information; 4) received other treatment prior to the current preoperative chemotherapy, including other course of chemotherapy, transcatheter arterial chemoembolization, portal vein embolization, and radiofrequency ablation.

Histologic changes in non-tumoral liver parenchyma, including steatosis, steatohepatitis, sinusoidal dilation, fibrosis, and nodular regenerative hyperplasia, were retrospectively reviewed by a pathologist who was blinded to patient clinical data. The pathologist reviewed all available H&E-stained slides and Masson trichrome-stained slides of non-tumoral liver parenchyma. Cases with inadequate liver parenchymal area for evaluation (less than 1 cm^2) were excluded.

Fatty change was evaluated based on the NASH Clinical Research Network Scoring System for Nonalcoholic Fatty Liver Disease.[20] Steatosis was diagnosed when >5% of the liver parenchyma was involved by fat. Steatohepatitis was diagnosed when steatosis, fibrosis, lobular inflammation, and hepatocellular ballooning degeneration were simultaneously present. Fibrosis stage was evaluated using the METAVIR scoring system.[21] Stage 2-4 fibrosis was considered advanced fibrosis. Sinusoidal dilation was recorded for scores >1 according to the Rubbia-Brandt grading system.[22] A patient was regarded as having liver injury if steatosis, steatohepatitis, or sinusoidal dilation was observed by microscopic evaluation.

Statistical analysis

Aloysius, *et al.*, reported that steatosis was found in 68% of patients receiving combined fluorouracil and oxaliplatin for colorectal liver metastasis, as compared with 20% in patients that did not receive chemotherapy.[23] The required sample size was calculated based on two estimated proportions of two groups method, with a 95% confidence interval (CI) and a 5% error. This calculation resulted in a required sample size of 16 patients.

Bower, *et al.*, found that 39% of obese patients (BMI ≥30 kg/m^2) had steatosis or steatohepatitis, as compared with a rate of 12.8% in non-obese patients.[24] The required

sample size from the same method was 43 patients.

Continuous data are described as mean ± standard deviation (SD), mean and range, or median and range, as appropriate. Number and percentage were used to express categorical data. Mann-Whitney U test or independent t-test was used to compare continuous data between groups. Categorical data were compared using chi-square test or Fisher's exact test. Factors of interest and factors with a *p*-value less than 0.1 in univariate analysis were entered into multiple logistic regression analysis. Data were prepared and analyzed using PASW Statistics version 18.0 (SPSS, Inc., Chicago, IL, USA). All tests of significance were two-tailed, and a *p*-value less than 0.05 was considered to be statistically significant.

RESULTS

During the 2006 to 2013 study period, 163 patients were diagnosed with colorectal liver metastasis from hepatic resection at our center. Sixty cases and 11 eleven cases were excluded for not meeting non-pathologic and pathologic inclusion criteria, respectively (Fig 1). The remaining 92 patients were included.

Mean age of patients was 59.4 years (range: 48.5-70.3). Seventy-six patients (82.6%) were aged older than 50 years, and 50 patients (54.3%) were male. Forty-eight patients (52.2%) had underlying disease(s), as described in Table 1. Twenty-nine patients (31.5%) were obese. Forty-four patients (47.8%) received preoperative chemotherapy (CMT), and the remaining 48 patients (52.2%) did not receive preoperative chemotherapy (non-CMT) (Table 1).

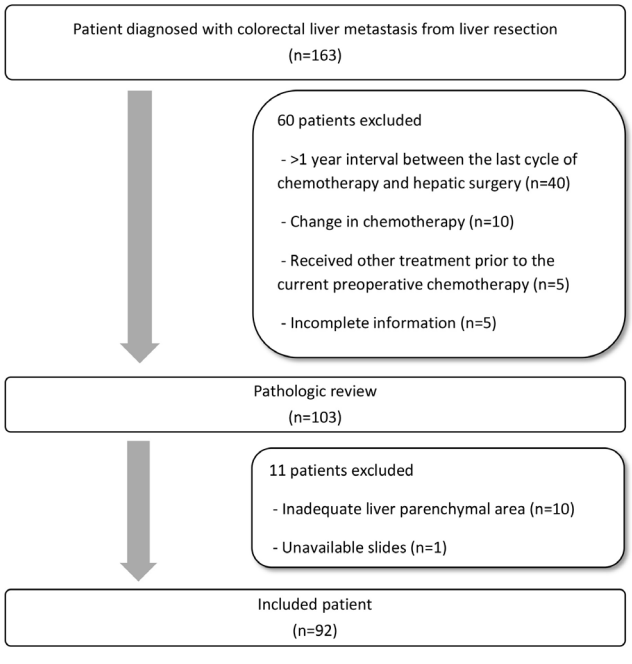


Fig 1. CONSORT flow diagram.

Association between obesity/CMT status and other clinical factors

Association between obesity/CMT status and other clinical factors was evaluated by Fisher's exact test. The other clinical factors included age, gender, and presence of underlying disease(s) (i.e., DM, HT, DLP, IHD, HBV, and HCV). Twenty-nine patients (31.5%) were obese (BMI ≥25 kg/m^2) (Table 2). Incidence of diabetes mellitus was significantly higher in obese patients than in non-obese patients (41.4% vs. 9.5%; p=0.001) (Table 2).

Preoperative chemotherapy regimen

Among the group of patients that received preoperative chemotherapy (CMT), fluorouracil or capecitabine combined with oxaliplatin was the most common regimen, followed by fluorouracil or capecitabine alone, and fluorouracil or capecitabine combined with irinotecan (Table 3). The mean duration of treatment was 4.4±1.4 months. The median number of cycles was 6 (range: 3-14). The mean interval between the last cycle of chemotherapy and liver resection was 2.3 months (range: 0.1-11.4).

Fifty-nine patients (64.1%) had liver injury, which was defined as presence of steatosis (Fig 1-3), steatohepatitis, or sinusoidal dilation (Table 4). Forty-one patients (44.6%) had steatosis, 9 patients (9.8%) had steatohepatitis, and 33 patients (35.9%) had sinusoidal dilation. Advanced fibrosis was found in 5 patients (5.4%), and nodular regenerative hyperplasia was observed in 8 patients (8.7%).

Incidence of liver injury was not significantly different between the CMT group and the non-CMT group (65.9% vs. 62.5%; p=0.902). However, incidence of liver injury was significantly higher in obese patients than in non-obese patients (82.8% vs. 55.6%; p=0.022). Steatosis, steatohepatitis, and fibrosis were significantly more commonly found in obese patients than in non-obese patients (79.3% vs. 28.6%, p<0.001; 24.1% vs. 3.2%, p=0.004; and, 17.2% vs. 0%, p=0.002, respectively) (Table 5).

Of the 10 factors included in univariate analysis, only obesity was found to be significantly associated with liver injury in patients with colorectal liver metastasis (p=0.015, odds ratio=3.84) (Table 6). Of the 4 factors of interest and the 1 factor that emerged from univariate analysis (age, DM, HT, preoperative CMT, and obesity, respectively), only obesity was once again found to be significantly associated with liver injury in our study population (p=0.022, odds ratio=3.95) (Table 6).

TABLE 1. Demographic and clinical characteristics of patients diagnosed with colorectal liver metastasis.

Characteristics	n (%)
Patients	92 (100%)
Age (years), mean (range)	59.4 (48.5-70.3)
Age ≤50 years	16 (17.4%)
Age >50 years	76 (82.6%)
Gender	
Male	50 (54.3%)
Female	42 (45.7%)
Underlying disease	
DM	18 (19.6%)
HT	39 (42.4%)
DLP	20 (21.7%)
IHD	7 (7.6%)
HBV infection	4 (4.3%)
HCV infection	1 (1.1%)
BMI (mg/m^2), mean (range)	23.9 (20.4-27.4)
Non-obese (BMI <25 mg/m^2)	63 (68.5%)
Obese (BMI ≥25 mg/m^2)	29 (31.5%)
Preoperative CMT	
No	48 (52.2%)
Yes	44 (47.8%)

Abbreviations: DM, diabetes mellitus; HT, hypertension; DLP, dyslipidemia; IHD, ischemic heart disease; HBV, hepatitis B virus; HCV, hepatitis C virus; BMI, body mass index; CMT, chemotherapy

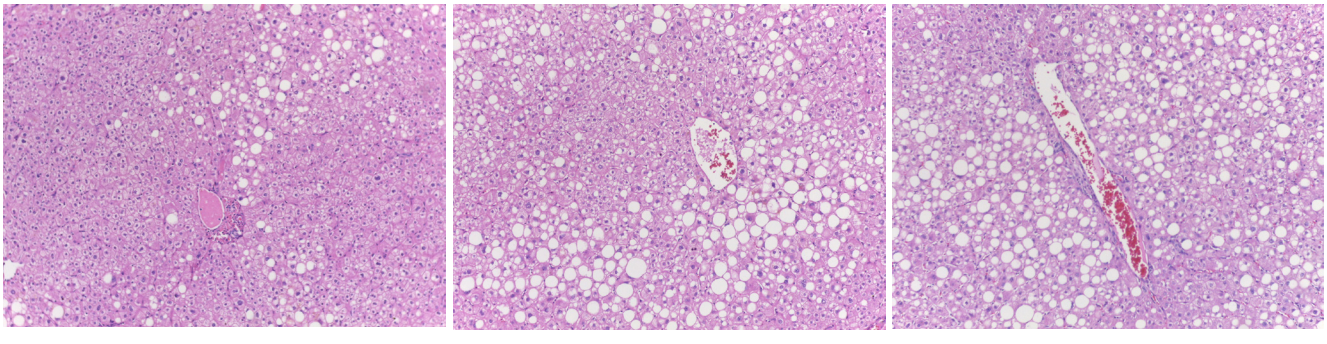


Fig 2-4: Hepatocytes show varying degree of steatosis from mild (fig 2), moderate (fig 3) and severe (fig 4). Steatosis shows characteristics large macrovesicular globules within cytoplasm of hepatocytes pushing the nuclei to the periphery.

TABLE 2. Obesity and chemotherapy status relative to other clinical factors in patients diagnosed with colorectal liver metastasis.

Characteristic	Non-CMT (n=48)	CMT (n=44)	*p*-value	Non-obese (n=63)	Obese (n=29)	*p*-value
Age (years)	61.5±9.7	57.1±11.8	0.052	59.1±10.4	60.6±12.0	0.557
Age ≤50 years	7 (14.6%)	9 (20.5%)	0.641	11 (17.5%)	5 (17.2%)	1.000
Age >50 years	41 (85.4%)	35 (79.5%)		52 (82.5%)	24 (82.8%)	
Gender						
Male	25 (52.1%)	25 (56.8%)	0.806	32 (50.8%)	18 (62.1%)	0.433
Female						
Underlying disease						
DM	11 (22.9%)	7 (15.9%)	0.560	6 (9.5%)	12 (41.4%)	0.001*
HT	23 (47.9%)	16 (36.4%)	0.363	22 (34.9%)	17 (58.6%)	0.056
DLP	14 (29.2%)	6 (13.6%)	0.121	11 (17.5%)	9 (31.0%)	0.232
IHD	3 (6.3%)	4 (9.1%)	0.706	4 (6.3%)	3 (10.3%)	0.674
HBV infection	2 (4.2%)	2(4.5%)	1.000	2 (3.2%)	2 (6.9%)	0.588
HCV infection	1 (2.3%)	0 (0.0%)	0.478	1 (1.6%)	0 (0.0%)	1.000
BMI (mg/m^2)	24.3±3.8	23.3±3.2	0.178	NA	NA	
Obese (BMI<25 mg/m^2)	19 (39.6%)	10 (22.7%)	0.130	NA	NA	
Non-obese (BMI ≥25 mg/m^2)	29 (60.4%)	34 (77.3%)		NA	NA	
Preoperative CMT						
Yes	NA	NA		34 (54.0%)	10 (34.5%)	0.130
No	NA	NA		29 (46.0%)	19 (65.5%)	

Data presented as number and percentage or mean ± standard deviation
*A *p*-value<0.05 indicates statistical significance
Abbreviations: CMT, chemotherapy; DM, diabetes mellitus; HT, hypertension; DLP, dyslipidemia; IHD, ischemic heart disease; HBV, hepatitis B virus; HCV, hepatitis C virus; BMI, body mass index

TABLE 3. Number of colorectal liver metastasis patients receiving each chemotherapy regimen.

Chemotherapy regimen	n (%)
Fluorouracil or capecitabine	15 (34.1%)
Fluorouracil or capecitabine + oxaliplatin	24 (54.5%)
Fluorouracil + irinotecan	5 (11.4%)

TABLE 4. Pathologic change of the liver in patients diagnosed with colorectal liver metastasis.

Type of pathologic change	n (%)
Liver injury	59/92 (64.1%)
Steatosis	41/59 (69.5%)
Steatohepatitis	9/59 (15.3%)
Sinusoidal dilatation	33/59 (55.9%)
Advanced fibrosis	5/92 (5.4%)
Nodular regenerative hyperplasia	8/92 (8.7%)

TABLE 5. Comparison of pathologic changes of the liver between the non-CMT/CMT and non-obese/obese groups.

Type of pathologic change	Non-CMT (n=48)	CMT (n=44)	*p*-value	Non-obese (n=63)	Obese (n=29)	*p*-value
Liver injury	30 (62.5%)	29 (65.9%)	0.902	35 (55.6%)	24 (82.8%)	0.022*
Steatosis	25 (52.1%)	16 (36.4%)	0.192	18 (28.6%)	23 (79.3%)	<0.001*
Steatohepatitis	4 (8.3%)	5 (11.4%)	0.732	2 (3.2%)	7 (24.1%)	0.004*
Sinusoidal dilatation	14 (29.2%)	19 (43.2%)	0.237	24 (38.1%)	9 (31.0%)	0.673
Advanced fibrosis	3 (6.3%)	2 (4.5%)	1.000	0 (0.0%)	5 (17.2%)	0.002*
Nodular regenerative hyperplasia	1 (2.1%)	7 (15.9%)	0.026*	6 (9.5%)	2 (6.9%)	1.000

Data presented as number and percentage
*A *p*-value<0.05 indicates statistical significance
Abbreviation: CMT, chemotherapy

CONCLUSION

In patients with unresectable colorectal liver metastasis, neoadjuvant chemotherapy can suitably reduce the size of the tumor to facilitate surgical resection.[4-6] In resectable cases, perioperative chemotherapy can increase progression-free survival.[25] Perioperative chemotherapy in colorectal liver metastasis is being used with increasing frequency, although toxicity to healthy liver tissue from these agents remains a concern.[4-6] Several previous studies reported that chemotherapy is associated with the development of pathologic lesions in liver tissue.[4-6] Peppercorn, *et al.*, found that 47% of patients treated with fluorouracil developed steatosis.[26] Vauthey, *et al.*, reported that irinotecan was associated with steatohepatitis in 20.2% of their patients.[27] In a study by Rubbia-Brandt, *et al.*, 78% of patients treated with oxaliplatin developed sinusoidal dilation.[22] Viganò, *et al.*, reported nodular regenerative hyperplasia (NRH) in 21.4% of patients who underwent liver resection for colorectal liver metastasis after administration of oxaliplatin.[28]

In this study, the incidence of liver injury was higher in the CMT group than in the non-CMT group, although the difference between groups was trivial (65.9% vs. 62.5%; p=0.902).

Importantly, the pathologic findings in patients with chemotherapy-induced steatosis/steatohepatitis are

TABLE 6. Univariate and multivariate analysis of factors associated with liver injury in patients with colorectal liver metastasis.

			Univariate		Multivariate	
	No injury	Injury	Odds ratio (95% CI)	*p*-value	Adjusted odds ratio[a] (95% CI)	*p*-value
Age (mean ± SD)	58.1±11.3	60.2±10.7	1.02 (0.98-1.06)	0.373	1.02 (0.97-1.08)	0.358
Gender						
Male	15 (45.5%)	35 (59.3%)				
Female	18 (54.5%)	24 (40.7%)				
Diabetes						
Yes	4 (12.1%)	14 (23.7%)	2.26 (0.68-7.53)	0.186	1.36 (0.35-5.31)	0.654
No	29 (87.9%)	45 (76.3%)				
Hypertension						
Yes	12 (36.4%)	27 (45.8%)	1.48 (0.62-3.54)	0.383	0.88 (0.29-2.7)	0.827
No	21 (63.6%)	32 (54.2%)				
Dyslipidemia						
Yes	5 (15.2%)	15 (25.4%)				
No	28 (84.8%)	44 (74.6%)				
IHD						
Yes	3 (9.1%)	4 (6.8%)				
No	30 (90.9%)	55 (93.2%)				
HBV infection						
Yes	1 (3.0%)	3 (5.1%)				
No	32 (97.0%)	56 (94.9%)				
HCV infection						
Yes	0 (0.0%)	1 (1.7%)				
No	33 (100%)	58 (98.3%)				
Obesity						
Obese	5 (15.2%)	24 (40.7%)	3.84 (1.30-11.35)	0.015*	3.95 (1.22-12.85)	0.022*
Non-obese	28 (84.8%)	35 (59.3%)				
Preoperative chemotherapy						
Yes	15 (45.5%)	29 (49.2%)	1.16 (0.49-2.73)	0.734	1.61 (0.64-4.08)	0.314
No	18 (54.5%)	30 (50.8%)				

[a]Analysis adjusted for age, diabetes, hypertension, obesity, and chemotherapy
Data presented as mean ± standard deviation or number and percentage
*A *p*-value<0.05 indicates statistical significance
Abbreviations: CI, confidence interval; SD, standard deviation; IHD, ischemic heart disease; HBV, hepatitis B virus; HCV, hepatitis C virus

similar and often indistinguishable from the pathologic findings caused by nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) caused by metabolic syndrome.[17] Metabolic syndrome has many definitions. All of those definitions include hyperglycemia or diabetes, hypertension, and obesity in their criteria.[29, 30] Several studies have reported a link between NAFLD and obesity. Wang, *et al.*, found that the percentage of subjects with NAFLD increased commensurately with increases in BMI (up to 81.9% in BMI >28.0 kg/m^2).[31] A study by Leite, *et al.*, reported that type 2 diabetes mellitus patients with NAFLD had a higher BMI and were more often obese than those without steatosis.[32]

This study has some mentionable limitations. First and consistent with the retrospective nature of this study, some patient data may have been missing or incomplete. Second, the size of the study population was relatively small. As a result, our study may have lacked sufficient power to identify all significant associations. Third, the patients enrolled in this study were from a single center. Finally, our center is Thailand's largest tertiary referral hospital, which means that our patients usually present with complicated and intransigent conditions. As such, it is possible that our findings may not be generalizable to patients with the same condition in other settings.

Of the ten factors evaluated for association in this study, including preoperative chemotherapy, obesity was the only factor found to be significantly associated with liver injury in patients with colorectal liver metastasis. The results of this study correspond with those reported from a previous study by Bower, *et al.*[24]

ACKNOWLEDGMENTS

The authors gratefully acknowledge Sasima Tongsai, Ph.D., of the Division of Clinical Epidemiology, Department of Health Research and Development, Faculty of Medicine Siriraj Hospital for assistance with statistical analysis.

Conflict of interest declaration: All authors declare no personal or professional conflicts of interest, and no financial support from the companies that produce and/or distribute the drugs, devices, or materials described in this report.

Funding disclosure: This study was funded by a grant from the Siriraj Research Fund, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (grant no. R015831020)

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A Novel Technique of Aluminum Multiplier Slide Culture for Fungal Identification

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ABSTRACT

Objective: Single slide culture technique (SSC) is the standard method of preparing fungal colonies for species identification. As contamination was frequently found, restarting the process wasted time and delayed diagnosis for patients. Aluminum slide culture (ASC) is the novel method, which aims to increase more fungal colonies in same time. Even through, some colonies showed contamination, there would be others left to use for identification. This study aimed to evaluate contaminate rate and time consumed in ASC technique to identify mold fungi compared to SSC.

Methods: ASC and SSC were used to identify 50 fungal colonies comprising 3 species of dermatophytes including *Trichophyton mentagrophytes, T. rubrum, Microsporum canis,* and 2 species of non-dermatophytes including *Neoscytalidium dimidiatum* and *Fusarium solani* which are common causative molds for superficial fungal skin and nail infection. Fungal colonies were microscopically examined every 5 days. Time to species identification and contamination rate were recorded and analyzed.

Results: This study demonstrated that ASC had equal efficacy in fungal identification with SSC. Median time from inoculation to fungal identification in ASC technique was 15 days which was significantly less than in SSC technique (18 days, p-value ≤ 0.05). At 15 days after inoculation, contamination rate revealed 2% in ASC whereas in SSC it was significantly higher at 16%.

Conclusion: ASC technique is an effective alternative method to prepare fungal colonies for identification. This study demonstrated that ASC uses less time to identify mold fungi and revealed lower contamination rate than SSC technique.

Keywords: Slide culture technique; fungal identification; morphological identification (Siriraj Med J 2018;70: 438-441)

INTRODUCTION

Superficial fungal infections such as skin and nail infections were caused by dermatophytes (DMPs) and nondermatophytes (NDMs). Prevalence of DMPs onychomycosis reported by previous studies was 75.9% in which *Trichophyton mentagrophytes* was the leading agent. Of patients who had NDMs onychomycosis, 17.3% were *Neoscytalidium dimidiatum* and 6.8% were *Fusarium* spp.[1] Clinical characteristics were not distinguishable between DMPs and NDMs except patients with NDMs onychomycosis showed absence of fungal glabrous skin infection in areas other than the feet. NDMs onychomycosis were considered recalcitrant to systemic treatment. Causative fungal identification was essential for making decision on patients' treatments. Early treatment could be initiated as soon as causative fungus was identified.[1,2]

Fungi culture remains the gold standard method to identify fungal species.[3] Specimens were collected from skin or subungual hyperkeratosis lesions and cultured. If fungal colonies were detected after specimen culture,

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Received 7 June 2017 Revised 18 September 2017 Accepted 20 September 2017
doi:10.14456/smj.2018.68

single slide culture (SSC) was performed for specific fungal detection. Fungus would take around 1-3 weeks to produce spores and colonies. Macroscopic and microscopic examinations were used to classify fungal species. SSC could provide well-preserved morphology of the fungus and the slide from this technique could be kept as the semi-permanent slide for studying later.[4,5] On the other hand, Tease slide and scotch-tape technique could be used to prepare colony for microscopic examination, but these techniques could disturb colony formation which resulted in difficulty to identify fungal species. Contamination was the main problem for SSC. If there was fungal contamination in SSC after culture, the process had to restart which resulted in time and money wasted. Moreover, prolonged fungal identification may lead to delay in specific treatment for patients.

Aluminum slide culture (ASC) technique is the novel innovative method, which aims to solve contamination problems in SSC. Generally, SSC contained one set of slide and cover glass for one colony formation. Conversely, ASC had 4 sets of 4 cover glasses for four colony formations. If contamination was found in one colony in ASC, there were others left to use for fungal identification without restarting process. This study aimed to evaluate time consumed for fungal identification and contamination rate of ASC, compared with SSC. This study will demonstrate the efficacy of ASC which will be a promising method for colony culture in general mycology laboratories.

MATERIALS AND METHODS

Specimen preparation procedures

Specimens were collected from scale or sunungual hyperkeratosis lesions of patients with superficial fungal infections. The specimens were placed in sabourauds dextrose agar (SDA) with cyclohexamide and chloramphenicol and SDA with chloramphenicol and inoculated at 25-30°C and 30% humidification. Normally, fungus takes around 1-3 weeks to form the specific colonies. Five fungal species, common causative species for superficial skin and nail infections including *Trichophyton mentagrophytes, T. rubrum, Microsporum canis, Neoscytalidium dimidiatum* and *Fusarium solani* were used in this study. Each species were performed 10 times therefore in total 50 plates were performed for ASC and 50 plates for SSC techniques.

Single slide culture technique (SSC)

A sterile glass slide and v-shaped glass rod on the sterile petri plate was prepared as Fig 1A. SDA, block sized 1x1 cm^3 was placed in the middle of the glass slide. Sterile needle was used to inoculate fungal colony to all 4 sides of SDA block and cover glass was put on the top of SDA block. Some sterile water was filled into the petri plate to prevent the drying of SDA block. The slide was incubated at 26°C.

Aluminum slide culture technique (ASC) 87

A sterile aluminum block with 4 cover glasses and 4 SDA blocks was placed on the sterile petri plate. Each SDA block sized 1x1 cm^3 were placed in the middle of each cover glass (Fig 1B). Sterile needle was used to inoculate fungal colony to all 4 sides of every SDA block and cover glass was put on the top of every SDA block. Some sterile water was filled into the petri plate to prevent the drying of SDA block. The slide was incubated at 26°C.

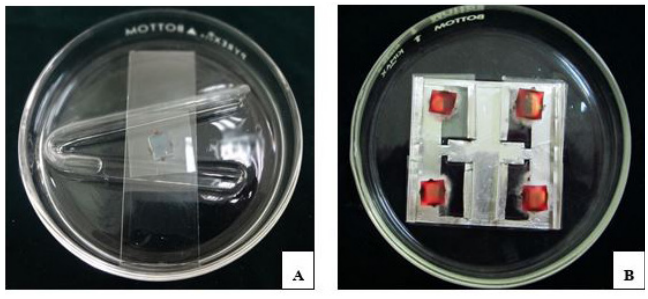


Fig 1. Single slide culture technique (SSC) and Aluminum slide culture technique (ASC).
A) SSC, the standard method of preparing fungal colonies for species identification. A sterile glass slide and v-shaped glass rod was put on the sterile petri plate as figure. Placed 1x1 cm^3 block SDA in a middle of the glass slide, inoculate 4 sides of SDA with fungal colony and put the cover glass on the top of SDA block. B) ASC, the novel method of preparing fungal colonies. A sterile aluminum block with 4 cover glasses and 4 SDA blocks was placed on the sterile petri plate as figure. Each SDA block sized 1x1 cm^3 were placed on a middle of each cover glass. Inoculate fungal colony to all 4 sides of every SDA block and cover glass was put on the top of every SDA block.

Interpretation and analysis of the test

Growth and contamination of the fungal colonies were examined every 5 days under low power microscopic examination (10x). After 15 days of inoculation, cover glasses were removed from sterile plate, placed on to the glass slide and examined under lacto phenol cotton blue and under light microscope.

Measurement of time consuming for fungal identification

During inoculation period, if fungal contamination was detected in SSC technique, restart of SSC technique was required to identify species. On the other hand, in ASC technique, even if some specimens were contaminated, the others would be left to use to identify fungal species. Except for all blocks of ASC were contaminated, restart of ASC technique was not performed. Duration from

inoculation to fungal identification was collected and analyzed.

Measurement of contamination rate

Contamination was reported every 5 days if there was other fungal growth in SDA blocks in both techniques. Regarding ASC, if any one block was contaminated, contamination rate was reported immediately. Contamination rate and the day that contamination was found were collected and analyzed.

Statistical analysis

Mann-Whitney U test and Fisher's exact test were used for analyses of time consumed to identify fungi species and contamination rate, $p \leq 0.05$ was considered as significant difference.

RESULTS

In total 50 plates of ASC and 50 plates of SSC were performed in this study. Fungal identification from ASC technique showed the same result with SSC techniques, which represented equal efficacy for both techniques. Median time from inoculation to fungal identification in ASC technique was 15 days while in SSC technique it was 18 days. Median time from inoculation to fungal identification in ASC technique was significantly less than in the SSC technique (p-value ≤ 0.05). From the SSC technique, there were 2, 3 and 3 specimens had contamination and had to restart the whole process on day 5th, 10th and 15th after inoculation.

Rate of contamination was shown in Table 1. After 5 days of inoculation, contamination was found in 2 (4%) *T. rubrum* slides in SSC and 1 (2%) *T. rubrum* slide in ASC technique. On the 10th day, contamination was revealed in 5 (10%) slides in SSC technique comprising 2 *T. rubrum* slides, 1 *T. mentagrophytes* slide, 2 *M. canis* slides; whereas there was only 1 (2%) *T. rubrum* slide in ASC technique. On the 15th day, contamination was demonstrated in 8 (16%) slides in SSC including 2 *T. rubrum* slides, 2 *T. mentagrophytes* slides, 4 *M. canis* slides which was significantly different from 1 (2%) *T.rubrum* slide in ASC technique with p-value =0.031.

DISCUSSION

SSC technique was the standard method of preparing fungal colonies for species identification. ASC is the novel method, aiming to produce more fungal colonies in the same time. This study demonstrated that ASC had equal efficacy on fungal identification as SSC. However, median time from inoculation to fungal identification in ASC technique was significantly shorter than in SSC technique. At 15 days after inoculation, contamination rate revealed 2% in ASC whereas in SSC it was found to be significantly higher at 16%. This study demonstrated that ASC technique would be an effective alternative method to prepare fungal colonies for identification.

SSC technique was recommended by Lewis and Hopper in 1943 as the standard method of preparing fungal colonies for species identification with well-preserved morphology of the fungi. Each SSC technique provided one fungal colony to use for identification. In clinical practice at mycology laboratory, contamination was the main problem in SSC technique. Time and materials would be consumed more when the whole process required restart. Delayed fungal identification leads to difficulty in diagnosis and treatment. Modified culture slide technique was previously reported by Rosana *et al.*,[6] which placed the agar directly on the sterile plate and inoculated fungal colony at 4 sides of agar then cover glasses were put on the top surface of the agar. Another technique used inoculation of the mole fungi directly on the sterile petri plate and place the agar on top later. However, ASC was different from other modified techniques. ASC was invented as the new technique to increase slides with the aluminium block at the same inoculation time.

TABLE 1. The comparison of contamination rate in SSC and ASC technique.

Day	Contamination rate (%)		*p*-value
	Slide culture technique (N=50)	Aluminum slide culture technique (N=50)	
Day 5	2 (4%)	1 (2%)	1.000
Day 10	5 (10%)	1 (2%)	0.204
Day 15	8 (16%)	1 (2%)	0.031*

*p-value <0.05 indicates statistical significance

Generally, SSC technique provided one slide for fungal identification; while ASC technique could produce 4 slides in the same time. Therefore, ASC had greater chances of success in mold fungi identification, but used less storage space, so the cost benefit should be greater.

According to efficacy for fungal identification, ASC technique was equivalent to the standard technique; SSC technique. This confirmed that ASC technique could be an alternative technique to culture fungi to use for identification. Median time from inoculation to fungal identification in ASC technique was significantly less than the SSC technique (p-value ≤ 0.05) in this study. Due to 4 slides available for colony formation in ASC technique, even if one slide got contaminated, the others slides could be continuously used for fungal documentation. Rapid and early fungal identification could be obtained from ASC which were valuable for patients' diagnosis and treatment. Cost reduction between ASC and SSC would need to be further elucidated.

DMPs and NDMs which were common causative agents for superficial skin and nail infections were studied in this report. Further study would be required to demonstrate efficacy for fungal identification in slow growing molds such as *Magnaporthe grisea* and *Exophiala jeanselmei* causing eumycetoma,[7-9] *Pocillopora verrucosa, Fonsecaea compacta* and *Fonsecaea pedrosoi* causing chromoblastomycosis.[9-12] and *Euphausea spinifera* causing phaeohyphomycosis. Due to slow growing molds, ASC is expected to significantly reduce time for fungal identification compared with SSC.

For the contamination rate, this study showed that ASC technique has significantly less contamination rate compared with SSC technique. In point of fact, ASC technique required to open the cover of petri plate for longer time than the SSC technique because inoculation had to be done for 4 SDA blocks in ASC, compared to only 1 SDA block in SSC. Formerly, contamination rate was expected to be higher in ASC due to prolonged opening of plate. However, this study did not show higher contamination rate in ASC, conversely this study reported that rates of contamination were the same on the 5th and 10th days and significantly lower in ASC on the 15th day. All processes in this study were done by one experienced technician and inoculations in the 2 techniques were done at the same time. Manual error was minimized in this study, so the reason for lower contamination rate with ASC may need further study. Unfortunately, from our literature review, data regarding contamination rate from other modified culture techniques was limited to comparisons with ASC technique.

CONCLUSION

The ASC technique is a novel method of preparing fungal colonies for species identification. The quality of identification of mold fungi is equivalent compared with the SSC technique, but better in less contamination rate and less time consumed to identify mold fungi species.

ACKNOWLEDGMENTS

The authors would like to thank Assistant Professor Dr. Orawan Supapueng for her advice about statistical analysis and Department of Dermatology, Siriraj Hospital for support for the research and laboratory in this study.

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Responsiveness and Minimal Clinically Importance Difference of Thai QuickDASH in Individuals with Adhesive Capsulitis

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ABSTRACT

Objective: To explore consistency between clinical outcomes derived from patient self-assessment and physical therapy evaluation. Also, to determine the minimal clinically importance difference (MCID), responsiveness, and optimal cut-off point specific to adhesive capsulitis for conditions of high and low disability, using the Thai version of QuickDASH.

Methods: A cohort study was conducted in two hospitals over six weeks of physical therapy. Eighty-seven adhesive capsulitis patients participated in this study. Clinical outcomes provided by participant self-assessment using Thai QuickDASH and Global Rating of Change (GRoC) scales were compared with irritability outcomes provided by a physical therapist. Thai QuickDASH is the validated outcomes measurement tool for adhesive capsulitis and GRoC is recommended as a standard external anchor. This anchor-based method and receiver-operating characteristics (ROC) were analysed to clarify MCID and responsiveness.

Results: The highest correlation was between the Thai QuickDASH and pain during passive shoulder elevation (rho = 0.638). MCID scores ranged from 9.1 to 20.4. The optimal cut-off point between high and low disability was 31.8, attained by a combination of two references; one was a score of at least GRoC+1 and the other was irritability outcome defined by pain during passive shoulder elevation.

Conclusion: Responsiveness and MCID measured by the Thai QuickDASH is specific to adhesive capsulitis. A combination of GRoC as a standard external anchor and irritability outcome gave a precise cut-off point for improvement. Overall management of adhesive capsulitis, including continuing evaluation, treatment justification, and decision planning, should be based on qualified confirmation of MCID and responsiveness.

Keywords: MCID; adhesive capsulitis; Thai QuickDASH; irritability outcome (Siriraj Med J 2018;70: 442-448)

INTRODUCTION

Adhesive capsulitis is a significant cause of pain and function deficit due to pathomechanics of the Glenohumeral (GH) joint and surrounding tissue.[1] Management of adhesive capsulitis needs to focus on change over time or responsiveness in the context of both pain and function outcomes. Responsiveness, identified by a combination of physical therapist evaluation and patient perception of improvement, corresponds to true change.[2] An outcome evaluation by diagnosing level of tissue irritability was used in the study; this is a clinical practice guideline for adhesive capsulitis,[3] providing assessment of motion and activity limitations associated with patient pain which enhances the effectiveness of outcomes and intervention plans. Moreover, the Global Rating of Change (GRoC) scale, a standard patient self-

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Received 4 April 2018 Revised 26 July 2018 Accepted 24 August 2018
doi:10.14456/smj.2018.69

reporting instrument for direct perception of improvement, was used as a reference standard or external criterion of change for clinical outcome measurement.[4] Despite the subjectivity of perception, clinical outcomes must take into consideration reliable sources of information including validating measurement tools. Consequently, the QuickDASH self-reporting questionnaire was used. QuickDASH is a short version of the disability of arm shoulder and hand (DASH) outcome measure, and the most widely used patient outcome measurement tool in both clinical practice and research, providing evaluation of symptoms and pain. Interpretation of responsiveness of outcome is confirmed for various shoulder pathologies[5] and is clarified by measuring against the minimal clinically importance difference (MCID),[6–8] except for adhesive capsulitis. The Thai version of QuickDASH[9,10] (Thai QuickDASH) has high reliability ($ICC_{2,1}$ = 0.83; 95% CI 0.515, 0.947), moderate correlation (rho = 0.733) with pain score,[11] and validation for adhesive capsulitis.[12] Clinical outcome, judged by patient self-evaluation and physical therapists, for responsiveness and discrimination of high and low disability in the context of adhesive capsulitis have not yet been confirmed. To determine clinically meaningful change, comparing the responsiveness of Thai QuickDASH to changes in other variable-related measures of outcome is a useful approach to this issue. The objectives of this study were to explore consistency between clinical outcomes derived from patient self-assessment and physical therapy evaluation and to use Thai QuickDASH to clarify MCID and responsiveness in adhesive capsulitis. For discrimination of high and low disability, an optimum cut-off point of Thai QuickDASH needed to be determined.

MATERIALS AND METHODS

Study design and participants

A cohort study was conducted in the physical therapy units of two hospitals in Thailand. Six weeks of physical therapy treatment were performed to reduce pain and increase range of movement, using modalities, mobilisation, and exercise therapy. For each participant, an average of one hour for each session of treatment and two treatment sessions per week were assigned by physical therapists. All participants signed informed consent documents before participation. A physical therapist (JR) with more than 10 years clinical experience enrolled participants using purposive sampling and conducted evaluation procedures for this study. Inclusion criteria were adhesive capsulitis as follows.[7]

1. The GH joint was limited in multiple directions, especially in passive range of motion (ROM) of external rotation (ER). Most movements were restricted whilst evaluating accessory movements or joint glides.
2. The GH joint had evenly decreased ROM in ER and internal rotation (IR) when the arm was abducted between 45 and 90°.
3. Pain was reproduced at the end ranges of passive GH motion.

Exclusion criteria were evidence of fractures of the humerus or clavicle or pathological disease in brain, central, or peripheral nervous system.

This study was approved by the Ethics Committees for Human Research in both hospitals (No.12/2558).

Procedure

For consistency of clinical outcomes, physical therapist assessment and participant self-assessment were conducted in parallel. Four assessments were recorded pre-treatment to provide a base line (in the 1st week) and after treatment in the 2nd, 4th, and 6th week. The physical therapist (JR) recorded 2 clinical outcomes, including GH active range of motions (AROMs), as standard examination and irritability outcomes. All AROMs were repeated three times and measured by a digital inclinometer with tolerances of ± 0.1°. The intra-rater reliability test of all AROMs was presented in a previous study.[12] Test-retest reliability results were good to excellent ($ICC_{3,1}$: 95% CI range from 0.835 to 0.987). The duration of symptoms from their onset was also recorded. Irritability outcome evaluation was adapted from diagnosis of tissue irritability level,[5] and was used as an objective outcome assessment in this study. Irritability outcome is composed of irritability level and pain pattern. Irritability level was categorised into three levels on a numeric rating scale comprising a 10 cm-straight line with even gaps; zero is no pain and 10 corresponds to extreme pain. High irritability level was 7-10, moderate level was 4-6, and low level was 0-3. Pain pattern was also categorised based on the extent of pain during the night and during passive shoulder elevation. Patterns of pain at night were subcategorised as: consistent throughout, intermittent, and no pain. Pain during passive shoulder elevation was also defined as aggravated before end range, at end range, and overpressure into end range. The data collection procedure is shown in Fig 1.

Clinical outcomes were evaluated by participant self-assessments consisting of Thai QuickDASH and GRoC. In the Thai QuickDASH scale, the minimum score of 0 is defined as no pain or tingling in the arm, shoulder, or hand, and no difficulty or limitations in actively using the limb, and the maximum score of 100 represents the most severe pain or tingling in the arm or shoulder, and the

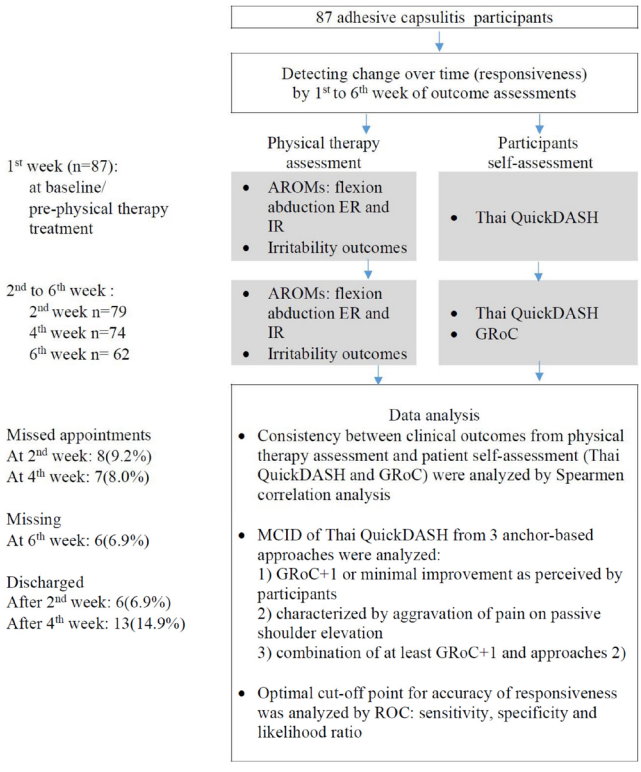


Fig 1. Flow chart of procedure.

most severe difficulty or inability to use the arm actively. The GRoC 7-point Likert scale was used as a standard external anchor to rate change after each treatment. A GRoC of zero was considered as unchanged. GRoC+1, +2, and +3 represented minimal, moderate, and large improvements respectively. In contrast, GRoC -3, -2, and -1 indicated perception of deterioration. New recording forms and Thai QuickDASH questionnaires were used for each assessment, thus previous outcomes were blinded to the physical therapist (JR) and participants.

Data analysis

Only completed Thai QuickDASH questionnaires were analysed, representing completion of 10 of the 11 items. All participants' outcomes were analysed for each follow-up. To determine improvements in irritability outcome from the adhesive capsulitis baseline, data was analysed using Chi square. The average AROM for each motion was recorded. Repeated measures for improvement of AROMs were analysed using ANOVA and Bonferroni correction methods with expected significance values of $p < 0.05$. Consistency between self-assessments and physical therapy assessment outcomes were analysed using Spearman's correlation. The MCID was reported by median and 95% CI on changes of Thai QuickDASH score. The anchor-based method was used to determine MCID and responsiveness. The anchor-based analysis used three approaches based on two specific criteria from this study. In the first approach, MCID was defined as GRoC+1, or minimal improvement as perceived by participants. In the second approach, MCID was defined by passive shoulder elevation, physical therapy and characterised as no pain or aggravation of pain (either before end range, at end range, or with over-pressure into end range). The third approach was a combination of a rating of at least GRoC+1 and the second approach. Comparisons of MCID and responsiveness from all approaches were analysed. To detect all measures of responsiveness in this study, MCID and ROC analysis were conducted on all outcomes of completed follow-up after treatment in the 2nd, 4th, and 6th week. ROC, area under the curve (AUC), sensitivity, specificity, and likelihood ratio (LR) analysis was used to produce an optimal QuickDASH cut-off point between high and low disability, for adhesive capsulitis during the six weeks of physical therapy.

RESULTS

The characteristics of the 87 adhesive capsulitis participants are shown in Table 1.

TABLE 1. Characteristics of the participants.

Characteristics of participants (n=87)		
Gender: Male/Female	n (%)	22 (25.3) / 65 (74.7)
Age (years)	Mean (SD)	55.1 (6.2)
Duration of symptom (weeks)		19.8 (15.2)
Pain intensity	Median (95%CI)	
Resting pain (NRS 0-10)		0 (0-2)
During activity (NRS 0-10)		6 (5-8)

Fig 2 shows that participants had irritability levels distributed from high to low levels, as shown by the baseline of irritability outcome (A). The overall presentation of pain pattern is shown in (B) and (C). Compared to baseline, significant improvements ($p < 0.001$) were found in irritability level, pain pattern, and AROM in flexion, abduction, ER, and IR after six weeks of treatment. Thai QuickDASH scores for each follow-up showed consistently significant values with regard to improvement ($p < 0.05$) (F). Spearman's correlation coefficients (rho) revealed significant correlation between participant self-assessments and physical therapy assessments ($p < 0.05$), these ranged from low to moderate (0.2-0.638 or less than 0.7), as shown in Table 2.

The MCID of Thai QuickDASH by participants who expressed minimal improvement (GRoC+1), ranged from a score of 9.1 to 13.6 (Fig 3). For cases of pain aggravated with overpressure into end range by passive shoulder elevation (lower irritability), the MCID was at least 15.9. For participants that showed improvement, but where pain was more easily aggravated than the pain presented before end range or at end range (higher irritability), the MCID score was at least 4.6. The score resembled the MCID of QuickDASH, defined by the combination criteria of pain presented before end range, or at end range with at least a score of GRoC+1. Alternatively, the combination criteria of at least GRoC+1 with physical therapy assessment of pain with overpressure into end range, the MCID was a trade-off and was at least 18.1.

All 215 of the completed follow-up outcomes were used in the ROC analysis (Fig 4). The QuickDASH cut-off point for disability of adhesive capsulitis by at least GRoC+1 was 38.4. Sensitivity and specificity were 60.0% and 73.3%, respectively (Fig 4a). For the next cut-off point, 31.8, based on the criteria of physical therapy assessment where pain presented with overpressure into end range, the sensitivity and specificity were 81.1% and 76.7%, respectively (Fig 4b). The score of 31.8 was the same value as the cut-off point defined by the combination criteria of at least GRoC+1 of pain presented with overpressure into end range, and here sensitivity and specificity were 76.6% and 78.7%, respectively (Fig 4c). In the comparison of AUCs, the highest AUC (95% CI) was 0.856 (0.802, 0.900) obtained by the third approach. LR+ (95% CI) was 3.6 (2.47, 5.25) and LR- was 0.3 (0.21, 0.42). During the 2nd and 4th week, 8 (9.2%) and 7 (8.0%) participants were absent, respectively, but they returned to physical therapy and continued through to the last follow-up, in the 6th week. In addition, 6 (6.9%) and 13 (14.9%) of the participants were discharged after the 2nd and 4th week of treatment because their improvement was acceptable. Their Thai QuickDASH values had reduced from baseline, the median (95% CI) scores of the changes which were 27.3 (52.3; 15.9) and 22.7 (43.2; 11.3), respectively. However, 6 (6.9%) participants who showed no improvement were absent at the last follow-up.

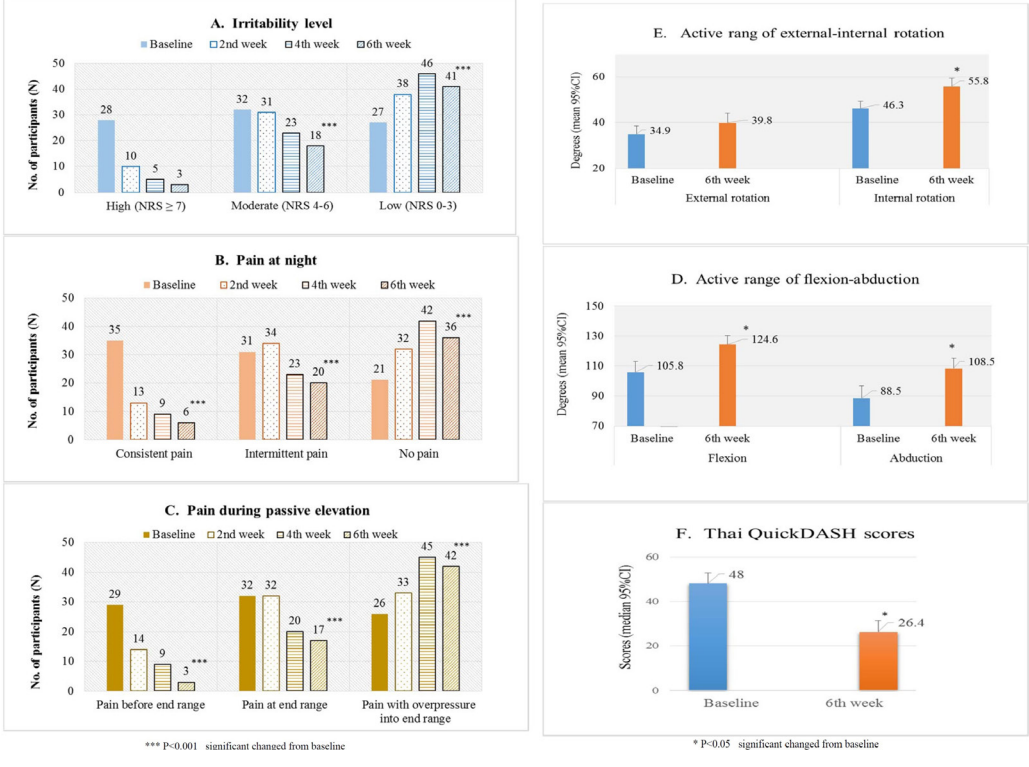


Fig 2. Clinical outcomes of adhesive capsulitis participants: at baseline and the follow-ups
A. Irritability level B. Pain at night C. Pain during passive elevation D.–E. Active range of motion of flexion-abduction and external-internal rotation F. Thai QuickDASH scores.

TABLE 2. Correlation between clinical outcomes of participant self-assessments and physical therapy assessments (total six weeks of follow-ups n = 302).

Self-assessment outcomes	Physical therapy assessment outcomes						
	AROM				Irritability outcomes		
	Flexion	Abduction	External rotation	Internal rotation	Irritability level	Pain during passive elevation	Pain at night
QuickDASH score	-0.541*	-0.566*	-0.358*	-0.338*	0.538*	0.638*	0.581*
GRoC+1	0.267*	0.332*	0.200*	0.237*	-0.346*	-0.350*	-0.341*

*significant p-value <0.05

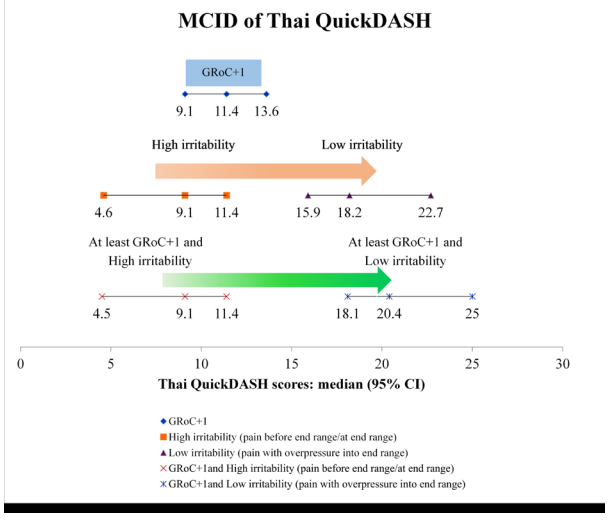


Fig 3. Comparison of the minimal clinically importance difference (MCID) of the Thai QucikDASH, measured by different approaches.

	Criteria		
	At least GRoC +1	Irritability: pain during passive elevation	Combination of at least GRoC +1 with Irritability: pain during passive elevation
All Thai QuickDASH scores of the completed follow-up outcomes (n= 215)			
	a	b	c
Area under curve (95% CI)	0.761 (0.700, 0.818)	0.850 (0.796, 0.896)	0.856 (0.802, 0.900)
Sensitivity/Specificity (%)	60.0/73.3	81.1/76.7	76.6/78.7
LR+/LR- (95% CI)	2.25 (1.56, 3.24)/ 0.55 (0.36, 0.83)	3.47 (2.48, 4.87)/ 0.25 (0.16, 0.38)	3.6 (2.47, 5.25)/ 0.3 (0.21, 0.42)
Cut-off points of Thai QuickDASH score	38.4	31.8	31.8
Discrimination of high and low disability by cut-off point: n (%)			
	High disability	Low disability	High disability	Low disability	High disability	Low disability
≥	21(30.4)	48(69.6)	77(73.3)	28(26.7)	82(78.1)	23(21.9)
<	14(9.6)	132(90.4)	18(16.4)	92(83.6)	25(22.7)	85(77.3)

≥ was at least or above the cut-off point
< was less than the cut-off point

Fig 4. Comparison of ROC and the Thai QuickDASH cut-off points for discrimination of high and low disability by at least GRoC+1, irritability (pain during movement), and combination of at least GRoC+1 with irritability (pain during movement).

DISCUSSION

Clinical outcome evaluation of adhesive capsulitis during six weeks of physical therapy was significantly improved in three ways. Firstly, significantly improved functional outcomes, represented by responsiveness, were greater than the Thai QuickDASH MDC. Secondly, there were three aspects of decreased irritability outcomes: significantly lowered irritability levels, more participants experiencing no pain at night, and pain with overpressure into end range of passive shoulder elevation. Thirdly, increased AROM was observed in flexion, abduction, and internal rotation (although, external rotation was not affected). All of these changes reinforced the consistency of the measurement methods.

The distribution of participant's characteristics in this study covered all three phases of adhesive capsulitis. Irritability outcomes represented by irritability level, pain at night, and pain during passive elevation showed significant improvement ($p < 0.001$). The highest degree of consistency between self-assessment outcomes and physical therapy evaluation was observed with Thai QuickDASH and pain during passive shoulder elevation, whereas GRoC revealed lower levels of correlation with all the physical therapy assessment outcomes. These findings suggest that, in adhesive capsulitis, pain during passive shoulder elevation may be another optimal external reference for the analysis of actual change.

In respect of the progression of adhesive capsulitis, the patient gains more function at stages of less irritability. The variety in condition of low to higher irritability which corresponds to the range of MCID may be a more practical measure to assess clinical change.[3] Therefore, we suggest that MCID scores between 9.1 and 20.4 may be a more suitable way to confirm that participants have shown true improvement from baseline, whilst perhaps still suffering from irritability. It is worth noting that this range of MCID included professional information on patient perception. The use of MCID values less than 9.1 are not recommended because they were in the range of MDC (MDC_{90}, or measurement error was 7.4)[11] and a valid MCID has to be at least as large as the MDC.[2] Compared to improvements from baseline for less severe conditions, or where pain exists with overpressure, the MCID score was at least approximately 18.1 (twice the lower bound of the MCID). The range of MCIDs in this study, were provided by the longitudinal study design that was suitable for assessing changes across time for adhesive capsulitis.

For discrimination between the high and low disability effects of adhesive capsulitis, the Thai QuickDASH cut-off point of 31.8 was recommended, this was consistently confirmed by both patient and physical therapy assessment during six weeks of treatment, as shown in the ROC results. The ROC provides an indication of the relationship between Thai QuickDASH and the criteria for defining improvement of outcome and decrease in disability. Three approaches were analysed in this study. The first approach was GRoC, which is recommended as an external standard for measuring change,[7] which showed high validation of clinician and patient-rated change (rho = 0.87).[4] The second approach, which was pain with overpressure into end range on passive elevation,[2] evaluated by the physical therapist, was adapted for quantifying objective assessment. The combination of a rating of at least GRoC+1 and the second approach achieved more consistent outcomes by adjusting the information from both participant perception and professional evaluation. Throughout, internal consistency was confirmed and error from recall bias or bias from over- or under-estimates was under control. As a consequence of the combination approach, specificity was improved to 78.7% and the highest AUC of 0.856 was achieved (95% CI 0.802, 0.900). This also represented true improvement by integrating the overall improvements and led to a decrease in the false positive rate to 21.9%. The AUC gives the range of values in which the true value lies. An AUC of less than 0.5 represents a high chance of incorrectly classifying participants with improvements as positive, and participants with non-improvements as negative; the lower limit of the 95% CI of AUCs for Thai QuickDASH of this study was at least 0.7.

In addition, the LR of any clinical finding is discrimination between patients with and without a specific condition. LR+ increases the probability of an event or outcome, once the test is positive. In contrast, LR- decreases the probability of an event or outcome, once the test is negative. Moreover, LR is defined in terms of sensitivity and specificity as: LR+ = sensitivity/(1-specificity) and LR- = (1-sensitivity)/specificity. The LR range is from 0 to infinity. In this study, the LR+ and LR- (with upper and lower limits of 95% CI) were 3.6 (2.47, 5.25) and 0.3 (0.21, 0.42), respectively, and this means that improvement during six weeks of physical therapy for adhesive capsulitis, determined by a Thai QuickDASH cut-off point of 31.8, is clinically significant. The discrimination of improved from non-improved participants may be of practical value in clinical situations, and applicable to the progression of adhesive capsulitis.

The responsiveness of QuickDASH in this study and Chester et al.,[13] were both evaluated by using the GROC 7-point Likert scale as an external standard. Moreover, both studies consistently supported the concept of dissimilar

responsiveness being found between improvers and non-improvers. However, some of the methods used were different since we rated clinical improvement of at least GRoC+1, but Chester et al., started with GRoC+2. ROC analysis in this study was used to discriminate high disability from low disability, whereas Chester et al., used this analysis to confirm MCID. In addition, our responsiveness results were anchored by a combination of irritability level and GRoC. Chester et al., used each increment of GRoC. Lastly, our MCID and responsiveness were specific to adhesive capsulitis, not revealed for shoulder pain of musculoskeletal origin as in Chester et al.

Limitations of the study

Our study had some limitations. Passive range of motion was not reported as a clinical outcome, although we implied actual movement of all patients by assessing pain during passive shoulder elevation. A further limitation was that the assessor was not blinded.

Weak correlation of AROMs, irritability outcomes, and GRoC may reflect relative differences of the expected outcomes by patient perception and physical therapist assessment.

CONCLUSION

The specific range of MCID established in this study represented true improvements in irritability outcome from baseline, while the ability to discriminate between high and low disability clarified the severity of functional disability. These MCIDs represented both clinical and statistical significance ($p < 0.05$). Moreover, these clinical outcomes were achieved by a combination of evaluations from patients' perception and professional experience. The specific range of MCID and the Thai QuickDASH cut-off point for adhesive capsulitis may optimise benefits for patients.

ACKNOWLEDGEMENTS

We are thankful to all participants, the Faculty of Physical Therapy of Srinakharinwirot University for funding, Asst. Prof. Junya Pattaraarchachai for advice on statistics, and Dr. Jeeranan Rapipong, Dr. Montana Buntragulpoontawee, Dr. Siam Tongprasert, Faculty of Medicine, Chiang Mai University, for permission to use translated Thai QuickDASH for this study. This research did not receive specific grants from funding agencies in the public, commercial, or not-for-profit sectors.

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Extra-Oral and Non-Genital Chronic Herpes Infection in an HIV-Infected Patient: A Case Report

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ABSTRACT

Objective: To report a case of uncommon site of chronic herpes infection in an HIV-infected male.

Case presentation: A 19-year-old Thai HIV-infected male who was diagnosed with chronic herpes simplex infection located on the scalp and face without lesion in oral and genital areas.

Conclusion: Chronic herpes simplex infection is predominantly located in oro-genital regions and presented as ulcerative lesions that frequently recur in immunocompromised patients. The prevalence is not known and HIV infection is a major risk factor. Misdiagnosis is common and might lead to delay of treatment. Oral acyclovir is the first line of treatment, although resistance to acyclovir has been found to be increasing.

Keywords: Chronic herpes infection; HIV; non-genital lesion (Siriraj Med J 2018;70: 449-451)

INTRODUCTION

Chronic herpes simplex infection (CHSV) is one of the atypical manifestations of herpes infection among immunocompromised host. CHSV manifests as wart-like and/or ulcerative mucocutaneous lesions lasting for at least 1 month.[1,2] The prevalence of CHSV is still unknown. In a cohort study, 0.2 percent of HIV-infected patients were reported to have this condition, and interestingly all lesions were located in genital area.[3] Misdiagnosis is common and might lead to delay of appropriate treatment.[4] To date, there have been a few cases of CHSV located outside oral and genital area.[4] We reported an HIV-infected patient diagnosed as CHSV whose lesions were located on the scalp and face without oral or genital lesions.

CASE REPORT

A 19-year-old heterosexual male was diagnosed HIV-positivite in 2014 which was controlled with triple anti-retroviral therapy (zidovudine, tenofovir and lopinavir/ritonavir). In July 2016, he presented to our skin clinic with two large well-demarcated ulcerated plaques that showed peripheral crusting on the scalp and right cheek. The lesions had developed over a period of one year (Fig 1). On this visit, his CD4 count and viral load respectively were 144 cells per microlitre and 1.8 million copies per ml.



Fig 1. Two large well-demarcated ulcerated plaques with peripheral crusting on the scalp and right cheek.

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Received 20 October 2017 Revised 5 June 2018 Accepted 10 August 2018

doi:10.14456/smj.2018.70

He had a history of complete course of pulmonary tuberculosis treatment in 2015 and also had herpes zoster infection on his right thigh 4 months ago that was successfully treated with oral acyclovir 800 mg five times a day for one week. Surprisingly, no history of herpes simplex had been reported before this visit.

On the date of visit, Tzanck smear at both lesions on his face showed negative finding for acantholytic cell or multinucleated giant cell. Direct fluorescent for HSV antigen detection assay from lesions on the scalp, forehead and cheek was positive, but negative for VZV. Blood for HSV and VZV IgG was positive, while blood for HSV and VZV IgM was negative. Tissue culture at scalp was positive for *Staphylococcus aureus* that was susceptible to methicillin and no growth for mycobacterium. Finally, this patient was diagnosed with CHSV with superficial bacterial skin infection. Treatment of oral acyclovir 400 mg per oral three times a day, dicloxacillin 2000 mg per day and topical antibiotics were prescribed. During 3 weeks of treatment, all lesions showed no improvement, but expansion of lesions was observed (Fig 2). Therefore, oral acyclovir 800 mg 5 times a day was given to the patient. After 2 months of treatment, the lesions gradually improved. All lesions were cleared with depressed scar and scarring alopecia was observed on scalp area. There was no new lesion developed after two months of follow up (Fig 3).



Fig 2. Chronic herpes simplex infection lesions after 3 weeks of oral acyclovir and oral/topical antibiotics treatments.



Fig 3. The lesions at follow-up visit after two months of high-dose oral acyclovir (zoster dosage regimen).

DISCUSSION

Chronic herpes simplex infections (CHSV) frequently recur with multiple small to extensive hyperkeratotic or ulcerative lesions in immunocompromised patients, including HIV infection.[5] However, no direct association between the CD4-positive count and the occurrence of CHSV had been observed.[5] The lesions are predominant at anogenital sites which persist for at least 1 month.[1] Wart-like CHSV is usually found on the skin. The ulcerative form, on the other hand, nearly always involves the mucosa.[4] Apart from oral and anogenital ulcerative lesions, they are typically asymptomatic.[5] These lesions are difficult to obtain the swab for cell culture or Tzanck smear; thus, DNA detection should be done to confirm the diagnosis.[6,7] The bacterial or Candida species colonization may present. Related internal dissemination or any fatal cases have not been reported.[5] The pathogenic mechanism remains unclear. A genetic predisposition seems likely.[3] An epidermal growth factor-dependent pathway and inadequate immune response have also been proposed.[8] Initially, CHSV should be treated with oral acyclovir at a dose of 200 mg five times a day for 1-2 weeks[5],although for disseminated or severe herpes infections, the treatment of choice is intravenous acyclovir 5-10 mg/kg every 8 hours. If acyclovir resistance is suspected, drug susceptibility test should be requested.[9] In case of treatment resistance, the dose should be increased to 800 mg at the same frequency and duration. If no improvement is seen, oral valacyclovir or famciclovir, topical foscarnet, and combination therapies (topical, oral, intravenous) should be

considered respectively. Cryosurgery, topical imiquimod, and oral thalidomide are the alternative steps. Resistance to thymidine kinase (TK)-dependent antiviral agents is one of the most common complications of CHSV. Hyperpigmentation, hypopigmentation and scarring are frequently observed after healing.[5] HIV-infected patients receiving suppressive acyclovir therapy were found to have lower HIV RNA levels,[10] but long-term prophylaxis can lead to the development of drug-resistance.[11]

As with our case, the patient had two large lesions of chronic herpes simplex infection lasting for a year. The lesions were typically asymptomatic and ulcerative, but located on an uncommon location. Tzanck smear was negative whereas HSV antigen detection by direct fluorescent antibody was positive. However, sensitivity of Tzanck smear and culture from ulcerative lesions are usually very low, so HSV DNA detection should be requested to confirm diagnosis. There was superimposed staphylococcal infection without any internal dissemination. The patient was prescribed with a standard dose of acyclovir, but extended for another week (totally 3 weeks) together with topical and oral antibiotics, but lesions progressed. Then, we doubled doses for 2 weeks according to treatment guideline. Without clinical improvement, the next step of treatment is oral famciclovir or valacyclovir,[5] but our patient could not afford. Therefore we continued the same acyclovir dose totally for 2 months. The lesions were smaller with no ulcer. There was a case report of a patient with chronic mucocutaneous herpes virus infection successfully treated with oral acyclovir up to 65 days.[12] After 3-month follow up, the lesions were obviously improved. HSV antigen detection was repeated and the result was negative. Hypopigmentation remained after healing. We advised the patient about the possibility of recurrence and the necessity of routine follow up under no suppressive acyclovir therapy.

Conflict of interest disclosure: The authors declare that there is no conflict of interest regarding the publication of this observation.

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Endoscopic Treatment of Bleeding Ileal Pseudo-diverticulum

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ABSTRACT

Objective: Jejunoileal bleeding is not common, with diverticular disease as the fourth common cause of bleeding. However, the diagnosis is sometimes challenging and easily overlooked.

Case presentation: This is a case of 71 years old lady presented with hematochezia. A bleeding ileal pseudodiverticulum was found and successfully controlled by endoscopic treatment.

Conclusion: Bleeding ileal pseudodiverticulum is an uncommon cause of lower gastrointestinal bleeding which can be treated endoscopically.

Keywords: Ileal pseudodiverticulum; lower gastrointestinal bleeding; endoscopic (Siriraj Med J 2018;70: 452-454)

INTRODUCTION

Diverticulum is the outpouching of mucosa of the hollow viscus organ, mostly in gastrointestinal tract. It is classified as, "true diverticulum", in which the outpouching sac is covered by the whole layer of gastrointestinal wall, and, "false or pseudodiverticulum", in which the outpouching sac is not covered by the whole layer. The latter is more common, and mostly encountered in the colon of elderly patients. Patient with diverticular disease usually are asymptomatic or might present with bleeding, infection or perforation.[1]

Although bleeding colonic diverticulum is the most common causes of lower gastrointestinal bleeding,[2] bleeding from ileal diverticulum is uncommon and might easily be overlooked. We present an uncommon case of lower gastrointestinal bleeding, in which the endoscopic treatment was successfully performed.

CASE PRESENTATION

A 71 years old woman with underlying type 2 diabetes, hypertension, and dyslipidemia receiving clopidogrel 75 mg once daily as a prophylaxis for cardiovascular event presented to the hospital with hematochezia. Her vital signs were normal and initial examinations revealed a low hemoglobin level of 9.6 mg/dl. Other laboratory examinations were remarkable. Emergency colonoscopy (PCF H190, Olympus Medical System, Tokyo, Japan) was performed after rapid colonic purge and a small diverticulum, 3 mm in size, located within 2 cm from the ileocecal valve was seen. Non-Meckel pseudodiverticulum was the most likely diagnosis due to the small size and the location just proximal to the ileocecal valve. Blood clot inside the diverticulum was removed (Fig 1A) and a visible vessel with blood oozing was seen (Fig 1B). Hemostatic clips (EZ clip HX-610-090L, Olympus Medical System, Tokyo, Japan) were applied across the mouth of the diverticulum and the bleeding stopped (Fig 1C). The patient did not experience any recurrent bleeding during several months of follow-up and her hemoglobin level has improved.

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Received 20 October 2017 Revised 12 March 2018 Accepted 14 March 2018
doi:10.14456/smj.2018.71



A B C

Fig 1. The blood clot within the terminal ileal diverticulum (A). After the clot was removed, a visible vessel with blood oozing was seen (B) and hemostatic clipping was performed and the bleeding was stopped (C).

Review of literature

Small intestine diverticular is much less common than colonic diverticular. The most common and well-known congenital diverticulum of the small intestine is Meckel diverticulum which may cause lower gastrointestinal bleeding, obstruction, perforation, inflammation, or volvulus.[3] Other false diverticulum were found mostly in duodenum surrounding the ampulla, followed by the jejunum and the ileum, respectively.[4,5] Most cases of small intestine pseudodiverticulum were asymptomatic, but several reports of complications of both acute complication (such as bleeding, perforation, and infection) and chronic (such as pain and malabsorption) have been published.[6] Gastrointestinal bleeding from the jejunoileal area is uncommon, with diverticular disease as the fourth common cause of bleeding.[7] The diagnosis of this condition is more difficult than colonic diverticulosis due to limited accessibility to the lesions by routine endoscopy. The identification of bleeding jejunoileal pseudodiverticular diseases using wireless capsule endoscopy, contrast-enhanced computed tomography (CT), and CT angiography were reported in several literatures.[8-10] Among the reports of bleeding ileal diverticulum, the most common cause is bleeding from Meckel's diverticulum. However, in this case, according to the location and the size, the diagnosis of pseudodiverticulum was more suggestive. Similar to the criteria for diagnosis of bleeding colonic diverticulum, retained blood clot was seen in the pseudodiverticulum, which is the definite diagnostic criteria for the diverticular bleeding.[1] However, sometimes the bleeding point might be difficult to identify especially in cases with multiple diverticulum. In a case report of bleeding from multiple jejunal diverticulums the bleeding point could not be identified, so no intervention was provided and the bleeding stopped spontaneously.[8]

Treatment of bleeding in small intestinal diverticular in most literatures included both surgery and endoscopic treatment such as injection therapy, hemocliping and heater probe.[9-11] Endoscopic treatment in these case series were delivered through upper GI endoscopy for duodenal diverticular bleeding and double balloon enteroscopy in ileal bleeding.[10,11] In another series, after the bleeding point was identified, either by CT scan or angiography, exploratory laparotomy was performed.[9] However, most of these reports were case reports or small case series. In the author's opinion, the treatment options should be individualized according to the location of the diverticulum and severity of bleeding. In cases who presented with GI bleeding, routine upper and lower endoscopies are usually performed and the endoscopic treatment could be applied if the lesion could be demonstrated. However, in those cases in which the bleeding point could not be reached by routine endoscopic examination, treatment attempts should be made based on the same principle as obscure overt GI bleeding patients. Capsule enteroscopy, device assisted enteroscopy, or angiography should be considered as the first diagnostic modality depending on the bleeding activity.[12] Fortunately, in this case, the lesion was located very close to the ileocecal valve so the endoscopic procedure was possible.

In conclusion, the author reported a case of ileal pseudodiverticular bleeding, which was successfully stopped by endoscopic clipping.

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A Rare Case of Obturator Nerve Schwannoma Resembling Ovarian Malignancy: Learning from our Lesson

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ABSTRACT

Objective: Obturator nerve schwannoma is an uncommon pelvic tumor and, therefore, is usually overlooked as a probable diagnosis per initial, preoperative, radiologic evaluations. This leads to an unanticipated difficult intraoperative management in a narrow operative field, surrounded by vital organs. This report describes a patient with obturator nerve schwannoma who presented with a pelvic mass and underwent surgical evaluation for possible ovarian malignancy.

Case presentation: A 58-year-old woman, presenting with asymptomatic right solid-cystic pelvic mass. Exploratory laparotomy, which was performed due to suspicion of ovarian cancer, revealed a 9-cm retroperitoneal mass located within the right obturator fossa, originating from the obturator nerve. Hysterectomy, bilateral salpingo-oophorectomy and enucleation of retroperitoneal mass were performed. The right obturator nerve was entirely preserved. Histopathological examination revealed a schwannoma. Postoperatively, the patient reported paresthesia at the medial aspect of the right thigh without weakness. No recurrence was detected at 6-month follow up.

Conclusion: Although obturator nerve schwannoma is rare and often not diagnosed preoperatively, a thorough inspection of CT scan and MRI can potentially reveal a continuity with its anatomical origin. Subsequently, a needle biopsy can be performed to obtain a diagnosis. Laparoscopy is a safe therapeutic approach for schwannomas located within the obturator fossa.

Keywords: Obturator nerve; ovarian tumor; pelvic mass; schwannoma (Siriraj Med J 2018;70: 455-458)

INTRODUCTION

Schwannoma is a rare peripheral nerve sheath tumor, composed of well-differentiated Schwann cells.[1] Schwannomas most commonly arise from cranial or main peripheral nerves, especially within the head and neck region.[1] Pelvic retroperitoneal schwannoma is even rarer, comprising only 0.5-3% of all schwannomas.[2] Due to their infrequent incidence, pre-operative diagnosis of pelvic retroperitoneal schwannomas is uncommon. In this circumstance, surgeon may face with intraoperative challenges in this narrow operative field which include adjacent major blood vessels and the preservation of nerve function. This study presents an unanticipated intraoperative event and management of obturator nerve schwannoma in a woman who underwent exploratory laparotomy for presumed ovarian cancer.

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Received 30 May 2018 Revised 21 September 201 Accepted 24 September 2018
doi:10.14456/smj.2018.72

CASE PRESENTATION

A 58-year-old healthy postmenopausal woman, Para 2, presented with an asymptomatic pelvic mass, which was detected during an annual check-up visit. Her previous pelvic examination performed 6 years ago was normal. Pelvic examination revealed a normal uterine size and an 8-cm, firm and fixed mass located at the right pelvic region. No other abnormalities were detected. Whole abdominal computed tomography (CT) scan with contrast revealed an 8.2x9.0x8.5-cm mixed solid and cystic mass located at the right ovary, suspicious of ovarian cancer (Fig 1). No metastasis was shown. Chest X-ray was normal. CA125, CA19-9 and CEA value were 12.1 U/mL (normal <35 U/mL), <0.6 U/mL (normal <40 U/mL), and 1.6 ug/L (normal <4 ug/L), respectively. Concerned about having ovarian cancer, the patient underwent exploratory laparotomy for surgical staging. There was no evidence of pelvic organ abnormality except for a 9-cm retroperitoneal mass located at right obturator fossa and extended to presacral area. The mass was attached laterally to right obturator nerve. Hysterectomy and bilateral salpingo-oophorectomy were initially performed since gynecologic malignancy could not be excluded. The mass was completely enucleated. Only the capsule remained unresected. The right obturator nerve was entirely preserved. During the procedure, the adjacent venous plexus was injured, leading to a massive bleeding (8,500 mL). Packed red blood cells 9 units, fresh frozen plasma 2,000 mL, platelet concentration 12 units and cryoprecipitate 12 units were transfused intraoperatively.

The gross examination of retroperitoneal mass revealed a 9x8x6-cm soft, lobulated, heterogeneous light brown and yellow tissue. There was an area of cystic degeneration measured 3x3 cm, with hemorrhagic tissue lining (Fig 2). Microscopically, there was a combination of two distinct growth patterns, Antoni A and Antoni B (Fig 3). Additionally, vascular hyalinization was observed, representing a degenerative change. The tumor exhibited immunoreactivity with S100 antibody, but not marked with CD34, SMA, desmin or CD117. Ki67 labeling index was less than 5%, indicating low proliferation (Fig 3). The histopathological findings and the anatomical location of the tumor supported the diagnosis of obturator nerve schwannoma. The patient was discharged on day 4. Besides having paresthesia at the medial aspect of the right thigh without evidence of weakness, she had an unremarkable recovery. At 6-month follow up, paresthesia persisted despite receiving rehabilitation, but no tumor recurrence was detected.

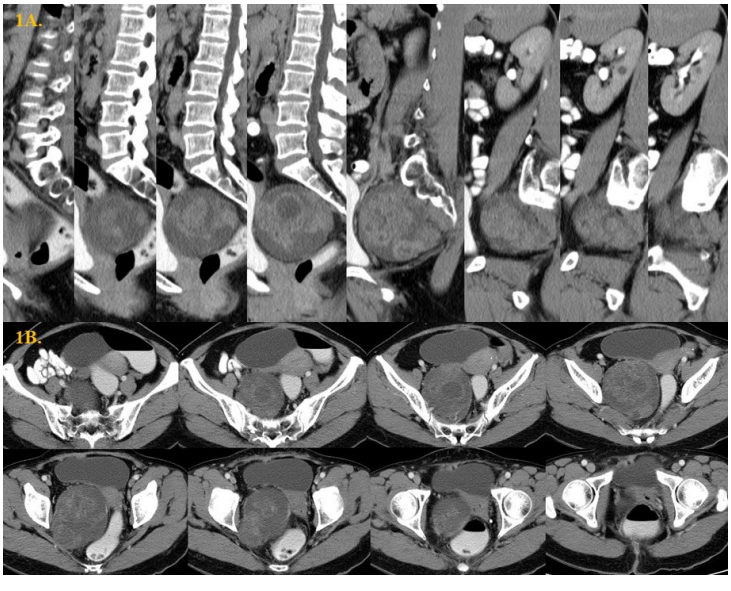


Fig 1. CT whole abdomen with contrast revealed a well-circumscribed heterogeneous solid-cystic mass at right pelvis (1A) sagittal view (1B) axial view



Fig 2. Gross findings of retroperitoneal mass

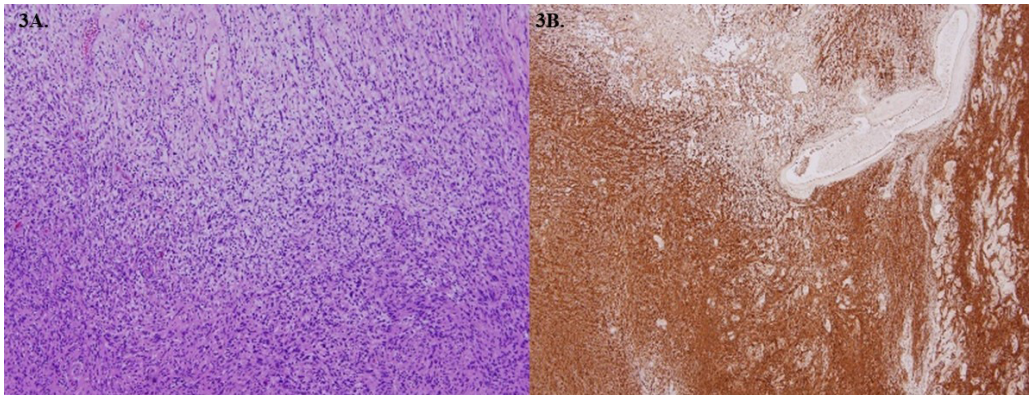


Fig 3. Microscopic appearance (3A) mixture of two growth pattern; Antoni A and Antoni B with area of vascular hyalinization (3B) diffuse reactivity for S-100 protein

DISCUSSION

Schwannomas are generally benign nerve sheath tumors. Patients diagnosed with schwannomas usually present with solitary and slow-growing masses that are otherwise asymptomatic. Compressive symptoms can occur due to tumor size. Neurological symptoms including pain and paresthesia have been reported in up to one third of the cases, particularly in large or deep-seated tumors.[3]

Preoperative diagnosis of schwannomas is extremely challenging due to their non-specific imaging characteristics. A previous study showed that only 15.9% (13/82) of retroperitoneal schwannomas were accurately diagnosed preoperatively using either ultrasonography, CT scan, or MRI[4]. Specifically for obturator nerve schwannomas, successful preoperative diagnosis is extremely rare, and to our knowledge, only one case has been reported to date.[5] Some studies proposed that presence of certain imaging features could raise the suspicion of nerve sheath tumors.[6] These findings included round and sharply demarcated solid tumors, which might exhibit cystic regions as signs of degenerative changes or, rarely, malignant transformation. MRI provides a better tissue analysis than CT and might be the superior imaging modality for the preoperative evaluation of schwannomas.[7] Schwannomas can appear as low signal intensity on T1-weighted images and high signal intensity on T2-weighted images.[6] Either central (target-like) or peripheral enhancement can be found.[6] However, the most reliable finding was presence of connecting between tumor and the originating nerve, in this case, the obturator nerve.[5]

Prior studies evidenced an advantage of needle biopsy. Strauss et al., showed that histopathological diagnosis of schwannoma were successfully obtained in 19 out of 28 patients who underwent needle biopsy.[8] This was further confirmed by the study of Ferretti et al., who showed that needle biopsy or fine needle aspiration can differentiate between benign and malignant tumor.[9]

For the presented case, since the CT scan suggested that the pelvic mass originated from the ovary, MRI was not performed for further evaluation. Because preoperative diagnosis is critical for the selection of surgical approaches and presurgical mapping, in situations where the diagnosis of a pelvic mass is uncertain, a combination of multiple imaging modalities with a needle biopsy might be necessary.

Surgical management of obturator nerve schwannoma presents a great challenge. These difficulties include a limited operative field in the obturator fossa, presence of adjacent structures such as the obturator nerve, the iliac vessels, and the venous plexus. Damages to obturator nerve can result in a transient or permanent weakness of hip adduction and outward rotation, as well as a sensory loss over the medial thigh. There are two major types of surgical management of obturator nerve schwannoma: enucleation or tumor resection.[5] Enucleation is the preferable method since it minimizes nerve damage while sufficiently removes the tumor.[10] In contrast, tumor resection is usually performed in rare cases that are suspicious for malignancy, such as patients with neurofibromatosis or Recklinghausen's disease, as well as tumors containing cystic changes.[5] Critically, if malignancy is suspected, tumor margins should be obtained and evaluated to prevent local recurrence.[5,11] Moreover, enucleation is usually performed on large schwannomas (>5 cm), whereas tumor resection should be performed on small tumors attached to one side of nerve.[5,6,10] Laparoscopic surgery is the recommended procedure since 1) its magnifying effect enhances the visualization of the structures surrounding the tumor, and 2) it is advantageous to conventional methods especially in small areas with limited access.[5,10] A recent study also showed that the use of robotic EndoWrist® and

3-dimensional viewing can successfully remove pelvic schwannomas while preserving neurological functions.[1]

In this case, enucleation was performed because of large tumor size. Massive bleeding caused by venous plexus injury was subsequently stopped by ligation. A number of strategies have been proposed to solve this life-threatening condition are packing, tacking, topical hemostatic agents, electrocoagulation and suture.[12] To prevent pelvic hemorrhage, preoperative embolization, internal iliac artery ligation or harmonic scalpel use are suggested.[1,10]

Recurrence is uncommon in benign schwannoma following a complete resection. In contrast, local recurrence was found in 20% and 72% of malignant schwannomas that underwent complete and incomplete resections, respectively, which emphasizes the importance of total excision in malignant schwannomas.[1]

CONCLUSION

Surgical management of obturator nerve schwannoma would be a great challenge avoiding injury to adjacent vital organs. Obtaining preoperative diagnosis using imaging modalities combined with needle biopsy can aid the selection of operative procedure and surgical planning. Although rare, schwannomas should always be considered a plausible diagnosis in patients who present with pelvic mass of uncertain origin since these tumors can be removed using a safe laparoscopic approach.

Conflict of interest declaration

All authors declare no personal or professional conflicts of interest, and no financial support from the companies that produce and/or distribute the drugs, devices, or materials described in this report.

Funding disclosure

This was an unfunded study.

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Circulating Cardiac Troponin T in Skeletal Muscle Disease: A Reflection of Re-expression Phenomenon?

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ABSTRACT

Determinations of cardiac troponin T (cTnT) and cardiac troponin I (cTnI) have replaced creatine kinase myocardial band (CK-MB) measurements as the "gold standard" biochemical methods for the detection of myocardial injury and infarction. Due to their high myocardial tissue specificity, it can can be assumed with a high probability that the troponins detected in the circulation are of cardiac origin. However, there have been several reports in recent years which showed increased serum concentrations of cTnT, but not of cTnI, in patients with skeletal muscle disorders, in whom no clinical evidence of myocardial cell damage were observed. Since cTnT isoform, in contrast to cTnI isoform, has been reported to be expressed in fetal skeletal muscle, there is the possibility that this fetal cTnT isoform is re-expressed in diseased and regenerating adult skeletal muscle. Therefore, the reported high myocardial tissue specificity of cTnT is to be questioned, and elevated cTnT concentrations found in patients with destructive skeletal muscle disease should be cautiously interpreted as biochemical evidence of myocardial cell injury.

This present review describes the physiological and pathophysiological aspects of cTnT and cTnI in health and disease, with special emphasis on the tissue expression of their isoforms in the fetal and adult hearts as well as in the fetal and adult skeletal muscles. Previous and recent data, obtained from international databases (PubMed, Scopus, Google Scholar), with reports on cardiac biomarkers in patients with acute and chronic skeletal muscle disorders are summarized and the clinical implications of these findings are also addressed and discussed.

Keywords: Cardiac troponins; expression; skeletal muscle (Siriraj Med J 2018;70: 459-465)

Physiology of cardiac troponin isoforms

Cardiac troponins, consisting of cTnT, cTnI and cTnC, represent parts of the troponin-tropomyosin-actin complex system in the myofilament which play an important role in the regulation of muscular contraction. The molecular structures of cardiac and skeletal muscle troponin C, in contrast to those of troponin T and I, are virtually identical, which prevent the use of cTnC as a specific marker of cardiac injury. With regard to cTnT, the presence of several troponin genes as well as alternative splicing provide for the existence of multiple troponin T isoforms.[1] Human cardiac muscle contains four isoforms of cTnT: three isoforms are expressed during the fetal period and one isoform is characteristic for adult heart.[2,3] In contrast, human fetal and adult heart muscles each contains only one cTnI isoform.

In physiological condition, the embryonic isoform of cTnT is found to be expressed in human fetal skeletal

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Received 4 July 2017 Revised 6 November 2017 Accepted 10 November 2017
doi:10.14456/smj.2018.73

muscle. After birth, there is down- regulation in the production of fetal cTnT isoform, resulting in the absence of cTnT in adult skeletal muscle. In contrast, there exists no expression of cTnI either in the fetal or adult skeletal muscle.[4]

Pathophysiology of cardiac troponin isoforms

The fact that cTnT and not cTnI is physiologically expressed in both the fetal heart and skeletal muscle has led to the assumption that the fetal cTnT isoform may be re-expressed in pathological conditions of the heart, and especially in the adult skeletal muscle. In this context, several animal experimental and human clinical studies have demonstrated the existence of messenger RNA (mRNA) and protein expression of cTnT in diseased myocardial and skeletal muscle tissues, along with the presence of cTnT which increases in the absence of elevated cTnI in the peripheral circulation.

Re-expression of CK-MB and cTnT in heart diseases

Several experimental studies performed on dogs and pigs have shown that chronic myocardial ischemia induced by ligation of coronary arteries resulted in several fold increases in myocardial CK-MB content.[5,6] Since an increase in myocardial CK-B subunit mRNA in ischemic myocardium of dogs was observed,[7] it was hypothesized that CK-MB is re-expressed in diseased myocardium. In contrast to CK-MB, coronary artery occlusion did not result in increased cTnT myocardial contents.[8-10] Similar results were obtained from studies on humans with ischemic injured myocardial tissue.[8] Since increases in both mRNA of fetal cTnT[11] and fetal isoforms of cTnT[2,11,12] were observed in diseased heart muscles of animals and humans, it was assumed that chronic loss of cardiac troponin from injured myocardium is not replenished through re-expression of genes that might increase protein synthesis.[13]

Re-expression of CK-MB and cTnT in skeletal muscle diseases

The dynamics of CK-MB and cTnT alterations have also been intensively investigated in chronic skeletal muscle disorders. By using marathon runners as subjects, Apple et al.,[14,15] studied the effects of chronic long distance running on the CK-MB content of skeletal muscle. It was found that skeletal muscle CK-MB in the biopsy specimens was increased by 2-fold. Similar observation was made in patients with Duchenne muscular dystrophy.[16] Apple[13] has suggested that skeletal muscle damage and subsequent regeneration of muscle resulted in the differentiation of CK isoenzymes into the embryonic skeletal muscle formation of CK-BB and CK-MB.

The expression of cTnT isoforms in diseased skeletal muscle has been debated for several decades. Expression of mRNA of the cardiac isoforms of TnT has been found in patients with diseased human skeletal muscles such as uremic myopathy of end-stage renal disease[17] and Duchenne muscular dystrophy.[17,18] By using immunocytochemistry and immuno- blotting technique, Saggin et al.,[19] have demonstrated re-expression of cTnT in regenerating muscle fibers of rats following cold injury as well as in mature fibers after denervation. Similar results were obtained from a study by Boder et al.,[20] who reported expression of cTnT in regenerating skeletal muscles of patients with polymyositis and Duchenne muscular dystrophy. However, Ricchiuti et al.,[21] have shown that the cTnT isoform expressed in diseased skeletal muscles of chronic renal disease patients is not detected by the 2nd-generation cTnT immunoassay. These results stand in contrast to those of Jaffe et al.,[22] who found increased cTnT, but normal cTnI serum concentrations in patients with neuromuscular diseases. Western blot analyses of biopsy specimens performed on some of these patients have revealed the presence of strong immunoreactive bands, with a molecular weight similar to that of cTnT. The authors concluded that there are forms in diseased skeletal muscles that could cause increases in levels of cTnT, and these increases could reflect the re-expressed isoforms which can be detected by a more sensitive cTnT immunoassay.

Circulating CK-MB and cTnT in chronic skeletal muscle diseases

Cardiac involvement represents a common complication that confers a worse prognosis in chronic destructive myopathies.[23] It has been reported that the presence of increases in circulating CK-MB and cTnT, which may indicate myocardial involvement, is frequently associated with several chronic skeletal muscle disorders including polymyositis, dermatomyositis and muscular dystrophy.[24] Table 1 summarizes previous and recent data on the frequency of elevated cardiac biomarker levels in patients with chronic destructive skeletal muscle diseases, most of which showed no clinical evidence of acute coronary ischemic events.

In a study on 28 patients with polymyositis and several forms of muscular dystrophy, increases in the activity of CK and CK-MB were found in 86 and 100 percent of patients, respectively. Elevated cTnT, measured with the 2nd-generation immunoassay, and cTnI levels were 23 and 14 percent, respectively.[25] In a more recent study on 39 patients with polymyositis and

TABLE 1. Percentage of increased cardiac biomarkers in patients with chronic destructive skeletal muscle disorders.

Author (year)	Diagnosis	Percentage of increase			
		CK	CK-MB	cTnT	cTnI
Braun et al. (1996)	PM, DMD, BMD, LGD, FSHD	86	100	23	14
Erlacher et al. (2001)	PM, DM	nd	51	41	3
Sribhen et al. (2002)	PM, DM, DMD,	100	100	57	nd
Aggarwal et al. (2009)	PM, DM, CTDM	82 71	nd nd	64 nd	nd 2
Fisher et al. (2010)	PM, DM, IBM	100	nd	100	nd
Cox et al. (2010)	IBM	82	82	78	2
Jaffe et al. (2011)	IBM, FSHD	nd	nd	100	0

Abbreviations: BMD = Becker muscular dystrophy; DM = Dermatomyositis; DMD = Duchenne muscular dystrophy; FSHD = Facioscapulohumeral-type dystrophy; IBM = Inclusion body myositis; LGD = Limb girdle dystrophy; CTDM = Connective tissue disease-associated myositis; PM = Polymyositis; nd = not determined

dermatomyositis, increased CK-MB and cTnT were reported to be 51 and 41 percent, respectively.[26] Since there were close correlations between both cardiac biomarkers and skeletal muscle damage markers including myoglobin and myosin heavy chain, and a close relationship of cTnT with disease severity, it has been suggested that cTnT was released from skeletal muscle. By using a more sensitive and specific 3rd-generation cTnT assay, Sribhen et al.,[27] reported the frequency of cTnT increase in these patients to be 57 percent. Recent studies[22,28-30] performed on patients with inflammatory myopathies have also demonstrated that most of these patients exhibited elevated cTnT concentrations. In contrast to the cTnT results, four of the studies have found that only 0 to 3 percent of the patients displayed increased cTnI levels (Table 1).

The results from these trials have recently been confirmed by 2 studies utilizing the latest generation of cardiac troponin immunoassay. In a male patient diagnosed as having limb-girdle muscular dystrophy, Mair[31] has demonstrated chronic elevations of high-sensitivity troponin T (hs-cTnT) over several days whereas hs-cTnI was in the normal range. Echocardiography, exercise stress test and renal function were all normal. In addition, analytical interference by heterophile antibodies was ruled out. The author suggested that patients with unexplained increased cTnT with normal cTnI should be evaluated for possible, clinically still asymptomatic chronic skeletal muscle diseases. Rittoo et al.,[32] have conducted a hitherto largest study on 52 hospitalised and ambulatory patients with 20 different types of acquired and inherited neuromuscular diseases. The main finding in that study was that these patients commonly had persistent elevation of CK-MB and cTnT in the absence of electrocardiographic, echocardiographic and cTnI evidence of myocardial injury. The authors concluded that re-expressed cTnT in diseased skeletal muscle might appear to be the source of the elevated cTnT detected in the circulation of these patients.

The discordant cardiac troponin results, with the presence of increased cTnT, but normal cTnI levels, in patients with destructive skeletal muscle disorders without clinical evidence of acute coronary events, have led to the assumption that elevations of circulating cTnT found in these patients may not originate from the heart. Of interest was the observation by Kiely et al.,[33] who have

demonstrated no increase in cTnI levels in any of their 6 patients with polymyositis and dermatomyositis, despite an increase in CK-MB index. Since CK-MB showed significant correlation with skeletal muscle troponin I (sTnI) and not with cTnI, it was assumed that skeletal muscle was the source of CK-MB. In this context, it has been shown in a more recent study[34] on 129 patients with Duchenne and Becker muscular dystrophy that, although increased CK activity and cTnI concentration were found in a significant number of these subjects, there was no correlation between the two biomarkers. This can be explained by the fact that high levels of CK are usually observed in the early stage whereas cardiac involvement with release of cTnI occurs at a later stage of the disease. Since cTnI is uniquely expressed in cardiac muscle and is not expressed in other non-cardiac tissues, these results point to the usefulness of this protein as a specific biomarker to detect early stage of cardiac damage in patients with chronic destructive skeletal muscle diseases.

The patterns of cardiac biomarker release in patients with polymyositis and dermatomyositis differ significantly from those of ischemic cardiac injury. In acute coronary syndromes, there are significant rises and falls in CK-MB and cTnT levels, which return to the normal ranges within 2-3 days and 7-10 days, respectively. In contrast, the patients with inflammatory myopathies[35-37] exhibit a delayed decrease in CK-MB and relatively constant levels of cTnT when determined several days apart (Table 2). These results, thus, are not indicative of acute myocardial injury and may reflect a non-cardiac source of CK-MB and cTnT elevations.

Circulating CK-MB and cTnT in acute skeletal muscle diseases

Reports on chronic elevations of CK-MB and cTnT in the absence of cTnI increases in patients with severe acute skeletal muscle damage are rare and, to our knowledge, exist in only 3 cases in the literature. In a patient with amphetamine-related rhabdomyolysis following an intestinal operation due to intestinal herniation with bowel gangrene, Sribhen et al.,[38] observed increases in both CK-MB and cTnT without concomitant cTnI elevation in the subacute regenerating phase of the disease that persisted throughout several weeks of hospital stay. The same authors have also found chronic increases in CK-MB and cTnT, but not cTnI, from elevated baseline levels to around 7-fold the upper limit of reference ranges in a patient with chronic renal failure who developed rhabdomyolysis after limb ischemia (Fig 1, personal observation). Another patient diagnosed as having sepsis-associated rhabdomyolysis exhibited persistent increases in CK-MB and cTnT throughout their stay in the rehabilitation unit.[39] Unfortunately, measurement of cTnI was not performed. Nontheless, it was theorized in both studies that the prolonged elevations of cTnT were most likely due to re-expression of cTnT isoform in regenerating skeletal muscle.

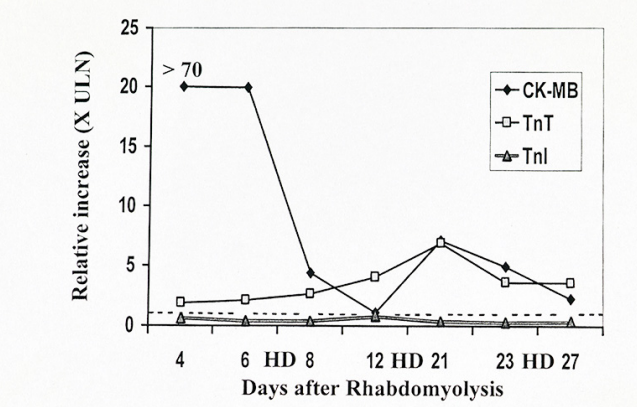
Circulating CK-MB and cTnT in cancer patients

There have been several reports of increased cardiac biomarker serum levels in cancer patients.[40-42] Even though, most of these studies have demonstrated the presence of macro CK type 1 and 2 as the non-cardiac cause of CK-MB elevation, ectopic production of CK-

TABLE 2. Serial cardiac biomarkers levels determined several days apart in patients with inflammatory myopathies.

Author (year)	Diagnosis	Cardiac biomarker levels			
		CK-MB (U/L)		cTnT (ng/ml)	
		S 1	S 2	S 1	S 2
White & Tideman (2001)	DM	243	293	0.52	0.54
Hamilton & Sharpe (2005)	DM	937	458	3.12	2.90
	PM	nd	nd	0.17	0.16
Rahim & Dave (2009)	DM	nd	nd	0.14	0.10

Abbreviations: DM = Dermatomyositis; PM = Polymyositis; S = sample; nd = not determined



CK (U/L)	161,500	80,100	15,050	529	192	272	112
CK-MB (%)	> 0.24	0.12	0.14	1.07	18.4	8.94	10.4
Creatinine	5.9	9.6	7.6	10.5	7.4	9.8	8.2

Fig 1. Patterns of cardiac biomarker release following rhabdomyolysis in a chronic renal failure patient.
Abbreviation: HD = hemodialysis. Creatinine level in mg/dl

MB by certain types of tumors has also been reported.[40] Isotalo et al.,[41] described a case of a patient with metastatic alveolar rhabdomyosarcoma in whom a series of increased concentrations of CK-MB mass and cTnT, were measured with the 2nd-generation assay, over several days. Since levels of cTnI, determined by two different immunoassays on the same blood specimens, were all in the normal ranges, a non-cardiac source of cardiac biomarkers elevations was suggested. In addition, since measurements of both CK-MB and cTnT were performed 5 months after the patient's last chemotherapy cycle, the possible toxic therapeutic effects could be ruled out. The authors theorized that rhabdomyosarcoma anaplasia leads to an immature skeletal muscle phenotype which may cause false-positive marker testing for myocardial infarction, secondary to expression of CK-MB and cTnT. Similar to these findings, persistent increases in both CK-MB and cTnT (3rd-generation assay) serum concentrations over several weeks have been found in a patient with uterine leiomyosarcoma.[42] Unfortunately, measurement of cTnI was not performed. Nevertheless, the author stated that the increases in cardiac biomarkers observed might be secondary to tumor expression of CK-MB and cTnT.

Cardiac troponins in amyotrophic lateral sclerosis

In recent years, several studies have demonstrated the presence of elevated serum cTnT concentrations in the absence of clinical evidence of an acute coronary event in amyotrophic lateral sclerosis (ALS), the most common form of motor neuron disease.[43-47] Hof et al.,[43] described a 65-year old man, diagnosed as having ALS, who presented with acute chest discomfort and nocturnal dyspnoe. Serial blood samplings have revealed slightly increased CK-MB activity and significantly elevated cTnT concentrations which persisted for several weeks. However, serum cTnI levels, determined in the same blood samples, were all in the normal ranges. Since there were no ischemic changes on electrocardiogram and the single-photon emission computed tomography (SPECT) with adenosine stimulation has shown a normal cardiac contraction and no evidence of ischemia or scarring, the initial suspicion of myocardial injury was rejected and a non-cardiac cause of cTnT elevation was assumed. Similarly, Graziani et al.[44] reported 3 cases of ALS patients with persistently high blood levels of cTnT, but normal values of cTnI in the absence of any cardiac disease. They suggested the hypothesis that non-inflammatory skeletal muscle degeneration could be the cause of re-expressed cTnT in diseased skeletal muscle and so the source of circulating cTnT. Similar observation was made in a study of ALS patients, of whom 27 (68%) patients had chronic elevations of cTnT, whereas their electrocardiographic and echocardiographic findings were negative for ischemia.[45] The study raised the possibility that cTnT originates from the atrophic skeletal muscle via re-expression of cTnT isoform.

Clinical implications for diagnosis of acute myocardial infarction

Introductions of sophisticated immunoassays for determining circulating cardiac troponins have led to cardiac biomarker testing with improved analytical sensitivity and precision. By utilizing the 99th percentile cut-off concentration that has been recommended by the international cardiology societies,[48] the diagnosis of acute coronary syndromes can be made in the early hours after the onset of symptoms, and so allowing for early risk assessment and appropriate management of the patients. The increased clinical sensitivity of the troponins, on the other hand, is associated with reduced specificity, and several conditions other than acute coronary events can cause troponin elevations.[49] These include congestive heart failure, myocarditis, stress-induced cardiomyopathy, pulmonary embolism, acute neurologic diseases (stroke, subarrhachnoid hemorrhage), sepsis and septic shock, as well as drug toxicity and drug abuse (doxorubicin, cocaine, amphetamine). Nevertheless, elevated levels of cardiac troponins found in these patients represent true-positive results indicating myocardial involvement. In contrast, increased cTnT serum concentrations in the absence of elevated cTnI found in patients with destructive skeletal muscle disorders may reflect a false-positive result, probably secondary to the re-expression of fetal cTnT isoform. Therefore, a single positive cTnT result

observed in these patients should be cautiously interpreted as biochemical evidence of cardiac involvement. In this context, it should be noted that serial measurements of cTnT which show significant rises and falls of cardiac marker points to acute coronary ischemic events, whereas chronic elevations are indicative of skeletal muscle source of cTnT. As stated by Jaffe et al.,[22] the unique specificity thought to occur for cTnT and cTnI might no longer be sustained for the current cTnT assay. The tissue specificity of cTnT could also be ensured by measuring with a sensitive cTnI assay to confirm or refute the increases in cTnT levels. Nontheless, it should be born in mind that cardiac troponin results represent only one of several criteria for the diagnosis of acute myocardial infarction and should be interpreted in conjunction with the presence of ischemic symptoms, as well as electrocardiographic, echocardiographic or other imaging results.

CONCLUSION

Measurements of cTnT and cTnI currently represent the most sensitive biochemical methods for diagnosis of myocardial injury and infarction. However, they are not disease specific, and increased cardiac troponin levels can be observed in several clinical conditions other than acute coronary syndromes. In patients with acute and chronic destructive skeletal muscle diseases, increased circulating cTnT may arise from regenerating skeletal muscle, probably as a result of re-expression of fetal cTnT isoform. In these patients, determination of cTnI levels may provide a more specific method for the early detection of myocardial involvement and a useful prognostic indicator of the diseases.

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Robotic Surgery in Thailand: Current Status and Future Development

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ABSTRACT

Robotic surgery, currently monopolized by the intuitive "daVinci Surgical System" (dVSS), has now established its strong foothold in the USA and a few other countries in Europe and Asia. However, because of its high cost and lack of evidence to support its superiority over the traditional laparoscopic technique, its utility especially in less affluent countries remains controversial. In this article, we studied the adoption and development of robotic programs in Thailand, a middle income country, that had its head started relatively early with the acquisition of the first generation daVinci system by the private international hospital. The systems have since been installed in 5 other institutions, 4 of which are academic centers. We analyzed and discussed the causes of success of the urological program and failure of the others. In the end, we concluded that instead of resisting the trend, it should be selectively and rationally embraced. We then suggested steps to help with the program development that are best suited to Thailand's financial and health care conditions.

Keywords: Robotic surgery; da Vinci Surgical System; Thailand (Siriraj Med J 2018;70: 466-470)

INTRODUCTION

Since its approval by the US FDA in 2000, the da Vinci Surgical System (dVSS) has so dominated the field of robotic surgery that its name is effectively synonymous with robotic surgery. Despite the exorbitant price tag and high annual maintenance fee, it is now installed in almost all major hospitals and medical centers in the USA. Although initial acceptance was tepid, the dVSS rapidly gained momentum as urologists began to deploy it. Compared to the laparoscopic approach, urologists found the dVSS—with its high definition 3D imaging, wristed instruments with 7-degree freedom of movement, and intuitive hand-eye coordination—to be effective on the one hand and to make minimally invasive prostatectomy possible on the other hand, and with much greater ease.[1] Adoption of the dVSS by general surgeons occurred at a much slower pace and initially was greeted with less enthusiasm and more skepticism. It has continued to be a controversial and contentious issue in general surgery because of the high cost, but similar outcomes, compared to the laparoscopic approach.[2] The dVSS has also prompted a heated debate over its role in the rising cost of national health care.[3] Despite sharp adversarial opinion, however, the dVSS has regained popularity in recent years due to its newfound potential roles and benefits in complex hernia repair surgery.[4] Hospitals are increasingly rushing to acquire the system and promote its use. For the moment, there seems to be no way to stop or reverse the trend, which has also begun to emerge elsewhere, including Thailand.

The development of robotic surgery in Thailand

The first dVSS - "The original da Vinci"

Robotic surgery was introduced into Thailand as early as 2003 through Transmedic (Singapore), which was a distributor for the dVSS in Southeast Asia at the

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Received 23 April 2018 Revised 25 April 2018 Accepted 27 April 2018
doi:10.14456/smj.2018.74

time. Bangkok Hospital, a premier international hospital with a nationwide network facilities, was the first to purchase the system for cardiac surgery. The first robotic symposium and live demonstration was organized and held at the same hospital in 2004 with an audience from all around the country. However, enthusiasm soon waned. Indeed the da Vinci was so infrequently used that it was eventually placed in a storage room at the hospital. During the same period in the USA, there was a surge in the popularity of the dVSS because of a report showing its benefits in prostatectomy in a large cohort study.[5] That and subsequent studies with similar outcomes helped to renew the interest in the dVSS among Thai urologists. Meanwhile, the dVSS distributor for Thailand changed and is now in the hands of Berli Jucker (BJC), a Thai firm.

Siriraj Hospital Robotic Surgery Program

In 2007 Siriraj Hospital—the oldest and largest hospital in Thailand—purchased the second generation da Vinci S from Berli Jucker Public Company Limited (BJC), then a new distributor in Thailand, and established a urological robotic surgery center. Their early experience of robotic prostatectomy showed outcomes similar to those in the US: less blood loss, less pain, shorter catheter indwelling time, shorter length of stay (but much higher cost), and no significant difference in erectile dysfunction.[6] Although with less enthusiasm, general surgeons also began to get involved and held their first robotic symposium on December 25, 2008, with participation by a US-based expert in the dVSS. The following year a deal was struck with a robotic surgery program in New Jersey, USA, and a number of junior attending surgeons from Siriraj Hospital went there for short-term training. On December 16, 2010, at the inaugural combined minimally invasive and robotic surgery conference held in Siriraj Hospital, the first live pancreatic surgery was performed. In the same year, the success of robot-assisted complete excision of choledochal cyst type I and hepaticojejunostomy was published in international journal by the minimally invasive surgical team.[7] Because of the success of the urological program and increased interest among general surgeons, the hospital, in 2012, invested in the third generation da Vinci Si. Since then surgeons at the Department of Surgery, Faculty of Medicine Siriraj Hospital have performed more than 1,500 procedures. The urology department,[8] which performed the majority of cases, has become the leading center for robotic prostatectomy in Asia. The success of the urology robotic program was due to a high volume of prostatectomy patients, most of whom were either self-paying or covered by insurance. In contrast the general surgical cases tended to be charity cases supported by a limited grant or charity fund allotted annually. Once the fund was depleted in any given year, surgeons were no longer able to schedule a case unless special provision was made to cover the cost. However, in 2016, the gynecological team join the robotic program and successfully performed the first total robotic hysterectomy of Thailand.[9]

King Chulalongkorn Memorial Hospital (KCMH)

In 2011 KCMH, another major university hospital in Bangkok, acquired its first da Vinci Si system with a strong push from the Department of Urology, Faculty of Medicine, Chulalongkorn University. Similar to Siriraj's experience, urologists quickly embraced the technology and have since been the main users of the robotics. In 2015 they published a landmark article looking at cost-utility aspects comparing laparoscopic prostatectomy and robotic prostatectomy (RP). They showed that RP was not cost-effective at 100 cases per year but could potentially be improved if the number were higher.[10] In essence, the more cases, the quicker the recoup of investment cost, and the higher the profitability margin.

On October 22, 2011, at the second minimally invasive and robotic surgery conference in Thailand, this time under the auspices of KCMUH, history was made with the world's first robotic surgery in soft cadaver.[11] The procedure was a pancreatoduodenectomy successfully performed by a local surgeon and proctored on a second console by an expert surgeon from the USA.

Other Institutions

Maharaj Nakorn Chiang Mai Hospital (2011), Ramathibodi Hospital (2012), and most recently **Bumrungrad International Hospital (2016)** also own the da Vinci Si system but use it primarily for urological procedures.

Justifications for Previous dVSS Procurements

Thailand is a middle-income country that offers universal health coverage to its citizens. Free services can be received in most public hospitals that are funded through public revenues and donation. With limited annual budgets, rationing of services becomes unavoidable, especially in rural hospitals. As such, there is still a significant disparity in the quality of care between urban and rural areas. Private hospitals, on the other hand, are financed by patient self-payment, private insurance, and, in some cases, investors and thus are well equipped to provide high-end services such as minimally invasive surgery. Although laparoscopic surgery has gradually trickled down to rural hospitals, it is still limited to a few larger

public hospitals which consistently have a long line of waiting patients. Rising health care costs associated with population growth and new technology will undoubtedly further squeeze limited universal healthcare resources resulting in widening of the inequality gap. With such a background, it is reasonable to question the rationale for acquiring the dVSS, which is priced at twice the cost of that in the USA, and so far has not been shown to be superior to the current conventional approaches.

To date the main justifications for acquiring the dVSS can be summarized as follows:

1. Marketing tool – this motive was obvious with the first dVSS acquired by the private-sector Bangkok Hospital. The business plan had a marketing focus and did not rely on evidence-based data. The goal was primarily to attract patients, in particular, high-paying cardiac patients. There were no experienced personnel to use the system. As a result, the dVSS ended up nothing more than an expensive marketing tool.
2. Keeping up with medical technology and its trends – this has been the common theme employed by teaching institutions, such as Siriraj and other university hospitals, to acquire advanced technology. Although the technology might not be practical for the larger community, it is believed to be the role of the higher institute to understand, teach, and prepare the next generation of surgeons to be ready for technology used in the more advanced and affluent countries.
3. Necessary technology for a private international hospital to compete in the era of medical tourism – in contrast to the first justification as above, Bumrungrad International Hospital's recent acquisition of the dVSS was in line with its business plan while also boasting experienced personnel ready to use the system. The dVSS also helped to reinforce the institute's progressive vision and commitment to state-of-the-art health care technology.
4. Higher reimbursement for a surgeon – this can be a powerful incentive for surgeons in the US or for Thai urologists who operate mostly on affluent patients. For the majority of Thai general surgeons, this rationale does not apply, since most of their procedures can be done laparoscopically and are neither high-end nor well-compensated.

Future Development of Robotic Surgery in Thailand

The technology is in and it is here to stay – that is the fact with which we must now live. The issues then are how to utilize and control the technology to benefit patients, surgeons, and society as a whole without bankrupting the healthcare system. The process must begin at the individual institutional level where a robotic surgery program is launched, its objectives defined, and guidelines established. A national robotic surgery association can then be formed through collaboration from all institutional programs to create a national consensus on issues related to robotic surgery.

How to Build a Robotic Surgery Program

Establishing a successful robotic surgery program whether in a public or private setting requires similar principles. These include defining the robotic program and setting achievable or realistic goals, formation of a surgical committee and robotic team, establishing a data collection and analysis center, and other relevant factors as outlined below.

Goals – a successful robotic surgery program which provides not only quality and diverse robotic procedures performed by highly skilled surgeons but also a center for education, training, and clinical research.

Surgical Committee

Led by a program director, the committee should consist of active robotic surgeons, a robotic nursing coordinator, and an OR administrator. The committee duties are to 1) establish credentialing, granting, and maintenance of robotic privileges; 2) perform critical evaluation and analysis of problem cases; 3) assess OR utilization, efficiency, and safety; and 4) collect and analyze data.

Administrative Office and Staff

In addition to the robotic program director, a program or office manager should be separately appointed to coordinate work and act as a liaison between surgeons and the hospital administrative staff in areas such as web design and management, marketing, public relations, patient education, and other non-clinical functions.

Surgical Team

An individual with experience who is widely recognized for robotic surgery practice is invaluable in the development of a top-tier program. Such an individual in the position of director performs not only the procedure proper, but also coordinates, trains, and oversees team performance. The director should also invite surgeons from other specialties or subspecialties to participate in the program. Restricting robotic practice to 1 or 2

specialties is not a cost-effective way of exploiting this expensive technology. All procedures holding potential for robotic surgery, regardless of specialty, should be explored. Table 1 shows a list of robotic surgical procedures performed worldwide. Of those, only procedures that can realistically be performed at the facility should be considered.

A multispecialty approach helps to increase volume, patient flow, and efficient usage of the system and operating room.[12] It reduces the cost of capital investment and maintenance per case, leading to early profitability. Furthermore, public awareness of a multispecialty robotic program can affect patient flow to other non-robotic programs, with the potential of greater market share through an increase in overall patient volume.

An Actual Robotic Surgery Center

While it is possible to promote a robotic surgery program "virtually", the importance of a physical facilities infrastructure cannot be overstated. An actual robotic surgery center is essential to familiarizing patients and families with robotic surgery. This is the place where a patient first encounters the surgeon who will counsel and explain in detail what robotic surgery entails. There should be a waiting room where patients and family can peruse robotic articles, brochures, or view multimedia materials. Facilities should also include a command center where a surgical director and program manager reside, as well as a data collection and audiovisual station for clinical research.

TABLE 1. Commonly performed robotic procedures.

Organ Systems	Procedures
Upper Gastrointestinal Tract	Esophagectomy Hiatal hernia repair Wedge resection Gastric bypass Bariatric sleeve gastrectomy Gastrectomy Enucleation
Hepato-Pancreatico-Biliary Tract	Cholecystectomy +/- CBD Exploration Liver cyst fenestration/unroofing Hepatectomy Pancreatic wedge resection Distal pancreatectomy Whipple's operation Splenectomy
Genitourinary Tract	Adrenalectomy Nephrectomy Prostatectomy
Lower Gastrointestinal Tract	Right/Left Hemi-Colectomy Coloproctectomy
Abdominal wall	Inguinal hernia repair Ventral hernia repair Incisional hernia
Gynecological System	Oophorectomy Hysterectomy
Head & Neck	Thyroidectomy/parathyroidectomy Tonsillectomy

Program Promotion and "Marketing"

Although the notion of marketing inevitably prompts complaints of conflict-of-interest in the academic sector, it is nonetheless an important factor in growing a robotic surgery program. The goal of program promotion in this case should be to expand the catchment territory and referral base while also distributing educational information to patients and physicians. Program promotion should begin within the institution through town-hall style lectures by the director and the team introducing the "robot" and "what it can do" so that news can spread in a factual fashion. The next step is to utilize various media outlets for the same purpose, such as newspapers or local TV networks. These strategies will help form public awareness of the existence of a robotic surgery program at any given institution. Equally important is the maintenance of a dedicated website providing information about the program, public outreach, and dedicated patient online interaction.

Program Growth and Maintenance

Clinical and financial data collection is essential to growing a program and making it economically sustainable and profitable.[13] On the clinical side, the collection and analysis of data provides insight into cost-effectiveness and outcomes and offers keys to further improvement. All procedures should be video-recorded for review and critique, self-improvement, and ongoing education of surgical staff and residents. Video-records are equally an important resource for clinical research and publication. On the financial side, data should be collected and analyzed with respect to the cost structure of the program, including average direct and total costs per case for hospital expenses (room, operating room services and supplies), as well as surgeons' fees. Targeted improvements, such as in operating time, number of instruments used, or length of hospital stay, can then be strategized based on analyzed data.[14]

CONCLUSION

Any robotic surgery program in Thailand is a luxury – that is an undeniable fact. It is not, by any evidence-based information we have to date, superior to the existing technology. However, the reality is that there is now no turning back on this technology on the local and international level. Moreover, it is no longer beyond the means of an increasingly affluent and internationalizing Thai medical community. But whatever goals we may set for robotic surgery in Thailand, the most important priority must be the safety of the patient and a good surgical outcome. These goals are definitely attainable through careful and realistic program design, selection of an exemplary surgical team, ongoing self-evaluation and analysis through prospectively collected data and videos, teaching and training of a new generation of robotic surgeons, and last but not least, a smart business plan.

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International Telemedicine Activities in Thailand

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ABSTRACT

The development of information and communication technology has had a dramatic impact on people's lives, including medical matters. The Internet has made it possible for telemedicine to be implemented with excellent image quality at low cost; such telemedicine was first applied between Japan and South Korea in 2002. The technology is not restricted to advanced countries: it can also be applied in developing nations, and it has expanded rapidly to other parts of Asia and beyond. In 2005 Thailand became the seventh country to be associated with the Telemedicine Development Center of Asia (TEMDEC). As of 2017, TEMDEC operates 144 programs in Thailand, mainly in endoscopy (55, 38%) and surgery (40, 28%): 17 hospitals or medical institutions are active members, and there are 165 telemedicine connections. Siriraj Hospital, Mahidol University was the first participant; it has 71 telemedicine connections; King Chulalongkorn Memorial Hospital became the second participant; it has 52 such connections. These two hospitals account for 74.5% (123/165) of all telemedicine activities in Thailand. Compared with outside Bangkok, the number of telemedicine connections is 14 times (154/11) greater and the number of such connections per hospital is 10 times (15.4/1.6) greater in the capital-even though the number of hospitals is only 1.4 times (10/7) greater in Bangkok. To efficiently meet local needs, we strongly hope that telemedicine will expand into rural parts of Thailand and into more medical specialties through ongoing technological development.

Keywords: Telemedicine; remote medical education; internet; endoscopy; surgery (Siriraj Med J 2018;70: 471-475)

Dawn of Practical Telemedicine Between Japan and South Korea

There have been many scientific and industrial revolutions in history. Just a few examples of major inventions include the origins of paper in ancient Egypt, the electric light bulb, usually credited to Thomas Edison, the steam engine and the industrial revolution it ushered in, and the first flight by the Wright brothers. It is undeniable that the recent development of information and communication technology represents another revolution: it has totally changed our way of living. The Internet has enabled humans to connect instantly with one another around the world, and many people cannot live without emails or smartphones.

Applying this new technology to medicine began in the 1990s. However, the start was not easy. That was mainly because medical imaging requires much higher resolution than that typically used in daily life. To demonstrate surgical procedures, for example, medicine demands videos at high frame rates of 30 frames per second; that is technically very demanding. Satellite technology offers some advantages over the Internet in terms of quality; however, the huge cost is prohibitive. This situation hampered the progress of telemedicine,

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Received 26 April 2018 Revised 16 May 2018 Accepted 20 June 2018
doi:10.14456/smj.2018.75

and for about a decade telemedicine remained at just an experimental stage.[1,2]

That situation changed completely in 2002, when the World Cup was cohosted by Japan and South Korea; then, the two countries became linked by an extremely high-speed Internet connection. The bandwidth rose to 2 Gbps, which was 250 times greater than the conventional bandwidth at the time. That development had a great effect on telemedicine: instead of practitioners struggling to find better compression technology so that medical images could be transmitted through confined channels, huge content, such as movies, could be sent as original, uncompressed files via the wide-broadband Internet. Accordingly, there was no loss in image quality at remote receiving sites; at that point, it became possible to apply telemedicine at a practical level.[3] Another essential technology that permitted the implementation of telemedicine is called the digital video transport system. It is easily downloadable, costs nothing, and it has eliminated the need to purchase expensive, specialized equipment to practice telemedicine.[4]

Expansion to Elsewhere in Asia and Beyond

After the two major problems of quality and cost were overcome, telemedicine underwent a dramatic rise: this occurred not only in Japan and South Korea but also in many other countries in Asia and around the world.[5] This development has been ascribed to the establishment of the high-speed part of the Internet called the Research and Education Network (REN) in many countries.[6]

As of December 2017, we at the Telemedicine Development Center of Asia (TEMDEC) have organized over 800 programs, involving collaborations with 566 institutions in 60 countries. Of those countries, 23 (38%) were in Asia; that proportion was followed by Europe (22%), Latin America (15%), and Africa (10%). The dominant fields were endoscopy (32%) and surgery (20%); however, the remaining 48% comprised various disciplines, such as pediatrics, gynecology, transplantation, cardiology, neurology, dentistry, nursing, and medical student education, among many others.[7]

Telemedicine Activities in Thailand

Thailand became associated with TEMDEC as the seventh country in 2005, when the first telemedicine was demonstrated with the support of ThaiREN, the REN in Thailand.[8] This program took place when the first meeting of the Asia-Pacific Advanced Network (APAN), the consortium of RENs in these regions, was hosted in Thailand.[9]

Table 1 lists the 17 hospitals and medical institutions in Thailand that had joined TEMDEC-organized telemedicine programs by the end of 2017. Those programs comprised 165 telemedicine connections. Siriraj Hospital, Mahidol University was the first institution to participate, and it has the largest number of telemedicine connections (71). Two years later, in 2007, King Chulalongkorn Memorial Hospital became the second hospital to join; it has 52 telemedicine connections. Vajira Hospital joined in 2013, and it has the third-highest number of telemedicine connections (14). The top two hospitals in terms of number of connections-Siriraj Hospital, Mahidol University and King Chulalongkorn Memorial Hospital-account for 74.5% (123/165) of all telemedicine activities in Thailand; when the third-placed institution, Vajira Hospital, is added, that proportion rises to 83.0% (137/165). In addition to the hospitals and medical institutions listed in Table 1, TEMDEC also has telemedicine connections with five hotels and convention centers (e.g., Amari Watergate Bangkok and Queen Sirikit National Convention Center); in all, 18 telemedicine sessions have taken place in medical meetings and congresses in Thailand.

Table 2 presents a geographic comparison of telemedicine activities with respect to locations in Bangkok and outside the metropolitan area. In Bangkok, the number of telemedicine connections is 14 times greater (154/11) than outside; the number of telemedicine connections per hospital is also around 10 times higher (15.4/1.6) in Bangkok-even though the number of hospitals is only 1.4 times greater (10/7). In Table 3, telemedicine activities are divided into two periods: before and after their expansion beyond Bangkok. It is evident in that table that the number of hospitals increased by a factor of 3.4 (17/5) and the number of telemedicine connections rose by a factor of 3.7 (35 to 130).

A breakdown by type of practiced telemedicine appears in Fig 1. Among 144 programs organized in Thailand, endoscopy (55, 38%) and surgery (40, 28%) constitute the two largest fields; the remaining 34% cover a variety of areas. Live demonstrations of endoscopy or surgery are performed in 30 (21%) programs. Photographs depicting one such program appear in Fig 2.

Future Direction

A characteristic feature of our worldwide telemedicine activity is that the number of sites connected within one program has increased dramatically in recent years. According to Kudo et al.,[10] although more than five institutions participated only in 21% of telemedicine programs in 2011, that figure rose to 54% in 2015 and in addition, 22% of the programs had more than 10 connected sites in one program. One reason for this

TABLE 1. Hospitals and medical institutions that joined telemedicine programs and number of telemedicine connections.

Institution	Location	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	Total
Siriraj Hospital, Mahidol University	Bangkok	1	4	4	4	3	1	1	6	10	7	6	12	12	71
King Chulalongkorn Memorial Hospital	Bangkok	-	-	3	1	3	3	4	4	2	8	8	10	6	52
Rajavithi Hospital	Bangkok	-	-	-	-	-	2	-	-	-	-	1	2	2	7
Phramongkutklao Hospital	Bangkok	-	-	-	-	-	1	-	1	-	-	-	-	-	2
Ramathibodi Hospital, Mahidol University	Bangkok	-	-	-	-	-	-	-	1	1	-	-	-	-	2
Chiang Mai University Hospital	Chiang Mai	-	-	-	-	-	-	-	1	-	-	-	-	-	1
Klang Hospital	Bangkok	-	-	-	-	-	-	-	-	1	-	-	-	-	1
Vajira Hospital	Bangkok	-	-	-	-	-	-	-	-	1	1	5	4	3	14
Prince of Songkla University Hospital	Hat Yai	-	-	-	-	-	-	-	-	-	1	1	2	1	5
Thai Red Cross Society	Bangkok	-	-	-	-	-	-	-	-	-	-	1	-	-	1
Samutprakarn Hospital	Samut Prakan	-	-	-	-	-	-	-	-	-	-	-	1	-	1
Paholpolpayuhasena Hospital	Kanchanaburi	-	-	-	-	-	-	-	-	-	-	-	1	-	1
National Cancer Institute of Thailand	Bangkok	-	-	-	-	-	-	-	-	-	-	-	1	2	3
Buriram Hospital	Buriram	-	-	-	-	-	-	-	-	-	-	-	1	-	1
Ratchaburi Hospital	Ratchaburi	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Rambhaibarni Rajabhat University	Chanthaburi	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Samitivej Sukhumvit Hospital	Bangkok	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Total		1	4	7	5	6	7	5	13	15	17	22	34	29	165

TABLE 2. Comparison of telemedicine activity between Bangkok and other cities.

	Bangkok	Other cities
Number of hospitals	10	7
Starting year	2005	2012
Number of telemedicine connections	154	11
Number of telemedicine connections per hospital	15.4	1.6

TABLE 3. Comparison of telemedicine activity before and after expansion beyond Bangkok.

	2005–11	2012–17
Duration (years)	7	6
Number of hospitals	5	17
Number of telemedicine connections	35	130
Number of telemedicine connections per hospital	7.0	7.6

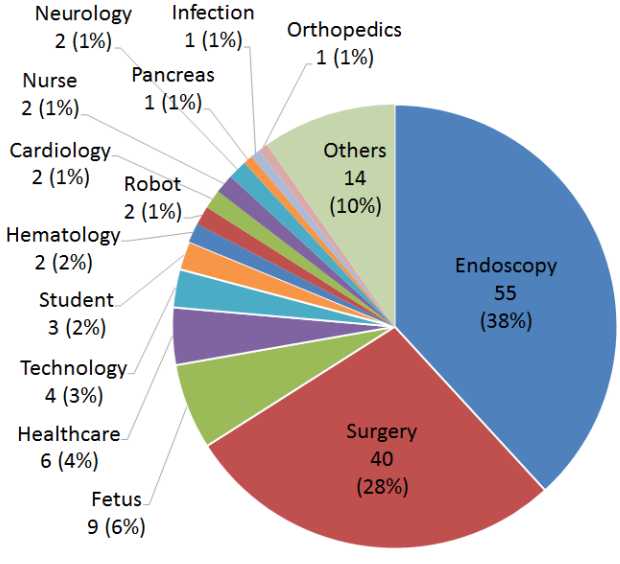


Fig 1. Type of telemedicine conducted in Thailand.



Fig 2. Teleconference view of minimally invasive surgery in 2015, organized by Siriraj Hospital, Mahidol University team in Thailand (f) and conducted with (a) the University of Malaya in Malaysia, (b) King Chulalongkorn Memorial Hospital in Thailand, (c) University of California, San Francisco in the United States, (d) Vajira Hospital in Thailand, and (e) St. Luke's Hospital in the Philippines.

development is the availability of satisfactory Internet bandwidth (both REN and commercial Internet)-even in developing countries and rural areas. Another reason is that new, handy teleconferencing systems have become available.[11,12]

With telemedicine in Thailand, the great majority of participating sites are located in Bangkok. However, telemedicine is now technically available in small cities; accordingly, its expansion into local areas should be expected. Telemedicine is also likely to expand into various medical specialties other than endoscopy and surgery.[13,14,15,16]

Toward initiating and improving telemedicine, workshops have been organized with the support of TEMDEC in such places as Indonesia and the Philippines, allowing doctors and engineers from around the country to discuss medical needs and technical issues. In Indonesia, that has resulted in monthly teleconferences being started,

involving 11 major university hospitals and remote islands. Similarly, in the Philippines, telemedicine programs have been launched and undergone expansion beyond metropolitan Manila.[17,18]

We at TEMDEC hope that telemedicine will expand across Thailand, offering its great benefits to medical providers and patients.

ACKNOWLEDGMENTS

The authors sincerely appreciate the technical expertise of all the engineering staff members involved in our telemedicine programs. This work was supported by JSPS Core-to-Core Program, B. Asia-Africa Science Platforms, and KAKENHI Grant Numbers 16H02773 and 15K08556. We thank the Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

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

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Current Status of GI Endoscopy in Thailand and Thai Association of Gastrointestinal Endoscopy (TAGE)

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ABSTRACT

Endoscopy practice in Thailand is being performed and serviced by both surgeons and gastroenterologists. The number of endoscopy performed by each specialty is very much comparable. Before 2000, the services were done independently. On 15th December 2005 the Thai Association of Gastrointestinal Endoscopy (TAGE) has been found there is a rapid progression in GI Endoscopy service, research, and teaching in Thailand and its neighbor. Since then the workload for the current number of endoscopists is still overwhelmed by day-to-day service. Thailand and TAGE require the support and collaboration from oversea to expand their need and to serve better for GI Endoscopy practice in Thailand.

Keywords: Thai Association of Gastrointestinal Endoscopy; endoscopist; Thailand Siriraj (Med J 2018;70: 476-478)

INTRODUCTION

Endoscopy practice in Thailand is being performed and serviced by both surgeons and gastroenterologists. The number of endoscopy performed by each specialty is very much comparable. Before 2000, the services were done independently. On 15th December 2005 the Thai Association of Gastrointestinal Endoscopy (TAGE) has been found (Fig 1). Since then, the collaboration between the two groups of endoscopists has been united and supervised directly by the Royal Colleges of Surgeons and the Royal Colleges of Physicians (Thailand), respectively.[1]



Fig 1. When the Thai Association of Gastrointestinal Endoscopy (TAGE) was found on December 15th, 2005.

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Received 20 August 2018 Revised 27 August 2018 Accepted 27 August 2018
doi:10.14456/smj.2018.76

Endoscopy Service

Due to a paucity of endoscopists in the country, in the early years the priority and workloads were based on emergency and urgent conditions such as upper or lower gastrointestinal bleeding and acute cholangitis. Over the last decade, the services have expanded to elective gastrointestinal conditions and more advanced procedures such as endoscopic ultrasonography (EUS), endoscopic submucosal dissection (ESD), and third space endoscopy. However, some of these special services are only available in university hospitals and large referral endoscopic centers. So far, 99 Endoscopic units in Thailand provided endoscopic retrograde cholangiopancreatography (ERCP) covering about 87% of healthcare-service area in the country. Meanwhile, EUS is being performed in 30 hospitals but limited to only large provincial hospitals and medical schools. ESD and third space endoscopy are limitedly available in a few hospitals and not in every university hospital. In 2017, the nationwide colon cancer screening by immunochemical fecal occult blood test (i-FOBT) has been launched and more colonoscopy performed due to positive i-FOBT is expected (Fig 2).[2-3] As a result, Endoscopic service in Thailand could face another challenge in the disproportion between workloads and human resource. The country definitely requires more number of endoscopists and their associates. In addition, the additional training to achieve a high level of quality in GI endoscopy is warranted.



Fig 2. Nationwide colonoscopy screening in Thailand has started in 2018.

Domestic GI Endoscopy training

The standard curriculum for gastroenterologist is a 2-year fellowship that continues after the 3-year of residency training in medicine. However, those who would like to pursue their career as the expert in GI endoscopy usually continue their third year at high volume centers. However, this third-tier does not require license or board examination. The other counter-part endoscopists, surgical training offers their basic endoscopy during 4-year surgical residency training and advanced endoscopic teaching through the minimally-invasive-surgery fellowship. To date, the overall number of endoscopists graduating from a formal training program is less than 50 graduates a year. This number is still far beyond the necessity of endoscopic workload in this country because the day-to-day workload requires at least 5 times more.

International GI Endoscopy training

According to the geography of Thailand, our country is in the perfect central area for trainees in the South-East Asia (SEA). All major cities in the SEA countries can reach Bangkok within one and a half hour by airplane. In 2006, the Siriraj Endocopy Unit – Faculty of Medicine Siriraj Hospital, Mahidol University has been recognized as one of the World Gastroenterology Organization (WGO) Training Center. Under the sponsorship of the Asia Pacific Digestive Endoscopy (APSDE) and other local supporters, many Thai endoscopy masters have joined other Asian faculties to teach many endoscopists from Myanmar, Laos, Vietnam, Indonesia and the Philippines. There are various training formats including 1) at the local site; outreach hands-on workshop, train the trainer on site, or 2) bringing the trainees to Thailand for observers in advanced procedures (Fig 3), training with the models at the sponsor-funded Endoscopy Training Center e.g. Olympus T-TEC. Moreover, the annual Live Endoscopy meeting organized by TAGE, involving all basic and advanced procedures performed by world experts, has been well attended by overseas endoscopists every year. Recently an Endoscopy Conference via internet called ECE (Endoscopic Club E Conference) has been used as the additional platform to teach other endoscopists who may miss the opportunity to participate face-to-face meeting in Thailand. The format of this meeting is using the internet to present and discuss the video clip on many interesting cases of GI Endoscopy. This platform has been evaluated as one of the excellent tools in GI Endoscopy teaching.[5]

TAGE History and its output

After the foundation of TAGE, there has been a rapid progress of its output in harmony by the two types of endoscopists. Up until now, TAGE has organized more than 15 International Live Endoscopy Demonstrations that attained with over 400 attendees including the oversea faculties from the US, EU, and Asia Pacific countries. In addition, TAGE has published 7 GI Endoscopy Atlases



Fig 3. International trainees from Myanmar and Vietnam attended the workshop and Live Endoscopy course in Thailand.

that can be downloaded freely from its website (http://www.thaitage.org/en/). Moreover, three small handbooks in Thai language that suitable for young endoscopists and GI Endoscopy nurses/associates are also available online. Under TAGE as co-author there are two GI endoscopy guidelines have been published in the leading GI Endoscopy peer-review journals.[6-7] Regard to the number of trainee, TAGE masters have conducted the hands-on teaching for more than 500 of domestic and international trainees in a variety of procedures including ERCP, EUS, ESD, POEM, etc.

CONCLUSION

Since TAGE has been found there is a rapid progression in GI Endoscopy service and teaching in Thailand and its neighbor. However, the workload for the current number of endoscopists is still overwhelmed by day-to-day service. Thailand and TAGE require the support and collaboration from oversea to expand their need and to serve better for GI Endoscopy practice in Thailand.

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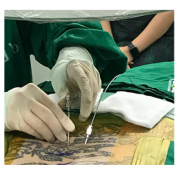
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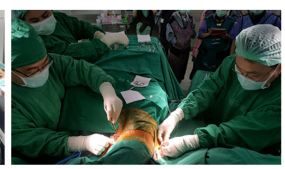
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