Breast milk benefits children as it reduces morbidity and mortality from many illnesses, and is considered the ideal food for newborn infants(1). Exclusive breastfeeding is adequate for infant growth during the first 6 months of age(2). In the developing world, breastfeeding promotion through the health care system has been a national priority in many countries. Practical strategies, which have been used to promote the success of breastfeeding, include effective prenatal education, a screening breast exam, the Baby Friendly Hospital Initiative and instruction on correct breastfeeding technique(3).

Several factors, which influence the incidence and duration of breastfeeding, have been studied, such as maternal education, prenatal care in the first trimester, previous experience of breastfeeding and maternal support(4). The mothers' perception of inadequate output of milk is a frequent reason for stopping breastfeeding in the first four months of life (5). However, prolonged breastfeeding beyond the first year of life may be associated with malnutrition (6,7). The duration of breastfeeding differs widely between populations, as do the consequences of these differences for the nutritional status of the child. This study aimed to evaluate current breastfeeding practices in remote rural communities of Chiang Mai province, Thailand and describe their association with child nutritional status.

METHOD

A cross-sectional study was conducted in 32 villages in a remote rural area of Chiang Mai, Thailand in 1999 with the permission of the Chiang Mai Public Health Office. All women with children aged less than 36 months were requested to participate. Mothers were interviewed for information about breastfeeding practices, weaning time, types of supplementary foods, level of education, types of houses and perception on family economic status. The children's weight was measured using a Salter scale in kilograms. Length was measured with a Starter measure mat in centimeters (Child Growth Foundation, UK). Z-scores for weight for height and height for age were calculated, and compared with a WHO reference population(8). Undernutrition, wasting and stunting were determined as a Z score less than -2 standard deviations for weight for age, weight for height and height for age, respectively(9). Correlation coefficients were estimated to describe relationship between age and Z-score for weight for height. The Chi-square test was used for comparisons of proportions of undernutrition, wasting and stunting between the groups. The statistical significance was considered at a p-value of < 0.05.

RESULTS

Three hundred and ninety-five women and their children were studied. This number was approximately 80 per cent of women with children less than 36 months of age in the study area. Maternal characteristics relating to breastfeeding are shown in Table 1. There was a higher prevalence of breastfeeding amongst hill-tribes than amongst Thai women (89.2% compared to 57.3%). Breastfeeding was also associated with younger age, higher parity and less education. The pattern of breastfeeding is shown in Table 2. Ninety two per cent of children aged up to six months were breastfed, with 52 per cent exclusively breastfed. For older children other foods complemented breastfeeding. Sixty six per cent (134/201) of children aged more than one year were still breastfed and 12 (5.9%) were exclusively breastfed (Table 2). Rice was the most frequently introduced in the first six months (61.9%), followed by egg (47.6%) and banana (28.5%). Meat, fish and vegetables were used as supplements in children older than six months (20.5%, 14.1% and 10.2%, respectively). Beans were supplemented late in the second year of life, although this was infrequent. The mean age (SD) for introduction of supplementary food was 3.8 (3.82) months. The mean age (SD) for weaning was 10.9 (4.68) months. The prevalence of undernutrition, wasting, and stunting is shown in Table 3. For children aged up to 6 months, the prevalence of undernutrition, wasting and stunting in the exclusively breastfed group was 0.0 per cent, 1.9 per cent and 7.7 per cent, respectively, compared to 2.1 per cent, 4.3 per cent and 8.5 per cent, respectively in partial/non-breastfed children. These differences were not significant. For children aged between 7-12 months, the undernutrition, wasting, and stunting in the exclusively breastfed group was 23.1 per cent, 15.4 per cent and 7.7 per cent, respectively, compared to 13.4 per cent, 7.3 per cent and 9.8 per cent, respectively in partial/non-breastfed children. These differences were not significant. For children older than one year, the number of exclusively breastfed was small (12/201). The undernutrition, wasting, and stunting in these children was 50 per cent, 0.0 per cent and 41.6 per cent, respectively, compared to 37 per cent, 14.8, and 37.5 per cent, respectively in partial/non-breastfed children. These differences were not significant.

Table 1. Maternal characteristics and prevalence of breastfeeding

Maternal characteristics	Number*	Still breastfeeding		P-value
		No.	%	
Age (years)				
15-20	85	78	91.8	
21-30	220	171	77.7	
≥ 31	87	64	73.6	< 0.01
Race				
Hill-tribe	278	248	89.2	
Thai	117	67	57.3	< 0.001
Parity				
1 to 2	279	213	76.3	
3 or more	116	102	87.9	< 0.01
Education				
None	217	192	88.5	
Primary school	176	121	68.8	
Economic status				
Poor	139	114	82.0	
Fair	244	191	78.3	> 0.05

* Group totals differ due to missing data for some characteristics.

Table 2. Breastfeeding pattern by age.

Age (months)	Number	Breastfeeding					
		Exclusive No.	%	BF with SF* No.	%	Not BF No.	%
0-6	99	52	52.5	40	40.4	7	7.1
7-12	95	13	13.7	76	80.0	6	6.3
13-18	97	10	10.3	64	66.0	23	23.7
19 or more	104	2	1.9	58	55.8	44	42.3
Total	395	77	19.5	238	60.2	80	20.3

* Breastfeeding with infant formula or supplementary food

Table 3. Undernutrition, wasting and stunting in exclusively breastfed and partial/non-breastfed children in relation to age.

Age Breastfeeding	No.	Prevalence of					
		Undernutrition[a] No.	%	Wasting[b] No.	%	Stunting[c] No.	%
0-6 months							
Exclusive	52	0	0.0	1	1.9	4	7.7
Partial/No	47	1	2.1	2	4.3	4	8.5
7-12 months							
Exclusive	13	3	23.1	2	15.4	1	7.7
Partial/No	82	11	13.4	6	7.3	8	9.8
> 12 months							
Exclusive	12	6	50.0	0	0.0	5	41.6
Partial/No	189	70	37.0	28	14.8	71	37.5

[a] Z-score for weight for age < -2, [b] Z-score for weight for height < -2, [c] Z-score for height for age < -2.
All categories were not significant different between exclusively breastfed and partial/non-breastfed for all age groups (p-value > 0.05)

DISCUSSION

This study showed that breastfeeding was highly prevalent amongst the hill-tribe population especially in uneducated multiparous women. The high prevalence of breastfeeding could relate to a previous positive experience with breastfeeding, as well as strong support from the family. These women also commonly introduce infant formula and supplementary foods during the early months after delivery. Only 53.6 per cent of children were exclusively breastfed in the first six months of life. Breastfeeding tended to be continue until or beyond the age of one year. A small number of breastfed children older than one year had never received other foods. This may occur due to ignorance of the mothers, as well as limitation of available food in poor families. The authors are uncertain about maternal perceptions on breastfeeding and food supplementation. Breastfeeding mothers may pay less attention to food supplementation, as there is no immediate need to secure a variety of supplemented foods. There was no significant differences in the prevalence of undernutrition, wasting and stunting between exclusively breastfed children and partial or non-breastfed children. The results showed that children were more likely to be malnourished as age increases in either the exclusively breastfed or partial/non-breastfed group. This may not be a breastfeeding issue but weaning practices. Appropriate food supplementation and correct weaning practices are essential in order to maintain nutritional status of children beyond six months of age.

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ภาวะโภชนาการในเด็ก และการเลี้ยงดูด้วยนมมารดา ในเขตพื้นที่ชนบทของจังหวัดเชียงใหม่

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การศึกษานาภาคตัดขวางนี้เป็นการศึกษาการเลี้ยงลูกด้วยนมแม่ของในพื้นที่ชนบทห่างไกลในจังหวัดเชียงใหม่ เก็บข้อมูลด้วยการสัมภาษณ์กลุ่มตัวอย่างมารดาที่มีบุตรอายุต่ำกว่า 36 เดือนจำนวน 395 คน และการประเมินภาวะโภชนาการของเด็กด้วยการชั่งน้ำหนักและวัดส่วนสูง กลุ่มตัวอย่างมารดาประกอบด้วยหญิงชาวไทยภูเขาและหญิงชาวไทยพื้นราบคิดเป็นร้อยละ 70 และร้อยละ 30 ตามลำดับ ผลการศึกษาพบว่า มารดาชาวไทยภูเขาที่ไม่มีการศึกษาและมีบุตรหลายคนมีการเลี้ยงลูกด้วยนมตนเองมากกว่ามารดาชาวไทยพื้นราบ มีเด็กร้อยละ 53.6 ที่ได้รับนมแม่เพียงอย่างเดียวในช่วงอายุ 0–6 เดือน การเลี้ยงลูกด้วยนมแม่พบว่ามีต่อเนื่องนานได้ถึงมากกว่า 1 ปี โดยมีการให้อาหารเสริมร่วมด้วย ความชุกของภาวะน้ำหนักตัวน้อย (Undernutrition) ภาวะการเจริญเติบโตช้า (Wasting) และภาวะเตี้ยแคระ (Stunting) ของเด็กที่มีอายุ 0–6 เดือนในกลุ่มที่กินนมแม่เพียงอย่างเดียวคิดเป็นร้อยละ 0.0, 1.9 และ 7.7 ตามลำดับ เปรียบเทียบกับกลุ่มที่ไม่ได้กินนมแม่คิดเป็นร้อยละ 2.1, 4.3 และ 8.5 ตามลำดับ ($p > 0.05$) สำหรับเด็กที่มีอายุระหว่าง 7–12 เดือน พบว่าภาวะน้ำหนักตัวน้อย ภาวะการเจริญเติบโตช้าและภาวะเตี้ยแคระในกลุ่มที่กินนมแม่เพียงอย่างเดียวมีร้อยละ 23.1, 15.4 และ 7.7 ตามลำดับ เปรียบเทียบกลุ่มที่ไม่ได้กินนมแม่มีร้อยละ 13.4, 7.3 และ 9.8 ตามลำดับ ($p > 0.05$) สำหรับเด็กที่มีอายุมากกว่า 1 ปี ($n = 201$ คน) พบว่ามีเด็ก 12 ใน 201 คน ที่ได้รับนมแม่เพียงอย่างเดียว และพบว่า 6 ใน 12 มีภาวะน้ำหนักตัวน้อย เปรียบเทียบกับกลุ่มที่ไม่ได้กินนมแม่พบ 70 ใน 189 ราย ($p > 0.05$) ผลการศึกษานี้ยืนยันว่า เด็กมีโอกาสเกิดภาวะทุพโภชนาการมากขึ้นตามอายุที่เพิ่มขึ้น ทั้งในกลุ่มที่กินนมแม่เพียงอย่างเดียวและกลุ่มที่ไม่ได้กินนมแม่ ซึ่งสาเหตุอยู่ที่การปฏิบัติในการเลี้ยงดูเด็กในระยะหย่านมมากกว่าที่จะเป็นเรื่องการได้รับนมแม่หรือไม่ได้รับ หรือระยะเวลาของการได้รับนมแม่ การดูแลเด็กอายุ 6 เดือนขึ้นไปต้องให้ความสำคัญ ของการเสริมอาหารที่เหมาะสมและเพียงพอเพื่อให้เด็กมีภาวะโภชนาการที่ดีต่อไป

คำสำคัญ : ภาวะโภชนาการ, การเลี้ยงลูกด้วยนมแม่, ภาวะน้ำหนักตัวน้อย

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Implementation of clinical practice guidelines for upper respiratory infection in Thailand

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KEYWORDS
Clinical practice guidelines; Implementation; Upper respiratory infection

Summary *Objective:* To determine the effectiveness of implementing clinical practice guidelines (CPG) on antibiotic prescribing for adults with upper respiratory infection (URI) in terms of the changes in diagnosis and prevalence and patterns of antibiotic prescribing.

Methods: The CPG on antibiotic treatments for adults with URI published in the Annals of Internal Medicine 2001; 134: 479–52 were considered to be of high quality and applicable to Thai patients. A one-page clinical practice protocol in Thai was prepared from these guidelines. The dissemination strategy provided CPG and clinical practice protocol to 12 general practitioners in Siriraj Social Security Program in Bangkok during interactive educational meetings in April 2001. The information on 837 URI episodes from January to March (pre-CPG phase) and 774 URI episodes during May to July (post-CPG phase) were extracted from the patients' medical records. Telephone follow up for patients without antibiotics in the post-CPG phase was also attempted.

Results: Changes in the post-CPG period included (1) The diagnosis of URI was used less frequently whereas the diagnosis of common cold, pharyngitis and acute bronchitis were used more frequently ($p < 0.05$). (2) Antibiotic use fell from 74.0% to 44.1% ($p < 0.001$). (3) Fewer prescriptions for amoxicillin, roxithromycin, co-trimoxazole and doxycycline, and more for penicillin V ($p < 0.05$). Patients ($n = 97$) not given antibiotics reported recovery in 83.5% and improvement in 16.5%.

Conclusion: A locally prepared clinical practice protocol based on US CPG for appropriate antibiotic use for URI combined with interactive educational meetings is effective in promoting appropriate diagnosis and antibiotic therapy in an ambulatory setting in a tertiary care hospital in Thailand.

Introduction

The prevalence of penicillin-resistant *Streptococcus pneumoniae* in Thailand increased to 42% in 2000.[1,2] Overuse of antibiotics for minor respiratory infections is found to be an important factor for the

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selection of resistant strains.[3–6] Antibiotics are prescribed to 51–76% of adults with upper respiratory infections (URI) in the United States.[7,8] We found that antibiotics were prescribed to 80% of adults with URI who visited the Social Security Program at Siriraj Hospital, Bangkok, Thailand in the year 2000. It seems reasonable that reducing the use of unnecessary antibiotics could decrease or at least halt the development of drug-resistant streptococci.[9–11] Effective interventions are needed in view of the high rates of antibiotic resistance and use in Thailand and other countries.

The objective of this study was to determine the effectiveness of a simple one-page clinical practice protocol and US CPG for appropriate antibiotic use for URI combined with interactive educational meetings with general practitioners to improve the diagnosis and the use of antibiotics for adults with URI.

Methods

The study was approved and endorsed by Faculty of Medicine Siriraj Hospital, Mahidol University. It was conducted at Siriraj Hospital in Bangkok, a 2000-bed tertiary care university hospital. There are about 80,000 adult outpatient visits annually to the Social Security Program in the hospital clinic. URI accounts for about 5% of these visits. The key recommendations presented in position papers on appropriate antibiotic use for URI in adults (Ann Intern Med 2001; 134: 479-529) were used to prepare a one-page clinical practice protocol in the Thai language (Figure 1).

The first part of the protocol emphasizes an importance for the diagnosis of specific clinical syndromes, i.e., common cold, rhinitis, non-specific upper respiratory tract infections, pharyngitis, tonsillitis, sinusitis, rhinosinusitis and acute bronchitis.

Clinical Practice Protocol on Antibiotic Use in Adults with Upper Respiratory Infections (URI) *

This protocol is intended for guiding general practitioners in making diagnoses and prescribing antibiotics for adults with upper respiratory infections in ambulatory care.

An adult who has no chronic or serious underlying diseases and presents to ambulatory care with symptoms and/or signs of upper respiratory infections should receive a more specific diagnosis of 'common cold' or 'rhinitis' or 'rhinopharyngitis' or 'pharyngitis' or 'tonsillitis' or 'sinusitis' or 'acute bronchitis' depending on his/her major symptoms and signs.
A diagnosis of 'URI' should be avoided.

The recommended treatments for each clinical syndrome of upper respiratory infections are:

Common cold/rhinitis/non-specific upper respiratory tract infections
- Symptomatic therapy such as an antipyretic should be given
- An antibiotic is not necessary since this syndrome is almost always caused by viruses.

Pharyngitis/tonsillitis
- Symptomatic therapy such as an antipyretic should be given
- An antibiotic should not be given routinely since most of the cases are caused by viruses. An antibiotic should be given to the patient who has at least three of the following criteria: fever, tonsillar exudate, tender anterior cervical lymphadenopathy, no cough. The antibiotic of first choice is penicillin V since group A streptococcus has not been resistant to penicillin. Erythromycin should replace penicillin V for the patient allergic to penicillin.

Sinusitis/rhinosinusitis
- The patient with mild symptoms should receive symptomatic therapy such as an antipyretic. An antibiotic may not be given
- The patient with severe symptoms or persistent symptoms longer than seven days should receive an antibiotic. The antibiotic of first choice is amoxicillin.

Acute bronchitis
- The patient should receive symptomatic treatment such as an antipyretic
- A β-agonist inhaler may relieve the symptoms
- An antibiotic is not necessary since this syndrome is usually caused by viruses.

* This protocol is modified from Principles of Appropriate Antibiotic Use for Treatment of Acute Respiratory Tract Infections in Adults: Background, Specific Aims, and Methods. *Annals of Internal Medicine* 2001;134:479–529

Figure 1 Translation into English from Thai of the Clinical Practice Protocol.

The second part focuses on the antibiotics recommended for each clinical syndrome. The clinical practice protocol and US CPG were presented to 12 general practitioners who provided care for the Social Security Program. Two sessions of interactive educational meeting were organized in April 2001. Each session lasted 1.5 hours.

One of the investigators (VT) presented the current situation on antibiotic use for adults with URI at the ambulatory care service of Social Security Program and the necessity for change. The rationale for a separate diagnosis of each clinical URI syndrome and the principles for prescribing antibiotics for each clinical syndrome were then explained. Evidence for each recommendation in the CPG was clarified. The physicians agreed to adhere to the clinical practice protocol.

Sample size was based on the following considerations. The antibiotic prescription rate for adults with URI at the ambulatory care service of Social Security Program at Siriraj Hospital in 2000 was approximately 80%. It was hypothesized that antibiotic prescriptions could be reduced to 50% or less. For a 5% type I error and 20% type II error, 50 episodes of URI for each general practitioner were required. Therefore at least 600 episodes of URI for each period were needed. The medical records of the patients who attended ambulatory care service from January to March 2001 (pre-CPG period) and May to July 2001 (post-CPG period) were retrieved. The inclusion criteria were that:

- The adult patients had no underlying diseases and that they received care from the participating general practitioners.
- Information was extracted on diagnoses and antibiotic prescriptions.
- The clinical outcomes for patients who received no antibiotics during the post-CPG period were assessed by telephone interviews at seven days following their visits.
- The data were analyzed by descriptive statistics. All comparisons were performed by a chi-square test using Epi-Info version 6. All statistical tests were 2-sided and considered significant at $p < 0.05$.

Results

The URI clinical syndromes identified by general practitioners during the two study periods are shown in Table 1. The diagnosis of URI was significantly reduced and pharyngitis, the common cold and bronchitis were diagnosed more often during the post-CPG period compared to the pre-CPG period. Time series analysis of clinical syndromes of URI revealed no significant difference among the months during each period. The antibiotic prescription rates were 74.0% in the pre-CPG period and 44.1% in the post-CPG period ($p < 0.001$, RR 0.6 with 95% CI 0.55–0.65).

The antibiotics prescribed during each period are shown in Table 2. There was a significant reduction in use of amoxicillin, co-trimoxazole, roxithromycin and doxycycline; and penicillin V was prescribed significantly more often during the post-CPG period compared with the pre-CPG period. Time series analysis of antibiotic prescription rates revealed no significant difference among the months during each period. Co-trimoxazole is not recommended in the URI antibiotic guidelines, nevertheless it accounted for 22.3% of the patients' prescriptions during the pre-CPG period and 17.1% during the post-CPG period. The correlation between the clinical syndromes of URI and antibiotic prescribing is shown in Table 3. The antibiotic prescription rate for the common cold was significantly less than for all other clinical syndromes of URI for both periods. The antibiotic prescription rates for URI, bronchitis and the common cold were significantly less during the post-CPG period when compared with those during the pre-CPG period.

Telephone interviews at seven days post-visit were attempted for 192 patients who received no antibiotics during the post-CPG period. Of these

Table 1 Clinical syndromes of URI made by general practitioners

Clinical syndrome	Pre-CPG period (837 episodes)	Post-CPG period (774 episodes)	p	Relative risk (95% confidence interval)
URI	720 (86.0%)	242 (31.1%)	<0.001	0.36 (0.33-0.40)
Pharyngitis	49 (5.9%)	192 (24.8%)	<0.001	4.24 (3.2-5.7)
Bronchitis	38 (4.5%)	99 (12.8%)	<0.001	2.6 (1.8-3.7)
Tonsillitis	24 (2.9%)	17 (2.2%)	0.5	0.8 (0.4-1.4)
Common cold	5 (0.6%)	223 (28.8%)	<0.001	48 (20-116)
Sinusitis	1 (0.1%)	1 (0.1%)	1	

Table 2 Antibiotic prescriptions made by general practitioners.

Antibiotic	Pre-CPG period (837 episodes)	Post-CPG period (774 episodes)	p	Relative risk (95% confidence interval)
Amoxicillin	289 (34.5%)	108 (14%)	<0.001	0.45 (0.37-0.55)
Cotrimoxazole	187 (22.3%)	132 (17.1%)	0.01	0.76 (0.62-0.93)
Roxithromycin	79 (9.4%)	22 (3%)	<0.001	0.3 (0.19-0.48)
Doxycycline	21 (2.5%)	4 (0.5%)	0.01	0.21 (0.07-0.6)
Penicillin V	16 (1.9%)	72 (9.3%)	<0.001	4.87 (2.86-8.29)
Cefuroxime	11 (1.3%)	0		
Erythromycin	3 (0.4%)	2 (0.3%)		
Spiramycin	6 (0.7%)	0		
Co-amoxiclav*	1 (0.1%)			
Lincomycin	3 (0.4%)	1 (0.1%)		
Cephalexin	2 (0.2%)	0		
Norfloxacin	1 (0.1%)	0		

*Amoxicillin-clavulanate.

Table 3 Prevalence of antibiotic prescribing in each clinical syndrome of URI.

Clinical syndrome	Prevalence of antibiotic prescription	
	Pre-CPG phase (%)	Post-CPG phase (%)
URI	73	49
Pharyngitis	81	78
Tonsillitis	92	94
Bronchitis	74	40
Common cold	20	10

†p < 0.01 when compared with other clinical syndromes.

97 (50.5%) were contacted after two attempts. Eighty-one (83.5%) of patients reported URI recovery, 16 (16.5%) reported that they had much improved.

Discussion

Clinical practice guidelines are tools for changing clinicians' behaviour. Success in promoting more appropriate healthcare behaviour in clinicians depends on the quality and relevance of clinical practice guidelines and the effectiveness of the strategy used to disseminate the information. It was found that the URI CPG published in the Annals of Internal Medicine to be of a high quality according to Shaneyfelt's criteria[12] and relevant to clinical practice in Thailand. They are however in English and are much too long and detailed to be useful for busy practitioners. It was felt that only a few key points were needed to construct a practical protocol. Two main issues were focused upon: diagnosis and antibiotic prescribing for healthy adults with URI. It was elected to use interactive educational meetings for this study because it has been demonstrated to be an effective dissemination strategy.[13,14]

The intervention used in the study was effective in changing clinicians' behaviour in the diagnosis and treatment of URI patients. Similar results have been obtained by different interventions.[15,16] It is believed that a major factor contributing to the success of the current intervention was the substantial increase in the diagnosis of the common cold. Most of the clinic physicians agreed that antibiotics are not needed for this condition. A relatively small proportion of the patients were diagnosed with pharyngitis or tonsillitis, but antibiotic prescription rates for these two syndromes were still high (78–94%). This appears to be excessive since only up to 30% of the healthy adults with pharyngo-tonsillitis were found to have a positive throat culture for *Streptococcus pyogenes* (Thamlikitkul V, unpublished data). Use of rapid diagnostic methods for this bacterium should help reduce rate of antibiotic use, but may not reduce costs.

Co-trimoxazole is not recommended in the URI antibiotic guidelines. Nevertheless it accounted for 22.3% of the patients' prescriptions during the pre-CPG period and 17.1% during the post-CPG period. This is explained by the use of this drug by one senior clinician for almost all his patients with URI. He did not change his prescribing behaviour after receiving the intervention. When this practitioner's practice was excluded, the

antibiotic prescription rates were reduced from 66.8% to 34.2% for the pre-CPG period and post-CPG period respectively ($p < 0.001$). He has now retired.

Several issues continue to be of concern. First, although the antibiotic prescription rates fell from 74.0% to 44.1%, they still remained high in the post-CPG period. Ideally the antibiotic prescription rate for healthy adults with URI should not exceed 10%, since more than 90% are not caused by bacteria. Given the uncertainty of clinical findings in differentiating bacterial from viral infection in pharyngitis and tonsillitis, the antibiotic prescription rate would be expected to exceed 10% for these conditions, but a 44.1% use during the post-CPG period still appears to be excessive. Second, although the selection of antibiotics during the post-CPG period tended to be more appropriate, the choice made by the general practitioners needs to be improved. Third, this intervention was successful for at least a three-month period. In order to maintain the effectiveness of our intervention, all general practitioners have been reminded every six months since January 2002. The prevalence of antibiotic prescribing in 100 consecutive adult patients with URI in June 2002 was 41%. Finally, evidence-based clinical practice guidelines may need to be shown to be safe as well as effective under field conditions. It was found that virtually all patients who did not receive antibiotics during the post-CPG period had improved and none required readmission.

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The effect of quicklime (calcium oxide) as an inhibitor of *Burkholderia pseudomallei*

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Summary Measurement of in vitro activity of quicklime against *Burkholderia pseudomallei* revealed that quicklime at concentrations of 10% or more was bactericidal for up to 35 d. The effect of quicklime as an inhibitor of *B. pseudomallei* in soil from a rice field was studied in a laboratory setting. The soil, collected from a rice field in north-eastern Thailand, was mixed with *B. pseudomallei*. In experiment 1, quicklime was mixed with the soil in different amounts. In experiment 2, quicklime was spread over the soil surface. In experiment 3, quicklime solution was poured onto the soil. It was found that the pH of the soil in experiment 1 was much higher than that in experiments 2 and 3. Only quicklime mixed with soil at a concentration of 40% or more (weight/weight) was effective in inhibiting the growth of *B. pseudomallei* for up to six weeks.

1. Introduction

Melioidosis is caused by *Burkholderia pseudomallei*, which is a saprophytic bacterium in the soil. The disease is prevalent in South-East Asia and northern Australia (Chaowagul et al., 1989; Dance, 1991; Suputtamongkol et al., 1994). Epidemiological studies of melioidosis and *B. pseudomallei* in Thailand have shown that the disease is more prevalent in north-eastern Thailand; arabinose-negative *B. pseudomallei* was also found in soil collected from north-eastern Thailand (Trakulsomboon et al., 2000; Vuddhakul et al., 1999). The organism can be readily isolated from environmental sources such as rice paddies, still or stagnant water and moist soils which predominate in the tropics, and it is believed that these habitats are the primary reservoirs (Ellison et al., 1969). Under laboratory conditions, it was found that *B. pseudomallei* survives best in an environment with a pH of 5.0–8.0, although it was also able to survive for a long period at pH 4.0 (Tong et al., 1996). Consequently, the survival of *B. pseudomallei* may be favoured by the relatively acidic environment of a rice paddy, which is usually pH 5.0–6.8 and is pH 4.4–7.7 in north-east Thailand (Kanai and Kondo, 1994). It was reported that *B. pseudomallei* was able to grow on glyphosphate, a non-selective herbicide,

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as a sole phosphorous source (Peñaloza-Vazquez et al., 1995). *Burkholderia pseudomallei* can reduce nitrate in order to grow in an anaerobic environment, and the use of nitrate fertilizers might thus contribute to its proliferation in agricultural land (Kanai and Kondo, 1994).

Calcium oxide (quicklime) is a strong base and a disinfectant. The active ingredient that exerts killing activity against the pathogens is calcium hydroxide, which is produced when calcium oxide is mixed with water. A rapid reduction in total coliforms, *Salmonella* and *Shigella* counts was observed after the addition of quicklime and cement dust to sewage sludge (Amer, 1997; Plachy et al., 1996). One kilogram of quicklime spread over the sawdust bedding of dairy cows reduced bacterial counts (Hogan and Smith, 1997). Application of quicklime to pasture areas of a dairy herd which was paratuberculosis-positive was associated with a 72% reduction in the number of test-positive cattle (Johnson-Ifearulundu and Kaneene, 1999). In Thailand, quicklime has been used for a long time in farming systems. In addition, farmers usually apply quicklime to the soil in order to adjust soil conditions and eliminate some plant parasites. It was reported that quicklime could adjust the acid-base balance of the soil (Stevens and Laughlin, 1996). These applications reduced the need for chemical fertilizers, increased the quality of calcium in the soil and also increased grass production. An application of quicklime to soil was used for commercial carrot production to reduce the incidence of cavity spot disease in carrots (El-Tarabily et al., 1996).

The objectives of this study were to determine the in vitro activity of quicklime against arabinose-negative *B. pseudomallei* and the effect of quicklime on inhibiting arabinose-negative *B. pseudomallei* in soil under experimental conditions.

2. Materials and methods

2.1. Bacterial strain

The arabinose-negative *B. pseudomallei* used in the study was that isolated from the blood of a patient. The organism was grown on modified Ashdown's agar at 37 °C for 48 h. Five colonies of *B. pseudomallei* were suspended in Tryptic Soy Broth (TSB) and incubated overnight on an orbital shaker at room temperature. The concentration of pathogens was measured with a spectrophotometer at a wave length of 500 nm. The cultured broth was kept at 4 °C, and suspended in sterile distilled water in order to produce a concentration of 5.5×10^5 cfu/ml. This broth was used for all experiments.

2.2. Quicklime (CaO)

Quicklime was purchased from a factory in Saraburi Province, Thailand and dissolved in water at 30% (weight/volume) in order to produce a solution with a pH of at least 12.0.

2.3. In vitro study of quicklime against *Burkholderia pseudomallei*

Quicklime was dissolved in sterile distilled water to make 200 ml suspension of varying concentrations (weight in grams/volume in ml) of 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50%. A bottle of 200 ml sterile distilled water served as the control. Each sample was inoculated with 1 ml of *B. pseudomallei* suspension in TSB with 5.5×10^5 cfu/ml on days 1, 7, 14, 21, and 35. The mixtures were kept at room temperature and 3 ml of suspension was withdrawn at 0 h, after 6 h of incubation and just prior to the next inoculation of *B. pseudomallei*, added to 10 ml of threonine basal salt solution containing 20 mg/l colistin (TBSS-C20) and incubated at 42 °C for 48 h. The samples were then subcultured onto Ashdown's medium plates and incubated at 37 °C for 3 d. *Burkholderia pseudomallei* was identified using standard biochemical tests. The pH of the suspension was also measured along with the subculture.

2.4. Study of the inhibitory effect of quicklime against *Burkholderia pseudomallei* growth in soil

2.4.1. Soil sample

Soil was collected from a rice field in Khon Kaen province, Thailand. The soil was then left to dry under sunlight for two weeks and a culture of the soil sample revealed no *B. pseudomallei*. Forty kilograms of soil was put in each container to a depth of 40 cm. The soil was inoculated with 2.5 l of *B. pseudomallei* solution at a concentration of 5.5×10^5 cfu/ml.

2.4.2. Study procedures

Three experiments were performed using the above described samples. In experiment 1, quicklime was mixed with the soil at 2.5, 5, 10, 15, 20, 25, 30, 35, 40 and 50% (weight/weight) concentrations. In experiment 2, quicklime was spread over the soil at amounts of 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 kg/m^2. In experiment 3, quicklime solution in distilled water at concentrations of 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50% (weight/volume) was poured onto the soil once daily. A control soil without quicklime was set up

for each experiment. All soil samples were soaked by spraying with water once daily and the containers of soil in all experiments were reinoculated with the same amount of *B. pseudomallei* every 7 to 10 d. Three grams of soil sample were collected at depths of 0, 5, 10, 20, and 30 cm on days 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 and every week for 6 weeks. The soil samples were taken to the laboratory for detection of *B. pseudomallei*. The pH of the soil sample was also recorded at the time of soil collection. A survival assay of *B. pseudomallei* was done by mixing the soil sample with distilled water for 2 min. Then 2 ml of the supernatant was transferred into 10 ml TBSS-C20. The mixture was incubated at 42 °C for 48 h, and subcultured onto modified Ashdown's agar. The plate was incubated at 37 °C for 48–72 h. *B. pseudomallei* was identified using standard biochemical procedures.

3. Results

3.1. In vitro activity of quicklime against *Burkholderia pseudomallei*

The pH values of the quicklime suspensions at different concentrations are shown in Table 1. All quicklime suspensions were alkaline. *Burkholderia pseudomallei* was consistently isolated from the control bottle without quicklime. The serial subcultures from the quicklime suspensions at all concentrations revealed no growth for up to 7 d. The quicklime suspension with a concentration of 5% was bactericidal for 21 d, whereas the quicklime suspension with a concentration of 10% or more exerted killing activity against *B. pseudomallei* for up to 35 d as shown in Table 1.

Table 1 The average pH and in vitro activity of quicklime suspensions against *Burkholderia pseudomallei*

Concentration of quicklime suspension (%) (weight/volume)	pH	Bactericidal activity at 6 h, 24 h, 7 d, 14 d, 21 d, and 35 d
0	7.8	No
2.5	8.81	Yes, up to day 7
5	9.77	Yes, up to day 21
10	12.14	Yes, up to day 35
15	12.41	Yes, up to day 35
20	12.45	Yes, up to day 35
25	12.59	Yes, up to day 35
30	12.61	Yes, up to day 35
35	12.66	Yes, up to day 35
40	12.72	Yes, up to day 35
45	12.72	Yes, up to day 35
50	12.73	Yes, up to day 35

Table 2 The average pH of the soil mixed with different amounts of quicklime (experiment 1)

Concentration of quicklime in soil mixture (%) (weight/weight)	pH
2.5	7.47
5	7.64
10	7.68
15	7.71
20	8.06
25	8.54
30	8.56
35	8.75
40	9.45
45	9.86
50	10.59

3.2. Inhibitory effect of quicklime against *Burkholderia pseudomallei* in soil

The pH of the soil without quicklime was 5 to 6 and *B. pseudomallei* was consistently detected. In contrast, the soil with quicklime had a pH higher than 6 as shown in Tables 2–4. The pH of the soil mixture with quicklime in experiment 1 was much higher than that in experiments 2 and 3, as also shown in Tables 2–4. In experiment 1, quicklime at 5% or more was effective in inhibiting the growth of *B. pseudomallei* for up to 7 d. However, only quicklime at 40% or more remained effective for up to 6 weeks. In experiments 2 and 3, *B. pseudomallei* could still be recovered from soil mixture at all concentrations of quicklime.

Table 3 The average pH of soil treated by surface-spreading of different amounts of quicklime (experiment 2)

Amount of quicklime in soil mixture (kg/m^2)	pH
0.25	6.63
0.5	6.79
1	6.97
1.5	6.84
2	7.17
2.5	7.14
3	7.26
3.5	7.18
4	7.29
4.5	7.25
5	7.32

Table 4 The average pH of soil treated with quicklime solution (experiment 3)

Concentration of quicklime solution (%) (weight/volume)	pH
2.5	7.10
5	7.14
10	7.29
15	7.39
20	7.41
25	7.24
30	7.27
35	7.36
40	7.23
45	7.47
50	7.38

4. Discussion

Our study demonstrated that quicklime was also bactericidal against *B. pseudomallei* in addition to other pathogens such as coliforms, *Shigella* spp., *Salmonella typhimurium*, *Pythium coloratum* and *Mycobacterium paratuberculosis* as has been observed in previous studies (Hogan and Smith, 1997; Johnson-Ifearulundu and Kaneene, 1999; Plachy et al., 1996). It was also shown that a quicklime suspension with a pH of less than 10 was not effective, whereas that with pH of 12 or greater was always bactericidal. Since quicklime has been used for adjusting soil pH and preventing infectious diseases in plants (El-Tarabily et al., 1996; Peñaloza-Vazquez et al., 1995; Stevens and Laughlin, 1996), it could therefore be a potential substance for reducing the burden of *B. pseudomallei* in the soil of rice fields. Although several studies have demonstrated that quicklime is a strong disinfectant against various pathogens when it is either mixed with or spread over contaminated materials such as sewage sludge (Amer, 1997; Hogan and Smith, 1997; Johnson-Ifearulundu and Kaneene, 1999; Plachy et al., 1996), the findings from our study revealed that only soil mixed with a large amount of quicklime could inhibit the growth of *B. pseudomallei*, whereas quicklime spread over the soil or quicklime solution poured over the soil surface was not effective. This could be explained by differences in the textures of the contaminated materials. Sewage sludge or animal sawdust bedding is semisolid, whereas soil is solid. It is unlikely that quicklime spread over or poured over the soil surface can inhibit the growth of the pathogens residing far beneath the surface. Another explanation for our observation is that only the soil mixed with a large amount of quicklime will have a strongly alkaline pH of more than 8, since the killing activity of quicklime is dependent on an alkaline pH. Although rice stems in a rice field can grow at pH 4–10, studies on the effect of quicklime (or strong alkali) on the growth of rice and the ecological changes of the surrounding environment need to be explored prior to recommending quicklime as a measure for environmental control of *B. pseudomallei*, since quicklime has to be used in large amounts to decontaminate the soil from the presence of *B. pseudomallei*.

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Randomized Controlled Trial of *Tinospora crispa* for Additional Therapy in Patients with Type 2 Diabetes Mellitus

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A randomized double blind placebo controlled trial was conducted to determine the efficacy of Tinospora crispa as additional treatment in patients with type 2 diabetes mellitus who did not respond to oral hypoglycemic drugs and refused insulin injection. Twenty patients were allocated to receive Tinospora crispa powder in capsule form at a dosage of 1 gram thrice daily for 6 months. Twenty patients received a placebo. The main outcomes were changes in fasting plasma glucose, glycosylated hemoglobin and insulin levels. The baseline characteristics of the patients in both groups were not significantly different. There were no significant changes in fasting plasma glucose, glycosylated hemoglobin and insulin levels among the patients within the group and between groups. Two patients who received Tinospora crispa showed marked elevation of liver enzymes that returned to normal after discontinuing Tinospora crispa. Moreover, patients in the Tinospora crispa group had significant weight reduction and cholesterol elevation while taking Tinospora crispa. It is concluded that there is no evidence to support the use of Tinospora crispa 3 grams a day for additional therapy in patients with type 2 diabetes mellitus who did not respond to oral hypoglycemic drugs. The patients receiving Tinospora crispa may have an increased risk of hepatic dysfunction.

Keywords : *Tinospora crispa, Diabetes mellitus*

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Tinospora crispa is a medicinal plant used as a remedy for patients with diabetes mellitus in Malaysia[1]. *Tinospora crispa* was found to have an anti-hyperglycemic effect in animals[2-4]. The hypoglycemic effect of *Tinospora crispa* is mediated by increasing insulin secretion from isolated rat and human islets of Langerhans[3]. *Tinospora crispa* is commonly used in diabetic patients in Thailand as well. Toxicological study of crude extract of *Tinospora crispa* revealed no obvious adverse effects[2]. However, animals of both sexes receiving the highest dose of *Tinospora crispa* extract had significantly higher alkaline phosphatase (ALP) levels, alanine aminotransferase (ALT) levels and liver weights than those of the water control and tragacanth control groups. Histopathological study of the liver indicated that male rats receiving the highest dose of the extract had significantly higher incidence of bile duct proliferation and focal liver cell hyperplasia than the two control groups. Blood chemistry studies revealed that both male and female rats receiving 1.28 g/kg. body weight of the extract had significantly higher cholesterol levels but significantly lower glucose levels than those of water control and tragacanth control groups. To our knowledge, there has been no controlled clinical trial of *Tinospora crispa* in patients with diabetes mellitus.

The objective of the study was to determine the efficacy of *Tinospora crispa* in patients with type 2 diabetes mellitus who did not respond to oral hypoglycemic drugs and refused insulin injection.

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Patients and Method

The study was a randomized double blind placebo controlled trial conducted at Siriraj Hospital.

The study was approved by the Institutional Review Board of the Faculty of Medicine Siriraj Hospital. The eligible study subjects were patients with type 2 diabetes mellitus older than 35 years who had received an adequate dose of oral hypoglycemic agents for at least 2 months and still had a glycosylated hemoglobin of greater than 8.5% and refused insulin injection. Patients with liver disease, heart disease, renal impairment or those who previously received traditional medicine were excluded. Eligible patients were randomly allocated to the study group or the control group. All subjects received oral hypoglycemic agents. The study group received additional *Tinospora crispa* powder in a capsule form at a dosage of 1 gram thrice daily for 6 months. *Tinospora crispa* powder was prepared by the Department of Medical Sciences, Ministry of Public Health. The control group was given placebo in an identical capsule to be taken in the same fashion as the study drug. Compliance with the medication was made by a pill count at each visit. The patients were interviewed, examined and blood was taken for complete blood count, fasting plasma glucose, liver enzyme profile and renal function at entry and every month during the study. Blood for glycosylated hemoglobin and insulin determination was collected at enrollment and every 2 months during the study.

A sample size of 16 patients per group was estimated according to the assumption that baseline mean glycosylated hemoglobin was 10% with a standard deviation of 2% and post treatment mean glycosylated hemoglobin in *Tinospora crispa* group was 8% or less with type I error 5% and type II error 20%. The data were analyzed by descriptive statistics, student t test, repeated measure ANOVA and chi-square test where appropriate. A p value of ≤ 0.05 indicates a statistically significant difference.

Results

There were 40 eligible patients. Twenty patients were in the study group and 20 in the control group. The baseline characteristics of the patients between the two groups were not significantly different as shown in Table 1. Six patients (3 in the *Tinospora crispa* group and 3 in the control group) were withdrawn from the study. One patient in the *Tinospora crispa* group had to receive insulin due to having active pulmonary tuberculosis. Two patients in the *Tinospora crispa* group had elevation of liver enzymes (SGOT and SGPT of greater than 200 u/L.) more than 3 times the baseline values after receiving it for 2 and 5 months. Liver enzymes in the aforementioned 2 patients returned to normal (less than 30 u/L.) after discontinuing *Tinospora crispa* for one month. One of them had evidence of hepatitis C infection. Two patients in the control group had to receive insulin due to having a subdural hematoma and being treated with prednisolone for Bell's palsy. One patient in the control group had to leave the study due to difficulty in returning to the clinic for follow up. Therefore, the authors were able to follow 34 patients until the end of the study. Fasting plasma glucose, glycosylated hemoglobin and insulin levels of the patients in both groups during 6 months were

Table 1. Baseline characteristics of the patients in the study

Characteristic	*Tinospora Crispa* Group(N=20)	Placebo Group(N=20)	P value
Gender, Male : Fema	7 : 13	5 : 15	0.7
Mean age, year (SD)	58.4 (9.2)	59.1 (10.7)	0.8
Mean body weight, kg (SD)	60.8 (10.0)	58.9 (10.0)	0.5
Mean BMI, kg./m² (SD)	27 (5.5)	26 (5.1)	0.7
Mean FPG, mg/dL. (SD)	214.9 (45.5)	227.3 (73.4)	0.5
Mean glycosylated Hb, % (SD)	10.4 (1.6)	10.0 (1.2)	0.4
Mean insulin level, uu/ml. (SD)	17.9 (9.5)	17.8 (13)	0.9
Mean hematocrit, % (SD)	38.7 (3.3)	39.7 (2.7)	0.3
Mean WBC (SD)	7,971 (2,072)	7,368 (1,586)	0.3
Mean BUN, mg./dL. (SD)	15.1 (5.2)	15.6 (4.9)	0.8
Mean creatinine, mg/dL. (SD)	1.0 (0.3)	1.0 (0.2)	0.6
Mean cholesterol, mg/dL (SD)	233.6 (51.9)	218.2 (31.7)	0.7
Mean triglyceride, mg/dL (SD)	204.8 (127.2)	183.9 (75.3)	0.5
Mean SGOT, u/L (SD)	28.36 (12.8)	25.8 (9.8)	0.4
Mean SGPT, u/L (SD)	30.3 (15)	27.5 (17.3)	0.6
Mean bilirubin, mg/dL (SD)	1.33 (0.26)	1.58 (0.27)	0.4

not significantly different as shown in Fig. 1 and Fig. 2. At the end of the study, all patients in the *Tinospora crispa* group had glycosylated hemoglobin values greater than 8.5% compared with 71% of the patients in the control group ($p = 0.04$). The body weight of the patients significantly decreased (approximately 2 kilograms) and the patients' cholesterol levels significantly increased (approximately 30 mg./dL.) after taking *Tinospora crispa*. Changes in hematocrit, white blood cells, triglyceride, renal function and liver profile of the remaining patients were not observed.

Discussion

This study was unable to demonstrate the efficacy of *Tinospora crispa* for therapy in patients with type 2 diabetes who did not respond to oral hypoglycemic drugs since there were no significant changes in fasting plasma glucose or glycosylated hemoglobin between those collected at baseline and during the study period in either group. Therefore, there is no evidence to support the use of *Tinospora crispa* in diabetic patients. However, there may be several explanations for being unable to detect any efficacy of *Tinospora crispa* in the present study. The authors recruited only type 2 diabetic patients who did not respond to an adequate dose of oral hypoglycemic agents. The insulin levels in the blood samples of the patients taking *Tinospora crispa* in the present study were not increasing. If the mechanism of action of *Tinospora crispa* is to stimulate insulin secretion, it is very unlikely that *Tinospora crispa* will be efficacious in these patients. A study that includes patients with mild diabetes who have never received oral hypoglycemic agents should be conducted in order to determine the efficacy of *Tinospora crispa*. Small sample size was not an explanation since 16 patients per group should be sufficient to detect the effect of at least 2% difference in glycosylated hemoglobin between the groups and there was no trend for any reduction in fasting plasma glucose or glycosylated hemoglobin in 17 patients who received *Tinospora crispa* for 6 months. In addition, all patients in the Tinospora crispa group still had glycosylated hemoglobin greater than 8.5% compared with 71% of those in the placebo group. An inadequate dosage or inadequate active ingredients of *Tinospora crispa* used in the study might explain the study results. A treatment duration of 6 months should be long enough to see the effect of treatment and this should not be the reason for negative results. Compliance with the medication was found to be satisfactory. Contamination was unlikely since this study included only patients who did not receive other traditional medicines. Co-intervention was considered insignificant since this study was double-blinded. *Tinospora crispa* is a well known appetite stimulant due to its bitterness and the patients in this group might consume more food after taking *Tinospora crispa* leading to uncontrolled diabetes and weight reduction. An explanation for the increase in cholesterol after taking *Tinospora crispa* is unclear. This observation was also found in animals[3]. Two patients (10%) who received *Tinospora crispa* at a dosage of 3 grams a day developed liver dysfunction and the study medication had to be discontinued. Although one patient had underlying chronic hepatitis, this

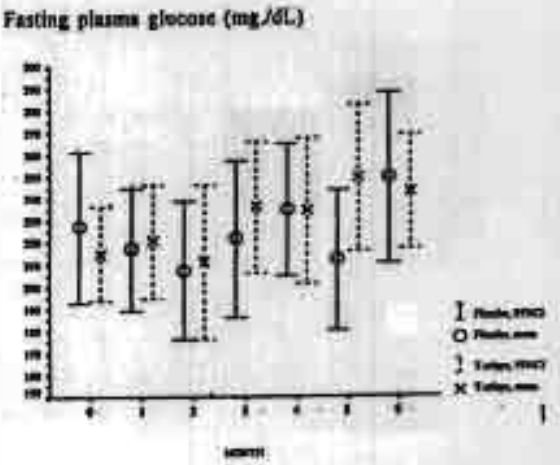

Fig. 1 Fasting plasma glucose in patients taking *Tinospora crispa* (X) and taking placebo (O)

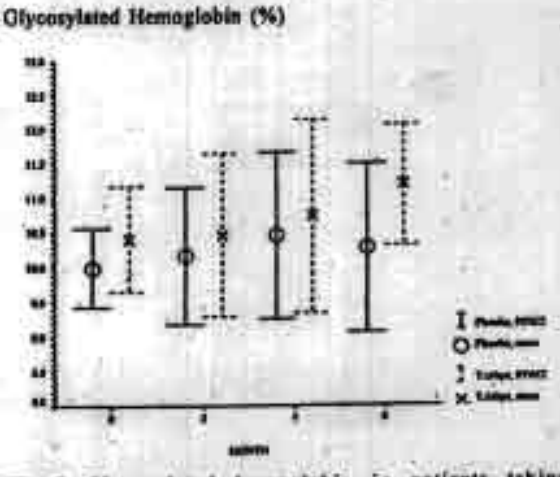

Fig. 2 Glycosylated hemoglobin in patients taking *Tinospora crispa* (X) and taking placebo (O)

observation suggests that hepatic dysfunction is an adverse effect of *Tinospora crispa*, and patients wanting to take *Tinospora crispa* and health care personnel who want to provide *Tinospora crispa* to the patients should be aware of this effect.

Acknowledgements

The authors wish to thank the Department of Medical Sciences, Ministry of Public Health for preparing the medications and supporting the study. Dr. Visanu Thamlikitkul is a senior researcher scholar, a recipient of the Thailand Research Fund.

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ประสิทธิผลของบอระเพ็ดในการรักษาผู้ป่วยเบาหวาน

ชวัญญา แสงสุวรรณ, สุทธิพล อุดมพันธุรัก, สาธิต วรรณแสง, วิษณุ ธรรมลิขิตกุล

คณะผู้วิจัยได้ศึกษาประสิทธิผลของการรักษาโรคเบาหวานในผู้ใหญ่ที่ไม่ตอบสนองต่อการรักษาด้วยยารับประทานและไม่ยินยอมรับการรักษาด้วยอินสุลินจำนวน 40 คนโดยแบ่งผู้ป่วยออกเป็น 2 กลุ่มแบบสุ่ม ผู้ป่วยจำนวน 20 คนได้รับการรักษาเดิมที่เคยได้รับร่วมกับบอระเพ็ดขนาด 1 กรัมรับประทานวันละ 3 ครั้งติดต่อกันนาน 6 เดือน ส่วนผู้ป่วยอีก 20 คนได้รับการรักษาเดิมที่เคยได้รับร่วมกับยาหลอก ลักษณะของพื้นฐานและความรุนแรงของโรคในผู้ป่วยทั้งสองกลุ่มไม่แตกต่างกัน ผลการศึกษาพบว่าระดับน้ำตาลในพลาสมาและระดับของ glycosylated hemoglobin ภายหลังได้รับบอระเพ็ดไม่ลดลงจากระดับก่อนได้รับบอระเพ็ด และไม่น้อยกว่ากลุ่มที่ได้รับยาหลอก ผู้ป่วย 2 ราย (ร้อยละ 20) ที่ได้รับบอระเพ็ดมีผลข้างเคียงที่ตับ ผู้ป่วยที่ได้รับบอระเพ็ดมีน้ำหนักตัวลดลงและมีระดับโคเลสเตอรอลในเลือดเพิ่มขึ้น การศึกษานี้แสดงว่าบอระเพ็ดไม่มีประสิทธิผลในการรักษาโรคเบาหวานในผู้ใหญ่ที่ไม่ตอบสนองต่อการรักษาด้วยยารับประทานและไม่ยินยอมรับการรักษาด้วยอินสุลินโดยอาจมีผลข้างเคียงต่อตับ

The Effect of *Thunbergia lauriforia Linn.* on Blood Alcohol Concentration after Consumption of Beer

Siriwan Sasithonrojanachai*
Suthipol Udompanthurak†
Visanu Thamlikitkul‡

Abstract Blood alcohol concentration was determined by alcohol breath test in nine adult healthy subjects after taking a bottle (630 ml) of Thai beer with an alcohol concentration of 8 per cent (vol/vol) and after taking 1.8 grams of *Thunbergia lauriforia Linn.* prior to consumption of Thai beer at the same concentration and amount. The concentration of alcohol in the blood after taking *Thunbergia lauriforia Linn.* was found to be statistically significantly lower (by 11.7 per cent) than that when was consumed alone.

(Intern Med J Thai 2004; 20:27-29)

Key words: *Thunbergia lauriforia Linn.*, blood, alcohol

Traffic accidents comprise one of the leading causes of death in Thailand and alcohol consumption has been found to be a major risk factor for road accidents[1-4]. There have been extensive campaigns against drunk drivers and breath testing in drivers has been officially employed since 1996. A blood alcohol concentration of 50 mg% or more is considered illegal for driving vehicles. In the year 2000, there was a claim publicized in the newspapers that taking *Thunbergia lauriforia Linn.* at the same time as alcohol could decrease the absorption of alcohol and therefore prevent testing positive on an alcohol breath test. *Thunbergia lauriforia Linn.* is a medicinal plant widely used in Thailand. The main chemical ingredients in this plant are flavonoids such as apigenin, cosmosin and delphinidin[5]. *Thunbergia lauriforia Linn.* has been found to produce the effect of anti-intoxication by organophosphate insecticides in animals[6,7]. To our knowledge, there is no information about the effect of *Thunbergia lauriforia Linn.* on alcohol absorption in human subjects.

The objective of the study was to determine the effect of *Thunbergia lauriforia Linn.* on blood alcohol concentration after consumption of beer in healthy adult volunteers.

SUBJECTS AND METHODS

The subjects were nine healthy adult volunteers, seven men and two women, average age 34.3 years (SD 7

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years), average height 165.9 cm (SD 6.5 cm) and average body weight 63.3 Kg (SD 10.3 Kg). They drank a bottle (630 ml) of Thai beer with an alcohol concentration of 8 per cent (vol/vol) on an empty stomach within 30 minutes. Blood alcohol concentration was measured by alcohol breath test every 15 minutes after commencing beer consumption for 180 minutes or until alcohol was undetectable. Several weeks later the same subjects took 1.8 grams of *Thunbergia lauriforia Linn.* dried leaf powder capsules orally prior to drinking the same amount of beer. Blood alcohol concentration was measured by alcohol breath test every 15 minutes after commencing alcoholic beverage consumption for 180 minutes or until alcohol was undetectable. The subject was instructed to thoroughly rinse the oral cavity with water to remove any residual alcohol in the oral cavity before measuring. All alcohol breath tests were performed using an Intoxilyser (model Alco-sensor IV, Intoximeters Inc., MO). The blood alcohol concentrations of the subjects in both experiments were analyzed by descriptive statistics and multiple regression analysis. A p value of 0.05 or less was considered statistically significant.

RESULTS

The blood alcohol concentration-time curves for both experiments are shown in Figure 1. The mean peak alcohol concentration in the blood of the subjects after beer consumption was 61.6 mg% (SD 10.2 mg%) at 30 minutes whereas that of the subjects who took of *Thunbergia lauriforia Linn.* prior to beer consumption was 54.4 mg% (SD 8.6 mg%). Consumption of *Thunbergia lauriforia Linn.* along with beer can reduce peak blood alcohol concentration by 11.7 per cent. The difference in blood alcohol concentration time curves between the two experi-ments was statistically significant ($p < 0.001$). No subjects reported any adverse reactions to *Thunbergia lauriforia Linn.*.

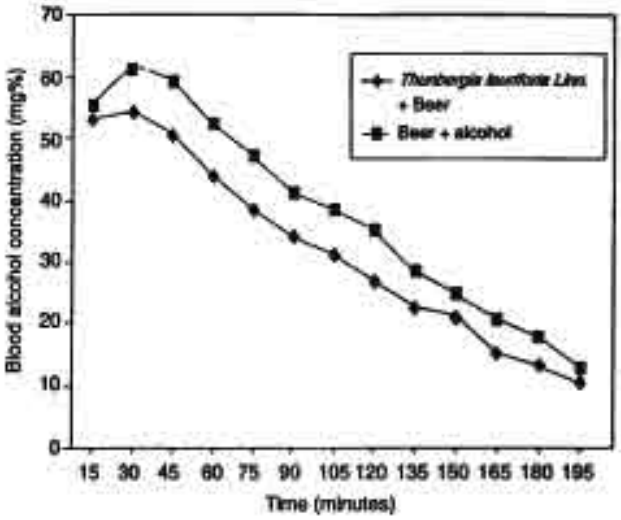

Fig. 1. Blood alcohol concentration time curves after beer consumption alone and consumption *Thunbergia lauriforia Linn.* and beer.

DISCUSSION

This study used an alcohol breath test to determine blood alcohol concentration since it had been demonstrated that the alcohol level measured by breath test was highly correlated with that directly measured from blood with a correlation coefficient of 0.987 ($p = 0.001$)[9]. The results of the study revealed that taking *Thunbergia lauriforia Linn.* at a dosage of 1.8 grams just prior to alcohol consumption could reduce the peak blood alcohol concentration. This effect is likely to be due to a decrease in absorption of alcohol from the gastrointestinal tract. However, the effect of *Thunbergia lauriforia Linn.* is quite minimal since it could only reduce the blood alcohol concentration by 11.7 per cent which might not be clinically important if a large amount of alcohol is consumed. The effect of a larger dose of *Thunbergia lauriforia Linn.* on blood alcohol concentration is unknown. It should be mentioned that our experiment was not performed in the subjects after taking meals instead of taking *Thunbergia lauriforia Linn.*; therefore, the findings observed in this study could be due either to a direct effect of *Thunbergia lauriforia Linn.* or a non-specific effect similar to taking alcohol after a meal.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Medical Sciences, Ministry of Public Health for supplying the Thunbergia lauriforia Linn. capsules for the study.

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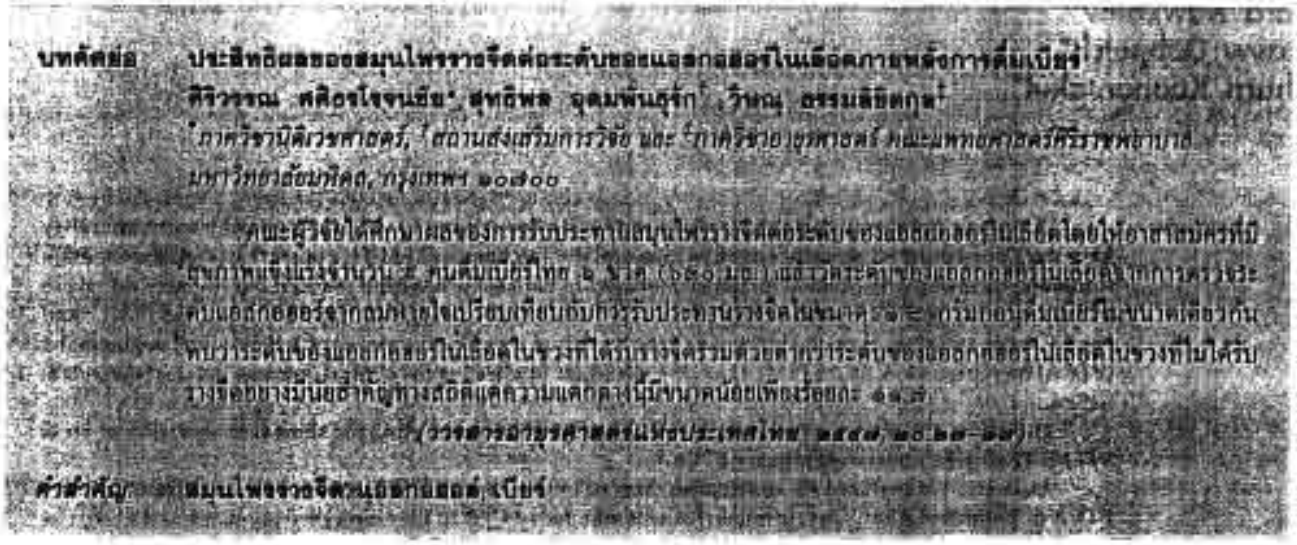

บทคัดย่อ **ประสิทธิผลของสมุนไพรรางจืดต่อระดับของแอลกอฮอล์ในเลือดภายหลังการดื่มเบียร์**

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การศึกษาวิจัยได้ศึกษาผลของการรับประทานสมุนไพรรางจืดต่อระดับของแอลกอฮอล์ในเลือดโดยให้อาสาสมัครที่มีสุขภาพแข็งแรงจำนวน [illegible] คน ดื่มเบียร์ไทย ๖ ขวด (๖๕๐ มล.) และวัดระดับของแอลกอฮอล์ในเลือดจากการตรวจวิเคราะห์ตัวอย่างเลือดที่เจาะจากปลายนิ้วมือ เปรียบเทียบกับการรับประทานรางจืดในขนาดเดียวกัน พบว่าระดับของแอลกอฮอล์ในเลือดในรางที่ได้รับการรางจืดร่วมด้วยต่ำกว่าระดับของแอลกอฮอล์ในเลือดในรางที่ไม่ได้รับรางจืดอย่างมีนัยสำคัญทางสถิติ และความแตกต่างนี้มีขนาดน้อยเพียงเรื่อยๆ [illegible]

(วารสารอายุรศาสตร์แห่งประเทศไทย ๒๕๔๗; ๓๐:๒๕-๓๗)

คำสำคัญ: สมุนไพรรางจืด, แอลกอฮอล์, เบียร์

Changes in Hematologic Markers in Patients with Mitral Stenosis after Successful Percutaneous Balloon Mitral Valvuloplasty

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Systemic embolism is a major complication of mitral stenosis which is usually related to a presence of left atrial thrombus. Percutaneous balloon mitral valvuloplasty (PBMV) was previously reported to reduce the incidence of this complication. However, the mechanisms of this beneficial procedure was under investigated. The aim of this study was to investigate the changes in coagulation activity, platelet activity and endocardial function in 29 patients with mitral stenosis after successful PBMV. All subjects had good left ventricular systolic function and 48.3% had atrial fibrillation. There was a significant reduction in thrombin-antithrombin complex (TAT) after a successful procedure and the level of thrombomodulin was also significantly higher one month after successful procedure. However, the level of platelet factor 4 (PF_4) and beta-thromboglobulin (β-TG) were increased after this procedure but not achieved the statistical significance.

In conclusion, successful PBMV can reduce the prethrombotic state in patients with mitral stenosis. In addition, it may improve endocardial function of the left atrium in those without atrial fibrillation.

Keywords : *Mitral stenosis, Balloon mitral valvuloplasty, Left atrial thrombus*

J Med Assoc Thai 2004; 87(6): 594-9

A serious complication of mitral stenosis is systemic embolism. Risk factors for systemic embolism in mitral stenosis patients are old age, atrial fibrillation, the presence of left atrial thrombus, large left atrial size, previous history of embolism and small mitral valve area[1-5]. The consequence of mitral valve obstruction is stasis of blood in the left atrium which may contribute to a local pre-thrombotic state due to abnormal activation of platelet activity and accumulation of circulating pre-thrombotic substances as reflected by the increased concentration of thrombin-anti-thrombin complex (TAT), Prothrombin activation F_{1+2} and D-dimer in the left atrium of these patients[6-10]. If this abnormal physiology persists and gradually increases, it may overwhelm the ability of the fibrinolytic system to maintain hemostasis, and thrombus formation may occur in the left atrium. Abnormal fibrinolysis has been found in patients with mitral stenosis as demonstrated by an increased level of PAI-I (plasminogen activator inhibitor-I) in the peripheral blood[11]. In addition, the level of thrombomodulin, reflecting the function of the endocardium of the left atrium was found to be decreased as a result of injury from high left atrial pressure which may predispose the patient to left atrial thrombus formation. One study reported that the patients who had previous percutaneous balloon mitral valvuloplasty (PBMV) had a lower incidence of thromboembolism[3]. However, there is little data concerning the effects of balloon mitral valvuloplasty on coagulation and platelet activity in these patients.

The objective of the present study was to determine the changes in coagulation activity, platelet activity and endocardial function of the left atrium in patients with mitral stenosis after successful balloon mitral valvuloplasty.

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Patients and Method

The study was conducted in 29 patients with moderate to severe symptomatic mitral stenosis who had successful balloon mitral valvuloplasty at Siriraj Hospital between March and November 2002. Exclusion criteria included continuing use of aspirin or anticoagulant, stopping antiplatelet drugs or anticoagulant drugs less than 2 weeks before the study, having a history of renal or liver disease, pulmonary embolism, deep vein thrombosis, malignancy or connective tissue disorder. Antiplatelet drugs (aspirin, dipyridamole and clopidogrel), warfarin and hemorheologically active drugs such as NSAIDs or estrogen containing drug were discontinued in all patients 10 days before the procedure. Transthoracic and transesophageal echocardiography were performed the day before the mitral valvuloplasty procedure to assess the presence or absence of left atrial thrombi, interatrial septum, mitral valve score, spontaneous echo contrast (SEC), mitral valve area, transmitral valve gradient and the severity of mitral regurgitation.

Percutaneous balloon mitral valvuloplasty procedure

Right and left heart catheterization were performed by a percutaneous approach with the right femoral vein and artery to obtain hemodynamic data before the process of dilatation. Subsequently, transeptal puncture was performed using the standard technique[12] and the Inoue balloon catheter was carefully inserted over a spring-coil wire into the left atrium and further manipulated into the left ventricle for mitral valve dilatation. A left ventriculogram was also performed before and after PBMV to assess left ventricular function and the degree of mitral regurgitation. A successful outcome was defined as a final mitral valve area, (determined by Gorlin's formulation) of more than 1.5 cm^2 without mitral regurgitation of more than grade 2 by Seller's Classification[13] with no major complications.

Blood sample collection and Assay procedure

A blood sample was collected from the patients on 2 occasions. The first blood collection was done by cautiously withdrawing 4.5 ml of blood from the vascular sheath placed in the femoral vein before heparin was administered. The second specimen was collected 1 month after the PBMV after discontinuing anti-platelet and anticoagulant drugs for at least 10 days. All blood samples were then analyzed for hematologic markers including platelet count, PT, aPTT, F1+2, thrombin-antithrombin III complex (TAT), plasminogen activator inhibitors-1 (PAI-1), platelet factor 4 (PF_4), beta-thromboglobulin (□TG), thrombomodulin, von Willebrand factor (vWF) and fibrinogen.

Plasma concentration of F1+2 and TAT were measured using enzyme immunoassay kits from DADE BEHRING (E' NOST F1+2, E' NOST TAT). Plasma concentration of □TG, PF_4 and PAI-1 was determined by enzyme immunoassay kits from Diagnostic Stage, France (ASSERACHROM □TG, ASSERACHROM PF_4 and ASSERACHROM PAI-1). D-dimer was measured by a latex-enhanced, turbidimetric test from DADE BEHRING (BC D-dimer). Monoclonal antibodies specific to each detected parameter were used in all of the test systems.

Statistical analysis

Continuous variables are expressed as mean ± SD and categorical variables as percent. A paired t-test was used to compare the hematologic markers pre-and post- mitral valvuloplasty if the data was normally distributed and a Wilcoxon Ranged test if the data were not normally distributed. A P value ≤ 0.05 was considered significant.

Results

Out of 29 patients who had a successful procedure, 72.4% were female and 27.6% were male. The mean age was 40 years and 48.3% of the patients had atrial fibrillation. A previous history of stroke was found in 6.9% of the patients. All of them had good left ventricular systolic function with a mean left ventricular ejection fraction of 65%. The mean mitral valve area and transmitral valve gradient before the dilatation procedure were 0.97 cm^2 and 12.35 mmHg respectively. After PBMV, the mean mitral valve area increased to 2.05 cm^2 and the mean transmitral valve gradient was reduced to 5.07 mmHg as shown in Table 1.

The hematologic markers before and after PBMV are shown in Table 1. There was a significant reduction in TAT after a successful procedure. In addition, the level of F_{1+2} also decreased but this was not statistically significant. Thrombomodulin was significantly higher one month after a successful procedure. The concentration of PF_4 and □TG were increased after successful dilatation but the differences were not significant. Finally, the level of PAI-1 was not significantly lowered after the valvuloplasty procedure.

Table 1. Hemodynamic data and hematologic markers pre-and post-PBMV in 29 patients

Hemodynamic data	Pre-PBMV	Post-PBMV	P
Mean ± SD of Mitral valve area (cm^2)	0.97 ± 0.28	2.05 ± 0.54	< 0.001
Mean ± SD of LA-LV gradient (mmHg)	12.35 ± 4.07	5.07 ± 2.22	< 0.001
Mean ± SD of Pulmonary artery pressure (mmHg)	33.31 ±11.42	31.93 ± 8.78	0.302
Mean ± SD of Cardiac output (l/min/M^2)	3.95 ± 0.84	4.70 ± 0.92	< 0.001
Mitral regurgitation			
Grade 0	55.2%	44.8%	
Grade 1	41.4%	48.3%	
Grade 2	3.4%	6.9%	
Hematologic markers			
Mean ± SD of PT (sec)	12.81 ± 2.34	15.86 ± 7.23	0.03
Mean ± SD of PTT (sec)	31.42 ± 5.78	34.67 ± 7.94	0.06
Mean ± SD of F1+2 (nmol/L)	0.91 ± 0.53	0.75 ± 0.78	0.3
Mean ± SD of TAT (ug/L)	12.84 ± 17.52	2.70 ± 1.89	0.01
Mean ± SD of PAI-I (ng/ml)	13.15 ± 12.55	11.60 ± 9.05	0.45
Mean ± SD of D-Dimer (ug/L)	233.72 ± 135.60	255.45 ± 169.01	0.21
Mean ± SD of Platelet ($x10^3$)	235.76 ± 57.83	225.34 ± 60.84	0.23
Mean ± SD of PF4 (IU/ml)	49.00 ± 34.44	56.38 ± 29.11	0.31
Mean ± SD of BTG (IU/ml)	121.10 ± 64.77	137.32 ± 52.62	0.33
Mean ± SD of Thrombomodulin(ng/ml)	6.43 ± 1.25	8.01 ± 2.07	0.001
Mean ± SD of VWF (IU/ml)	100.68 ± 27.64	108.91 ± 22.41	0.05
Mean ± SD of Fibrinogen (mg/dl)	393.45 ± 129.67	414.31 ± 115.90	0.27

NB: PT = Prothrombin time; PTT = Partial thromboplastin time; F1+2 = Prothrombin activation fragment 1+2; TAT = Thrombin-antithrombin complex; PAI-1 = Plaminogen activation inhibitor-1; BTG = Beta-thromboglobulin; PF_4 = Platelet factor-4; vWF = Von Willebran factor

Discussion

Thromboembolic events in patients with mitral stenosis are associated with left atrial thrombus formation which is related to various potential mechanisms such as the accumulation of pre-thrombotic substances, an abnormality of endocardial function, increased platelet activation, decreased fibrinolysis and rhythm abnormality (atrial fibrillation)[6-11]. All of these abnormalities may be reduced by PBMV as shown in previous studies[14-16].

In the present study, the authors demonstrated that the level of thrombin-antithrombin complex (TAT), a marker of thrombus formation, was significantly lower one month after successful balloon mitral valvuloplasty. In addition, the level of PAI-1 and F_{1+2} were also decreased but this did not achieve statistical significance. These results confirm those of Zaki A et al who also reported that balloon mitral valvuloplasty caused a significant reduction of TAT in the right atrium 30 minutes after the procedure in a subgroup with a left atrial pressure of less than 10 mmHg[16]. The beneficial effect of PBMV on the level of TAT was demonstrated significantly in the subgroup with atrial fibrillation as shown in Table 2. However, no significant changes of F_{1+2} were observed in the present study. One reason may in part be explained by the different stage of the formation of TAT and F1+2 which gives different information about coagulation activity. Considering the level of D-dimer as shown in Table 1, the present study could not demonstrate a significant change of this marker after successful PBMV. The reason might be related to the method of measuring the level of this marker which is a latex-enhanced, turbidimetric method that is probably not as good as an ELISA technique. Another reason might be related to the small sample size.

Looking at the levels of vWF and thrombomodulin, which reflect endocardial function in the left atrium, these were also increased after a successful procedure especially in patients without atrial fibrillation as shown in Table 3. This was a new finding that has never previously been reported. It may indicate that successful PBMV can improve

Table 2. Hemodynamic data and hematologic markers pre-and post-PBMV in 14 patients with atrial fibrillation (AF)

Hemodynamic data	Patients with AF		
	Pre-PBMV	Post-PBMV	P
Mean ± SD of Mitral valve area (cm^2)	0.91 ± 0.33	1.97 ± 0.62	< 0.001
Mean ± SD of LA-LV gradient (mmHg)	11.29 ± 4.45	5.00 ± 2.80	< 0.001
Mean ± SD of Pulmonary artery pressure (mmHg)	35.29 ± 13.21	33.43 ± 8.80	0.385
Mean ± SD of Cardiac output (l/min/M^2)	3.47 ± 0.61	4.17 ± 0.66	< 0.001
Hematologic markers			
Mean ± SD of PT (sec)	13.36 ± 3.09	19.43 ± 8.35	0.02
Mean ± SD of PTT (sec)	32.69 ± 7.55	38.73 ± 6.32	0.07
Mean ± SD of F1+2 (nmol/L)	0.96 ± 0.68	0.75 ± 1.02	0.46
Mean ± SD of TAT (ug/L)	15.54 ± 21.02	2.46 ± 1.46	0.04
Mean ± SD of PAI-I (ng/ml)	17.62 ± 15.80	13.86 ± 11.51	0.37
Mean ± SD of D-Dimer (ug/L)	223.43 ± 83.90	235.00 ± 160.80	0.69
Mean ± SD of Platelet ($x10^9$)	257.21 ± 69.32	229.21 ± 73.93	0.05
Mean ± SD of PF4 (IU/ml)	49.97 ± 34.78	46.56 ± 26.95	0.74
Mean ± SD of β-TG (IU/ml)	134.00 ± 69.37	119.67 ± 49.37	0.58
Mean ± SD of Thrombomodulin(ng/ml)	6.52 ± 1.42	7.28 ± 1.46	0.18
Mean ± SD of VWF (IU/ml)	110.66 ± 21.86	114.53 ± 18.63	0.6
Mean ± SD of Fibrinogen (mg/dl)	403.08 ± 124.41	445.17 ± 93.02	0.08

NB: PT = Prothrombin time; PTT = Partial thromboplastin time
F1+2 = Prothrombin activation fragment 1+2; TAT = Thrombin-antithrombin complex
PAI-1 = Plaminogen activation inhibitor-1; β-TG = Beta-thromboglobulin
PF_4 = Platelet factor-4; vWF = Von Willebran factor

Table 3. Hemodynamic data and hematologic markers pre-and post-PBMV in 15 patients without atrial fibrillation (AF)

Hemodynamic data	Patients with AF		
	PrePBMV	PostPBMV	P
Mean ± SD of Mitral valve area (cm^2)	1.03 ± 0.22	2.13 ± 0.46	< 0.001
Mean ± SD of LA-LV gradient (mmHg)	13.33 ± 3.56	5.13 ± 1.60	< 0.001
Mean ± SD of Pulmonary artery pressure (mmHg)	31.47 ± 9.57	30.53 ± 8.81	0.593
Mean ± SD of Cardiac output (l/min/M^2)	4.39 ± 0.80	5.21 ± 0.87	< 0.001
Hematologic markers			
Mean ± SD of PT (sec)	12.29 ± 1.24	12.53 ± 3.87	0.8
Mean ± SD of PTT (sec)	30.23 ± 3.27	30.88 ± 5.22	0.57
Mean ± SD of F1+2 (nmol/L)	0.85 ± 0.36	0.75 ± 0.51	0.43
Mean ± SD of TAT (ug/L)	10.33 ± 13.76	2.93 ± 2.25	0.07
Mean ± SD of PAI-I (ng/ml)	8.98 ± 6.69	9.48 ± 5.56	0.66
Mean ± SD of D-Dimer (ug/L)	243.33 ± 173.31	274.53 ± 179.75	0.13
Mean ± SD of Platelet ($x10^9$)	215.73 ± 36.58	221.73 ± 47.96	0.54
Mean ± SD of PF4 (IU/ml)	48.10 ± 35.31	65.93 ± 28.75	0.11
Mean ± SD of β-TG (IU/ml)	109.06 ± 60.01	153.79 ± 51.68	0.03
Mean ± SD of Thrombomodulin(ng/ml)	6.34 ± 1.12	8.70 ± 2.35	0.001
Mean ± SD of VWF (IU/ml)	91.36 ± 29.86	103.67 ± 24.91	0.010
Mean ± SD of Fibrinogen (mg/dl)	384.47 ± 138.12	385.51 ± 130.34	0.97

NB: PT = Prothrombin time; PTT = Partial thromboplastin time
F1+2 = Prothrombin activation fragment 1+2; TAT = Thrombin-antithrombin complex
PAI-1 = Plaminogen activation inhibitor-1; β-TG = Beta-thromboglobulin
PF_4 = Platelet factor-4; vWF = Von Willebran factor

endocardial function in these patients. Thus, the possibility of left atrial thrombus formation can be reduced. Regarding platelet activity, in contrast to a previous study[16], the present study found that platelet activity, reflected by the level of □-TG and PF_4, was not significantly decreased after successful PBMV. Moreover, when the authors analyzed only the subgroup without atrial fibrillation, it was noted that the level of □-TG was significantly higher after successful PBMV (Table 3). Therefore, it is plausible that in patients with mitral stenosis and no atrial fibrillation, this procedure might provoke thromboembolic events one month after a successful procedure via the mechanism of increased platelet activity. However, this finding should be further investigated in the future with a larger sample size. It should be mentioned that there are some limitations to the present study. First, the authors included only patients with moderate to severe mitral stenosis, thus, it may not be appropriate to apply the present results to those with mild mitral stenosis. Second, from the present study, the impact is not known of PBMV on patients with adequate mitral valve dilatation who develop mitral regurgitation of greater than grade 3 (by Seller's Classification) because of the small size of this subgroup. Third, knowledge of TAT regarding to the level which is prone to thrombosis is still lacking.

In summary, successful PBMV can reduce the pre-thrombotic state in patients with severe mitral stenosis as demonstrated by the decreased level of TAT after a successful procedure. In addition, PBMV may improve the endocardial function of the left atrium in those without atrial fibrillation as shown by the increased level of vWF and thrombomodulin.

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การเปลี่ยนแปลงของปัจจัยการเกิดลิ่มเลือดในห้องหัวใจเอเตรียมซ้ายในผู้ป่วยลิ้นไมตรัลตีบหลังได้รับการขยายลิ้นหัวใจด้วยบอลลูน

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ภาวะแทรกซ้อนที่สำคัญของโรคลิ้นหัวใจไมตรัลตีบคือการที่มีลิ่มเลือดจากหัวใจกระจายไปอุดตามหลอดเลือดต่าง ๆ ในร่างกายโดยเฉพาะที่ระบบประสาทส่วนกลางทำให้ผู้ป่วยเกิดอัมพาต ลิ่มเลือดดังกล่าวมักเกิดขึ้นในห้องหัวใจเอเตรียมซ้าย มีรายงานว่าการขยายลิ้นไมตรัลตีบด้วยบอลลูนสามารถลดอุบัติการณ์ของภาวะแทรกซ้อนดังกล่าวได้ อย่างไรก็ตามกลไกที่ช่วยลดอุบัติการณ์ดังกล่าวยังไม่ทราบแน่ชัด วัตถุประสงค์ของการวิจัยนี้คือเพื่อทราบการเปลี่ยนแปลงของการแข็งตัวของเลือด การทำงานของเกร็ดเลือด รวมทั้งการทำงานของเยื่อบุห้องหัวใจเอเตรียมซ้ายในผู้ป่วยโรคลิ้นหัวใจไมตรัลตีบจำนวน 29 คนที่ได้รับการขยายลิ้นไมตรัลตีบด้วยบอลลูนและประสบความสำเร็จพบว่าระดับของทรอมบิน-แอนติทรอมบิน คอมเพล็กซ์ที่หนึ่งเดือนลดลงอย่างมีนัยสำคัญทางสถิติและระดับของทรอมโบมอดูลินก็เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติด้วย ส่วนระดับของเพล็ตเล็ตแฟคเตอร์สี่และเบต้าทรอมโบกลอบบูลินมีแนวโน้มจะเพิ่มขึ้นแต่การเปลี่ยนแปลงดังกล่าวไม่มีความแตกต่างอย่างมีนัยสำคัญ

การศึกษานี้สรุปได้ว่าการขยายลิ้นไมตรัลตีบทำให้ปริมาณสารที่ก่อให้เกิดลิ่มเลือดลดลง และอาจทำให้การทำงานของเยื่อบุหัวใจเอเตรียมซ้ายดีขึ้นในผู้ป่วยลิ้นไมตรัลตีบที่มีจังหวะการเต้นของหัวใจเป็นปกติ

Effectiveness of Physical Therapy for Patients with Adhesive Capsulitis: a Randomized Controlled Trial

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Objective : *To compare the effectiveness of a combined technique of physical and ibuprofen for the treatment of adhesive capsulitis with ibuprofen alone.*

Material and Method : *122 subjects were randomly allocated to have 3 weeks treatment either with ibuprofen (n=61) or ibuprofen and a combined technique of physical therapy (n=61). Outcome measures were carried out 3 weeks and 12 weeks after randomization. Primary outcome measures were the success of treatment measured by improvement in the Shoulder Pain and Disability Index, and global rating.*

Results : *At 3 weeks, 21 (35.0%) of 60 patients in the study group were considered to have had successful treatment compared with 11 (18.6%) of 59 in the control group (difference between groups 16.4%, 95% CI: 4.0-31.3, p=0.044). There was no significant difference in the success rate between the two groups at the 12th week follow-up.*

Conclusion : *The results of this study support the use of physical therapy for patients with adhesive capsulitis.*

Keywords : *Randomised controlled trial, Physical Therapy, Non-steroidal anti-inflammatory drugs, Adhesive capsulitis*

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Adhesive capsulitis (so-called "frozen shoulder") is a common problem in general practice, rheumatologic, orthopaedic and rehabilitation clinics. It is characterised by shoulder pain that is aggravated by movement and limitation of the range of shoulder motion and daily activities. Several different therapeutic regimens have been used for the purpose of increasing the extent and speed of recovery. Conventional management includes patient advice, analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), steroid injection and a wide variety of physical therapy methods. Manipulation while anaesthetised can be effective, but significant complications have been documented and publication reports protracted recovery[1]. Arthroscopic release done under general anaesthesia is invasive and few patients' outcomes have been reported[2-3].

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Various physical therapy regimens are used conventionally. Systematic reviews have shown that there is insufficient data to draw a conclusion about the effectiveness of physical therapy[4-5]. However, previous studies usually compared the efficacy of one component of physical therapy which were unlike routine use. For example, comparing the effect of ultrasound alone[6-7] or mobilisation alone[8]. Winter et al studied the effects of "classic" physical therapy, manipulation and corticosteroid injection. Their survival analysis showed that the duration of shoulder complaint in patients with a synovial problem was shortest in the corticosteroid injection group. However, the "classic" physical therapy in this study comprised exercise therapy, massage and physical application but no mobilisation techniques were allowed[9]. Van de Windt et al tried to enhance the external validity of this study by adding passive mobilisation in their PT protocol[10] but they used a superficial modality instead of the deep heat modality

that is usually recommended in a chronic condition like adhesive capsulitis[11].

The primary objective of this prospective, randomised, controlled trial was to study the effectiveness of a combined technique of PT, which is similar to the usual clinical practice in patients with primary adhesive capsulitis in terms of success rate. The secondary objectives were to compare the mean quantity of analgesic used, the mean change in The Shoulder Pain and Disability Index (The SPADI)[12], the mean change in the range of motion, and patients' satisfaction between the two groups including any adverse effects of PT.

Material and Method

Subjects

All patients who had shoulder pain and limitation of a passive range of shoulder motion in all directions that interfered with their activities of daily living and attended the orthopaedic and rehabilitation clinic at Siriraj Hospital were eligible for the study. Exclusion criteria included patients with secondary adhesive capsulitis; with intrinsic causes of shoulder problems such as a history of fracture, or dislocation or extrinsic causes such as neuromuscular disorders (stroke, parkinsonism), generalised arthritis, bilateral involvement, contraindication for NSAIDs, or who had bleeding tendencies.

Randomization

The patients who gave informed written consent were randomly allocated to a 3-week treatment protocol by simple randomisation using a random numbers table and allocation concealed within an opaque envelope.

Assessments

The outcomes of the intervention were assessed at 3 weeks. The patients were asked to rate one global rating on pain and disability on a five point Likert scale; disappearance of shoulder complaints, some pain or limitation but which does not interfere with everyday life, minimal inconvenience to everyday life, moderate inconvenience, and marked inconvenience. For measuring the primary outcome, patients were counted as a success if they rated themselves as having disappearance of shoulder complaints or some pain/ limitation which does not interfere with everyday life. The following secondary outcome measures were included:

1. The Shoulder Pain and Disability Index (the SPADI) score change. The SPADI is a 13 item, self-administered instrument developed by Roach KE et al in 1991[12]. It consists of two separate scales: one for pain and the other for functional activities. The score varies from 0 to 100. A higher score indicates worse problems. The change in score for each patient was calculated for each patient by subtracting the result at baseline from the follow-up at the end of the 3rd week.

2. Range of shoulder motion measured with a goniometer according to the method advocated by Clarke by a investigator blinded to the type of treatment[13]. The goniometer was attached by a Velcro® strap to the upper arm with the patient sitting upright for total abduction. External rotation of the shoulder was assessed while lying supine with the shoulder in 90 degrees of abduction and the goniometer attached to the dorsal aspect of the forearm. Internal rotation range was quantified by measuring the distance between the spine of C7 and the tip of the thumb with the arm fully internal rotated. An independent study demonstrated that the inter-ratter reliability for abduction, external rotation and internal rotation was 0.98, 0.92, and 0.99 respectively.

3. Patients' satisfaction was rated concerning the treatment regimens on a four point Likert scale "very satisfied, moderately satisfied, unsatisfied, very unsatisfied".

4. The quantity of analgesic used was calculated from the number prescribed minus the number of pills left.

5. Adverse reactions recorded by the patients who received the PT program for the questions "Do you have pain that persisted more than 2 hours after treatment or more disability the next morning or not?" Moreover, at each follow-up, an investigator, blinded to treatment modality asked all patients "Have the trial drugs and/or treatment program upset you in any way?" and examined the patient for any signs of echymosis or burn during range of motion evaluation.

Additional follow-up assessments were scheduled to evaluate the primary outcome only at 6, 12, and 24 weeks. The assessments at 12 and 24 weeks were by telephone or postal questionnaire.

Intervention

The patients in the control group had ibuprofen 400 mg three times daily for 3 weeks and they also received an information sheet containing advice on protection of the shoulder from vigorous

activities such as pushing and pulling. They were encouraged to use their arms in a normal fashion for reaching and other activities of daily life. All the subjects were asked to have no other adjuvant therapy during the study except for oral acetaminophen (up to 6 g/day). All of them were asked to record if they received any additional treatment.

The patients in the study group had ibuprofen and general advice, which was same as the control group in addition to the combined technique of PT. A hospital-based PT program was carried out 3 times a week by each of the three research physical therapists whose performance had been standardised. Each session comprised short wave diathermy (20 minutes), mobilisation and passive glenohumeral joint stretching exercises up to the patient's tolerance. On the days they did not receive the hospital-based PT program, they were advised to perform pulley exercises (actively assisted exercises for 5 minutes). Active non-assisted exercises using a towel and wall (5 minutes after applying a hot pack for 20 minutes). The exercise guideline was based on Cyriax[14]. If, during the passive movements the patients felt pain before the therapist reached the end of the range, exercise was contraindicated. If pain was experienced at the end of the range then exercise was attempted. Subjects were asked to complete a diary documenting the number of hospital-based PT they actually received and the number of home exercise programs they performed. The number of patients needing additional treatment after three weeks and the types of treatment received are shown in Table 3.

Statistical analysis

Intention to treat analysis was used to evaluate a statistical difference between the two groups. Chi-square was used in comparing the proportions of patients. Using Student - t test, compared the difference in the mean improvements in The SPADI score and range of motion between the two groups. The Man-Whitney U test was used to compare the median of patients' satisfaction between the two groups. Multiple logistic regression was used to detect any effects of the difference in baseline.

Sample size calculation was based on the ability to detect a clinically important difference in success rate of 25 % between two groups. The authors assumed a success rate of 40% in the group having the least successful treatment and, thus, estimated a target sample size of 60 patients in each group. (two-tailed, a = 0.05, b = 0.02).

Results

From January 2001 to September, 2001, 255 patients with adhesive capsulitis attended the orthopaedic clinic and rehabilitation at Siriraj Hospital. There was a total of 122 patients with adhesive capsulitis who fulfilled the eligible criteria and were willing to join the present study. Of the 133 subjects not recruited, it was inconvenient for 83 cases because they lived far away from Bangkok, so they were instructed to receive treatment and to be followed up at the hospital in their hometown instead of the coming to Bangkok, 28 had secondary adhesive capsulitis, 16 had contra-indications for NSAIDs, and 6 had bilateral involvement. At the end of the 3rd week, 2 subjects dropped out from the study; 1 from the control group and 1 from the study group. The total number of cases included in the analysis was 59 in the control and 60 in the study group. By the end of the 24th week, a total of 12 cases (10.1%) had withdrawn from the study (Fig. 1). All of them lost to follow-up for unknown reasons and the investigators could not contact them.

Details of the baseline characteristics of the patients are shown in Table 1. The study group tended to have a greater male/ female ratio, more subjects who had a history of minor trauma before onset, less association with neck pain and less personal preference as to randomisation. However, these differences were not statistically significant.

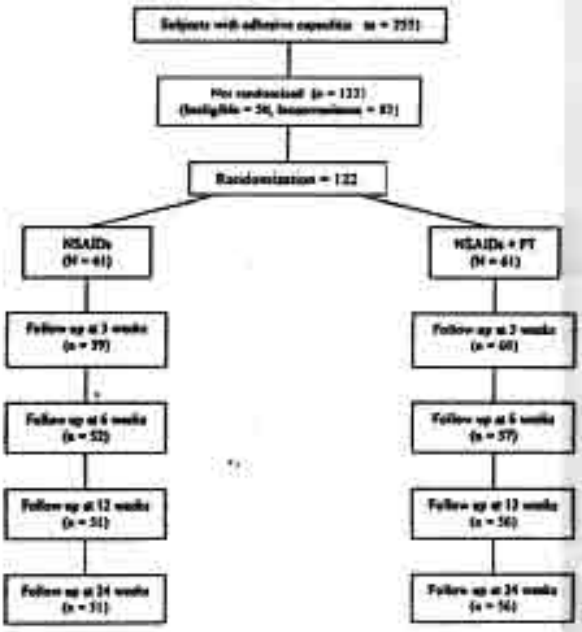

Fig. 1 Summary data for study recruitment and completion.

Table 1. Baseline characteristics of patients with adhesive capsulitis by group. Values are numbers (percentages) unless indicated otherwise

Baseline variables	The control group (n = 59)	The study group (n = 60)
Mean (SD) of age (years)	57.7 (10.0)	56.3(10.6)
Gender (male, female)	14 (23.7%), 45 (73.3%)	24 (40.0%), 36 (60.0%)
Duration of disability;		
- < 6 weeks	6 (10.2%)	13 (21.7%)
- 6 weeks but < 12 weeks	20 (33.9%)	20 (33.3%)
- ≥12 weeks	33 (55.9%)	27 (45.0%)
Dominant shoulder involvement	31 (52.5%)	28 (46.7%)
History of minor trauma before onset	11 (18.6%)	17 (28.3%)
Associated with DM	10 (16.9%)	10 (16.7%)
Concomitant neck pain	12 (20.3%)	8 (13.0%)
Patient's preference as randomisation	50 (89.8%)	45 (76.7%)
Global rating of pain and disability		
- No shoulder complaint	0	0
- Some pain or limitation but does not interfere with everyday life	0	0
- Minimal inconvenience	12 (20.3%)	9 (15.0%)
- Moderate inconvenience	34 (57.6%)	35 (58.3%)
- marked inconvenience	13 (22.3%)	16 (26.7%)
Mean (SD) of the SPADI score*	50.6 (16.6)	54.93 (21.3)
Range of motion		
- Mean (SD) glenohumeral abduction (degree)	121.3 (27.8)	121.9 (27.8)
- Mean (SD) glenohumeral external rotation (degree)	75.3 (16.0)	74.8 (22.1)
- Mean (SD) of distance between tip of thumb and C7 spine (cm)**	41.1 (10.3)	41.2 (10.6)

* Pain and disability as rated on the SPADI score in which scores range from 0-100; the higher scores indicate more severe pain and disability

** Internal rotation was quantified by measuring distance between thumb and tip of C7 spine in hand behind back position

At the end of the 3rd week, 21 cases (35.0%) in the study group (n = 60) had successful treatment, whereas, 11 cases (18.6%) in the control group (n = 59) were successful. The difference between groups was 16.4% (95% CI: 4.0-31.3, p = 0.040).

For secondary outcome variables, the number of analgesics used, changes of the SPADI scores, and range of motion improvement (glenohumeral abduction, external rotation and internal rotation) were continuous data. All of these variables were tested for normality of distribution. It was found that improvements in the SPADI scores, and ranges of motion were normally distributed. These changes were tested for differences between the 2 groups by Student's t-test. The quantity of analgesics used in both groups was tested by Mann-Whitney U test due to the fact that this parameter was not normally distributed.

The mean (standard deviation) changes of the SPADI scores of the study group and the control group were 11.9 (14.2) points and 20.4 (15.4) points, respectively. The subjects in the study group showed a mean improvement in the score of 8.6 points more than the control group (95% CI: 3.1-13.9 points, p = 0.002).

Regarding range of motion, the study group showed a mean improvement in glenohumeral abduction 7.2 degrees more than the control group (95% CI: 1.2-14.2 degrees, p = 0.005) (Table 2). For glenohumeral external rotation, the mean improvement in the study group was 3.0 degrees more than the control group but the difference was not statistically significant (95% CI: -2.0 to 8.6, p = 0.085). The distance between the thumb to the tip of C7 spine (cm) was used to quantify glenohumeral internal rotation. The analysis showed that the study group showed a significantly greater improvement than the control group (p = 0.015). The magnitude of the difference was 3.3 centimetres (95% CI was 0.7 cm to 6.0 cm).

Table 2. Outcome variables of patients with adhesive capsulitis by group of treatment at the end of the 3rd week with additional follow- up of primary outcome. Values are numbers (percentages) unless indicated otherwise

Outcome variables	The control group (n = 59)	The study group (n = 60)	Difference (95% CI)	P value
Had successful treatment				
- 3 weeks	11/59 (18.6%)	21/60 (35.0%)	16.4% (4.0%–31.3%)	0.044
- 6 weeks	22/52 (42.3%)	35/57 (61.4%)	19.1% (4.0%-36.1%)	0.046
- 12 weeks	31/51 (60.8%)	43/56 (76.8%)	16.0% (-1.50%-32.5%)	0.073
- 24 weeks	42/51 (82.4%)	45/56 (80.4%)	-2.0% (-16.6%-13.1)	0.791
Mean (SD) Of the SPADI score improvement	11.9 (14.2)	20.5 (15.4)	8.6 (3.1 to 13.9)	0.002
Mean (SD) of improve in abduction (degree)	14.7 (18.1)	21.9 (21.0)	7.2 (1.2 to 14.2)	0.005
Mean (SD) of improvement in external rotation (degree)	18.3 (15.4)	21.3 (15.3)	3.0 (-2.6 to 8.6)	0085
Mean (SD) of improvement in internal rotation (cm)	3.0 (7.0)	6.3 (7.7)	3.3 (0.7 to 6.0)	0.040
Mean rank of number of analgesic use (tab)	58.59	61.38		0.652*
Satisfaction;				
- Very satisfied	1		5	
- Moderately satisfied	1	7		< 0.001
- Unsatisfied	13		24	
- Very unsatisfied	45		23	

Mann-Whitney U test found that the median quantity of analgesics used did not differ significantly between the two groups (p = 0.652).

For ordinal secondary outcomes, Man-Whitney U test was used to compare the results between the two groups. It was found that the subjects in the study group rated their satisfaction better than the subjects in the control group, which was significant (P<0.001) (Table 2).

During the 3-week period, the patients in the study group reported a total of 10 episodes of pain that persisted more than 2 hours after treatment from 4 subjects. There were no other complications recorded. Regarding NSAIDs, 15 subjects (12.6 %) had gastrointestinal side effects; the number of those who had severe dyspepsia and had to stop NSAIDs was 6 (4.2%). There were 2 report of severe oedema and 1 case with a severe headache, which rapidly subsided after the drug was discontinued.

Compliance, Contamination and Co-Intervention

About three-quarters of the subjects of both groups received NSAIDs as prescribed. The reasons why some patients received fewer NSAIDs than the others was due to gastrointestinal discomfort, forgetting to take them or a misunderstanding about the schedule. In the study group, 7 cases (11.7%) received fewer than 6 sessions of hospital-based PT, 5 cases (8.3%) performed the home programme exercises fewer than 6 sessions. Two cases from the control group reported that they had additional treatment; 1 had Chinese herbal medicine and 1 received analgesics from a private clinic. No patient in the control group had hospital-based PT or home exercise therapy for their shoulder. The number of patients needing additional treatment after three weeks and the types of treatment received are shown in Table 3.

Table 3. Number (percentage) of patients with adhesive capsulitis needing treatment for residual pain and disability at the fourth week follow-up (treatment no longer restricted to interventions as described in protocol)

Additional treatment	The control group (n = 52)	The study group (n = 57)
Non-steroidal anti-inflammatory drugs	18 (34.6%)	13 (22.8%)
Non-steroidal anti-inflammatory drugs and physical therapy	12 (23.1%)	17 (29.8%)
Physical therapy	3 (5.4%)	5 (8.8%)
Corticosteroid injections	3 (5.4%)	3 (5.3%)
Home exercise	13 (25.0%)	21 (36.8%)

At the 6th week, 35 cases (61.4%) in the study group (n=57) were counted as successful, whereas 22 (18.6%) cases in the control group (n=52) were successful. The study group had a greater success rate than the control group by 19.1% (95% confidence interval: 4.0-36.1, p=0.044). There was no significant difference between the two groups at the 12th and 24th week follow-up (Table 2).

Discussion

This randomised, controlled trial demonstrated that the 3-week treatment regimen comprising a combined technique of PT and ibuprofen produced more beneficial effects than the use of ibuprofen alone for the treatment of (primary) adhesive capsulitis in terms of success rate, improvement in the SPADI score, patients' satisfaction and improvement in the range of motion. At the end of the 6th week, the success rate of patients who received physical therapy was more than the success rate of the control group. After that, the differences were not statistically significant. The results were analysed by intention to treat analysis even though the treatments actually received were modified from the protocol, because it was found that the reasons for modifying the treatment were strongly related to the results of allocated interventions[12].

The results of the present study are different from previous studies in which systematic reviews concluded that there was insufficient data to draw conclusions about the effectiveness of PT[4-5]. The reasons might be due to the fact that the PT regimen in the present study comprised important components. Deep heat modality was introduced in order to increase the tissue temperature and its extensibility; making a passive range of motion more effective[11]. To use this combined technique of PT in addition to NSAIDs can make the patients more comfortable.

One important limitation in the present study was the lack of the blinding process. It was not possible to keep the subjects blinded as to the experimental conditions for each subject and as the primary outcome was a subjective measurement, it was probably directly influenced by the subjects preconceived idea regarding the effectiveness of intervention. Patients' preferences can be an important determinant of the outcomes[16-17]. Participants who were randomised to their treatment of choice may have a better outcome irrespective of the physiological effects of the intervention. The placebo treatment, which theoretically would have alleviated this threat to internal validity, was not convenient in the present study. Therefore, the differences of primary outcome between the two groups in the present study could be due to a placebo effect. However, this problem might have been partly ameliorated because the patients' treatment preferences were elicited after randomisation and it was found that the patients in the control group had a tendency to prefer their allocated treatment compared with the patients in the study group. This would make it unlikely that the difference in primary outcome at the end of the study was due to the patients' preference.

The deviation from the protocol in the present study might not reverse the results. On the contrary, the differences of the outcomes at the end of the study should be elicited more easily if there was no protocol deviation. Because the patients in the study group received fewer treatments than the schedule determined (six cases had fewer than 6 sessions of hospital-based PT and 6 cases performed home exercise fewer than 6 sessions), while the subjects in the control group received more treatment than the schedule (one case had Chinese herbal medicine and 1 case had analgesics from a private clinic).

In conclusion, the results of the present study give us evidence to support the use of physical therapy for patients with adhesive capsulitis from the beginning of the treatment.

However, because a combined technique of physical therapy needs a wide variety of resources such as people, time, facilities and equipment, it is necessary to carry out a further study to evaluate the economic aspect of this study to provide a balance sheet of the benefits, harms and costs for making the choice for a combined treatment regimen. If the combined technique of physical therapy is not cost-effective, a home-programme of physical therapy should be an alternative intervention to be studied in a further trial.

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ศึกษาเปรียบเทียบประสิทธิผลระหว่างการทำกายภาพบำบัดร่วมกับการใช้ยาต้านการอักเสบกับการใช้ยาต้านการอักเสบอย่างเดียวในผู้ป่วยเอ็นข้อไหล่อักเสบ

กึ่งแก้ว ปาจรีย์, นวพร ชัชวาลพาณิชย์, สมลักษณ์ เพียรมานะกิจ, จันทร์จิรา เกิดวัน, พัชรินทร์ พุทธรักษา, ญาณณี วงศรานุชิต

วัตถุประสงค์ : เพื่อเปรียบเทียบประสิทธิผลระหว่างการทำกายภาพบำบัดร่วมกับการใช้ยาต้านการอักเสบกับการใช้ยาต้านการอักเสบอย่างเดียวในผู้ป่วยเอ็นข้อไหล่อักเสบ

วิธีการ : คัดเลือกผู้ป่วยที่มีปัญหาข้อไหล่อักเสบตามเกณฑ์ที่กำหนด แล้วสุ่มให้เข้ากลุ่มรับการรักษานาน 3 สัปดาห์ กลุ่มควบคุมรับประทานยา ibuprofen กลุ่มที่ศึกษาได้รับยา ibuprofen ร่วมกับการทำกายภาพบำบัดที่โรงพยาบาล สัปดาห์ละ 3 ครั้ง ประเมินผลเมื่อสิ้นสุดสัปดาห์ที่ 3, 6, 12 และ 24 ความสำเร็จในการรักษาประเมินจากแบบสอบถาม The Numeric Shoulder Pain and Disability Index (ภาคภาษาไทย) และ global rating of improvement นำตัวแปรทั้งสองกลุ่มมาเปรียบเทียบกันด้วยวิธี intention to treat analysis

ผลการศึกษา : เมื่อครบ 3 สัปดาห์ พบว่าร้อยละ 35.0 (21 รายจากจำนวน 60 ราย) ของผู้ป่วยกลุ่มศึกษาประสบความสำเร็จในการรักษา มากกว่ากลุ่มควบคุม ซึ่งประสบความสำเร็จร้อยละ 18.6 (11 รายจากจำนวน 59 ราย) คิดเป็นร้อยละ 16.4 (ค่าร้อยละ 95 ของความเชื่อมั่น = ร้อยละ 4.0 - 31.3, ค่า p = 0.044) เมื่อติดตามครบ 6 สัปดาห์ อัตราความสำเร็จของกลุ่มศึกษามากกว่ากลุ่มควบคุมร้อยละ 19.1 (ค่าร้อยละ 95 ของความเชื่อมั่น = ร้อยละ 4.0 – 36.1, ค่า p = 0.046)

สรุป : การศึกษานี้สนับสนุนการทำกายภาพบำบัดในผู้ป่วยเอ็นข้อไหล่อักเสบ