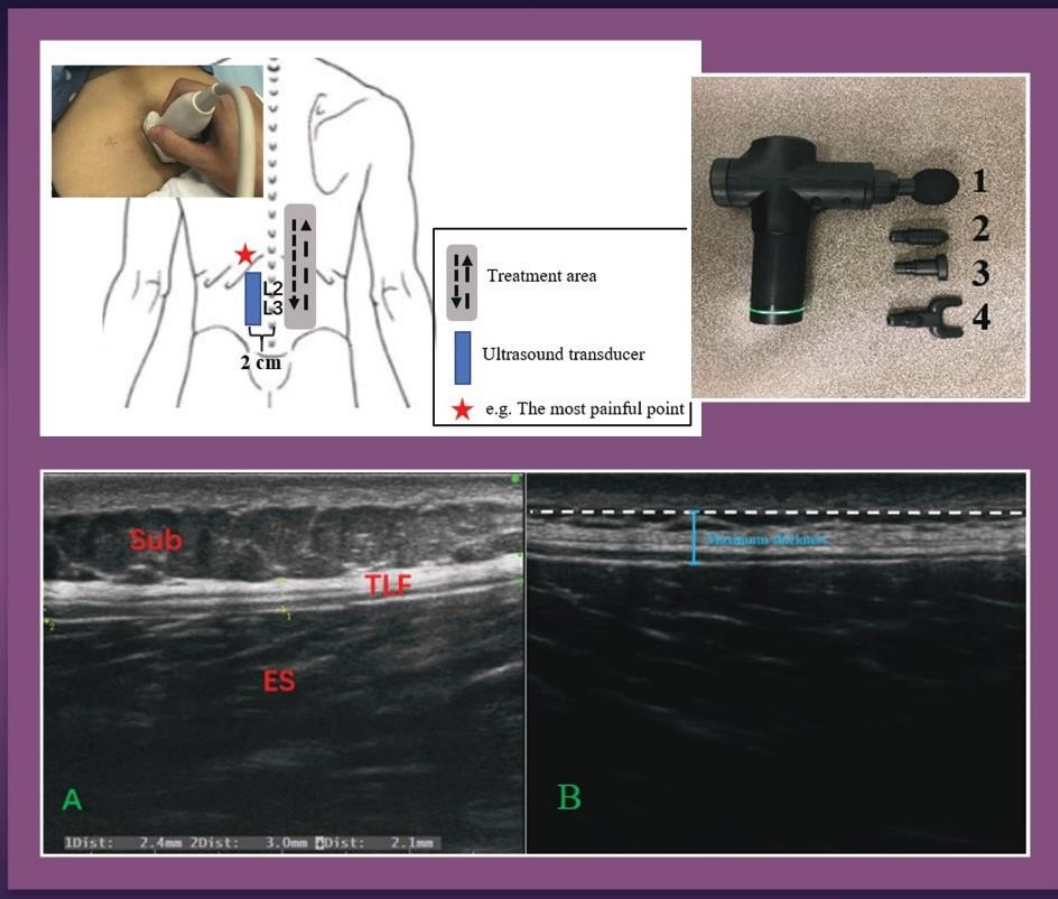
ARCHIVES OF ALLIED HEALTH SCIENCES

Archives of ALLIED HEALTH SCIENCES

Arch AHS
Volume 34
Issue 3
2022



Arch AHS
ARCHIVES OF ALLIED HEALTH SCIENCES

ISSN 2730-2008 (Online)

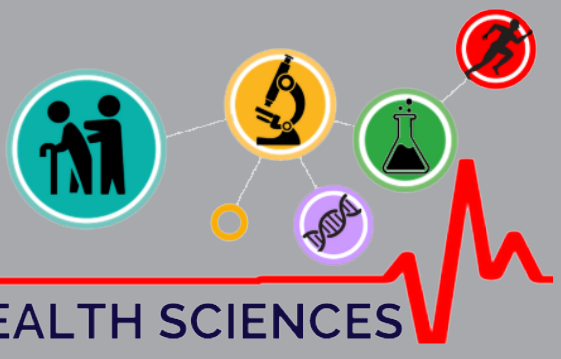
https://www.tci-thaijo.org/index.php/ams

ARCHIVES OF ALLIED HEALTH SCIENCES

Volume 34, Issue 3, 2022

Arch AHS

ARCHIVES OF ALLIED HEALTH SCIENCES



The **Archives of Allied Health Sciences (Arch AHS)** is an international peer-review multi-disciplinary journal published in English. It is owned by the Faculty of Associated Medical Sciences, Khon Kaen University, Thailand. The Arch AHS was formally known as *Journal of Medical Technology and Physical Therapy (JMTPT)*, which was founded in 1989. The title of the journal was changed to ***the Archives of Allied Health Sciences (Arch AHS)*** from 2020 (volume 32 issue 2: May - August) onward.

The Arch AHS aims to be a leading forum for research and knowledge in evidence-based practice relating to Allied Health Sciences. Contributions from all parts of the world and from different professionals in Allied Health Sciences are encouraged. Original articles, reviews, special reports, short communications, and letters to the editor are published 3 regular issues per year, online and in print.

Production and editorial assistance:
Arpassanan Wiyanad
Roongnapa Intaruk
Syarifah Fatima Yasmin

All correspondence concerning manuscripts, editorial issues and subscription should be addressed to:
Editorial Officer: ArchAHS.TH@gmail.com
Faculty of Associated Medical Sciences,
Khon Kaen University, Thailand.

Publication information:
Arch AHS (ISSN: 2730-1990; eISSN: 2730-2008)
appears 3 issues a year.
Issue 1 January – April;
Issue 2 May – August;
Issue 3 September – December.

Indexing:
Arch AHS is indexed in Thai Citation Index (TCI tier 1) and ASEAN Citation Index (ACI) databases.

Manuscript preparation:
Please review author guideline for manuscript preparation: https://drive.google.com/drive/folders/1kO5FijEnuLSYwzgKcnQ9mxO74ZZ0wGRA

Link to website

Arch AHS
ARCHIVES OF ALLIED HEALTH SCIENCES

Contents

REVIEW ARTICLE

Arch AHS

Archives of Allied Health Sciences 2022; 34(3): 1-13.

The effectiveness of home-based exercise with and without tracking for people with knee osteoarthritis: a systematic review

Pattaridaporn Saengpromma[1], Nathaphon Jirasakulsuk[1], Santhanee Khruakhorn[1*]

[1] Department of Physical Therapy, Faculty of Allied Health Sciences, Thammasat University, Thailand.

KEYWORDS
Osteoarthritis; Home-based exercise; Tracking; Pain; Function.

ABSTRACT

Knee osteoarthritis (KOA) is the most common inflammatory arthritis, involving inflammation and significant structural changes in the knee joint and causing pain and disability at work. Exercise and education are often recommended for KOA patients. However, it is often not reasonable to adhere to home exercise programs in elderly. Using digital communication can be inexpensive and accessible to help promote adherence and the effects of exercise. This systematic review was to find the evidence of randomized controlled trials (RCTs) on the effectiveness of home-based exercise with tracking and home-based exercise alone for patients with KOA. We searched Cochrane, MEDLINE, PubMed, and PEDro. We selected randomized controlled trials published in the English language, which were undertaken to identify interventions that used home exercise with or without tracking for KOA. Two reviewers independently extracted data. The risk of bias in the included studies was evaluated using the Cochrane 'Risk of Bias Tool 2.0'. As a result, a total of 1868 studies were found in the search. Of these, eight studies met the inclusion criteria and were further analyzed. All studies have a low risk of bias. In these studies, home exercise programs with tracking provide significant improvements in pain and function and more adherence and changes in behavior in elderly with KOA. In conclusion, home-based exercise with tracking in people with KOA is essential for increasing adherence and improving pain and physical function compared to the untracked group.

***Corresponding author:** Santhanee Khruakhorn, PT, PhD. Department of Physical Therapy, Faculty of Allied Health Sciences, Thammasat University, 99 Moo.18 Phahonyothin Road, Khlong Nueng, Khlong Luang, Pathum Thani 12120, Thailand. Email: santhanee.k@allied.tu.ac.th
Received: 10 March 2022/ Revised: 18 May 2022/ Accepted: 7 July 2022

Introduction

Knee osteoarthritis (KOA) is the most common arthritis, involving inflammation and significant structural changes of the knee joint, causing knee pain[1], muscle weakness[2,3], and decreased knee mobility[4], resulting in decreased body function and quality of life[1,5-7]. KOA prevalence is approximately 16.7% in people aged 45 and older[8]. The incidence of the disease increases rapidly from age 50 and older[9,10], and the prevalence increases with age[8,9]. Previous studies indicated that exercises and education are often recommended for patients with KOA[11-13]. Home exercise is often recommended for people with osteoarthritis[14,15]. The home exercise aims to enhance muscle strength, balance, increase range of motion and endurance[16]. The effects of home exercise are reducing pain and improving function in adults with KOA[17]. A systematic review found that adherence to long-term home exercise program resulted in better performance outcomes[18]. The effectiveness of a home exercise program for elderly with KOA depends on the patient's ability to follow the program[19].

Consequently, consistent home-based exercise program compliance may improve functioning and reduce pain and disability[20,21]. However, adherence to home-based exercise program in the elderly is often poor[22]. This unsuccessful home-based exercise program is often caused by poor adherence and lack of motivation to exercise[23,24]. However, ongoing clinician involvement may be unfeasible or impractical for many patients due to access to service and costs such as distance, travel time, and travel costs[25]. Previous studies have found that patient follow-up during treatment increases motivational strategies and improves adherence to their exercise program[26]. Instead, digital communications may be inexpensive and accessible options to help promote exercise adherence[27].

Therefore, we performed a systematic review of randomized controlled trials (RCTs) on the effectiveness of home-based exercise with tracking and home-based exercise alone for people with KOA.

Materials and methods

Search strategy

The following electronic databases were searched from the earliest date available until April 30, 2021: Cochrane, MEDLINE, PubMed, and PEDro. We used a search strategy that combined medical subject heading (MeSH) and free keywords and connected them with Boolean conjunctions (OR/AND). The keyword terms "home-based exercise," "home exercise," "self-exercise," "exercise," and "knee osteoarthritis" were used. The limitations placed on the search included the following: English, Human Studies, and Randomized Control Trials.

Inclusion and exclusion criteria

Limits were by design as we included only randomized clinical trials (RCTs), published in the English language prior to September 1, 2014. The intervention of interest was a home-based exercise program for KOA with tracking. Trials were required to compare home exercise programs with tracking and home-based exercise without tracking. Studies that did not include home exercise programs in their interventions were excluded. The outcome measures of interest were pain, physical function, and quality of life in patients with KOA. Randomized clinical trials were excluded if the publication was in the abstract form only.

Data extraction

Two reviewers independently extracted data from authors, publication year, some participants, age, study design, diagnosis, control intervention, outcomes, included/excluded. Consensus on extracted data was reached by discussion; furthermore, conflictive data were discussed with a third member of the study team.

Assessment of methodological quality

Two reviewers independently assessed the risk of bias of the included studies using the Cochrane 'Risk of Bias Tool 2.0'[28]. Within each domain, the two reviewers answered one or more signaling questions which led to judgments of "low risk of bias," "some concerns," or "high risk of bias." The judgments within each domain lead to an overall risk-of-bias judgment for the outcome being assessed.

Statistical analysis

The outcome measures chosen for this review were continuous level aggregate data on pain, physical function, or quality of life. Mean change scores (posttreatment-baseline) were used. Standardized mean differences (SMD) with their 95% confidence intervals (CI), calculated from the change score and baseline standard deviation, for the effects of exercise intervention above control intervention were estimated for each study. The estimates were combined using a fixed-effects model.

Results

Identified studies

The initial search resulted in 1,868 research studies. Nine hundred and eighty-one studies that appeared in more than one database or did not meet predetermined inclusion criteria were excluded. A total of 887 studies were assessed for eligibility. Seven hundred and nineteen studies were eliminated because they did not match the inclusion criteria or were not available in full text (Figure 1). The final selection, made by consensus, resulted in the inclusion of eight studies in the quality assessment phase.

All eight studies have a low risk of bias, according to the Cochrane 'Risk of Bias Tool 2.0' (Figure 2).

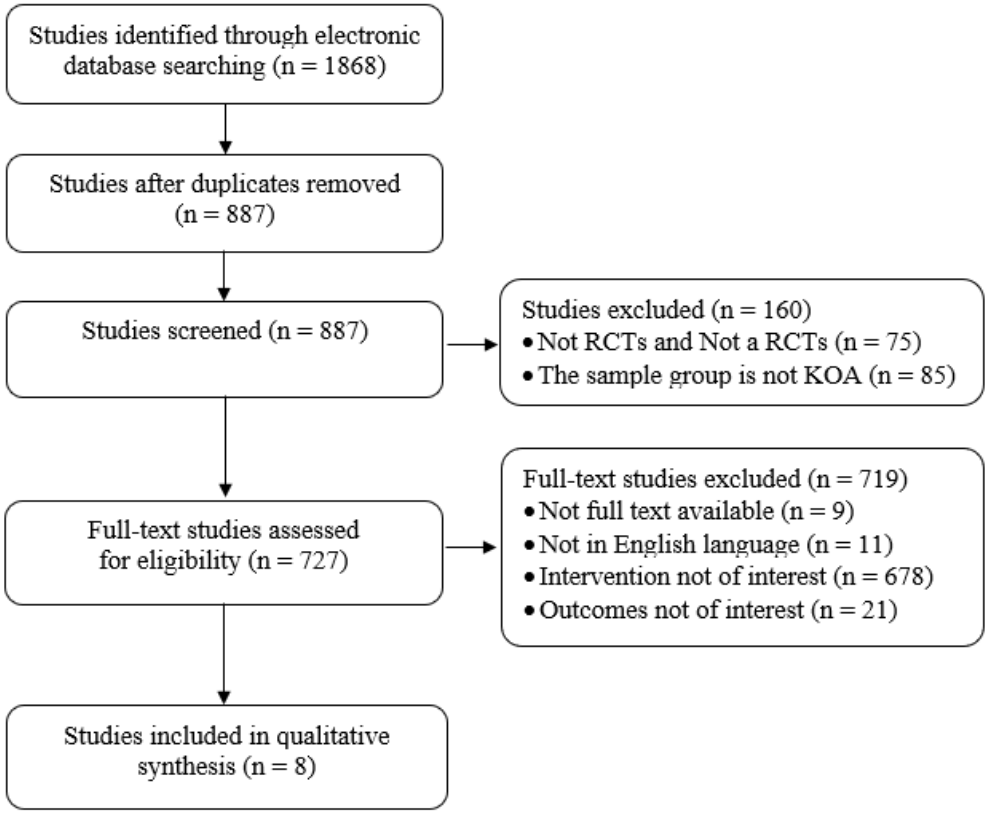


Figure 1 Flow diagram of the study procedure.

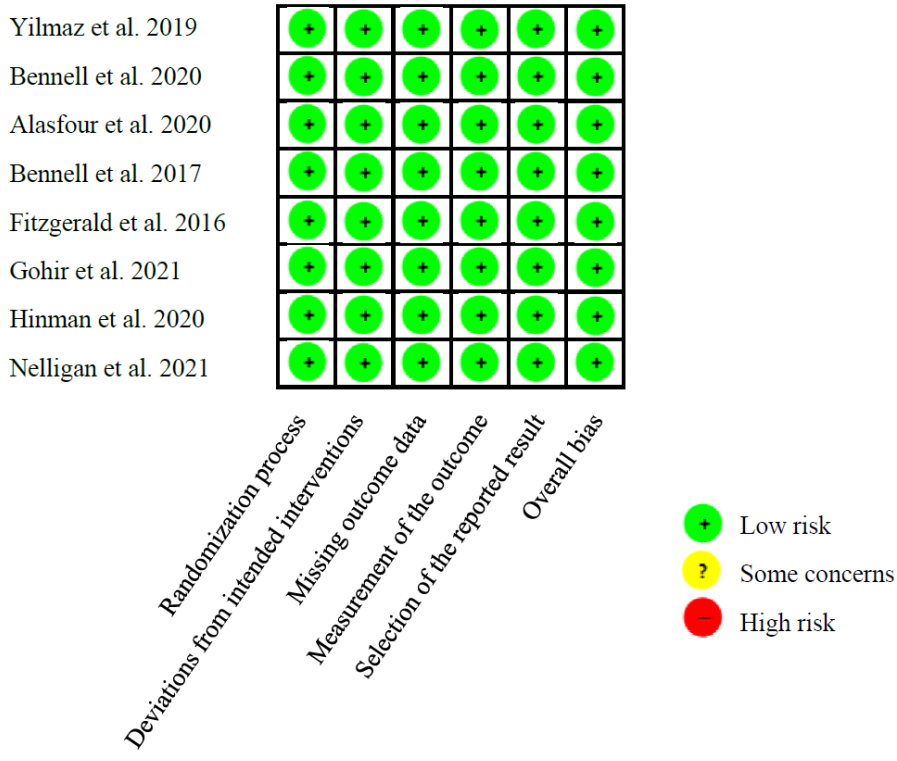


Figure 2 Risk of bias assessment according to the Cochrane Collaboration's tool (RoB 2.0) for randomized controlled trials.

General data about the selected studies

Table 1 Basic characteristics included randomized controlled clinical trials using the following information: author, subjects, design, diagnosis, intervention, tracking, control, duration, outcomes, and result. Regarding the effects found in most studies, a significant effect of home exercise programs with tracking was found on adherence rate, pain, and knee function.

Table 1 Basic characteristics of included randomized controlled clinical trials

Author	Subjects	Age - range	Diagnosis	INT	Tracking	CON	Duration	Outcomes	Main result
Yilmaz et al[17]	KOA (n = 80)	60.22 ± 9.5 years	KOA clinical criteria	Home exercise program (n = 41)	Telephone	Home exercise program (n = 39)	6 weeks	- VAS - ROM - Muscle strength - WOMAC - SF-36	INT was significantly improved in pain and function of the knee.
Bennell et al[27]	KOA (n = 110)	62.30 ± 6.75 years	Self-report	Home exercise program unsupervised (3 sessions per week) (n = 56)	SMS	Home exercise (n = 54)	24 weeks	- Adherence to prescribed home exercise - Number of days on which home exercises were completed in the past weeks - Adherence to home - Weekly NRS - NRS - KOOS - AQoL - ASES	Home exercise with the SMS greater adherence rate (mean 16.5, SD 6.5 vs mean 13.3, SD 7.0; mean difference 3.1, 95% CI 0.8-5.5; *p*-value = 0.01) than CON
Alasfour et al[29]	KOA (n = 40)	54.40 ± 4.33 years	X-ray	Home exercise on application (n = 20)	The app automatically sends alerts	Home exercise (n = 20)	6 weeks	- Self-reported exercise adherence. - ANPRS - ArWOMAC - FTSST	INT was significantly greater in adherence rate (26.60%) and pain (*p*-value = 0.015) than CON
Bennell et al[30]	KOA (n = 148)	61.15 ± 7.05 years	ACR	Home exercise (n = 74)	Skype	Home exercise and education (n = 74)	3 months and 6 months	- NRS overall - WOMAC - NRS walking - ASES -AQoL -PCS -CSQ	INT significantly greater than CON in all outcomes except coping attempt in 3 months

Table 1 Basic characteristics of included randomized controlled clinical trials (cont.)

Author	Subjects	Age - range	Diagnosis	INT	Tracking	CON	Duration	Outcomes	Main result
Fitzgerald et al[31]	KOA (n = 300)	58.35 ± 9.35 years	Self-report	Home exercise (booster) (n = 76)	Meet PT at the clinic	Home exercise (n = 75)	9 weeks and 1-yr	-WOMAC - Knee pain - TUG - 30-second chair stand - 40-meter walk test	There was no difference between groups in 9 weeks. However, there was significant booster*-times interaction for knee pain between 9 weeks and one year.
Gohir et al[32]	KOA (n = 146)	66.7 ± 9.2 years	ACR	Home exercise on application (n = 67)	Asynchronous chat	Management for KOA (n = 79)	6 weeks	- NRS - WOMAC - TUG - 30-second chair stand - MSK-HQ - Quadriceps and hamstring strength	Application for KOA management program was more excellent routine self-managed care in the primary outcome, NRS pain score, and functional performance.
Hinman et al[33]	KOA (n = 175)	65.45 ± 8.6 years	ACR and X-ray	Existing service + exercise (n = 87)	Telephone	Existing service (including exercise) (n = 88)	6 months	- NRS overall - WOMAC function scale - NRS walking - ASES -Brief Fear of Movement Scale for OA - PASE - Barriers to Physical Activity Scale - Benefits of Physical Activity Scale - AQoL- 8D	Telephone-delivered physiotherapist-led exercise advice and support resulted in improved functional outcomes at 6 months.

Table 1 Basic characteristics of included randomized controlled clinical trials (cont.)

Author	Subjects	Age - range	Diagnosis	INT	Tracking	CON	Duration	Outcomes	Main result
Nelligan et al[34]	KOA (n = 206)	60.0 ± 8.4 years	Self-report	Website access to education and home exercise (n = 103)	Automated text messages	Education and home exercise (n = 103)	24 weeks	- NRS - WOMAC - KOOS - AQoL - PASE - ASES - SEE	INT significantly greater improvement than CON in pain and function

Note: ANPRS, Arabic Numeric Pain Rating Scale; ArWOMAC, The Western Ontario and McMaster Universities Osteoarthritis Index (Arabic Version); AQoL, The Assessment of Quality of Life; ASES, Arthritis Self-Efficacy Scale; CSQ, Coping Strategies Questionnaire; CON, Control group; FTSST, Five Times Sit to Stand Test; INT, Intervention group; KOOS, Knee injury and Osteoarthritis Outcome Score; KOA, Knee osteoarthritis; NRS, numeric rating scale; PASE, Physical Activity Scale; PCS, Pain Catastrophizing Scale; SEE, Self-efficacy exercise; SF-36, Short Form -36; SMS, Short message service.

Home exercise programs included in studies

The interventions used as home exercise programs in the reviewed studies included strengthening exercise program for lower-limb muscles[17,27,29-34], muscle stretching[17,31], range of motion exercise[17], balance training,[31,32] and core exercise[31,32] as home exercise programs.

Outcome measures

The outcome measures of interest were pain and function in patients with KOA. Four studies used Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain score[17,30,32,33], eight studies used the Knee Pain Rating Scale (NPRS)[17,27,29-34]. Six studies used WOMAC for measuring function[17,29,30,32-34], One study used the Knee Injury and Osteoarthritis Outcome Score (KOOS)[27], and one study used Timed Up and Go (TUG), 30-second chair stand and 40-meter walk test for measuring function[31].

Program monitoring and tracking

We identified types of program monitoring and tracking: automated text messages[27,29,34], asynchronous chat via the app or telephone[32], telephone[17,33], Skype[30], and meeting physiotherapist at the clinic[31].

Main results

Adherence rate

Bennell et al[27] reported home exercise with tracking by SMS greater adherence rate (mean 16.5, SD 6.5 vs mean 13.3, SD 7.0; mean difference 3.1, 95% CI 0.8-5.5; *p*-value =0.01) than control group. Alasfour et al[29] reported home exercise with tracking by the application automatically sends alerts was significantly greater in adherence rate (26.60%) and pain (*p*-value = 0.015) than control group.

Pain

Alasfour et al[21] compared the home exercise program the application automatically sends alerts and home exercise with a hard copy sheet. The results showed that pain as measured by ANPRS was significant between groups (Mean -1.08, 22.26%, *p*-value = 0.015), with the application group having better pain improvement at the end of week 6. However, there was a significant reduction in pain scores for the application group across time, and the mean difference within-group was statistically significant (*p*-value < 0.001).

Bennell et al[21] found no difference in pain using NRS between home exercise with automated text messages and paper-based home exercise. Fitzgerald et al[31] showed no difference in pain by NRS between home exercise with booster group and home exercise only.

Five studies[17,30,32-34] showed that a home program with a tracking group improved in pain to a greater extent compared with self-home exercise by using WOMAC pain score[17,30,32,33] or NRS[34].

Function

Alasfour et al[29] showed that the mean difference between groups was insignificant (*p*-value = 0.619) at week 6 using the WOMAC score. There was a significant reduction in physical function scores for the application group across time; the mean difference was statistically significant with repeated measures ANOVA (*p*-value < 0.001).

Five studies[17,30,32-34] showed that a home program with a tracking group improved function to a more extraordinary than self-home exercise by using a WOMAC function score[17,30,32-34]. Gohir et al[24] studied the effect of home exercise encouraged by daily emails or smartphone notifications, or by the physical therapist via asynchronous chat or telephone during the study period and found that it improved statistically significantly more than the control group in function by using the 30-second sit-to-stand test (between-group difference, 3.4 [95% CI, 2.2 to 4.5]; *p*-value < 0.001) and the TUG (between-group difference, -1.8 seconds [95% CI, -3.0 to -0.5]; *p*-value = 0.007).

Fitzgerald et al[31] showed no difference in function by TUG, chair rise, and 40m walk test between home exercise with booster group and home exercise only at nine weeks or one year.

Quality of life

Bennell et al[30] showed significant between-group differences favoring the intervention in quality of life at month 3 (p-value ≤ 0.001) and month 9 (p-value = 0.003). Likewise, the study of Yilmaz et al[17] found that in the follow-up group, there was a significant change in the quality of life, and when compared between groups, there was an improvement in the tracking group greater than the control group.

Discussion

This study is the first systematic review on the effectiveness of home-based exercise with tracking and home-based exercise alone for patients with KOA. The present review evaluated eight studies (eight RCTs, 1,205 subjects with KOA) to examine evidence regarding the effectiveness of home exercise programs with or without tracking in the management of KOA on pain and physical function at short term and long term. The analysis indicated that home exercise programs with and without tracking are commonly used in KOA. The eight studies evaluated using the 'Risk of Bias Tool 2.0'[28] were considered of high methodological quality. Based on the present review, the home exercise programs with tracking used in the reviewed literature can be considered treatment possibilities for KOA individuals.

There were considerable variations in the content and duration of the exercise programs included in our systematic review. Length of intervention ranged from six weeks to one year, while the home exercise programs included exercises such as strengthening the hip, quadriceps, and hamstring muscle, stretching, and range of motion exercise. However, most high-quality studies included open and closed kinematic chain exercises as a home exercise program with consistently positive outcomes such as reduced pain and improved function[17,27,29-34]. Except for a study by Fitzgerald et al[31], the group that was followed by physical therapist visits was not significantly different from the group who did exercise alone. This may be due to the long intervals between visits to each physical therapist, so there were no differences in the long-term measurements between groups.

In our included trials, home exercise programs with tracking provide more adherence and behavior change than without tracking group when given the same home exercise program. Nicolson et al[22] found that adherence of the elderly to home-based exercise was relatively low. Previous studies have found that barriers to access, such as distance, travel time, and travel costs, contribute to the decline in adherence and motivation to exercise[25]. Each track can alert and provide more explanation about the exercise program, or serve as a communication channel between elderly and therapist that increases accessibility, adherence, and motivation for home exercise program[17,27,29,30,32-35]. In this study, it was found that in the tracking group, the rate of adherence was higher than that in the without tracking group[27,29]. Therefore, tracking during a home exercise program in people with KOA to better adhere to exercise and individual trials supported the use of motivational strategies and behavior change. In their review, Teeter et al[36] identified that telephone-based motivational interviewing could help improve medication adherence and behavior change. Each study had different channels used to track participants, and each channel can improve consistency and behavior change. On the other hand, Bennell et al[30] found that exercising at home exercise with tracking did not improve coping attempts in people with osteoarthritis in three months, but at 9-month follow-up, coping attempts showed a significant improvement. This is because this variable requires adherence and time to improve behavior.

This review highlighted the variety in the tracking of exercise programs as automated text messages[27,34], asynchronous chat via the application or telephone[29,32], telephone[17,33], Skype[30], and meeting physiotherapist at the clinic[31]. According to the reviews, each tracking channel can improve exercise adherence in elderly with osteoarthritis. In addition, remote tracking can help reduce costs, reduce travel time, and increase access to healthcare in people living in remote or rural areas[30,32,34].

The advantages of each track are as follows: automated text messages are one-way communication, often used as reminders of workout days, to add motivation or to explain more about an exercise program, which is a channel that is easy to access and reduce cost[27,34]. Bennell et al[27] used automated text messages to alert and track exercise at home in people with KOA. It was found that exercise adherence was significantly higher in the receiving message group than in the non-messaging group. However, there were no differences in pain and knee function variables. The effect of this exercise tracking may be because the automatic messaging did not provide sufficient details in the exercise content to make a difference in pain and physical function. Nelligan et al[34] using web-based exercises supported by automated text messages in subjects with KOA showed significant differences in pain, knee function, and quality of life compared to the web-based exercise with the same content without automated text messages. The website used by both groups describes how people with KOA are managed, details about exercise for those with KOA, and knowledge that is communicated both in text, image, or video formats to help participants understand better. Technology-based program applications provide guidelines for exercise performance. They are designed to appeal to the elderly and are easy to use. Exercises are shown using colorful animations to make it easier for patients to follow. The application supports exciting features, such as alerts, and monitoring systems controlled by physical therapists. This application provides an automatic recording of exercise compliance. Combining time and session completion can improve compliance with a home-based exercise program and improve the management of patients with KOA[29]. Telephone-based exercise advice for people with KOA without supervision, one-to-one, communicated only by the voice from the call alone. Hinman et al. (2019) determined the effectiveness of adding exercise advice and support by physiotherapists to an existing nurse led musculoskeletal telephone service on pain and function in adults with KOA[17]. They found that telephone-delivered physiotherapist-led exercise advice and support modestly improved physical function. Video conferencing refers to the clinical application of consultative, preventative, diagnostic, and therapeutic services via two-way interactive telecommunication technologies[35]. It enables the physiotherapist to provide individualized instructions, feedback, and training programs for each patient in real-time[35]. Bennell et al[30] evaluated the effectiveness of an innovative internet-based intervention combining physiotherapist-prescribed home exercise delivered via videoconferencing (Skype) and automated pain coping skills training (PCST) in person with KOA and found that Skype groups are improved in pain and function that is sustained for at least six months. Naeemabadi et al[35] reported that video conference–based programs can be considered as the well-established solution for the conventional rehabilitation program for the target group. Therefore, these studies reported that home-based exercise with tracking in people with KOA is essential for increasing adherence and improving pain and physical function when compared to the untracked group.

A limitation in the current study was that a meta-analysis was not performed due to the small number of studies participating and variations in the trials.

Conclusion

Based on the high quality of studies included in this systematic literature review, it can be concluded that home-based exercise with tracking in people with KOA is essential for increasing adherence and improving pain and physical function compared to the untracked group.

Take home messages

Tracking during home-based exercise program in people with KOA is essential for increasing adherence and improving pain and physical function.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

We acknowledge my SR team's personal and academic support and advisor, Asst. Prof. Dr.Santhanee Khruakhorn for guiding us at every step for SR, my co-researcher Nathaphon Jirasakulsuk for conducting each step with my diligence.

References

1. Abramoff B, Caldera FE. Osteoarthritis: pathology, diagnosis, and treatment options. Med Clin North Am 2020; 104(2): 293-311.
2. Slemenda C, Brandt KD, Heilman DK, Mazzuca S, Braunstein EM, Katz BP, et al. Quadriceps weakness and osteoarthritis of the knee. Ann Intern Med 1997; 127(2): 97-104.
3. Alnahdi AH, Zeni JA, Snyder-Mackler L. Muscle impairments in patients with knee osteoarthritis. Sports Health 2012; 4(4): 284-92.
4. Bade MJ, Kohrt WM, Stevens-Lapsley JE. Outcomes before and after total knee arthroplasty compared to healthy adults. J Orthop Sports Phys Ther 2010; 40(9): 559-67.
5. Alkan BM, Fidan F, Tosun A, Ardıçoğlu ÖJMr. Quality of life and self-reported disability in patients with knee osteoarthritis. Mod Rheumatol 2014; 24(1): 166-71.
6. Cubukcu D, Sarsan A, Alkan H. Relationships between pain, function and radiographic findings in osteoarthritis of the knee: a cross-sectional study. Arthritis 2012; 2012: 1-5.
7. Salaffi F, Carotti M, Stancati A, Grassi W. Health-related quality of life in older adults with symptomatic hip and knee osteoarthritis: a comparison with matched healthy controls. Aging Clin Exp Res 2005; 17(4): 255-63.
8. Lawrence RC, Felson DT, Helmick CG, Arnold LM, Choi H, Deyo RA, et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. Arthritis Rheumatol 2008; 58(1): 26-35.
9. Cross M, Smith E, Hoy D, Nolte S, Ackerman I, Fransen M, et al. The global burden of hip and knee osteoarthritis: estimates from the global burden of disease 2010 study. Ann Rheum Dis 2014; 73(7): 1323-30.
10. Zhang Y, Jordan JM. Epidemiology of osteoarthritis. Clin Geriatr Med 2010; 26(3): 355-69.
11. Bruce-Brand RA, Walls RJ, Ong JC, Emerson BS, O'Byrne JM, Moyna NM. Effects of home-based resistance training and neuromuscular electrical stimulation in knee osteoarthritis: a randomized controlled trial. BMC Musculoskelet Disord 2012; 13(1): 118.
12. McCarthy CJ, Mills PM, Pullen R, Roberts C, Silman A, Oldham JA. Supplementing a home exercise programme with a class-based exercise programme is more effective than home exercise alone in the treatment of knee osteoarthritis. Rheumatology (Oxford) 2004; 43(7): 880-6.
13. Rogers MW, Tamulevicius N, Semple SJ, Coetsee MF, Curry BF. Comparison of clinic-based versus home-based balance and agility training for the symptoms of knee osteo-arthritis. S Afr J Sports Med 2011; 23(3): 80-3.
14. Petrella RJ, Bartha C. Home based exercise therapy for older patients with knee osteoarthritis: a randomized clinical trial. J Rheumatol 2000; 27(9): 2215-21.
15. Deyle GD, Allison SC, Matekel RL, Ryder MG, Stang JM, Gohdes DD, et al. Physical therapy treatment effectiveness for osteoarthritis of the knee: a randomized comparison of supervised clinical exercise and manual therapy procedures versus a home exercise program. Phys Ther 2005; 85(12): 1301-17.
16. Fitzgerald GK, Oatis C. Role of physical therapy in management of knee osteoarthritis. Current opinion in rheumatology. Curr Opin Rheumatol 2004; 16(2): 143-7.
17. Yilmaz M, Sahin M, Algun ZC. Comparison of effectiveness of the home exercise program and the home exercise program taught by physiotherapist in knee osteoarthritis. J Back Musculoskelet Rehabil 2019; 32(1): 161-9.

18. Pisters MF, Veenhof C, Schellevis FG, Twisk JWR, Dekker J, De Bakker DH. Exercise adherence improving long-term patient outcome in patients with osteoarthritis of the hip and/or knee. Arthritis Care Res (Hoboken) 2010; 62(8): 1087-94.
19. Anwer S, Alghadir A, Brismée J-M. Effect of home exercise program in patients with knee osteoarthritis: a systematic review and meta-analysis. J Geriatr Phys Ther 2016; 39(1): 38-48.
20. O'Reilly SC, Muir KR, Doherty M. Effectiveness of home exercise on pain and disability from osteoarthritis of the knee: a randomised controlled trial. Ann Rheum Dis 1999; 58(1): 15-9.
21. Thomas KS, Muir KR, Doherty M, Jones AC, O'Reilly SC, Bassey EJ. Home based exercise programme for knee pain and knee osteoarthritis: randomised controlled trial. Brit Med J 2002; 325(7367): 752.
22. Nicolson PJA, Hinman RS, Kasza J, Bennell KL. Trajectories of adherence to home-based exercise programs among people with knee osteoarthritis. Osteoarthr Cartil 2018; 26(4): 513-21.
23. Campbell R, Evans M, Tucker M, Quilty B, Dieppe P, Donovan JL. Why don't patients do their exercises? Understanding non-compliance with physiotherapy in patients with osteoarthritis of the knee. J Epidemiol Community Health 2001; 55(2): 132-8.
24. Çolak TK, Kavlak B, Aydoğdu O, Şahin E, Acar G, Demirbüken İ, et al. The effects of therapeutic exercises on pain, muscle strength, functional capacity, balance and hemodynamic parameters in knee osteoarthritis patients: a randomized controlled study of supervised versus home exercises. Rheumatol Int 2017; 37(3): 399-407.
25. Carrillo JE, Carrillo VA, Perez HR, Salas-Lopez D, Natale-Pereira A, Byron AT. Defining and targeting health care access barriers. J Health Care Poor Underserved. 2011; 22(2): 562-75.
26. Nicolson PJA, Bennell KL, Dobson FL, Van Ginckel A, Holden MA, Hinman RS. Interventions to increase adherence to therapeutic exercise in older adults with low back pain and/or hip/knee osteoarthritis: a systematic review and meta-analysis. Br J Sports Med 2017; 51(10): 791-9.
27. Bennell K, Nelligan RK, Schwartz S, Kasza J, Kimp A, Crofts SJ, et al. Behavior Change Text Messages for Home Exercise Adherence in Knee Osteoarthritis: randomized trial. J Med Internet Res 2020; 22(9): e21749.
28. Higgins JPTTJ, Chandler, J., Cumpston, M., Li, T., Page, M.J., Welch, V.A. Cochrane Handbook for Systematic Reviews of Interventions version 6.0. Cochrane 2019. Available from: Available from www.training.cochrane.org/handbook.
29. Alasfour M, Almarwani M. The effect of innovative smartphone application on adherence to a home-based exercise programs for female older adults with knee osteoarthritis in Saudi Arabia: a randomized controlled trial. Disabil Rehabil 2020; 44(11): 2420-7.
30. Bennell KL, Nelligan R, Dobson F, Rini C, Keefe F, Kasza J, et al. Effectiveness of an internet-delivered exercise and pain-coping skills training intervention for persons with chronic knee pain: a randomized trial. Ann Intern Med 2017; 166(7): 453-62.
31. Fitzgerald GK, Fritz JM, Childs JD, Brennan GP, Talisa V, Gil A, et al. Exercise, manual therapy, and use of booster sessions in physical therapy for knee osteoarthritis: a multi-center, factorial randomized clinical trial. Osteoarthr Cartil 2016; 24(8): 1340-9.
32. Gohir SA, Eek F, Kelly A, Abhishek A, Valdes AM. Effectiveness of internet-based exercises aimed at treating knee osteoarthritis: the iBEAT-OA randomized clinical trial. JAMA Netw Open 2021; 4(2): e210012.

33. Hinman RS, Campbell PK, Lawford BJ, Briggs AM, Gale J, Bills C, et al. Does telephone-delivered exercise advice and support by physiotherapists improve pain and/or function in people with knee osteoarthritis? Telecare randomised controlled trial. Br J Sports Med 2020; 54(13): 790-7.
34. Nelligan RK, Hinman RS, Kasza J, Crofts SJC, Bennell KL. Effects of a self-directed web-based strengthening exercise and physical activity program supported by automated text messages for people with knee osteoarthritis: a randomized clinical trial [with consumer summary]. JAMA Internal Medicine 2021; 181(6): 776-85.
35. Naeemabadi M, Fazlali H, Najafi S, Dinesen B, Hansen JJJBE. Telerehabilitation for patients with knee osteoarthritis: a focused review of technologies and teleservices. JMIR Biomed Eng 2020; 5(1): e16991.
36. Teeter BS, Kavookjian J. Telephone-based motivational interviewing for medication adherence: a systematic review. Transl Behav Med 2014; 4(4): 372-81.

Arch AHS

ORIGINAL ARTICLE

Archives of Allied Health Sciences 2022; 34(3): 14-21.

Diagnostic performance of immunochromatographic assay and fluorescence immunoassay for the detection of acute dengue infection

Rinjong Kendal[1], Piyaporn Sonthimoon[1], Siriwan Hrongbutsri[1], Kanjanan Chainam[1], Patcharaporn Tippayawat[2*]

[1] Department of Medical Technology, Khon Kaen Hospital, Thailand.

[2] Department of Medical Technology, Faculty of Associated Medical Science, Khon Kaen University, Thailand.

KEYWORDS
Immunochromatographic assay (ICT); Fluorescent immunoassay (FIA); Sensitivity; Specificity

ABSTRACT

Dengue is a crucial public health issue worldwide. The clinical manifestation of dengue infection is nonspecific. The high performance of the dengue diagnostic test has led to prompt and well-organised treatment. To detect dengue infection in routine laboratories, the immunochromatographic rapid test is generally used. Nowadays, high-performance commercial kits are distributed by many manufacturers. A new rapid fluorescent immunoassay (FIA) for the detection of acute dengue infection was declared to reduce technical errors (naked-eye detection) and produced high sensitivity and specificity. Herein, we evaluated two features of the acute dengue infection test kit, including an immunochromatographic assay (ICT) and new rapid fluorescence immune assay (FIA) compared to retrieved clinical data. Twenty plasma samples were tested for dengue NS1 Ag and dengue IgM/IgG by ICT and FIA against clinical data. The results showed a higher sensitivity of FIA compared to ICT (81.8% and 72.7%). In contrast, the specificity of ICT was greater than FIA (66.7% and 44.4%). Moreover, ICT provided 72.7% PPV and 66.7% NPV, while IFA provided 64.2% PPV and 66.7% NPV. However, the performance of commercial test kits may be dependent on dengue serotypes, the day of onset, the manufacturer, and the tested sample size. Due to limited resources, only twenty samples were included in this study. For more precise information, the sample size should be increased. Nevertheless, this study provided sufficient fundamental efficacy information on the test kits for purchasing decisions.

***Corresponding author:** Patcharaporn Tippayawat, MT, PhD. Department of Medical Technology, Faculty of Associated Medical Science, Khon Kaen University, Khon Kaen 40002, Thailand. Email: patchatip@kku.ac.th
Received: 31 March 2022/ Revised: 5 May 2022/ Accepted: 13 June 2022

Introduction

Dengue virus (DENV), which causes dengue fever, is transmitted by Aedes spp. mosquitoes including Aedes aegipti, Aedes albopictus etc. It is an arbovirus belonging to the Flaviviridae family and Flavivirus genus. There are four serotypes including DENV1, DENV2, DENV3, and DENV4 [1,2]. Following the World Health Organisation (WHO) criteria, dengue case classification for diagnosis and management can be categorised as asymptomatic and symptomatic cases. Symptomatic dengue infections are classified as undifferentiated fever, dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS)[3,4]. DENV infection is endemic in tropical and subtropical regions, and is critically known as a public health concern. The WHO estimated that 3.9 billion people in 128 countries were at risk for dengue infection[5]. In 2019 and 2020, the Thailand Ministry of Public Health reported 13.25, 27.41 cases of dengue infection per 100,000 population and 0.15% and 0.13% death, respectively[6]. The severity of a patient's condition depended on the severity of dengue infection and DENV serotype, as well as patient age, host conditions, and pre-existing dengue virus or other flavivirus infection[7,8]. However, delayed diagnosis may cause morbidity and mortality. To enable prompt and well-organised treatment, precise and early diagnosis tools are required. At present, several methods are used for DENV detection, e.g. viral nucleic acid testing, ELISA-based and immunochromatography-based techniques for DENV NS1 Ag and DENV IgM/IgG[9]. DENV NS1 Ag is the non-structural protein of DENV; it releases from infected cells. It plays an essential role in viral replication and triggers humoral and cell-mediated immunity. NS1 is also an important marker for early diagnosis of the disease[10,11]. The nucleic acid technique and the combination of DENV Ag and Ab were used for confirmation testing, but the techniques involve time-consuming procedures and require more complicated tools. Therefore, rapid diagnostic testing for DENV NS1 Ag and DENV IgM/IgG based on the immunochromatography technique (ICT) is more common and appropriate for routine approaches. The technique is rapid, easy to use and provides high sensitivity and specificity results[9,12]. Recently, ICT commercial kits have been widely available from different manufacturers. However, the new rapid fluorescence immune assay (FIA) is thought to reduce human error and provide higher sensitivity, specificity, and accuracy compared to the present ICT kits[13,14]. Furthermore, the performance data between ICT and FIA against clinical data are limited. Therefore, the evaluation of ICT and FIA is required to facilitate the selection of more efficient detection devices. Thus, this study aimed to evaluate the DENV screening test between the recently used-rapid ICT test and FIA test compared to the retrieved clinical data. Clinical data such as date of onset and final diagnosis were provided by clinicians. The results of this study provided fundamental efficacy information for the DENV diagnosis test kit concerning purchasing decisions.

Material and methods

Sample collection

This study was performed from June to August 2020 at Khon Kaen Hospital, Thailand. Inclusion criteria were: (1) Leftover specimens from routine dengue detection, (2) Samples detected by both dengue antigen (Dengue NS1 Ag) and dengue antibody (Dengue IgM/IgG). Exclusion criteria were: (1) Haemolysis sample, (2) Insufficient sample (< 500 µL). After routine determination by regular ICT (DENV NS1 and DENV IgM/IgG), patient samples were separated and stored at -20°C for further determination by rapid FIA. Samples were examined following the manufacturer's protocol. Clinical data including final diagnosis and date of onset were retrieved from medical records. Herein, the confirmation test was not performed due to the limitation of resources, and the clinical data, date of onset and final diagnosis were provided by clinicians. Ethical approval was received from Ethics Review Committee, Khon Kaen Hospital (Approval number KEXP63050).

Rapid diagnostic test kits

Rapid immunochromatographic test

DENV NS1 Ag Rapid Card Bio Tracer (NanoEn Tek, Gyeonggi-do, Korea) and DENV IgM/IgG Rapid Test (Lungene, Hangzhou, China) are one-step immuno-chromatographic assays aimed at the detection of Dengue NS 1 antigens and Dengue IgM/IgG in human plasma or serum.

Rapid fluorescent immunoassay test

FIA is the detection principle using a fluorescence dye, which absorbs light at a specific wavelength and then emits light that is measured by an analyser. The advantages are higher sensitivity detection and reduced human error by naked-eye reading. DENV NS1 Ag and DENV IgM/IgG Fluorescence Apoti Dengue Test (ACRO Biotech Inc., CA, USA) are based on fluorescence immunoassay for in vitro detection of DENV NS1 Ag and DENV IgM/IgG in human plasma or serum. The technique produces a cut-off index (COI) output, in which COI ≥ 1.0 will be interpreted as positive, whereas COI < 1.00 is determined as negative.

Interpretation

DENV NS1 positive and/ or DENV IgM positive were interpreted as the primary infection. Whether DENV NS1 positive/DENV IgM positive/ DENV IgG positive, DENV NS1 negative/DENV IgM positive/ DENV IgG positive were interpreted as secondary infections. Both primary and secondary infections are acute dengue infections. Additionally, DENV IgG positive only is assumed a past infection, which is a non-acute dengue infection.

Statistical analysis

The results of rapid ICT (DENV NS1 and DENV IgM/IgG) and the new rapid FIA (Fluorescence Apoti Dengue) were compared to clinical diagnosis. Percentages of sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) were computerised, and statistical analysis was performed using SPSS software (Version 18.0. Chicago: SPSS Inc.; 2009).

Results

DENV NS1 and DENV IgM/IgG by rapid ICT and rapid FIA test compared to medical records

Twenty samples were determined as DENV NS1 and DENV IgM/IgG by ICT and FIA. Medical records including date of onset and final diagnosis were retrieved. Eleven of 20 samples were diagnosed as acute dengue infection, while nine cases were identified as Kaposi's sarcoma with underlying thrombocytopenia, acute febrile illness and autoimmune haemolytic anaemia, systemic infection, Chikungunya infection, acute febrile illness, fever, non-specific fever, acute pharyngitis, adenomyosis, and unidentified (non-DENV). Furthermore, two of 11 acute dengue infections had complications including thalassemia and scrub typhus infection with hepatitis (Table 1). In addition, there were various dates of onset from one day to five days, as shown in table 1. DENV infection was classified into four categories including DENV primary infection, DENV secondary infection, past infection, and non-DENV infection by using rapid ICT and rapid FIA detection systems. The results demonstrated that detection by rapid ICT system could be grouped as DENV primary infection, secondary infection, past infection, and non-DENV infection for 6, 5, 4, and 5 cases, respectively. Additionally, DENV infection classification by rapid FIA system were 3, 11, 6, and none were classified as non-DENV infection, respectively (Table 2).

Table 1 Results of DENV NS1, DENV IgM/IgG by ICT and FIA compared with clinical data

No.	ICT for DENV			Classification of infection by ICT	FIA for DENV (Cut off >1.0)			Classification of infection by FIA	Medical records	
	NS1	IgM	IgG		NS1	IgM	IgG		Date of onset	Clinical Diagnosis
1	-	-	-	Non-DENV	> 20.0	0.11	7.74	Secondary infection	ND*	Thrombocytopenia, acute febrile illness, autoimmune haemolytic anaemia, Kaposi's sarcoma
2	-	-	+	Past infection	0.98	0.19	21.94	Past infection	3	Dengue fever with thalassemia
3	-	-	-	Non-DENV	1.56	0.33	0.12	Primary infection	3	Dengue fever
4	-	-	+	Past infection	0.81	0.38	78.1	Past infection	5	Dengue haemorrhagic fever
5	-	-	-	Non-DENV	0.85	0.25	3.75	Past infection	1	Systemic infection
6	-	-	-	Non-DENV	0.81	0.34	15.87	Past infection	2	Chikungunya infection
7	-	-	-	Non-DENV	0.3	0.57	7.9	Past infection	1	Acute febrile illness
8	-	-	+	Past infection	4.7	1.24	42.52	Secondary infection	4	Fever
9	-	+	+	Secondary infection	> 20.0	0.57	8.74	Secondary infection	3	Scrub infection, Dengue fever and hepatitis
10	-	-	+	Past infection	1.01	0.35	78.95	Secondary infection	3	Acute pharyngitis
11	+	-	+	Secondary infection	4.52	3.39	63.38	Secondary infection	4	Dengue fever
12	+	-	+	Secondary infection	> 20.0	0.54	103.37	Secondary infection	5	Dengue haemorrhagic fever
13	+	-	+	Secondary infection	12.07	1.26	57.06	Secondary infection	ND*	Adenomyosis
14	+	+	-	Primary infection	2.77	1.17	0.11	Primary infection	2	Viral gastroenteritis
15	+	-	+	Secondary infection	1.29	0.31	195.3	Secondary infection	3	Dengue haemorrhagic fever
16	+	-	-	Primary infection	4.97	1.26	159.07	Secondary infection	5	Dengue haemorrhagic fever
17	+	-	-	Primary infection	0.94	0.12	2.43	Past infection	ND*	Non DENV
18	+	+	-	Primary infection	13.07	7.97	12.75	Secondary infection	3	Dengue fever
19	+	-	-	Primary infection	> 20.0	0.49	46.75	Secondary infection	3	Dengue fever
20	+	-	-	Primary infection	1.82	0.79	0.1	Primary infection	4	Dengue fever

Note: *ND, No data.

Table 2 Classification of dengue infection by ICT and FIA

Classification of DENV infection	Number of samples (%)	
	ICT	FIA
Non-dengue infection	5(25)	0
Past infection	4(20)	6(30)
Primary infection	6(30)	3(15)
Secondary infection	5(25)	11(55)
Total	20	20

Performance of DENV NS1 DENV IgG/IgM ICT and FIA to detect acute dengue infection

DENV NS1 and DENV IgG/IgM were detected by using two platforms between present ICT and FIA compared to clinical data. DENV NS1 and DENV IgM positive were classified as acute infections, either primary infection or secondary infection. DENV NS1 and/or DENV IgM negative was non-dengue infection, either DENV IgG positive or negative. The results are shown in table 3.

Table 3 Numbers for DENV NS1 Ag and DENV IgM/IgG detection by ICT and FIA for acute dengue infection

	ICT (N = 20)		FIA (N = 20)	
	Acute-DENV	Non-DENV	Acute DENV	Non-DENV
Positive (Primary and secondary dengue infection)	8	3	9	5
Negative (Past infection and non-dengue infection)	3	6	2	4

The sensitivity, specificity, accuracy, PPV and NPV of ICT to diagnose acute dengue infection were 72.7%, 66.7%, 70.0%, 72.7%, and 66.7%, respectively. The sensitivity, specificity, accuracy, PPV and NPV of rapid FIA were 81.8%, 44.4%, 65.0 %, 64.2%, and 66.7%, respectively, as shown in table 4.

Table 4 Performance of ICT and FIA to detect acute dengue infection

Test performance	ICT (%)	FIA (%)
Sensitivity	72.7	81.8
Specificity	66.7	44.4
Accuracy	70.0	65.0
PPV	72.7	64.2
NPV	66.7	66.7

Comparison of date of onset in suspected DENV detection by ICT and FIA

DENV NS1 Ag and IgM are acute infection markers. Herein, we compared the ability of two principal kits to detect acute dengue infection on the day after onset. The rapid FIA test kit showed greater detection on the third day of fever compared to ICT. Data are shown in table 5.

Table 5 Comparison of detection of suspected acute dengue infection between ICT and FIA

Date onset	Number of samples (N = 17)	NS1 Positive		IgM Positive	
		ICT	FIA	ICT	FIA
1	2	0	0	0	0
2	2	1	1	1	0
3	7	3	6	2	1
4	3	2	3	0	2
5	3	2	2	0	1

Discussion

This study demonstrated the performance of two diagnostic tools to detect acute dengue infection (DENV NS1, DENV IgM/IgG), including the rapid immunochromatographic test (ICT), which was available in the hospital, and the new rapid fluorescent immunoassay (FIA). Individually, ICT and FIA results could classified dengue infection and compared to clinical diagnoses retrieved from medical records (Table 1). This is the first evaluation of DENV NS1 Ag and DENV IgM/IgG ICT compared with FIA to diagnose recent dengue infection. Twenty samples were examined for DENV NS1 and DENV IgM/IgM with both ICT and FIA compared to medical diagnoses. The sensitivity of FIA was higher than that of ICT (ICT=72.7%, FIA =81.8%). In contrast, the specificity of FIA was lower than that of ICT (ICT=66.7%, FIA =44.4%, FIA =81.8%) (Tables 3 and 4). According to previous studies, the performance of several commercial diagnostic tests (ICT) was different. Humanis, SD bioline and CareUS by using DENV NS1 RT-PCR and DENV antibodies ELISA are the gold standard. The results showed variable sensitivities for DENV NS1 Ag ranging from 42.9 % to 100%, DENV IgM from 38.1-90.5% and DENV IgG from 65.7-100%[9,15]. The specificities ranged from 88-100%. Paulo Sousa Prado et al. (2018) showed that SD Bioeasy Dengue Duo NS1/IgM combined had moderate sensitivity and high specificity[16]. Kok-Siang Yow et al[17] demonstrated the sensitivity of DENV NS1 and DENV IgM in several commercial kits including Standard Q, SD bioline, Multisure, and CareUS in recent dengue infection. Standard Q had the highest sensitivity at 99.1%, while Multisure had the lowest at 92.6%. All enrolled kits were highly specific for dengue NS1 and IgM (96.7% to 100%). Lorenzo et al[14] evaluated a new rapid fluorescence immunoassay for the combination of DENV NS1 and DENV IgM to detect acute infection and showed a sensitivity of 100%. Positive predictive values varied from 98.4% to 100%, and the negative predictive value was 96.8%. Our study showed that the sensitivity of the two diagnostic tools in recent dengue infection was satisfactory (ICT=72.7%, FIA =81.8%) In contrast, the specificity of ICT and FIA tools was inadequate (66.7% and 44.4%) when compared to previous studies. However, the reaction conditions from patients that have heterophile antibodies, underlying autoimmune and inflammation may cause interference of the experiment test[18] and cause interaction with the immunoassay procedure, which might lead to false-positives and misinterpretation[19]. Herein, some complications might affect the performance of FIA test kits (Table 1). Therefore, an appropriate cut-off index might be required in order to assess the high performance of the kit. The positive predictive value and negative predictive value of both ICT and FIA were (72.7%, 66.7%) and (64.2%, 66.7%), respectively. Interestingly, the FIA method enabled early detection in cases numbers 4, 12, 15 and 16 (Table 1), which might be the advantage of FIA in that it can predict severe conditions of DHF, leading to effective management and reduced fatalities. In addition, the detection of acute DENV infection by FIA at 3 days onset is faster than ICT at 5-6 days onset (Table 5); the consequence of well-organised treatment is a decline in mobility and mortality[11,16]. Thus, new rapid FIA might benefit the early diagnosis of dengue infection and

reduce human error from ICT test kits. However, different factors that affect the performance of the detection tools include sample size, date onset, age of patients, pre-exposure *flavivirus*, DENV serotype, and original performance of the chosen commercial kit. This study used the left-over samples from routine work. The samples were kept at -20°C until they were taken for study, and then reusing the sample will be the first thaw. Frozen and thawed samples that have been stored for a long time can be reused without affecting the test[20-22]. Practically, the gold standard method was limited; clinical data and other routine laboratory results, including PT, PTT, CBC, DENV NS1, DENV IgM/IgM, were mostly utilised for diagnosis. This study might be the appropriate model for real situations in hospitals with limited resources. However, more reliability by increasing the sample size and comparison with the gold standard ELISA-based techniques are recommended. In addition, a different set of samples, study design, laboratory settings, and other technical conditions could affect the results. Therefore, verification of the new test kit in other laboratories should be accomplished.

Conclusion

The sensitivity of the new rapid fluorescent immunoassay (FIA) is greater than the rapid immunochromatographic test (ICT), and the specificity of FIA is lower than ICT for the detection of acute dengue infection.

Take home messages

The performance evaluation of the rapid diagnostic test revealed that the new rapid fluorescent immunoassay (FIA) enabled higher sensitivity when compared to the immunochromatographic assay (ICT), although it presented low specificity. Thus, new rapid FIA might benefit the diagnosis of early dengue infection and reduce human error from the ICT test.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgement

The authors wish to express their gratitude to all the patients and personnel who participated in and supported this study.

References

1. Halstead SB. Pathogenesis of dengue: challenges to molecular biology. Science 1988; 239(4839): 476-81.
2. Kurane I. Dengue hemorrhagic fever with special emphasis on immunopathogenesis. Comp Immunol Microbiol Infect Dis 2007; 30(5-6): 329-40.
3. Sri Rezeki S. Hadinegoro. The revised WHO dengue case classification: does the system need to be modified? Paediatr Int Child Health 2012; 32(1): 33-8.
4. Srikiatkhachorn A, Rothman AL, Gibbons RV, Sittisombut N, Malasit P, Ennis FA, et al. Dengue-how best to classify it. Clin Infect Dis 2011; 53(6): 563-7.
5. Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG, et al. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis 2012; 6(8): 1-15.
6. Department of disease control, Ministry of public health Thailand. Dengue fever report [online] 2020 [cited 2021 Sep 25]. Available from: http://phanhospital.go.th/phanhospital/images/Disease 20situation/DHF_Wk18 2004282563.pdf
7. Hasan Khan MI, Anwar E, Agha A, Saleh N, Ullah E, Syed IA, et al. Factors predicting morbidity in patients with dengue fever in a tertiary care hospital. Mediterr J Hematol Infect Dis 2013; 5(1): 1-6.
8. Soo KM, Khalid B, Ching SM, Chee HY. Meta-analysis of dengue severity during infection by different dengue virus serotypes in primary and secondary infections. PLOS ONE 2016; 11(5): 1-16.

9. Lee H, Ryu JH, Park H-S, Park KH, Bae H, Yun S, et al. Comparison of six commercial diagnostic tests for the detection of dengue virus non-structural-1 antigen and IgM/IgG antibodies. Ann Lab Med 2019; 39(6): 566-71.
10. Muller DA, Young PR. The flavivirus NS1 protein: Molecular and structural biology, immunology, role in pathogenesis and application as a diagnostic biomarker. Antiviral Res 2013; 98(2): 192-208.
11. Fan J, Liu Y, Yuan Z. Critical role of Dengue Virus NS1 protein in viral replication. Virol Sin 2014; 29(3): 162-9.
12. Darwish NT, Sekaran SD, Khor SM. Point-of-care tests: a review of advances in the emerging diagnostic tools for dengue virus infection. Sens Actuators B Chem 2018; 255(3): 3316-31.
13. Linares EM, Pannuti CS, Kubota LT, Thalhammer S. Immunospot assay based on fluorescent nanoparticles for Dengue fever detection. Biosens Bioelectron 2012; 41(2013): 180-5.
14. Zammarchi L, Colao MG, Mantella A, Capobianco T, Mazzarelli G, Ciccone N, et al. Evaluation of a new rapid fluorescence immunoassay for the diagnosis of dengue and Zika virus infection. J Clin Virol 2019; 112: 34-9.
15. Jang WS, Kwak SY, May WL, Yang DJ, Nam J, Lim CS. Comparative evaluation of three dengue duo rapid test kits to detect NS1, IgM, and IgG associated with acute dengue in children in Myanmar. PLOS ONE 2019; 14(3): e0213451.
16. Prado PS, Almeida JT, Abreu LT, Silva CG, Souza LD, Gomes MC, et al. Validation and reliability of the rapid diagnostic test 'SD Bioeasy Dengue Duo'for dengue diagnosis in Brazil: a phase III study. Memórias do Instituto Oswaldo Cruz 2018; 113(8): 1-8.
17. Yow KS, Aik J, Tan EYM, Ng LC, Lai YL. Rapid diagnostic tests for the detection of recent dengue infections: an evaluation of six kits on clinical specimens. PLOS ONE 2021; 16(4): 1-11.
18. Martins TB, Pasi BM, Litwin CM, Hill HR. Heterophile antibody interference in a multiplexed fluorescent microsphere immunoassay for quantitation of cytokines in human serum. Clin Vaccine Immunol 2004; 11(2): 325-9.
19. Gaće M, Špacir Prskalo Z, Dobrijević S, Mayer L. Most common interferences in immunoassays. Croat J Oncol 2015; 43(1-3): 23-7.
20. DiMagno EP, Corle D, O'Brien JF, Masnyk IJ, Go VL, Aamodt R. Effect of long-term freezer storage, thawing, and refreezing on selected constituents of serum. Mayo Clin Proc 1989; 64(10): 1226-34.
21. Torelli A, Gianchecchi E, Monti M, Piu P, Barneschi I, Bonifazi C, et al. Effect of Repeated Freeze–Thaw Cycles on Influenza Virus Antibodies. Vaccines (Basel) 2021; 9(3): 267-6.
22. Shurrab FM, Al-Sadeq DW, Amanullah F, Younes SN, Al- Jighefee H, Younes N, et al. Effect of multiple freeze-thaw cycles on detection of anti-SARS-CoV-2 IgG antibodies. medRxiv. J Med Microbiol 2021; 70(8): 1-4.

Arch AHS

ORIGINAL ARTICLE

Archives of Allied Health Sciences 2022; 34(3): 22-32.

Immediate effects of percussive massage treatment on thoracolumbar fascia thickness: a quasi-experimental design in healthy individuals

Chao Yang[1], Wichai Eungpinichpong[2,3*], Wiraphong Sucharit[2,3], XingYu Huang[4]

[1] Department of Exercise and Sport Sciences, Faculty of Graduate school, Khon Kaen University, Khon Kaen, Thailand.
[2] School of Physical Therapy, Faculty of Associated Medical Sciences, Khon Kaen University, Thailand.
[3] BNOJPH Research Center, School of Physical Therapy, Faculty of Associated Medical Sciences, Khon Kaen University, Thailand.
[4] Department of Human Movement Sciences, Faculty of Physical Education, Gan Nan noramal University, Gan Zhou, China.

KEYWORDS
Percussive massage;
Vibration massage;
Massage gun;
Thoracolumbar fascia;
Fascia thickness.

ABSTRACT

In recent years, numerous studies have pointed to the integral role of fascial tissue in developing lower back pain. Percussion massage therapy could stretch muscles and connective tissues by generating muscle waves, which may improve the fascial structures and muscle functions and provide new ideas for preventing and treating lower back pain. However, no studies have been conducted to investigate the effects of percussion massage therapy on fascial structure, skin and muscular responses, and lumbar flexibility. This pilot study aimed to preliminarily investigate the effects of percussive massage therapy on thoracolumbar fascia (TLF) thickness, skin temperature, muscle stiffness and pain, and lumbar mobility. A quasi-experimental before-and-after design was obtained in 12 healthy participants aged 20-40. A 15-minute percussion massage was performed on the participants' TLF in the lower back region. All parameters were measured before and immediately after the intervention. The results showed that the maximum thickness value of the TLF decreased significantly after the intervention (p-value < 0.05), whereas the mean thickness value did not change. Shortly after the intervention, skin temperature increased (p-value < 0.05), perceived stiffness decreased (p-value < 0.05), visual analog scale (VAS) and press pain threshold (PPT) decreased (p-value < 0.05), and perceived stiffness decreased (p-value < 0.05). No change in lumbar flexibility was observed (p-value > 0.05). The results indicated that percussive massage therapy may reduce the maximum thickness value of TLF and improve tissue hardness, skin temperature, and pain intensity. A randomized controlled study with a large sample size is suggested to verify these effects.

***Corresponding author:** Wichai Eungpinichpong, PT, PhD. School of Physical Therapy, Faculty of Associated Medical Sciences, Khon Kaen University 123, Mitraphab Highway, Muang, Khon Kaen 40002, Thailand. E-mail: wiceun@gmail.com
Received: 12 May 2022/ Revised: 23 August 2022/ Accepted: 18 September 2022

Introduction

More than half of the population worldwide has suffered from low back pain in their lifetime, and it is second only to upper respiratory problems as a symptom-related reason for seeking medical care[1]. The traditional view of lower back pain is attributed to specific sources of pain, such as nerve roots, spinal joints, sacroiliac, and intervertebral disc sources of pain[2]. However, an increasing number of studies have shown that fibrosis and densification of the thoracolumbar fascia (TLF) play an integral role in back pain[3,4]. Previous studies found that patients with chronic lower back pain have thicker TLF and lower TLF shear strain compared to people without lower back pain[5,6] and that disorganization of TLF structure was associated with high levels of lower back pain and disability[7]. Besides, Andrea et al[8] evaluated the quadriceps, Achilles, and plantar fascia in 82 healthy volunteers and found that even among healthy volunteers, 51% had varying degrees of connective tissue thickening. The thickening of the fascia was associated with a negative impact on the mechanical properties and function of the fascial tissue and resulted in limited flexibility[9]. Earlier studies found that the fascia in the sternocleidomastoid area thinned in patients with neck pain after they were treated with manipulation[10]. This may be because mechanical stimulation increased local strain modifying the mechanical properties of the extracellular matrix[4].

The efficacy of manual therapy depends on the level and experience of the therapist, and not everyone has sufficient conditions to receive the treatment of the therapist[11]. Therefore, it is particularly important to find a tool for self-physiotherapy. Handheld percussive guns have been shown to have similar effects to manual therapy and are an effective way to improve muscle tissue[12]. Due to its benefits, the handheld percussive gun (Figure 1) has become popular in both therapeutic and sports communities and has been widely used by professional athletes and sports enthusiasts around the world. It has the advantages of being easy to use, portable, and inexpensive[13], which allow users to treat or care for themselves at home under the guidance of a professional (doctor/physiotherapist/exercise physiologist). Percussive massage therapy has the potential ability to treat muscles and connective tissues (fascia) because it combines the physiological mechanisms of conventional massage and vibration therapy. Percussive massage therapy allows the application of a particular vibration frequency to the muscles, which induces a tonic vibration reflex that promotes profound tissue improvement through changes in muscle tone[14]. In addition, since the fascia is composed of fibroblasts, high-frequency percussion may cause creeping changes in the collagen fibers by continuous cyclic loading, and reversing the fascia's densification[15].

To our knowledge, there are no studies on the effects of percussive massage therapy in the lumbar region. It is necessary to verify the effect of percussive massage therapy on the back in a healthy population before conducting studies on patients with lower back pain. This study aimed to preliminarily investigate the immediate effects of percussive massage treatment on the TLF structural, and mechanical properties of the lower back, as well as the lumbar flexibility in healthy adults. We hypothesized that the percussive massage treatment would result in a decrease in thoracolumbar fascia thickness, and tissue hardness, as well as perceived stiffness and pain intensity, and an increase in skin temperature, and lumbar flexibility.

Materials and methods

Study design

This was a quasi-experiment utilized pre- and post-study design. The experiments were conducted at the Department of Physiology, Khon Kaen University, Thailand from November 25 to December 4, 2020. Baseline data before the intervention and immediately post-intervention (a percussive massage treatment) were collected. Experimental measurements and interventions were performed in a room with the temperature of 26.5 degrees Celsius. The study proposal was approved by the Research Ethics Committee of Khon Kaen University, Number: HE642185.

Participants

The inclusion criteria for participants in this study were 20 – 40 years old males and females, BMI (body mass index) < 30 kg/m^2, and VAS scale of pain intensity ≤ 3 cm. The conditions for exclusion were based on a review of past medical history. Participants with previous health problems and contraindications to have massage (such as low-back pathology, documented disabilities, and pregnancy) were excluded. Finally, twelve participants (6 males, and 6 females) were included. Their mean height, weight, and BMI were 168.17 ± 7.26 cm, 69.6 ± 13.8 kg, and 24.4 ± 3.99 kg/m^2 respectively.

Percussive massage therapy protocol

To maintain the consistency of the intervention, the percussive massage treatments were all conducted by a researcher who had a certified exercise prescription from the Chinese National Sports Administration and was supervised by a physiotherapist. During the percussive massage treatment, participants lay prone on a standard massage bed with a small pillow on the abdomen to ensure relaxation of the back muscles. The researcher used a percussive massage gun (35Hz) to perform 15-minute percussive massage on the erector spinae muscles of the lower back (7.5 minutes per side). He applied the percussive massage treatment on the outermost side of the treated muscle and moved the massage gun in a straight line from the distal end to the proximal end within 30 s. Then he moved the percussive massage gun laterally and then in a straight line from the proximal end to the distal end (Figure 2). The researcher kept trying to apply the same pressure to the skin, and the massage gun never left the skin to ensure continuous percussion vibration.

Figure 1 The handheld percussive massage gun with the different attachment heads: (1) hard ball head, (2) spinal head, (3) hard plain head, and (4) double-spinal head.

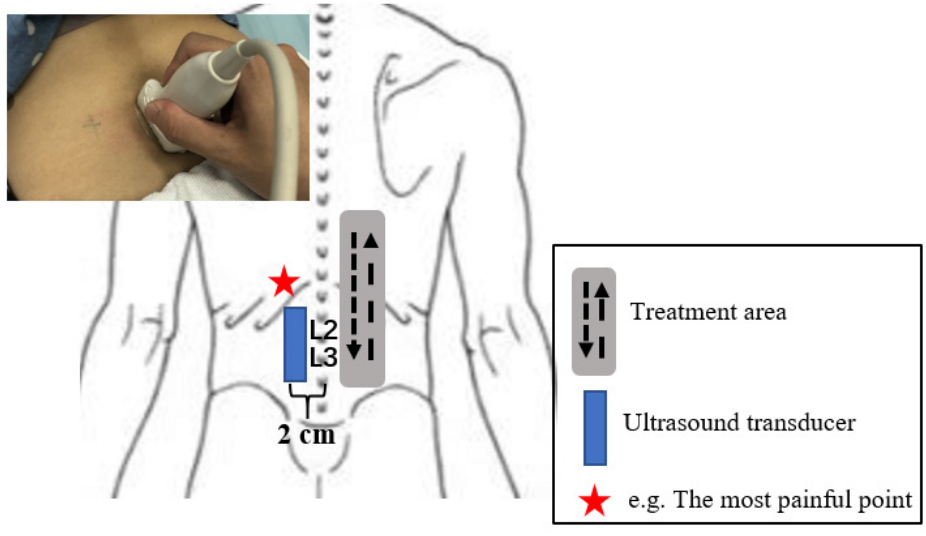


Figure 2 Schematic diagram of the treatment area and measurement points
Note: The intervention was bilateral; the measurement points on the other side are the mirror image of the schematic position. Five-pointed star: measure point of PPT (press pain threshold), tissue hardness.

Outcome measurements

To reduce the confounding effects of measurement, the outcomes were measured in the order starting from skin temperature, then VAS (visual analog scale), PPT (pressure pain threshold), tissue hardness, fascia thickness, perceived stiffness, and lumber flexibility. Measurements of fascia thickness using ultrasound images and PPT were performed by a physiotherapist with eight years of experience and training in ultrasound, and other outcome measures were performed by a trained researcher.

Skin temperature

Skin temperature was measured by using an infrared thermal imaging camera featuring 320·240 pixels with an infrared spectral band of 7.5 – 14.0 mm (Ti10 Fluke Thermal Imaging Camera; Fluke Corporation, Washington). The region of interest (ROI) was the same as the area of the treatment area. The assessor held the instrument so that the lens was parallel to the skin and 40 cm away from the measurement area to obtain the skin temperature[16]. The measurement was repeated three times and the average value was used on both sides for data analysis.

Pain intensity

PPT and VAS were used to assess participants' pain intensity before and after the intervention. The PPT was the minimum amount of pressure that caused discomfort or pain. We measured the PPT using a tissue algometer (Algometer Combo, OE-220 Japan) by vertically placing a 1 cm^2 circular plastic tip over the participant's designated pain point. A physiotherapist palpated the participants' lower back regional provocation points and marked the provocation points where the participants' pain was most pronounced by pressure. Then he gradually applied the pressure to the measurement point at approximately 1 $kg/cm^2/s$ until the participant began to feel pain and pressed the switch button attached to the dynamometer and recorded the corresponding force value (kg/cm^2). The participant was asked to remember this pain level and to apply the same criteria to the subsequent measurement. The lower the score of VAS, the greater PPT felt in the test area[17]. Measurements were repeated three times at 10-second intervals at each side of the measurement point, and the average value of the two sides was taken for data processing. The VAS[18] was used to measure

the intensity of perceived pain by drawing a 10 cm straight line on paper with a ruler and telling participants to draw a line perpendicular to the line according to their perceived back pain during the test, where the leftmost end of the line represented no pain and the rightmost end represented extreme pain. The VAS test was measured before and immediately after the intervention.

Tissue hardness

The measurement of tissue hardness was used with a tissue hardness meter/algometer (Algometer Combo, OE-220 Japan), and measured by placing a plastic disk with a diameter of 10 cm vertically on the painful point (The measurement points were in the same location as PPT. The tissue hardness meter/algometer automatically recorded the tissue stiffness value after low pressure[17], repeated three times, and the average value on both sides was used for data processing.

Fascia thickness

Ultrasound images were acquired by an ultrasound-trained physiotherapist using Hitachi ARIETTA Prologue, Japan, with a 4-cm, 14 MHz linear array transducer for ultrasound imaging of the lumbar spine region. The location of the paravertebral muscles was first determined during real-time ultrasound imaging, and next the ultrasound focus area was adjusted to a superficial border close to the connective tissue. A paraspinal image was acquired bilaterally from the bilateral transducer centered 2 cm from the middle of the L2-L3 interspinous ligament (Figure 1)[5]. The reason was that the fascia was more parallel to the skin at this location than at other areas such as the L4-L5 level, and there was less angular variation between the skin surface and the TLF. This ultrasound-described protocol was a reliable method to determine fascial thickness concerning both intra-observer (ICC: 0.67 – 0.77) agreement and inter-observer (ICC: 0.82 – 0.92) agreement[9,19]. The mean and maximum values of the fascia thickness at that location were recorded separately, and the mean value was evaluated by taking the average of three measurements of the fascial thickness in the proximal, middle, and distal portions of the transducer (Figure 3A). The maximum value of the fascia was averaged by repeating the measurement three times for the thickest part of the fascia on the image (Figure 3B). The thickness of the TLF was collected separately from the left and right sides of the back, and the thickness of the TLF was further calculated by obtaining the mean value of the left and right sums. The measurements were performed with the transducer gently placed on the skin without compressing the soft tissue. The sequence of measurement of bilateral TLF in the left and right order was randomly obtained.

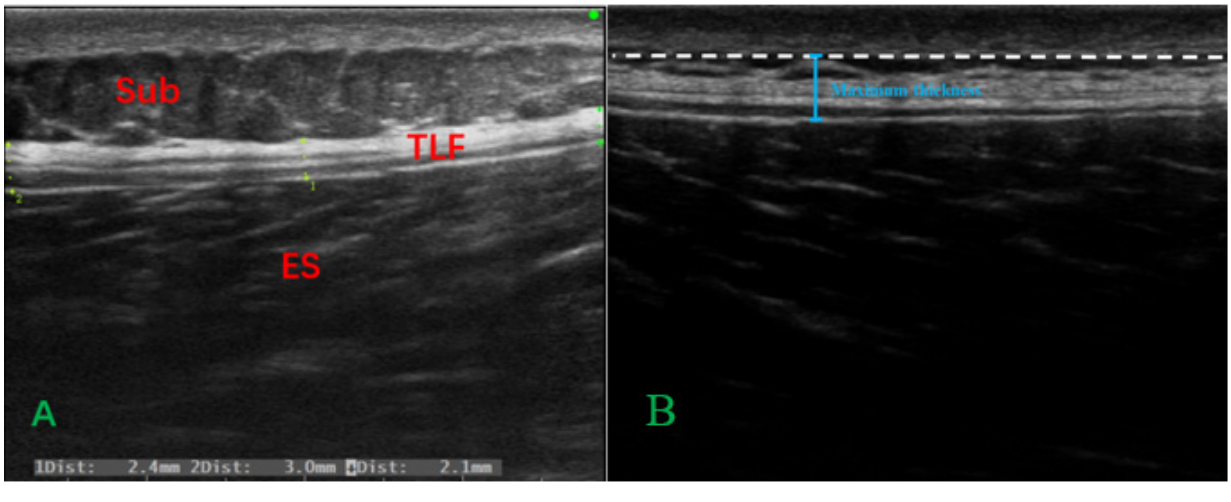


Figure 3 Ultrasound image measurement of mean and maximum values of TLF thickness. (A) Mean value of TLF thickness, (B) Maximum value TLF thickness.

Note: Sub, Subcutaneous layer; TLF, Thoracolumbar fascia; ES, Erector spinae.

Perceived stiffness

Each participant was asked to indicate his or her perceived stiffness scale, a perceived stiffness scale measure consistent with the VAS, ranging from 0 = no stiffness to 10 = most stiffness. Previous research has also shown that this method can be a valid measure of perceived stiffness[20].

Lumbar flexibility

We used a modified Schober test to measure lumbar flexibility because this test could eliminate the errors in the identification of the lumbosacral junction. The measurement covers the entire lumbar spines[21]. The physiotherapist asked the participants to stand up straight and drew a horizontal line connecting the participants' bilateral posterior superior iliac crest marker points. After completing the marking, participants were instructed to bend forward at the waist as if to touch their toes and to ensure that their knees were not bent. The distance between the bottom and the top horizontal line marks was measured using a soft ruler as the participant reached the maximum bend. Fifteen cm subtraction (the original length between the two points) from the collected data for the participant lumbar flexibility value was performed. The validity of the modified Schober test against radiographs was found to be strong (r = 0.97) in a previous study[22].

Statistical analysis

All statistical analyses were performed using the SPSS (version 26.0, SPSS IMB), and the Shapiro-Wilk test was used to verify the normal distribution of data of all variables. Subsequently, if the data were normally distributed, a paired sample t-test was used to detect pre- and post-intervention differences. Wilcoxon sign-rank test was used to detect pre- and post-intervention differences for non-normally distributed data. An alpha level was set at 0.05 to define for the statistical significance of all the tests.

Results

Fascia thickness

Paired samples t-test showed a 9% reduction (p-value < 0.05) in the TLF maximum thickness value after the intervention (Table 1). This indicated that after 15 minutes of percussive massage treatment, the participants' TLF maximum thickness was significantly reduced. For the remaining indicators, we did not find significant changes between before and after the intervention.

Table 1 Comparison of the thoracolumbar lumber fascia measure between baseline and post-test

Fascia Thickness	Baseline (Mean ± SD)	Post-intervention (Mean ± SD)	Differences (Post-Baseline)	95% CI	p-value
Max (mm)	3.85 ± 0.68	3.52 ± 0.47	-0.34 ± 0.53	0.13 – 0.67	0.049*
Mean (mm)	3.07 ± 0.52	3.06 ± 0.47	-0.02 ± 0.42	-0.28 – 0.26	0.901
Lt. Max (mm)	3.91 ± 0.74	3.65 ± 0.53	-0.26 ± 0.47	-0.03 – 0.56	0.077
Lt. Average (mm)	3.23 ± 0.74	3.08 ± 0.54	-0.14 ± 0.39	-0.1 – 0.39	0.226
Rt. Max (mm)	3.79 ± 0.96	3.39 ± 0.48	-0.41 ± 0.87	-0.14 – 0.96	0.125
Rt. Average (mm)	3.06 ± 0.59	2.89 ± 0.35	-0.17 ± 0.63	-5.7 – 0.22	0.352

Note: Values showed means ± standard deviation, (*) is statistically significant, was p-value < 0.05 (2-tailed). Max, maximum value; Mean, mean of the value; Lt., left side of the body; Rt., right side of the body; 95% CI, 95% confidence interval.

Skin temperature

The skin temperature increased by a large magnitude following the percussive massage treatment by 2.34 ± 0.81°C (+8.8%, p-value < 0.01). The results, as shown in table 2, indicated that after 15 minutes of percussive massage treatment, the back skin temperature could be increased.

Pain intensity

For the PPT, the value between pre- and post-intervention was significantly different. (*p*-value < 0.01). The VAS decreased by 48% after percussive massage (*p*-value < 0.01). The results of pain intensity indicated that after 15 minutes of percussive massage treatment, the pain intensity of the participants was significantly relieved.

Tissue hardness

The tissue hardness of the measuring point was a significant difference after intervention (*p*-value < 0.05) (Table 2). Moreover, the tissue hardness change (decrease) between pre- and post-intervention was 1.96 ± 2.88 % (*p*-value < 0.05).

Lumbar flexibility

After the intervention, Schober test value increased by 10% (Table 2), but within-group pre- and post-comparisons did not reveal a statistical significance (*p*-value > 0.05).

Perceived stiffness

For the stiffness scale, statistical analysis showed that participants perceived stiffness decreased by 2 ± 1.46 cm (*p*-value < 0.01). This result indicated that the participants perceived the stiffness improved after the intervention.

Table 2 Comparison of the clinical outcomes measures between baseline and post-test

Parameters	Baseline (Mean ± SD)	Post-intervention (Mean ± SD)	Differences (Post-baseline)	95%CI	*p*-value
ST (ºC)	33.3 ± 1.02	35.6 ± 1.09	2.34 ± 0.81	-2.86 – -1.83	0.001*
PPT (kg/cm^2)	3.65 ± 0.93	4.58 ± 1.11	0.93 ± 0.95	-1.54 – -0.33	0.006*
TH (%)	61.5 ± 4.80	59.8 ± 5.79	-1.96 ± 2.88	-0.95 – 4.52	0.038*
LF (cm)	7.97 ± 3.45	8.59 ± 3.25	0.63 ± 1.59	-1.64 – 0.38	0.199
VAS (cm)	1.77 ± 1.10	0.93 ± 0.64	-0.85 ± 0.77	0.36 – 1.33	0.003*
PS (cm)	3.54 ± 2.08	1.53 ± 1.99	-2.00 ± 1.46	1.08 – 2.93	0.003*

Note: Values showed means ± standard deviation, (*) is statistically significant, was *p*-value < 0.05 (2-tailed). ST, skin temperature; PPT, pressure pain threshold; TH, tissue hardness; LF, lumbar flexibility; VAS, visual analog scale; PS, perceived stiffness; 95% CI, 95% confidence interval.

Discussion

This study aimed to preliminarily examine the immediate effects of percussive massage treatment on TLF thickness, and mechanical properties of the lower back, as well as the lumbar flexibility in healthy adults. We found that after the percussive massage treatment the participants showed a decrease in the maximum thickness value of TLF (*p*-value < 0.05), an increase in skin temperature, and significant improvements in pain intensity, muscle stiffness, and perceived stiffness, but no changes were found in the mean thickness of fascia or lumbar mobility (*p*-value > 0.05).

Abnormalities in the body's movement patterns can lead to local imbalances in muscle tone, with the fascia adapting to the increased regional tension in a denser and more parallel fiber arrangement. Prolonged abnormalities in the fascia can induce inflammation, fibrosis, and densification[4]. In this condition, the concentration of hyaluronic acid in the loose connective tissue becomes viscous, increasing the distance between the fascial layers (fascial thickening)[23], resulting in adhesions in the tissue structure, further affecting the stiffness and mobility of the tissue[5]. In the current study, the maximum, and mean TLF thicknesses observed at baseline values were 3.85 ± 0.68 mm, and 3.06 ± 0.47 mm respectively. These

results were similar to the TLF thickness values of 3.7 ± 0.04 mm for males and 4.1 ± 0.03 mm for females reported by Langevin et al[6].

The single most striking observation to emerge from the data comparison was the maximum value of TLF thickness was decreased immediately after the percussive massage treatment. However, these data must be interpreted with caution, as we did not find changes in the remaining fascial indicators. Firstly, one possible reason for the decrease in maximum fascial thickness could be that percussive massage therapy stretches muscle fibers and fascia by generating continuous muscle waves through constant tapping[24]. Xiong et al[25] found stretching could decrease skin thickness and increase subcutaneous tissue motility in mice (n = 48), possibly due to reduced expression of CCL2 and ADAM8 in the skin. Secondly, stretching also affected connective tissue of inflammation regression. Thirdly, the densification of loose connective tissue was a factor in the thickening of TLF[3,4]. Previous research has established that the structural properties of the loose connective tissue change with increasing temperature, and the three-dimensional superstructure of the HA chains stabilized gradually decomposes when the temperature rises to about 40 ºC[26]. This was consistent with the results we found that we observed a significant increase (p-value < 0.01) (Table 2) skin temperature after a percussive massage treatment, which could support this hypothesis. Statistical analysis showed a significant decrease in tissue stiffness (p-value < 0.05) and perceived stiffness (p-value < 0.01) (Table 2), probably because percussive massage therapy reduced the viscosity of the sparse connective tissue within the fascia. It might increase sliding between the collagen fibrous layers of the deep fascia, and reduce the surrounding tissue stiffness[4].

This study did not find a significant difference in lumbar flexibility after a percussive massage treatment. This outcome was contrary to that of Konrad et al[13] who found the range of motion (ROM) of plantar flexor muscles increased significantly by 5.4% after 5 minutes of percussive massage treatment (54HZ) on the calf muscles. On the one hand, this discrepancy could be attributed to the frequency used in our study was 35HZ, which was much lower than that in the study of Andreas et al[27]. On the other hand, due to the present study ultrasound image acquisition was centered 2 cm from the middle of the L2-L3 interspinous ligament (located on the erector spinae muscle), for better observing the immediate effects of percussive massage treatment, we intervened only on the erector spinae muscles of the lower back. In fact, the TLF blends aponeurotic and fascial planes, which cover the multifidus, lumbar square, and erector spinae muscles, and also connects the abdominal muscles to the gluteal muscles. Thus, the absence of differences in the flexibility before and after the intervention may be due to the incomplete area of the TLF intervention.

Concerning the pain intensity, the percussive massage treatment shown in results had positive changes in pressure pain threshold and VAS (p-value < 0.01) (Table 2). This finding was in consistent with Romero-Moraleda[28] who observed after 5 minutes of vibratory foam rolling on the thigh muscles of a healthy female, and found a significant decrease in both PPT and VAS. This result may be explained by the Gate control theory[29]. The vibratory stimuli pass through large size afferent fibers to establish inhibitory control of pain pathways[30], and the increase in blood circulation and temperature[31] after a percussive massage could accelerate the turnover of pain mediators, such as substance P[30]. The present study also had some limitations, since this was our initial pilot study and did not have a randomized control group. We did not know whether any changes in each of the parameters were due to the intervention or resting posture. However, we may use the results to calculate the sample size and conduct a randomized controlled trial in the future. The participants in this study were healthy adult. Although thickening of connective tissue also occurs in healthy individuals[8], it was still only a small amount compared to patients with chronic lower back pain[5]. It would be interesting to further study the effects of this intervention

in lower back patients. Moreover, TLF thickening is due to fibrosis of the connective tissue, which is a long-term process[27]. Further, longer-term intervention studies are needed to determine long-term effects. Lastly, concerning the frequency of vibration, we only used the frequency of 35HZ for 15 minutes of intervention on the erector spinae of the lower back and found some beneficial effects. It would be interesting to investigate whether different frequency of intervention would produce different results or not.

Conclusion

This novel evidence suggests that 15 minutes of percussion massage therapy could improve maximum TLF thickness values, tissue stiffness, perceived stiffness, and pain intensity in healthy adults but could not reveal changes in mean TLF thickness or lumbar spine flexibility. Although this study has provided some new knowledge of percussive massage therapy on healthy participants, its effects on patients are not well known. Further study with randomized controlled trial in low back pain patients are recommended to explore the effects of vibration therapy.

Take home messages

The present study provides preliminary evidence that a 15-minute back percussion massage therapy reduced the maximum thickness values of the thoracolumbar fascia in healthy adults whereas there were no changes in the mean TLF thickness values. Also, a significant increase in skin temperature was found, as well as improvements in tissue stiffness, perceived stiffness, and pain intensity. Further studies in low back pain patients are warranted.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

We would like to thank the Faculty of Graduate School and Faculty of Associated Medical Sciences, Research Center in Back Neck and Other Joint Pain Research Group, and Khon Kaen University for financial supporting this project. Also thanks to Prof. Dr. Wichai Eungpinichpong for the design of this study, and physiotherapist Wiraphong Sucharit for his help in collecting the ultrasound data, and Assoc. Prof. Dr. XingYu Huang for the analysis of the data in this article.

References

1. Deyo RA, Weinstein JN. Low back pain. N Engl J Med 2001; 344(5): 363-70.
2. Allegri M, Montella S, Salici F, Valente A, Marchesini M, Compagnone C, et al. Mechanisms of low back pain : a guide for diagnosis and therapy. F1000Res 2016; 5: 1-11.
3. Wilke J, Schleip R, Klingler W, Stecco C. The lumbodorsal fascia as a potential source of low back pain: A Narrative Review. Biomed Res Int 2017; 2017: 1-6.
4. Pavan PG, Stecco A, Stern R, Stecco C. Painful connections: Densification versus fibrosis of fascia. Curr Pain Headache Rep 2014; 18(8): 1-8.
5. Langevin HM, Stevens-Tuttle D, Fox JR, Badger GJ, Bouffard NA, Krag MH, et al. Ultrasound evidence of altered lumbar connective tissue structure in human subjects with chronic low back pain. BMC Musculoskelet Disord 2009; 10(1): 1-9.
6. Langevin HM, Fox JR, Koptiuch C, Badger GJ, Greenan- Naumann AC, Bouffard NA, et al. Reduced thoracolumbar fascia shear strain in human chronic low back pain. BMC Musculoskelet Disord 2011; 12(203): 1-11.
7. Almaz J, Daniel L, Romero-morales C, Bravo-aguilar M, Calvo-lobo C. Quantitative ultrasound imaging differences in multifidus and thoracolumbar fasciae between athletes with and without chronic lumbopelvic pain : a case-control study. J Clin Med 2020; 9(8): 1-20.

8. Di Matteo A, Filippucci E, Cipolletta E, Martire V, Jesus D, Musca A, et al. How normal is the enthesis by ultrasound in healthy subjects? Clin Exp Rheumatol 2020; 38(3): 472-8.
9. Wilke J, Macchi V, De Caro R, Stecco C. Fascia thickness, aging and flexibility: is there an association? J Anat 2019; 234(1): 43-9.
10. Stecco A, Meneghini A, Stern R, Stecco C, Imamura M. Ultrasonography in myofascial neck pain: Randomized clinical trial for diagnosis and follow-up. Surg Radiol Anat 2014; 36(3): 243-53.
11. Moyer CA, Rounds J, Hannum JW. A meta-analysis of massage therapy research. Psychol Bull 2004; 130(1): 3-18.
12. García-Sillero M, Benítez-Porres J, García-Romero J, Bonilla DA, Petro JL, Vargas-Molina S. Comparison of interventional strategies to improve recovery after eccentric exercise-induced muscle fatigue. Int J Environ Res Public Health 2021; 18(2): 1-11.
13. Konrad A, Glashüttner C, Reiner MM, Bernsteiner D, Tilp M. The acute effects of a percussive massage treatment with a hypervolt device on plantar flexor muscles' range of motion and performance. J Sport Sci Med 2020; 19(4): 690-4.
14. Guang H, Ji L, Shi Y. Focal vibration stretches muscle fibers by producing muscle waves. IEEE Trans Neural Syst Rehabil Eng 2018; 26(4): 839-46.
15. Comeaux Z. Dynamic fascial release and the role of mechanical/vibrational assist devices in manual therapies. J Bodyw Mov Ther 2011; 15(1): 35-41.
16. Chatchawan U, Jarasrungsichol K, Yamauchi J. Immediate effects of self-thai foot massage on skin blood flow, skin temperature, and range of motion of the foot and ankle in type 2 diabetic patients. J Altern Complement Med 2020; 26(6): 491-500.
17. Zheng Z, Wang J, Gao Q, Hou J, Ma L, Jiang C, et al. Therapeutic evaluation of lumbar tender point deep massage for chronic non-specific low back pain. J Tradit Chinese Med 2012; 32(4): 534-7.
18. Downie WW, Leatham PA, Rhind VM, Wright V, Branco JA, Anderson JA. Studies with pain rating scales. Ann Rheum Dis 1978; 37(4): 378-81.
19. De Coninck K, Hambly K, Dickinson JW, Passfield L. Measuring the morphological characteristics of thoracolumbar fascia in ultrasound images: An inter-rater reliability study. BMC Musculoskelet Disord 2018; 19(1): 1-6.
20. Siegmund LA, Barkley JE, Knapp D, Peer KS. Acute effects of local vibration with biomechanical muscle stimulation on low-back flexibility and perceived stiffness. Athl Train Sport Heal Care 2014; 6(1): 1-9.
21. Wright V. The measurement of back movement. Rheumatology 1983; 22(4): 193-6.
22. Rezvani A, Ergin O, Karacan I, Oncu M. Validity and reliability of the metric measurements in the assessment of lumbar spine motion in patients with ankylosing spondylitis. Spine (Phila Pa 1976) 2012; 37(19): 1189-96.
23. Tadmor R, Chen N, Israelachvili JN. Thin film rheology and lubricity of hyaluronic acid solutions at a normal physiological concentration. J Biomed Mater Res 2002; 61(4): 514-23.
24. Caliandro P, Celletti C, Padua L, Minciotti I, Russo G, Granata G, et al. Focal muscle vibration in the treatment of upper limb spasticity: A pilot randomized controlled trial in patients with chronic stroke. Arch Phys Med Rehabil 2012; 93(9): 1656-61.
25. Xiong Y, Berrueta L, Urso K, Olenich S, Muskaj I, Badger GJ, et al. Stretching reduces skin thickness and improves subcutaneous tissue mobility in a murine model of systemic sclerosis. Front Immunol 2017; 8(124): 1-11.
26. Matteini P, Dei L, Carretti E, Volpi N, Goti A, Pini R. Structural behavior of highly concentrated hyaluronan. Biomacromolecules 2009; 10(6): 1516-22.
27. Schilder A, Magerl W, Hoheisel U, Klein T, Treede RD. Electrical high-frequency stimulation of the human thoracolumbar fascia evokes long-term potentiation-like pain amplification. Pain 2016; 157(10): 2309-17.

28. Romero-Moraleda B, González-García J, Cuéllar-Rayo Á, Balsalobre-Fernández C, Muñoz-García D, Morencos E. Effects of vibration and non-vibration foam rolling on recovery after exercise with induced muscle damage. J Sport Sci Med 2019; 18(1): 172-80.
29. Melzack R, Wall PD. Gate Control-Pain mechanisms - a new theory. Science 1965; 150: 971-9.
30. Guieu R, Tardy-Gervet MF, Giraud P. Substance P-like Immunoreactivity and Analgesic Effects of Vibratory Stimulation on Patients Suffering From Chronic Pain. Can J Neurol Sci 1993; 20(2): 138-41.
31. Lu X, Wang Y, Lu J, You Y, Zhang L, Zhu D, et al. Does vibration benefit delayed-onset muscle soreness?: a meta-analysis and systematic review. J Int Med Res 2019; 47(1): 3-18.

ORIGINAL ARTICLE

Arch AHS

Archives of Allied Health Sciences 2022; 34(3): 33-43.

Pain-related self-efficacy, pain catastrophizing, and function in individuals with chronic low back pain: further evaluation of the validity of the T-UW-PRESE6 and T-UW-CAP6

Nipaporn Akkarakittichoke[1], Kornkanok Khutok[2], Rotsalai Kanlayanaphotporn[3], Mark P. Jensen[4], Prawit Janwantanakul[3*]

[1] Inter-Department Program of Biomedical Sciences, Faculty of Graduate school, Chulalongkorn University, Bangkok, Thailand.
[2] School of Allied Health Sciences, Walailak University, Nakhonsithammarat, Thailand.
[3] Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand.
[4] Department of Rehabilitation Medicine, University of Washington, USA.

KEYWORDS
Pain catastrophizing; Self-efficacy; Quality of life; Musculoskeletal disorder.

ABSTRACT

Evidence shows the important role of pain-related cognitions, such as catastrophizing and self-efficacy beliefs, on quality of life in patient with chronic low back pain. Thai versions of two new measures of psychological factors: the Thai 6-item short form of the University of Washington Pain Related Self-Efficacy scale (T- UW-PRSE6) and the Thai 6-item short form of the University of Washington Concerns About Pain scale (T-UW-CAP6) have been developed. Reliable and valid measures of such measurements are important to evaluate the catastrophizing and self-efficacy on this domain as well as to understand its role in quality-of-life domain of individuals with chronic pain. The aim of this study was to evaluate the reliability and validity of T-UW-PRSE6 and T-UW-CAP6. A total of 424 individuals with chronic low back pain completed three questionnaires assessing (1) pain self-efficacy (T-UW-PRSE6), (2) catastrophizing (T-UW-CAP6), and (3) seven quality of life domains (Thai version of Patient-Reported Outcomes Measurement Information System-29 scale; T-PROMIS-29). Cronbach's alphas were calculated to estimate internal consistency of the T-UW-PRSE6 and T-UW-CAP6, and multiple linear regressions were used to estimate the contributions of each measure to the association of pain intensity and the seven quality of life domains. The Cronbach's alphas of the T-UW-PRSE6 and T-UW-CAP6 were 0.84 and 0.89, respectively. T-UW-PRSE6 and T-UW-CAP6 each made significant and independent contributions to the association of each quality-of-life domain assessed by the T-PROMIS-29 (p's < 0.01). The findings support the reliability and validity of the T-UWPRSE6 and T-UW-CAP6 as measures of pain-related self-efficacy and catastrophizing, respectively. These brief measures appear to provide viable alternatives to the legacy measures of these important constructs.

***Corresponding author:** Prawit Janwantanakul, PT, PhD. Department of Physical Therapy, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, 10330, Thailand. Email: prawit.j@chula.ac.th
Received: 25 May 2022/ Revised: 20 July 2022/ Accepted: 31 July 2022

Introduction

Chronic low back pain is the most common chronic pain problem, with an annual prevalence in the working population from 24% to 61%[1]. In Thailand, chronic low back pain affects between 27% and 30% of the adult population annually[2] and the number of people with chronic low back pain conditions is expected to increase substantially over the next decades[3]. Low back pain leads to a great socioeconomic burden on both individual and society.

Chronic low back pain is a multidimensional syndrome affecting many qualities of life domains, including physical activity, physical function, and psychological function. Theory (i.e., biopsychosocial models) supports the conclusion that psychological factors play an important role in the adjustment to chronic low back pain severity and pain-related disability[4]. Two psychological factors that have been consistently shown to play an important role in function in individuals with chronic pain are pain self-efficacy (i.e., a belief that one is able to manage pain and its effects on function) and pain catastrophizing (i.e., a pattern of negative cognitive-emotional responses to pain that includes rumination, magnification, and helplessness)[5-8].

Research has shown that individuals with chronic musculoskeletal pain who endorse higher levels of pain-related self-efficacy possess higher levels of physical function, self-perceived health status, and employment status, and lower levels of pain intensity, disability, depressive symptoms, and fatigue, than individuals who endorse lower levels of pain-related self-efficacy[8]. Moreover, pain self-efficacy has also been shown to mediate the effects of interdisciplinary pain treatment, supporting this construct as key mechanism variable in effective pain treatment[9]. Pain self-efficacy has also be shown to mediate the association between psychological function (e.g., fear and depression) and disability, again supporting the role of self-efficacy as a central mechanism variable that explains the differences in function observed in individuals reporting similar levels of pain intensity[10].

Support for the important role that pain catastrophizing plays in function in individuals with chronic pain comes from research showing that measures of catastrophizing have been shown to be positively associated with pain severity, disability, poor treatment outcomes for patients with chronic low back pain[11]. Moreover, catastrophizing has been shown to predict both (1) the development of chronic pain in previously pain-free individuals, and (2) those with acute back pain[11], and, like pain self-efficacy has been shown to mediate the beneficial effects of interdisciplinary pain treatment[10].

Research to evaluate the effects of self-efficacy and catastrophizing on pain requires the availability of reliable and valid measures of these constructs. Previous studies have used a variety of such measures, including the Pain Self-Efficacy Questionnaire (PSEQ)[12] to assess pain self-efficacy, and the Pain Catastrophizing Scale (PCS)[13] to assess catastrophizing. However, each of these legacy measures was developed using classic measure development theory, which is associated with a number of weaknesses. These include the requirement that all of the items be administered, which can be challenging in situations where assessment burden is an issue. In addition, measures developed using classic measure development theory are not usually scored into a common metric (e.g., a T-score, with a mean of 50 and SD of 10 in the development sample), which limits the ability to easily interpret scale scores and compare them between different samples[14]. Item response theory (IRT), a statistical analysis technique used to develop and evaluate questionnaire-based measurement tools, addresses these limitations. With IRT, banks of items can be created, any combination of which can be used to assess the domain of interest and create a standardized score that can be directly compared to scores obtained using any other combination of items from that same item bank. In addition, the items from the item banks can be used to either create static scales of varying number of items, or can be administered using computer assisted testing (CAT), with each subsequent item selected based on an individual's responses to previous items.

Recently, item banks to assess pain-related self-efficacy and catastrophizing were created using IRT: the University of Washington Pain Related Self-Efficacy Scale (UW-PRSE) and the University of Washington Concerns About Pain Scale *(*UW-CAP*)*[15]. Static 6-item versions of these measures have been translated into Thai (T-UW-PRSE6 and T-UW-CAP6)[16,17], and preliminary evidence supports the psychometric strengths of these static measures, including internal consistency, test–retest reliability, and ability to detect changes over time in individuals with chronic low back pain[16-18]. Khampanthip et al[16] showed that the T-UW-PRSE6, a measure of pain-related self-efficacy, was negatively correlated with fear avoidance and positively associated with a number of key quality of life domains (i.e., general health, physical functioning, role limitation related to physical and emotional problems, social functioning, bodily pain, vitality, and mental health) in individuals with chronic low back pain. In the same study sample, Youprasart et al[17] found that the T-UW-CAP6, a measure of pain-related catastrophizing, was positively correlated with fear avoidance and negatively associated with social functioning, vitality, and mental health. As a group, these studies provide preliminary support for the validity of the T-UW-PRESE6 and T-UW-CAP6. However, drawing conclusions regarding the psychometric properties of new measures requires multiple studies, especially when those measures are being considered in light of the existence of legacy measures. Thus, further evaluation of the psychometric properties of the T-UW-PRSE6 and T-UW-CAP6 is needed before they can be recommended for use over the legacy measures of these constructs. In particular, to date, no study has investigated the associations between T-UW-PRSE6/T-UW-CAP6 and a variety of additional quality-of-life domains; namely pain intensity, pain interference, fatigue, depressive symptom severity, anxiety, and sleep disturbance.

The aim of this study was to provide additional evaluations of the reliability and validity of the UW-PRSE and UW-CAP; in this case, the static 6-item Thai versions of these measures: the T-UW-PRSE6 and T-UW-CAP6, in individuals with chronic low back pain. We hypothesized that if the measures were reliable, their internal consistency coefficients (Cronbach's alphas) would be ≥ 0.70 in both samples. We also hypothesized that if valid, the T-UW-PRSE6 and T-UW-CAP6 would make independent contributions to each of six domains of quality of life (i.e., measures of pain intensity, pain interference, fatigue, depressive symptoms severity, anxiety, and sleep disturbance). Finally, we hypothesized the opposite pattern of associations of the two measurement scales with two-domain of quality-of-life measures, including physical function and perceived ability to participate in social roles and activities.

Materials and methods

Subjects and study design

This study used a cross-sectional design. Data for the current analyses came from two studies of individuals with chronic low back pain[16,19]. One sample was recruited from August 2018 through February 2019 (n = 241)[16]. The other was recruited from November 2018 through October 2019 (n = 183)[19]. Both samples were recruited via referrals from physical therapy clinicians working in the outpatient physical therapy departments of seven large public hospitals and one physical therapy clinic in the Bangkok metropolitan area. Of the 424 participants, 267 participants received one or more of a variety of standard physical therapy treatments for low back pain (e.g., physical therapy, self-exercise, or massage), tailored to their specific needs, and which therefore varied from patient to patient. The remaining 157 participants did not receive any treatment for low back pain.

Study inclusion criteria included being a native Thai speaker who could read, write, and speak in the Thai language, being aged 18 years or older, and having chronic low back pain, as defined by the NIH Task Force on Research Standards for chronic Low Back Pain as "a back-pain problem that has persisted at least 3 months and has resulted in pain on at least half the days in the past 6 months"[20]. Exclusion criteria included

having a serious medical condition or complication in addition to low back pain that might affect the ability to participate in the study procedures.

Measures

Thai version of the University of Washington Pain Related Self-Efficacy scale

As noted previously, the UW-PRSE item banks contains 29 items[15]. A static 6-item short form has been developed, and translated into Thai (T-UW-PRSE6)[16]. The T-UW-PRSE6 items assess the respondent's perceived ability to: (1) perform daily activities despite pain, (2) manage pain, (3) engage in valued activities despite pain, (4) keep pain from interfering with their social life, (5) stay in a good mood despite pain, and (6) get a good night's sleep, despite pain. Respondents indicate their agreement with each self-efficacy item on a 5-point Likert scale with 1 = "Not at all," 2 = "A little bit," 3 = "Somewhat," 4 = "Quite a bit," and 5 = "Very much." The total raw score when all six items are administered can range from 6 to 30. Higher scores indicate higher levels of pain-related self-efficacy. The raw scores were transformed to a T-score, with a mean of 50 and SD of 10 in the normative sample (in this case, consisting of individuals with a variety of chronic pain conditions). The T-UW-PRSE6 had shown good internal consistency (i.e., Cronbach's alpha = 0.85) and adequate test–retest stability ($ICC_{(2,1)}$ = 0.72)[16].

Thai version of the University of Washington Concerns About Pain scale

The University of Washington Concerns About Pain Scale *(*UW-CAP*)* is an item bank consisting of 24 items[15]. A static 6-item short form has been developed, translated into Thai (T-UW-CAP6)[17]. The T-UW-CAP6 asks respondents to rate the frequency with which they have the catastrophizing response represented by each item in the past 7 days using a 5-point Likert scale, ranging from 1 ("Never") to 5 ("Always"). Sample items include "My life will only get worse because of my pain" and "My pain is more than I can manage." The total raw score for the T-UW-CAP6 potentially range from 6 to 30. Higher scores indicate more catastrophizing. The raw scores were transformed to a T-score metric, with a mean of 50 and SD of 10 in the original normative sample. The T-UW-CAP6 has evidenced good internal consistency (i.e., the Cronbach's alpha = 0.89) and adequate test-retest stability (i.e., $ICC_{(2,1)}$ = 0.72)[17].

Study criterion variables

Pain intensity, pain interference, fatigue, depressive symptom severity, anxiety, sleep disturbance, physical function, and perceived ability to participate in social roles and activities were assessed using the Thai version of the 29-item Patient-Reported Outcomes Measurement Information System-29 (PROMIS-29)[21]. Twenty-eight of the measure's items (excluding the Pain Intensity item) ask respondents to rate the symptom or item using 1 to 5 Likert scales; the single item assessing pain intensity is measured using a 0 to 10 numerical rating scale with 0 = "No pain" and 10 = "Worst pain imaginable." The T-PROMIS-29 scale scores were transformed into T-scores (means 50 and SD 10) according to the PROMIS adult profile instrument guideline (http://www.healthmeasures.net). A translated and cross-cultural adapted Thai version of the PROMIS-29 that has demonstrated good to excellent reliability as measured by the Cronbach's alphas (range, 0.84 to 0.94) and adequate stability as measured by the $ICC_{(2,1)}$ (range, 0.57 to 0.74)[21].

Procedures

After signing the informed-consent form, participants were asked to provide demographic information (i.e., age, sex, height, weight, pain location, duration of pain, diagnoses, and employment status) and were asked to complete paper-and-pencil version of the study measurement (i.e., the T-UW-PRSE6, T-UW-CAP6, and T-PROMIS-29 items). They returned completed measures to the researchers at the hospital/clinic or by mail, if they elected to complete them at home. All measurements were collected only once and were used to assess internal consistency as well as construct validity. The study participants were at various stages of treatment when they completed the study questionnaires. Ethical approval was obtained from the Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group,

Chulalongkorn University (COA No. 156/2018) and Lerdsin Hospital Human Research Ethics Committee, Lerdsin Hospital (No. 112/2019).

Statistical analysis

Descriptive statistics for the demographic and pain history variables were reported as means and standard deviations (SDs; continuous variables) or as number and percentages (categorical variables). In order to determine if the two samples could be combined into a single sample for purposes of analyses here, the two samples were compared with respect to all demographic variables and study measures using a series of chi-square (categorical variables) and t-test (continuous variables) analyses. In the event that the two samples differed to a great extent, we planned to test the study hypotheses in the two samples separately. With nonsignificant difference, the two samples would be combined into a single sample.

In order to test the study hypothesis regarding the reliabilities of the two scales, we computed the Cronbach's alpha for both. Next, to test the study hypothesis regarding the validity of two scales, we conducted a series of eight multiple linear regression analyses. In these analyses, the eight variables assessed by the T-PROMIS-29 were the criterion variable (i.e., pain intensity, pain interference, fatigue, depressive symptom, anxiety, sleep disturbance, physical function, and perceived ability to participate in social roles and activities). In order to evaluate the extent to which each of the scales made independent contributions (i.e., when controlling for the other) to the association of the criterion variables, we entered the two variables a block in the regression analyses. All analyses were conducted using SPSS statistical software, version 22.0 (SPSS Inc, Chicago, IL, USA). Statistical significance was set at the 5% level.

Results

Baseline comparisons between participants from the two samples indicated no significant differences in any demographic variable, except for low back pain treatment received status. The participants from the study that was conducted earlier reported higher percent of treatment received (n (%) = 175 (73%) versus 92 (50%), *p*-value < 0.001). The two samples had similar scores on the T-UW-CAP6, T-UW-PRSE6, and most of the T-PROMIS-29 score. Exceptions were pain intensity and sleep disturbance domains. The participants from the study that was conducted earlier reported less pain intensity (mean (SD) = 4.7 (2.0) versus 5.2 (1.8), *p*-value = 0.009) and reported having a lower levels of sleep disturbance (mean (SD) = 48.8 (7.7) versus 50.2 (7.2), *p*-value = 0.014) compared to participants from the study that was conducted later. Nevertheless, these statistically significant differences were trivial as the values were less than the minimal clinically important differences of the T-PROMIS-29 which were 1.03 points for pain intensity and 5.0 points for sleep disturbance[19]. Given the similarity of the two samples (i.e., similar on 6 (75%) out of 8 measures), the two samples were combined into a single sample to test the study hypotheses. The 424 participants had a mean age of 46.9 (SD = 17.2) years (Table 1). The majority of the sample were women (69%). Their average BMI 24 (4.4) kg/m^2 was at the upper limit of normal ranges for Asians[22]. Their average low back pain duration was 50 months.

Table 1 Characteristics of study population (n = 424)

Demographic Characteristics	Total sample n = 424	Sample 1 n = 241	Sample 2 n = 183	*p*-value
	n (%) or mean ± SD			
Gender				0.297
Male	130 (31)	69 (29)	61 (33)	
Female	294 (69)	172 (71)	122 (67)	
Age (years; mean ± SD)	46.9 ± 17.2	46.2 ± 16.9	47.7 ± 17.5	0.375
BMI (kg/m^2; mean ± SD)	24.2 ± 4.4	23.9 ± 4.4	24.6 ± 4.2	0.103
Employment status				0.058
Working full- or part-time	332 (78)	194 (80)	138 (75)	
Unemployed	92 (22)	47 (20)	45 (25)	
Duration of chronic low back pain (months)	49.7 ± 70.2	52.3 ± 76.4	46.2 ± 61.1	0.377
Being treated for chronic low back pain?				< 0.001
Yes	267 (63)	175 (73)	92 (50)	
No	157 (37)	66 (27)	91 (50)	
T-UW-PRSE6 (T-score)	52.9 ± 7.5	53.3 ± 7.6	52.4 ± 7.4	0.489
T-UW-CAP6 (T-score)	53.7 ± 8.4	53.5 ± 8.4	54.1 ± 8.3	0.225
T-PROMIS-29 (all scores on a T-score metric, except pain intensity, which can have a range of 0 to 10)				
Pain intensity (0-10)	4.9± 1.9	4.7 ± 2.0	5.2 ± 1.8	0.009
Physical function	43.3 ± 7.3	43.6 ± 7.2	42.9 ±7.4	0.338
Anxiety	57.3 ± 9.2	57.0 ± 9.3	57.6 ± 9.1	0.510
Depression	49.3 ± 9.4	48.9 ± 8.7	50.7 ± 9.1	0.292
Fatigue	51.4 ± 3.46	51.2 ± 7.8	51.6 ± 8.9	0.678
Sleep disturbance	49.2 ± 7.6	48.8 ± 7.7	50.2 ± 7.2	0.014
Ability to participate in social roles and activities	51.3 ± 7.9	51.9 ± 7.7	50.5 ± 8.1	0.073
Pain interference	57.6 ± 6.2	57.3 ± 6.2	58.0 ± 6.2	0.226

Internal consistency

The Cronbach's alphas of the T-UW-PRSE6 and T-UW-CAP6 were 0.84 and 0.89, respectively.

Construct validity

Multiple linear regression analyses showed that the T-UW-PRSE6 and T-UW-CAP6 scales made independent and statistically significant contributions to the association of each one of the eight criterion variables (Table 2). T-UW-PRSE6 was associated negatively with pain intensity, anxiety, depression, fatigue, pain interference, and sleep disturbance (R^2's range, 0.19 to 0.40, ß's range, -0.14 to -0.33, all *p*'s < 0.004), and associated positively with physical function and ability to participate in social roles and activities (R^2's range, 0.31 to 0.33, ß's range, 0.23 to 0.31, all *p*'s < 0.001). The T-UW-CAP6 was associated negatively with physical function and perceived ability to participate in social roles and activities (R^2's range, 0.19 to 0.40, ß's range, -0.35 to -0.39, all *p*'s < 0.001), and positively with pain intensity, anxiety, depression, fatigue, pain interference, and sleep disturbance (R^2's range, 0.31-0.33, ß's range, 0.17 to 0.49, all *p*'s < 0.001).

Table 2 Results of multiple linear regression analyses for prediction of patient function in a sample of individuals with chronic low back pain (n = 424)

	Pain self-efficacy (T-UW-PRSE6)						Pain catastrophizing (T-UW-CAP6)					
	R^2	*β*	95% CI	*SE*	*t*	*p*-value	R^2	*β*	95% CI	*SE*	*t*	*p*-value
Pain intensity	0.26	-0.14	-0.06 to -0.01	0.01	-2.89	0.004	0.26	0.42	0.08 to 0.12	0.01	8.70	< 0.001
Physical function	0.31	0.23	0.07 to 0.15	0.02	5.07	< 0.001	0.31	-0.39	-2.0 to -0.12	0.02	-8.34	< 0.001
Anxiety	0.40	-0.22	-15 to -0.07	0.02	-5.12	< 0.001	0.40	0.49	0.17 to 0.25	0.02	4.9	< 0.001
Depression	0.25	-0.29	-0.17 to -0.09	0.02	-5.87	< 0.001	0.25	0.29	-08 to -16	0.02	5.99	< 0.001
Fatigue	0.19	-0.19	0.13 to -0.04	0.02	-3.72	< 0.001	0.19	0.31	0.08 to 0.17	0.02	5.99	< 0.001
Pain interference	0.38	-0.27	-0.17 to -0.09	0.02	-6.16	< 0.001	0.38	0.43	0.14 to 0.22	0.02	9.70	< 0.001
Sleep disturbance	0.19	-0.33	-0.19 to -0.10	0.02	-6.47	< 0.001	0.19	0.17	0.03 to 0.11	0.02	3.97	0.001
Ability to participate in social roles and activities	0.33	0.31	0.10 to 0.19	0.02	6.75	< 0.001	0.33	-0.35	-0.18 to -0.11	0.02	-7.65	< 0.001

Note: T-UW-PRSE6, Thai university of Washington pain-related self-efficacy scale; T-UW-CAP6, Thai university of Washington - concerns about pain scale; β, standardized coefficients; CI, confidence interval, *SE*, standard error.

Discussion

The results support the reliability and validity of both the T-UW-CAP6 and T-UWPRSE6 scales, as evidenced by good internal consistency reliability coefficients and by their ability to make statically significant and independent contributions to the association of a variety of pain-related quality of life domains in individuals with chronic low back pain. Pain self-efficacy appeared to be more strongly associated with sleep disturbance, while pain catastrophizing was more strongly associated with pain intensity, physical function, anxiety, fatigue, and pain interference. Both factors were similarly associated with depression and perceived ability to participate in social roles and activities in the study sample.

Both the T-UW-PRSE6 and T-UW-CAP6 evidenced at least adequate internal consistency (i.e., 0.80 to 0.89). This finding supports the conclusion that the items in each scale assess a single over-arching domain (i.e., self-efficacy and catastrophizing, respectively), and that the items together provide a fairly precise measure of these domains. These internal consistency coefficients are similar to those found by previous researchers assessing the internal consistency of legacy measures (e.g., PSEQ assess self-efficacy, range 0.70 to 0.95[23] and PCS assessing catastrophizing, range 0.53 to 0.92)[24,25]. The internal consistency findings reported here are also consistent with previous studies of the T-UW-PRSE6 and T-UW-CAP6[16,17].

The findings also support the association of the T-UW-PRSE6 and both physical and psychological function in a sample of chronic low back pain individuals. This finding is in line with previous studies which have examined the validity of primary legacy self-efficacy measure (i.e., the PSEQ), with respect to its negative associations with measures of pain intensity, pain interference, fatigue, depression, anxiety, and sleep disturbance[12,26] and positive associations with measures physical function and perceived ability to participate in social roles[8,26,27]. The finding not only provides additional support for the role that self-efficacy plays in patient function but for the ability of the T-UW-PRSE6 to assess pain self-efficacy in a way that demonstrates that role. The current results also suggest that pain self-efficacy as assessed by the T-UW-PRSE6, is more strongly associated with sleep disturbance than is the T-UW-CAP6. This finding is consistent with prior research, showing a negative association between sleep quality and self-efficacy[28] and the mediating role of self-efficacy to the relationship between sleep disturbance and musculoskeletal symptom severity[29]. Because causal conclusions cannot be drawn from cross-sectional data, we are unable to conclude that pain self-efficacy has an influence on sleep quality (or vice versa). An important next step would be to determine if treatments which target this domain specifically might be viable as treatments for sleep disturbance in individuals with chronic pain.

The directions and magnitudes of the associations between the T-UW-CAP6 and patient function are consistent with the findings from previous studies that have examined the associations between the primary legacy measure of catastrophizing (i.e., the PCS) and measures of pain-related quality of life[13,30]. Catastrophizing has been established as a fairly consistent predictor of patient function across many pain populations in many countries[27,31]. The current findings replicated this well-established finding in a new sample of individuals from a country (and culture) that differs from all of the other samples that have examined these associations to date, providing support for their reliability and generalizability. The findings also support the UW-CAP6 items as being valid for evaluating these associations. The current results revealed that pain catastrophizing assessed by T-UW-CAP6 is more strongly associated with a number of pain-related quality of life domains with chronic low back pain individuals, including pain intensity, physical function, anxiety, fatigue, and pain interference, than is pain self-efficacy, as assessed by the T-UW-PRSE6. An important next step would be to evaluate the extent to which catastrophizing as measured by this scale mediates the beneficial effects of pain treatments that target this domain for change, such as cognitive behavior therapy.

To our knowledge, this was the first study to evaluate the association and compare the ability of measures of both catastrophizing and self-efficacy to patient function in the same sample of chronic low back pain individuals, while controlling for the effects of the other. The fact that each made *independent* contributions to the association of each quality-of-life domains provides strong support for the importance of both measures. Within the limitation of the study, the findings are consistent with the possibility that low back pain treatments should not focus only on decreasing catastrophizing cognitions or increasing self-efficacy beliefs, but instead focus on both. These findings also indicate that research to evaluate the causal role of both variables in the same sample, i.e. research that would allow for a direct head-to-head comparison of the relative importance of each, is warranted.

A number of limitations should be considered when interpreting the results. First, as noted several times already, all of the measures were administered at a single time point. Thus, no causal conclusions about the associations among the study variables can be drawn. Second, the study sample included patients who had not received any physical therapy treatment yet (37%) as well as patients who had received a variety of different physical therapy treatments (63%). It is possible that having already received some treatment may have impacted on how the participants responded to the study measures. Different results may therefore have emerged if all of the study participants had either no treatment or some treatment. Third, the study sample was one of convenience (i.e., the sample was limited to individuals with chronic low back pain who were eligible and willing to participate in the original studies). The majority of the sample (67%) were women. The sample was middle-aged people with mean age of 46.9 years old. All of the individuals with the study were residents of Bangkok and nearby provinces. Thus, the sample is not representative of general Thai population, or even the Thai population with chronic pain. Because of this, we are unable to determine the extent to which the findings could be generalized to men with low back pain, to younger or older individuals, and to individuals from Thailand living outside of Bangkok and nearby provinces. That said, the fact that the findings were consistent with those from other studies examining self-efficacy and catastrophizing in other samples around the world suggest that the findings are reliable. Still, further research to evaluate the relative contribution of pain-related self-efficacy and pain catastrophizing to pain intensity, physical function, pain interference, fatigue, depression, anxiety, sleep disturbance, and ability to participate in social roles and activities in other samples of individuals from Thailand with chronic pain conditions is needed to confirm the generalizability of the current findings.

Conclusions

This study showed the two new measures of pain-related self-efficacy and catastrophizing, i.e. the T-UW-CAP6 and T-UW-PRSE6, are reliable and valid. The results support the conclusion that the pain-related cognitions, specifically catastrophizing and pain self-efficacy, are significantly and independently associated with a variety of quality-of-life domains in individuals with chronic low back pain. They replicate findings from other studies in different countries that used legacy measures of these constructions, supporting the generalizability of the importance of both domains in adjustment to chronic pain across countries and cultures. Research to evaluate the relative causal role of both domains in additional samples of individuals with chronic pain is warranted.

Take home messages

The findings provide further support of the reliability and validity of the Thai 6-item short form of the University of Washington Pain Related Self-Efficacy scale and the Thai 6-item short form of the University of Washington Concerns About Pain scale as measures of pain-related self-efficacy and catastrophizing, respectively. These brief measures appear to provide viable alternatives to the legacy measures of these important constructs.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

The authors would like to thank Angkana Khampanthip, Polake Rawang, and Sukhonwaroch Youprasart for their contributions to data collection. This work was funded by the 90th anniversary of Chulalongkorn University (Ratchadaphiseksomphot Endowment Fund, Chulalongkorn University) and the Graduate School, Chulalongkorn University to commemorate the 72th anniversary of his Majesty King Bhumibol Adulyadej.

References

1. Grotle M, Brox JI, Vøllestad NK. Functional status and disability questionnaires: what do they assess? A systematic review of back-specific outcome questionnaires. Spine (Phila Pa 1976) 2005; 30(1): 130-40.
2. Hoy DG, Smith E, Cross M, Sanchez-Rier L, Blyth FM, Buchbinder R, et al. Reflecting on the global burden of musculoskeletal conditions: lessons learnt from the Global Burden of Disease 2010 Study and the next steps forward. Ann Rheum Dis 2015; 74(1): 4-7.
3. Yiengprugsawan V, Hoy D, Buchbinder R, Bain C, Seubsman SA, Sleigh AC. Low back pain and limitations of daily living in Asia: longitudinal findings in the Thai cohort study. BMC Musculoskelet Disord 2017; 18(1): 1-7.
4. Jensen MP, Moore MR, Bockow TB, Ehde dM, Engle JM. Psychosocial factors and adjustment to chronic pain in persons with physical disabilities: a systematic review. Arch Phys Med Rehabil 2011; 92(1): 146-60.
5. Miles CL, Pincus T, Carnes D, Taylor SJC, Underwood M. Measuring pain self-efficacy. Clin J Pain 2011; 27(5): 461-70.
6. Sullivan MJL, Bishop SR, Pivik J. The pain catastrophizing scale: development and validation. Psychol Assess 1995; 7(4): 524-32.
7. Keefe FJ, Rumble ME, Scipio CD, Giordano LA, Perri LM. Psychological aspects of persistent pain: current state of the science. J Pain 2004; 5(4): 195-211.
8. Martinez-Calderon J, Zamora-Campos C, Navarro-Ledesma S, Luque-Suarez A. The role of self-efficacy on the prognosis of chronic musculoskeletal pain: a systematic review. J Pain 2018; 19(1): 10-34.
9. Schumann ME, Coombes BJ, Gascho KE, Geske JR, McDermott MC, Morrison EJ, et al. Pain catastrophizing and pain self-efficacy mediate interdisciplinary pain rehabilitation program outcomes at posttreatment and follow-up. Pain Med 2022; 23(4): 697-706.
10. Varela AJ, van Asselt KW. The relationship between psychosocial factors and reported disability: the role of pain self-efficacy. BMC Musculoskelet Disord 2022; 23(1): 1-14.
11. Yarnitsky D, Crispel Y, Eisenberg E, Granovsky Y, Ben-Nun A,Sprecher E, et al. Prediction of chronic post-operative pain: pre-operative DNIC testing identifies patients at risk. Pain 2008; 138(1): 22-8.
12. Karasawa Y, Yamada K, Iseki M, Yamaguchi M, Murakami Y,Tamagawa T, et al. Association between change in self-efficacy and reduction in disability among patients with chronic pain. PloS One 2019; 14(4): e0215404.
13. Meints SM, Mawla I, Napadow V, Kong J,Gerber J, Chan St, et al. The relationship between catastrophizing and altered pain sensitivity in patients with chronic low-back pain. Pain 2019; 160(4): 833-43.
14. Deyo RA, Katrina R, Buckley DI, Michaels L, Kobus A, Eckstrom E, et al. Performance of a patient reported outcomes measurement information system (PROMIS) short form in older adults with chronic musculoskeletal pain. Pain Med 2016; 17(2): 314-24.
15. Amtmann D, Liljenquist K, Bamer A, Bocell F, Jensen MP, Wilson R, et al. Measuring pain catastrophizing and pain-related self-Efficacy: expert panels, focus groups, and cognitive interviews. Patient. 2018; 11(1): 107-17.

16. Khampanthip A, Kanlayanaphotporn R, Jensen MP, Janwantanakul P. Cross-cultural adaptation, test-retest reliability, and construct validity of the Thai version of the university of Washington pain-related self-efficacy scale. Pain Rep 2019; 4(6): e787.
17. Youprasart KR, Janwantanakul P, Jensen MP. Cross-cultural adaptation, reliability, and construct validity of the Thai version of the university of Washington-concerns about pain scale in individuals with chronic low back pain. J Med Assoc Thai 2020; 103(7): 658-67.
18. Akkarakittichoke N, Janwantanakul P, Kanlayanaphotporn R, Jensen MP. Responsiveness of the UW Concerns-About-Pain and UW Pain-related self-efficacy scale in individuals with chronic low back pain. Clin J Pain 2022; 38(7): 476-83.
19. Khutok K, Janwantanakul P, Jensen MP, Kanlayanaphotporn K. Responsiveness of the PROMIS-29 scales in individuals with chronic low back pain. Spine (Phila Pa 1976) 2021; 46(2): 107-13.
20. Deyo RA, Dworkin SF, Amtmann D, Andersson G, Borenstein D, Carragee E, et al. Report of the NIH task force on research standards for chronic low back pain. Pain Med 2014; 15(8): 1249-67.
21. Rawang P, Janwantanakul P, Correia H, Jensen MP, Kanlyyanaphotporn R. Cross-cultural adaptation, reliability, and construct validity of the Thai version of the patient-reported outcomes measurement information system-29 in individuals with chronic low back pain. Qual Life Res 2020; 29(3): 793-803.
22. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 2004; 363(9403): 157-63.
23. Dubé M-O, Langevin P, Roy JS. Measurement properties of the pain self-efficacy questionnaire in populations with musculoskeletal disorders: a systematic review. Pain Rep 2021; 6(4): e972.
24. Fernandes L, Storheim K, Lochting I, Grotle M. Cross-cultural adaptation and validation of the Norwegian pain catastrophizing scale in patients with low back pain. BMC Musculoskelet Disord 2012; 13: 1-9.
25. Monticone M, Baiardi P, Ferrari S, Foti C, Mugnai R, Pillastrini P, et al. Development of the Italian version of the Pain Catastrophising Scale (PCS-I): cross-cultural adaptation, factor analysis, reliability, validity and sensitivity to change. Qual Life Res 2012; 21(6): 1045-50.
26. Nicholas MK, McGuire BE, Asghari A. A 2-item short form of the pain self-efficacy questionnaire: development and psychometric evaluation of PSEQ-2. J Pain 2015; 16(2): 153-63.
27. Jensen MP, Turner JA, Romano JM. Changes after multidisciplinary pain treatment in patient pain beliefs and coping are associated with concurrent changes in patient functioning. Pain 2007; 131(1-2): 38-47.
28. Simonetti V, Durante A, Ambrosca R, Arcadi P, Graziano G, Pucciarelli G, et al. Anxiety, sleep disorders and self-efficacy among nurses during COVID-19 pandemic: a large cross-sectional study. J Clin Nurs 2021; 30(9-10): 1360-71.
29. Tighe CA, Youk A, Ibrahim SA, Weiner DK, Vina ER, Kwoh CK, et al. Pain catastrophizing and arthritis self-Efficacy as mediators of sleep disturbance and osteoarthritis symptom severity. Pain Med 2020; 21(3): 501-10.
30. Travaglini LE, Highland KB, Rojas W, Buckenmaier CC, Kent M. Identification of functioning domains in the presurgical period and their relationships with opioid use and pain catastrophizing. Pain Med 2019; 20(9): 1717-27.
31. Martinez-Calderon J, Jensen MP, Morales-Asencio JM, Luque-Suarez A. Pain catastrophizing and function in individuals with chronic musculoskeletal pain: a systematic review and meta-analysis. Clin J Pain 2019; 35(3): 279-93.

Arch AHS

ORIGINAL ARTICLE

Archives of Allied Health Sciences 2022; 34(3): 44-52.

Establishment of in-house telomere length measurement using qPCR

Buntarawan Turapra[1], Jureerut Daduang[2], Molin Wongwattanakul[2,3], Chul-Young Bae[4], Patcharee Jearanaikoon[2,3*]

[1] Faculty of Graduate School, Khon Kaen University, Khon Kaen, Thailand.
[2] School of Medical Technology, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand.
[3] Center for Innovation and Standard for Medical Technology and Physical Therapy, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand.
[4] Department of Mediage R&D Center, Corporation Growth Center, Seong-nam, South Korea.

KEYWORDS
Telomere length; Chronological age; Quantitative polymerase chain reaction (qPCR).

ABSTRACT

Telomere is a nucleoprotein complex at the ends of chromosomes which can be used as a biomarker for aging and health condition. The gold standard method for telomere length measurement is quite a complication. Recently, quantitative polymerase chain reaction (qPCR) is a widely used method for molecular study with high throughput and cost-effectiveness. This study aimed to develop the in-house qPCR for estimating telomere length in kilobase in healthy samples. The in-house qPCR was established for the telomere gene and 36B4 gene. The analytical performance was verified prior to applying to 190 healthy participants, 139 females and 51 males. The telomere length (kilobase) was calculated and compared to the reference value. The results for telomere length by in-house qPCR method were 7.48 ± 1.78 kb for males and 7.53 ± 1.45 kb for females with the range of 4.66 – 10.69 kb and 4.03 – 11.50 kb, respectively. A total of 190 participants showed moderate correlation with a reference value at R^2 = 0.5672. The Bland-Altman analysis from two different methods showed only 5.26% (10 out of 190) were out of ± 25% bias in females. The in-house qPCR was successfully demonstrated for telomere length measurement with an acceptable performance compared to the reference values. However, the validation in more clinical samples should be performed in further study.

***Corresponding author:** Patcharee Jearanaikoon, MT, PhD. School of Medical Technology, Faculty of Associated Medical Sciences, Khon Kaen University 123, Mitraphab Highway, Muang, Khon Kaen 40002, Thailand. E-mail: patjea@kku.ac.th
Received: 20 August 2022/ Revised: 3 October 2022/ Accepted: 23 November 2022

Introduction

Telomeres are nucleoprotein structures composed of short tandem repeats, TTAGGG, at the ends of chromosomes. The average telomere length is about 10 – 15 kb in humans[1]. Telomeres reduce during each cell division, due to an end-replication problem that leads to cell death. The shorter telomeres imply a higher biological age. Therefore, telomere length has been proposed as a biomarker of aging and general health condition. Terminal restriction fragment (TRF) is a gold standard for telomere length measurement by southern blot[2]. However, the TRF method requires a large amount of DNA and expertise, and uses more time-consuming. Nowadays, several methods have been developed for telomere length measurement including quantitative fluorescence in situ hybridization (q-FISH), flow cytometry fluorescent in-situ hybridization (flow-FISH) and quantitative polymerase chain reaction (qPCR). Q-FISH and flow-FISH can determine the average telomere length using a fluorescent peptide nucleic acid (PNA) probe. The labor-intensive, requiring high skill level, expensive and technically demanding system are the limitations of these techniques[3].

Quantitative polymerase chain reaction (qPCR) is a widely used technique for population-based comparisons. The qPCR measurement for telomere length was first reported by Cawthon et al[4]. The principle is based on the abundance of telomere (T) signals per genome representing the average telomere length in a DNA sample. The single copy gene (S) is used to normalize the signal from the telomere reaction. Therefore, the T/S ratio shows the average telomere length per genome. The advantage of this method is the small amount of DNA requirement and high throughput performance[5]. Previous studies reported the T/S ratio for relative telomere length in biological age prediction and many diseases[6]. In this study, we aimed to establish in-house qPCR to estimate the telomere length (kilobase) using convert calculation from T/S ratio in a healthy population.

Materials and methods

Participants and DNA extraction

Blood samples were drawn from 190 healthy volunteers under written informed consents approved by the Ethics Committee of Khon Kaen University, Thailand with the approval no. HE622269. The inclusion criteria of volunteers were 18-80 years old with no history of high blood pressure or any chronic diseases such as diabetes, cancers, cardiovascular disease, kidney disease and liver disease. Moreover, participants who are taking any drugs for chronic diseases, having waist circumference more than 41 inches and having body mass index (BMI) more than 30 kg/m^2 were excluded. DNA was extracted using Qiagen® DNA Blood Kit (Qiagen® GmbH, Hilden, Germany) according to the manufacturer's protocol and kept frozen at -80 °C until use.

Telomere length analysis by quantitative polymerase chain reaction (qPCR)

The qPCR for telomere length measurement was modified from the Cawthon et al[4]. Primers and standard oligomers sequences of Telomere (T) and a single copy gene, 36B4, as an internal control synthesized by *Integrated DNA Technologies* (IA, USA) are listed in table 1. Two separate reactions for T and 36B4 gene were prepared to amplify each of an individual sample. A total of 6.25 ng of DNA template was added into the 25 µL of PCR reaction mixture, which was composed of 10 µmol of each primer and 12.5 µL of Maxima SYBR Green qPCR Master Mix (2X) (Thermo Fisher Scientific, MA, USA). The reactions were amplified under thermocycler (*Rotor-Gene Q, QIAGEN*, Germany) for telomere and single copy gene with initial denaturation at 93 °C for 10 min followed by 40 cycles of 93 °C for 15 sec and 54 °C for 1 min.

Telomere length calculations in Kilobases

The cycle threshold of telomere in each sample was relatively normalized with single copy gene (36B4) as telomere gene/single copy gene ratio (T/S) regarding the formula:

ΔCt telomere = [Ct (telomere of DNA sample) - Ct (telomere of DNA control)]

ΔCt single copy gene
= [Ct (single copy gene of DNA sample)
- Ct (single copy gene of DNA control)]
ΔΔCt sample = [ΔCt telomere - ΔCt single copy gene]
Relative telomere (T/S ratio) = $2^{-\Delta\Delta Ct\ sample}$

To control the precision between assays, a large pooled genomic DNA of one donor was performed within the assay as the internal control throughout this study.

The absolute telomere length in Kilobase (Kb) was further estimated from the T/S ratio of the calibrator obtained from the Absolute Human Telomere Length Quantification qPCR Assay Kit (ScienCell™ Research Laboratories, CA, USA).

Evaluation of assay performance of telomere length measurement

The performance in precision and accuracy of in-house telomere length qPCR assay was conducted in this study. A pooled DNA control was used to determine within-run (n = 18) and between-run (n = 20 days) imprecision of Telomere and 36B4 gene and indicated as the coefficient of variation (CV). The acceptable performance of qPCR should be less than 15%[8,9]. For the accuracy of in-house telomere length by qPCR, the telomere length of 190 samples was compared with the qPCR results obtained from Teloage® analysis (Mediage, South Korea) as reference values. The Bland-Altman analysis was used to compare the differences between the two methods with 95% limits of agreement. The mean difference less than 25% was used as an acceptable inaccuracy.

Table 1 Oligonucleotide sequences for telomere length measurement using qPCR

Primer Name	Oligomer sequences* (5' - 3')	Amplicon size
Telomere (Forward)	CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT	76
Telomere (Reverse)	GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT	
36B4 (Forward)	CAGCAAGTGGGAAGGTGTAATCC	75
36B4 (Reverse)	CCCATTCTATCATCAACGGGTACAA	

Note: *Oligomer sequences were followed from the previous study of Callaghan et al[7].

Results

Characteristic data of participants

A total of 190 healthy participants with 139 females and 51 males were included in this study. The overall age ranged from 20 to 76 years old with an average at 40.41 ± 13.53 years. The physical and blood parameters are listed in table 2. The upper and lower range of biochemical parameters were followed the criteria of the previous study which covered the biochemical values in older normal adult[10].

Table 2 Characteristic data of participants

Parameters	Mean (SD)	Median (min - max)
Age (years)	40.41 (13.53)	41 (20-76)
Weight (kg)	56.17 (8.77)	55 (40.8-92.6)
Height (cm)	161.95 (8.15)	160 (145-189)
Body mass index; BMI (kg/m^2)	21.31 (2.15)	21.2 (17-29)
Systolic blood pressure; SBP (mmHg)	115.69 (13.00)	115 (75-149)
Diastolic blood pressure; DBP (mmHg)	71.10 (8.15)	70 (50-89)
Fasting glucose (mg/dL)	83.92 (7.09)	83 (63-107)
Blood urea nitrogen; BUN (mg/dL)	12.58 (2.84)	12 (6-19)
Creatinine (mg/dL)	0.84 (0.18)	0.8 (0.4-1.4)
Cholesterol (mg/dL)	190.67 (25.37)	189 (119-253)
Triglycerides; TG (mg/dL)	77.17 (38.25)	67 (17-274)
High-density lipoproteins; HDL (mg/dL)	57.98 (11.61)	57 (32-97)
Low-density lipoproteins; LDL (mg/dL)	117.22 (24.09)	118 (50-172)
Aspartate aminotransferase; AST (U/L)	26.60 (5.19)	27 (16-47)
Alanine aminotransferase; ALT (U/L)	19.57 (8.01)	18 (9-68)
Alkaline phosphatase; ALP (U/L)	50.96 (15.32)	50 (16-92)
Albumin (g/dL)	4.68 (0.37)	4.68 (2.9-6.1)

The imprecision of the In-house telomere length qPCR method

The coefficient of variation (CV) for within-run and between-run are summarized in table 3. For the within-run assay, the %CV of cycle threshold of telomere gene and 36B4 gene was found with 3.75% and 1.16%, respectively, whereas the %CV of the between-run assay showed slightly higher than within-run assay as 3.38% of telomere gene, and 3.19% for 36B4 gene.

Table 3 Analytical imprecision of cycle threshold (Ct) of telomere gene and 36B4 gene using qPCR

Precision	Ct value of Telomere gene Mean ± SD (%CV)	Ct values of 36B4 gene (S) Mean ± SD (%CV)
Within-run (n = 18)	11.78 ± 0.44 (3.75)	18.28 ± 0.21 (1.16)
Between-run (n = 20)	12.07 ± 0.40 (3.38)	18.26 ± 0.58 (3.19)

The regression analysis of telomere length between the in-house qPCR method and the reference values

To evaluate the better correlation of telomere length between in-house qPCR method and the reference values, the gender separation was analyzed compared to all participants. The result showed that the correlation in male samples (R^2 = 0.6846) was better than that in females (R^2 = 0.5687) and all 190 participants (R^2 = 0.5672), respectively (Figure 1). The average converted telomere length measured by the in-house qPCR method was 7.48 Kb for males and 7.53 Kb for females, whereas the telomere length for males and females measured by the reference method was 7.44 Kb and 8.30 Kb, respectively (Table 4). In this study, the telomere length was calculated from the linear regression formula. To estimate the lower limit and upper limit or detectable range of this calculation, we have verified with the data from the values of 190 samples. Therefore, the lower detection of telomere length with our established method was obtained from the elderly group over 60 years with 4.66 kb in male and 4.03 kb in female as shown in table 4. The detectable range of telomere length in our in-house method was obtained at 4.66 – 10.69 Kb in males and 4.03 – 11.50 Kb in females compared to the reference method at 4.28 – 10.57 Kb in males and 4.79 – 11.94 Kb in females.

Table 4 The correlation analysis of telomere length between in-house qPCR and the reference method

	Telomere length (Kilobase)	
	In-house qPCR method	Reference method
Male (n = 51)		
Mean (SD)	7.48 (1.78)	7.44 (1.52)
Range (min - max)	4.66 – 10.69	4.28 – 10.57
Female (n = 139)		
Mean (SD)	7.53 (1.45)	8.30 (1.48)
Range (min - max)	4.03 – 11.50	4.79 – 11.94

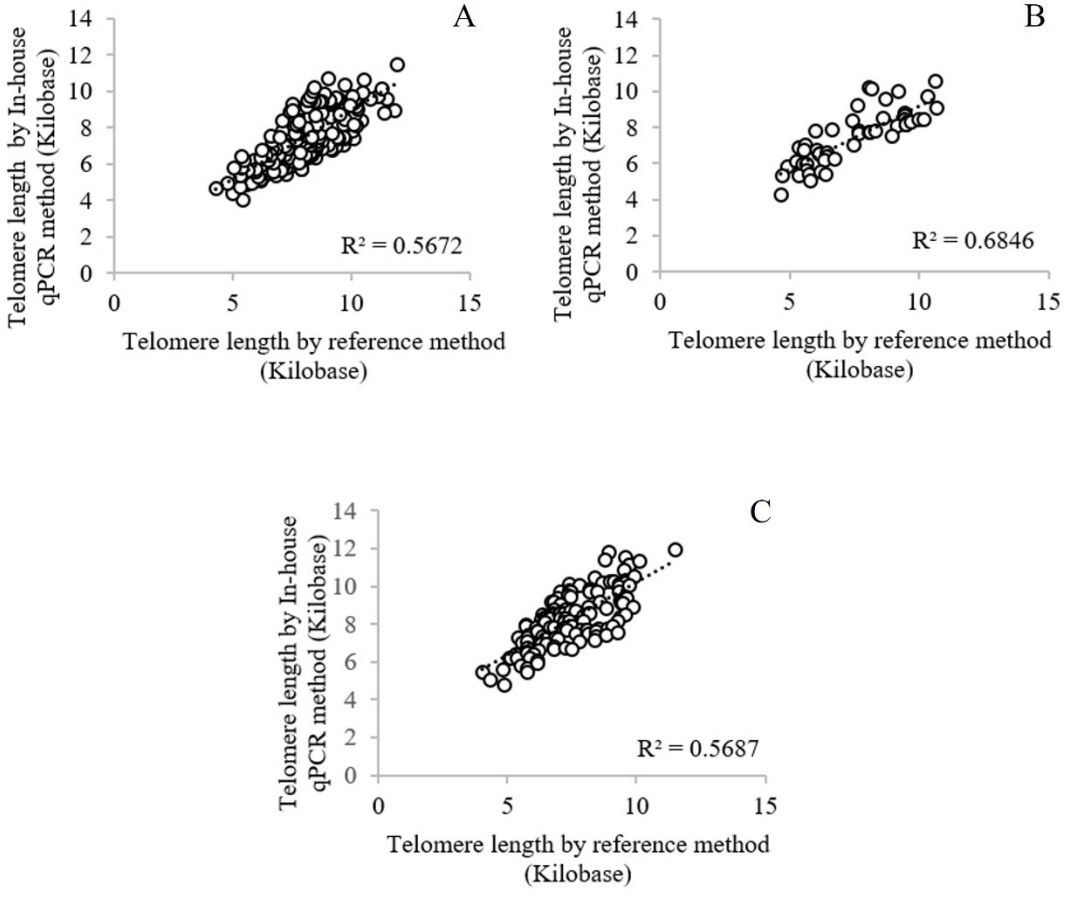


Figure 1 Linear regression plot of telomere length measured between in-house qPCR method and the reference method among A) total participants (n = 190), B) males (n = 51), and C) females (n = 139).

The Bland-Altman analysis of telomere length

To evaluate the difference of telomere length between two methods, Bland-Altman plot was performed as shown in figure 2. For total 190 samples, the mean of difference percentage of telomere length was -6.37 with 95% confidence interval at -31.7% to 18.9%. Moreover, the mean of difference percentage of more than ± 25% was set as an unacceptable inaccuracy. Only 5.26% (10 out of 190) were out of ± 25% which was obtained from females whereas no data from males was out of this range indicating approximately 95% of the study samples with acceptable accuracy.

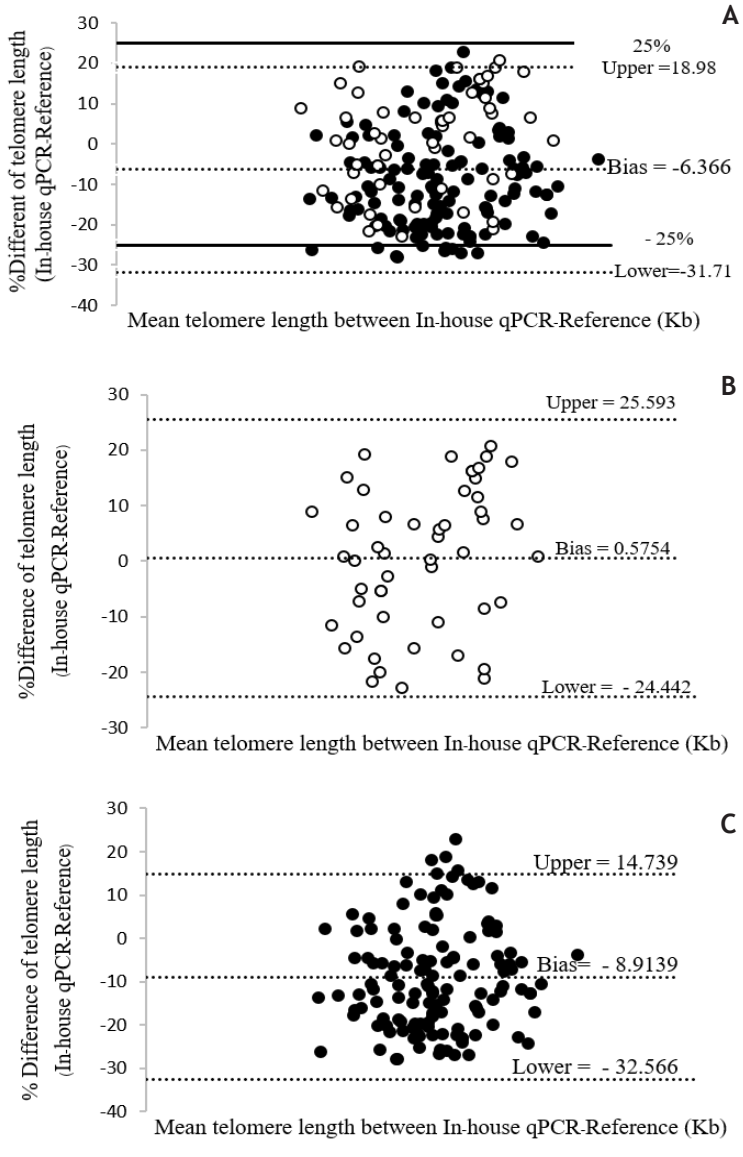


Figure 2 Bland-Altman plot for mean difference and 95% confidence upper and lower interval of telomere length measurement between in-house qPCR and reference method among A) total participants, B) males and C) females. A white circle indicates males and a black circle indicates females.

Discussion

Telomere length can be measured in many types of cells which have nucleus such as white blood cells (WBCs), buccal cells and tissues. However, the best types of cells are controversial among these reports. Thomas et al[14] investigated the telomere length using extracted DNA from WBCs, buccal cells, and brain tissues. Their results showed that absolute telomere length which was extracted from buccal cells was significantly shorter than that from WBCs even in control group or Alzheimer's group. On the other hand, Gadalla

et al[15] reported that the telomere length from blood cells was shorter than that of buccal cells and fibroblast from patient with dyskeratosis congenita and inherited bone marrow failure syndrome. In this study, WBCs were used according to their advantages in terms of high DNA yields and purity[13].

Nowadays, there is a commercial assay kit for human telomere length using qPCR available in the market; however, its high cost renders its limited uses. This study showed the successful establishment of in-house qPCR to estimate the telomere length using convert calculation from the T/S gene copy ratio.

The analytical performance of the developed method showed an excellent precision for both within-run and between-run CV of < 5% regarding the recommended imprecision for qPCR of less than 15%[8,9]. The minimal imprecision was achieved by including well internal control correction in every assay.

Regarding the reference value of comparison study, the telomere length measured from Teloage® analysis (Mediage, South Korea) has already been published[10]. In accordance with gender-related telomere length in table 3, longer telomere length was also observed in females which can be explained by the estrogen enhancement of telomerase activity[11]. Moreover, telomere shortening is correlated with many other factors, such as smoking, alcohol consumption, obesity, and lack of exercise exerting as the main role in increasing oxidative stress and inflammatory[12].

The comparison study between in-house qPCR and reference values using the Bland-Altman plot showed a significant and higher correlation in males than in females with 0% and 5.26% inaccuracy. The result illustrated that male group showed the lowest difference of telomere length between two methods compared to females and total participants; hence the estimation for males is more accurate than for other groups. This may be caused by an unequal number of males compared to females. Therefore, more sample sizes with equal number should be obtained for accurate estimation among gender difference in further study.

Conclusion

The development method of in-house qPCR for telomere length measurement was achieved with an acceptable performance of precision and accuracy compared to the reference values. However, this study was performed in a small sample size. Therefore, to apply the in-house qPCR, more sample size and aging status prediction from telomere length should be examined in a further study.

Take home messages

According to spontaneous cellular division, telomere length was known as aging biomarker in human. Shortening of telomere can be used to estimate the cellular aging of individual. To date, qPCR is concerned as one of the most convenient methods. The established in-house qPCR for telomere length provided acceptable performance compared to the reference values.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This work was financially supported by Center of Excellence on Medical Biotechnology (CEMB), S&T Postgraduate Education and Research Development Office (PERDO), Office of Higher Education Commission (OHEC), Thailand and National Research Council of Thailand (NRCT).

References

1. Jafri MA, Ansari SA, Alqahtani MH, Shay JW. Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. Genome Med 2016; 8(1): 69.
2. Vera E, Blasco MA. Beyond average: potential for measurement of short telomeres. Aging 2012; 4(6): 379–92.
3. Mensà E, Latini S, Ramini D, Storci G, Bonafè M, Olivieri F. The telomere world and aging: Analytical challenges and future perspectives. Ageing Res Rev 2019; 50: 27–42.

4. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res 2002; 30(10): 47.
5. Montpetit AJ, Alhareeri AA, Montpetit M, Starkweather AR, Elmore LW, Filler K, et al. Telomere length: a review of methods for measurement. Nurs Res 2014; 63(4): 289–99.
6. Alhusseini NF, Madboly AG. Telomere length measurement by quantitative real-time PCR: a molecular marker for human age prediction. Am J Biochem Biotechnol 2016; 12(1): 64–71.
7. O'Callaghan NJ, Fenech M. A quantitative PCR method for measuring absolute telomere length. Biol Proced Online 2011; 13(1): 3.
8. Karlen Y, McNair A, Perseguers S, Mazza C, Mermod N. Statistical significance of quantitative PCR. BMC Bioinformatics 2007; 8: 131.
9. Gutierrez-Rodrigues F, Santana-Lemos BA, Scheucher PS, Alves-Paiva RM, Calado RT. Direct comparison of flow-FISH and qPCR as diagnostic tests for telomere length measurement in humans. PloS One 2014; 9(11): 113747.
10. Bae CY, Kang YG, Piao MH, Cho B, Cho KH, Park YK, et al. Models for estimating the biological age of five organs using clinical biomarkers that are commonly measured in clinical practice settings. Maturitas 2013; 75(3): 253–60.
11. Zhou C, Steplowski TA, Dickens HK, Malloy KM, Gehrig PA, Boggess JF, et al. Estrogen induction of telomerase activity through regulation of the mitogen-activated protein kinase (MAPK) dependent pathway in human endometrial cancer cells. PLoS ONE 2013; 8(2): e55730.
12. Vidacek NŠ, Nanic L, Ravlic S, Sopta M, Geric M, Gajski G, et al. Telomeres, nutrition, and longevity: can we really navigate our aging? J Gerontol A Biol Sci Med Sci 2017; 73(1): 39–47.
13. Zanet DL, Saberi S, Oliveira L, Sattha B, Gadawski I, Côté HCF. Blood and dried blood spot telomere length measurement by qPCR: assay considerations. PLoS ONE 2013; 8(2): e57787.
14. Thomas P, O' Callaghan NJ, Fenech M. Telomere length in white blood cells, buccal cells and brain tissue and its variation with ageing and Alzheimer's disease. Mech Ageing Dev 2008; 129(4): 183–90.
15. Gadalla SM, Cawthon R, Giri N, Alter BP, Savage SA. Telomere length in blood, buccal cells, and fibroblasts from patients with inherited bone marrow failure syndromes. Aging 2010; 2(11): 867–74.