Journal of Southeast Asian Medical Research

www.jseamed.org
Vol.5 No.1 January-June 2021
ISSN 2586-8241

Introduction

The Journal of Southeast Asian Medical Research is a peer-reviewed journal with printing every 6 months. The main goal of this collaboration project is to distribute new knowledge in medical sciences to medical communities and scientists, as well as encouraging scientific collaborations within Southeast Asia and also other nations around the world. The journal publishes original research in the medical sciences: clinical and basic. We welcome original articles from across the world. The editorial board consists of international experts in various fields of medicine, ranging from internal medicine to a variety of surgeries. The full text of the journal is available online at http://www.jseamed.org

It is our aim to publish the most up-to-date and useful research information in medical sciences. In Southeast Asia, there are some unique problems in health care and diseases, such as tropical diseases, and it is crucial that health professionals can access, share and exchange knowledge promptly. In this region, there is still a gap of knowledge in health sciences that needs to be closed by scientific research, which we are hoping to close after this collaboration project. We hope that the journal will fulfill the objectives and will provide benefit to all, both medical practitioners and researchers alike.

Editorial board

JOURNAL OF
SOUTHEAST ASIAN
MEDICAL RESEARCH

JOURNAL OF SOUTHEAST ASIAN MEDICAL RESEARCH

www.jseamed.org

Table of Contents

A.Original Article

DOES THE PLATELET CONCENTRATION IN PLATELET RICH PLASMA INFLUENCE THE OUTCOMES OF PRIMARY KNEE OSTEOARTHRITIS?

Brang Mai, Maung Mg Htwe, Than Win, Zaw Min Han, Aung Myo

Department of Orthopaedic Surgery, University of Medicine, Mandalay, Myanmar

Abstract

Background: Growth factors in platelets have been extensively studied and were reported to be used to stimulate cartilage regeneration in osteoarthritis (OA)**.**

Objective: This study aimed to observe the influence of platelet concentration in platelet rich plasma (PRP) on the outcomes of primary knee OA.

Methods: Eighty-nine patients undergoing PRP injection in unilateral primary knee OA were assessed using the Western Ontario and McMaster Universities Arthritis Index (WOMAC) questionnaire and visual analog scale (VAS) before intervention at 3 weeks, 3 months, 6 months and 12 months after treatment. A small aliquot of PRP was sent for bacteriologic examination and evaluation of the platelet count. A student t-test was conducted to compare WOMAC and VAS score among patients before PRP injection (baseline) and at each follow-up. The platelet count and their influence on outcomes were also analyzed using Pearson`s correlation coefficient.

Results: Statistically significant differences were observed in the WOMAC score between baseline (M=47.08, SD=8.50) and 3 weeks (M=20.37, SD= 10.09, $p< 0.001$), 3 months (M= 23.24, SD= 11.39, $p<0.001$), 6 months (M= 29.89, SD=14.95, $p <0.001$), and final follow-up at 12 months (M= 27.78, SD= 16.56, $p<0.001$). Also a significant difference was observed in VAS between baseline (M=69.02, SD= 9.58) and 3 weeks (M= 36.23, SD= 15.72, $p <0.001$), 3 months (M= 37.04, SD= 17.30, $p <0.001$), 6 months (M= 42.58, SD=22.15, $p <0.001$) and 12 months (M=39.15, SD= 23.96, $p <0.001$). The mean platelet count in PRP injection was $1000.66x10^3$platelets/mL ($402x10^3$platelets/ml to $1630x10^3$platelets/mL). Positive correlations were discovered between the concentration of the platelet and the mean improvement WOMAC scores and VAS at 3 weeks (r =0.31, r=0.40), 3 months (r=0.10, r=0.23), 6 months (r=0.08, r=0.30) and 12 months after intervention (r=0.12, r=0.23), respectively.

Conclusion: Higher concentrations of platelets in the PRP had a better effect on outcomes of primary knee OA especially at three weeks after injection.

Keywords: Concentration of platelets, Platelet rich plasma, Primary knee OA

J Southeast Asian Med Res 2021: 5(1): 1-10
http://www.jseamed.org

Correspondence to: Brang Mai, Department of Orthopaedic Surgery, University of Medicine, Mandalay, Myanmar
E-mail: dr.brangmai@gmail.com

Received: 11 January 2021
Revised: 30 April 2021
Accepted: 10 May 2021

Introduction

Platelet rich plasma (PRP) is orthobiologic and several studies have reinforced that PRP has positive clinical results in treating osteoarthritis (OA).[1-8] Platelet rich plasma is the volume of concentrated platelets extracted from plasma usually 3 to 5 times above baseline. The platelets contain alpha granules that are rich in several growth factors such as platelet derived growth factor (PDGF), transforming growth factors-β, insulin like growth factors, vascular endothelial growth factor, epidermal growth factors and the release of these biologically active proteins from platelets is part of the natural healing process and tissue regeneration.[9-12] Growth factors in platelets have been extensively studied in OA and have been reported to be used as an attractive method to stimulate cartilage regeneration in OA.[11,13]

The concentration of growth factors depends on the platelet count[14-17] and may affect the outcomes of the disease. Few studies have estimated the growth factors involved[18, 19] as well as platelet count in PRP which is used to inject into the knee joint.[6, 18, 19] The platelet concentration in PRP is essential for fracture healing and the optimal biological effects on pain relief and functional recovery are associated with platelet concentrations of approximately $1{,}000x10^3$ platelet/mL.[20, 21] However, to our knowledge no report have determined the number of platelets required for effective regenerative therapy as well as the influence of platelet concentration in PRP on the outcomes of primary knee OA. This raises the question whether an increased platelet concentration in PRP has better efficacy in knee OA. This study observed the influence of platelet concentration in PRP on the outcomes of knee OA.

Methods

The study was approved by the Academic Committee of the University of Medicine Mandalay, Myanmar and informed consent from all patients was obtained. The following diagnostic criteria were applied: patients with primary unilateral knee OA with the history of chronic knee pain lasting at least 12 months and the radiologic signs of the knee OA grades 1, 2 or 3 according to Kellgren & Lawrence Radiographic Knee OA classification (K&L). Exclusion criteria were OA secondary to inflammatory diseases, patients with generalized OA, bilateral OA knee, advanced staged of knee OA, patients receiving intra articular injection within three months or any surgery to the knee joint, patients receiving anticoagulant therapy, patients with hemoglobin less than 10 g/dL infection around the knee joint, patients with known thrombocytopenia, and patients with crystal arthropathy or tumor.

The sample size for paired means t-test was calculated using Stata 11 with 85% power, 5% alpha, WOMAC mean difference of -13.3 (from a pilot study), standard deviation for WOMAC mean difference of 9.5 (from a pilot study) and estimated drop-out rate of 20%. The minimal required sample size was 81. From September 2015 to August 2020, 168 primary unilateral knee OA cases met the inclusion criteria and received the single intra-articular injection of PRP. Those patients were assessed with WOMAC and VAS scores before PRP injection, at 3 weeks, 3 months, 6 months and 12 months follow-up. A total of 89 patients who completed the one-year follow were included into this study.

Platelet rich plasma preparation

The blood (27 ml of venous blood) sample was extracted in six 6 ml sterile tubes containing 0.7 ml of CPD-A1. The samples were gently shaken to thoroughly mix the anticoagulant with the blood. The blood samples were placed in a centrifuge and centrifuged for 12 minutes at 3500 rpm resulting in the three following layers. The inferior layer was composed of erythrocytes, the intermediate layer consisted of leucocytes with platelets and the superior layer comprised plasma. The superior layer consisting of platelet poor plasma was first discarded. The intermediate buffy coat layer, consisting of platelets mixed with white blood cells was then gently aspirated with an 18 G epidural needle syringe in a volume of 3 ml of PRP and used for intra-articular injection within 30 minutes. An aliquot of product was sent to the laboratory for platelet concentration & bacteriological examination. All procedures were performed by the researcher at the outpatient department.

Table 1. Baseline characteristics of the study subjects

Age, mean± SD(years)	57.56±6.01	
Sex, M: F	19:70	
BMI, mean± SD	25.92±3.91	
Kellgren & Lawrence grade(K&L)	Male	Female
1	4	26
2	6	22
3	9	22
WOMAC	47.08±8.50	
VAS	69.02±9.58	
Platelet concentration in PRP, mean± SD (x10^3platelet/ml)	1000.66±291.91	

WOMAC; Western Ontario and McMaster Universities Arthritis Index, VAS; visual analog scale, PRP; platelet rich plasma

Treatment procedure and follow-up

The patient was placed in the supine position with the knee fully extended. Under aseptic condition, 2.5 cc of PRP was injected in the suprapatellar pouch using a superolateral approach with a 22-gauge needle without local anaesthesia. At the end of the procedure, the patient was invited to bend and extend the knee for a few seconds to distribute the PRP itself throughout the joint. The patient was discharged after 30 minutes of observation. They were reassessed with WOMAC score and VAS at the end or 3 weeks, 3 months, 6 months and 12 months after treatment. During the follow-up period, NSAIDs were not allowed and paracetamol 500 mg three times daily was prescribed in case of discomfort. All patients were asked to stop analgesic medications 24 hours before follow-up reassessment.

Data collection and statistical analysis

Data were collected through pre-structured pro-forma by medical personnel unaware of the procedure. All statistical analyses were performed using Microsoft Excel 2016 (Microsoft Inc., Seattle, WA, USA). A p value of less than 0.05 was considered statistically significant. The WOMAC and VAS scores before and after intervention were compared using paired-sample t-tests. Pearson`s correlation coefficient (r) and regression analysis were calculated between the platelet concentration in PRP and improved outcomes.

Results

All patients in this study had a mean age of 57.5 years (range, 50-80), mean BMI of 25.92 (range, 18.45-34.13) and other base line characteristics were reported (**Table 1**). Among 89 patients with unilateral primary knee OA treated by single injection of PRP, no major adverse events were observed during the procedure and the follow-up period. Statistically significant improvement of all functional and pain scores was observed through-out the follow-up compared with the baseline scores (**Table 2**). All patients were satisfied with their results.

The mean WOMAC score improved from 47.08 points (range, 28-66) before intervention to 20.37 points (range, 5-45) at 3 weeks, 23.24 points (range, 6-50) at 3 months, 29.89 points (range, 5-60) at 6 months and 27.78 points (range, 5-70) at 12 months and final follow-up showing a statistically significant improvement (p <0.001) at each of the follow-ups with respect to baseline (**Figure 1**). Mean VAS score also improved from 69.02 points (range, 50-85) before intervention to 36.23 points (range, 10-80) at 3 weeks, 37.04 points (range, 10-80) at 3 months, 42.58 points (range, 5-80) at 6 months, 39.15 points (range, 5-75) at 12 months, showing a statistically significant difference (p <0.001) at each of the follow-ups with respect to baseline (**Figure 1**).

Table 2. Clinical outcome scores before and after PRP injection

Outcomes	Baseline Mean±SD	3 weeks Mean±SD *p*-value	3 months Mean±SD *p*-value	6 months Mean±SD *p*-value	12 months Mean±SD *p*-value
WOMAC	47.08±8.50	20.37±10.09 <0.001	23.24±11.39 <0.001	29.89±14.95 <0.001	27.78±16.56 <0.001
VAS	69.02±9.58	36.23±15.72 <0.001	37.04±17.30 <0.001	42.58±22.15 <0.001	39.15±23.93 <0.001

WOMAC; Western Ontario and McMaster Universities Arthritis Index, VAS; visual analog scale p <0.001 (compared to baseline score)

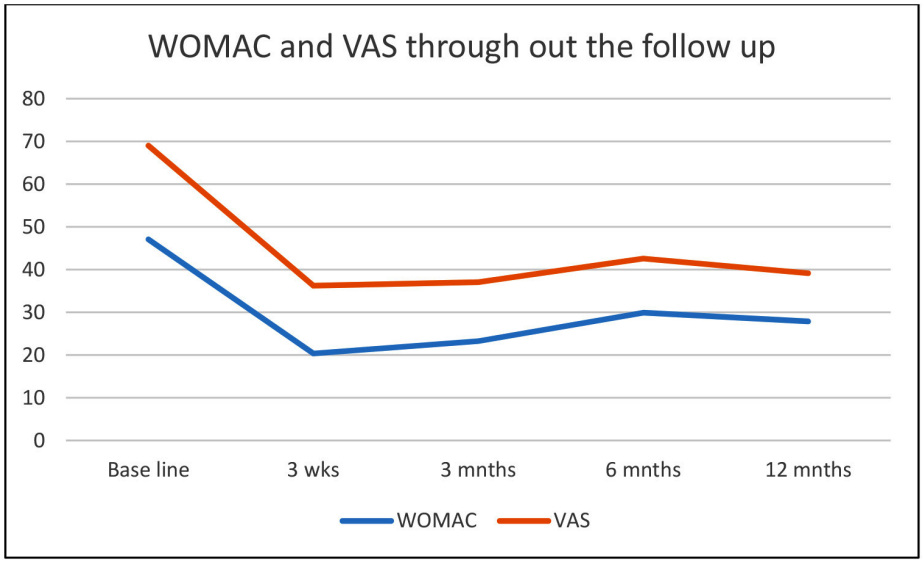


Figure 1. Overall assessment with VAS and WOMAC scores before injection and through to the follow-up period

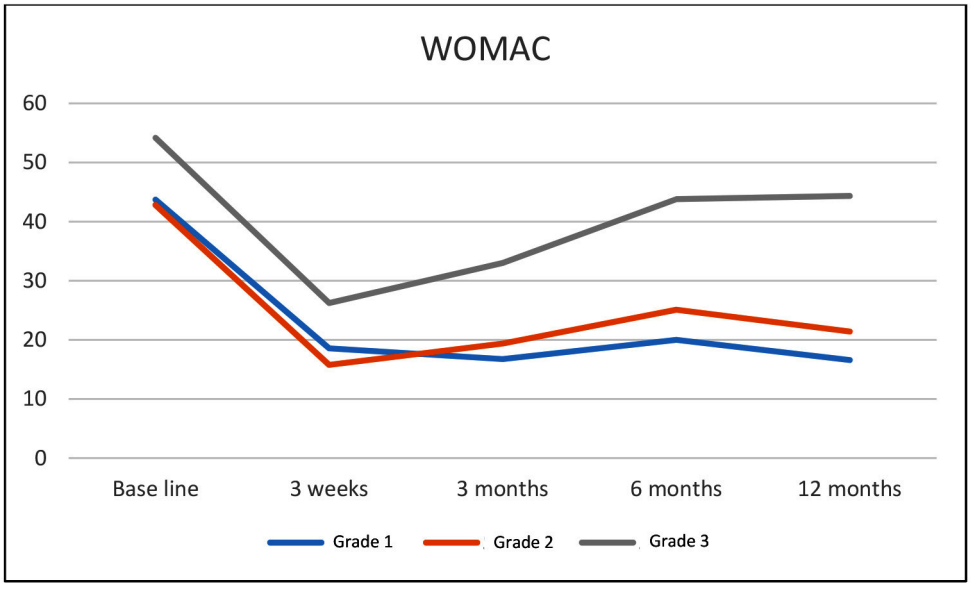


Figure 2. Comparison of WOMAC scores, All the stages improved significantly more in terms of WOMAC at 3 weeks compared with the baseline but the difference of improvement declined at 6 months postintervention. At 12 months after intervention, the scores of both grades 1 and grade 2 were significantly better than those of grade 3.

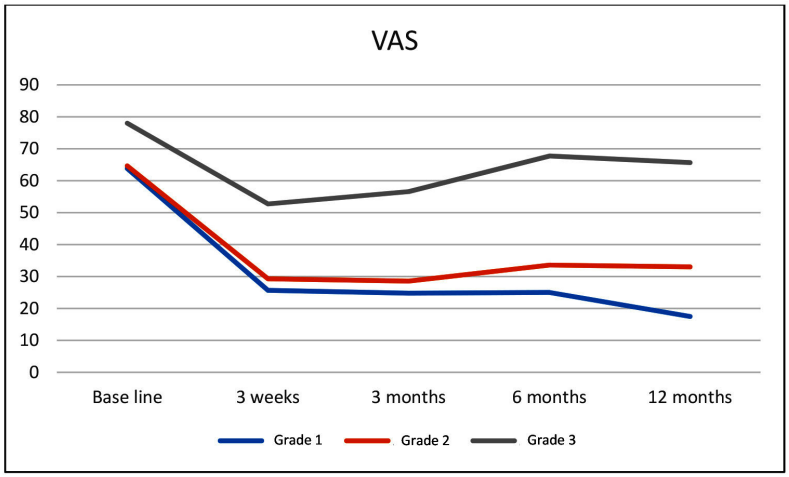


Figure 3. Comparison of VAS scores, All the grades improved significantly more in terms of VAS at 3 weeks compared with the baseline but the difference in improvement declined at 3 months postintervention in grades 2 and 3. At 12 months after intervention, the scores of grade 1 were better than those of 3 weeks whereas in grades 2 and 3, both the improvement pain scores decreased more than those at 3 weeks but were still better than baseline.

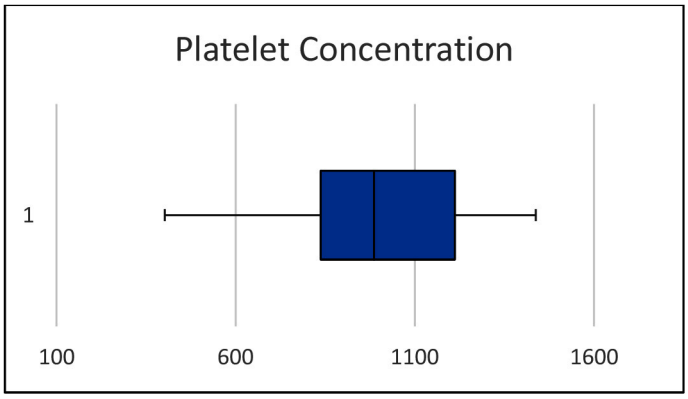


Figure 4. Box plot showing variability of the concentration of platelet in PRP

According to the K&L grade of knee OA, all grades in this study improved significantly in terms of WOMAC score at 3 weeks compared with that of baseline but the difference of improvement declined at 6 months postintervention. At 12 months after intervention, the WOMAC scores of both grades 1 and 2 were better than those of grade 3 (**Figure 2**). Regarding VAS, all grades significantly improved at 3 weeks compared with the baseline but the difference in improvement declined at 3 months postintervention in grades 2 and 3. In the grade 1 OA knee group, the VAS scores at 12 months were better than those at 3 weeks whereas in grades 2 and 3, both the improved pain scores decreased more than those at 3 weeks but were still better than baseline (**Figure 3**).

The platelet concentration was evaluated in every case of PRP injection. A high variability in platelet concentration in PRP was found. We observed that the mean concentration of platelet in PRP was 1000.66 $x10^3$ platelets/mL with a range of 402 $x10^3$platelets/mL to 1630 $x10^3$ platelets/mL without outlier (**Figure 4**). Average platelet concentration yielded 5.2 fold more than baseline. Correlation between the mean improvement scores (difference between each follow up score and the baseline) of both WOMAC and VAS with platelet concentration was determined (**Table 3**). Although positive correlations were found between platelet concentration and mean improvement scores of WOMAC and VAS at 3 weeks (r=0.31, r=0.40), 3 months (r=0.10, r=0.23), 6 months (r=0.08, r=0.30) and 12 months at final follow-up (r=0.12, r=0.23), the most significant results were seen at 3 weeks postintervention (**Figures 5, 6**).

Table 3. Correlation coefficient (r) between clinical improvement scores and platelet concentration

	K&L Grade 1		K&L Grade 2		K&L Grade 3	
	r	*p*-value	r	*p*-value	r	*p*-value
WOMAC						
3 weeks	0.40	0.02	0.31	0.10	0.24	0.19
3 months	0.004	0.98	0.16	0.39	0.15	0.39
6 months	0.111	0.55	0.09	0.61	0.20	0.26
12 months	0.15	0.42	0.29	0.12	0.19	0.28
VAS						
3 weeks	0.63	<0.01	0.52	<0.01	0.19	0.29
3 months	0.28	0.13	0.28	0.13	0.16	0.36
6 months	0.42	<0.01	0.33	0.08	0.34	0.05
12 months	0.15	0.41	0.43	0.01	0.25	0.16

WOMAC; Western Ontario and McMaster Universities Arthritis Index, VAS; visual analog scale, K&L; Kellgren & Lawrence Radiographic Knee OA classification

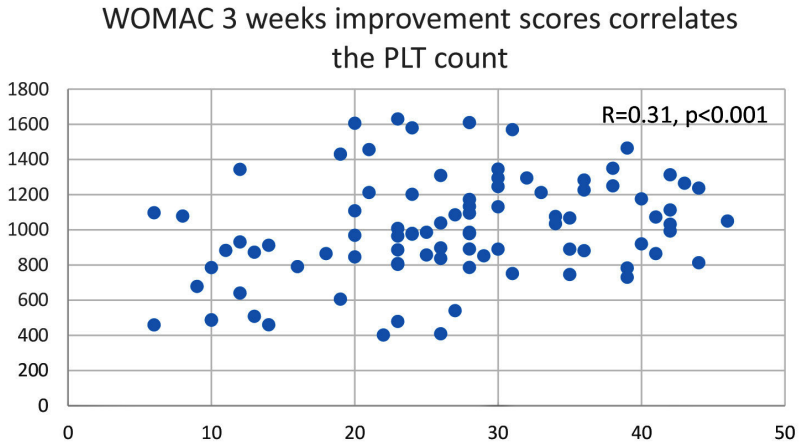


Figure 5. Correlation between improvement in WOMAC at 3 weeks and platelet concentration, WOMAC improvement on the X-axis and concentration of the platelet was plotted on the Y-axis.

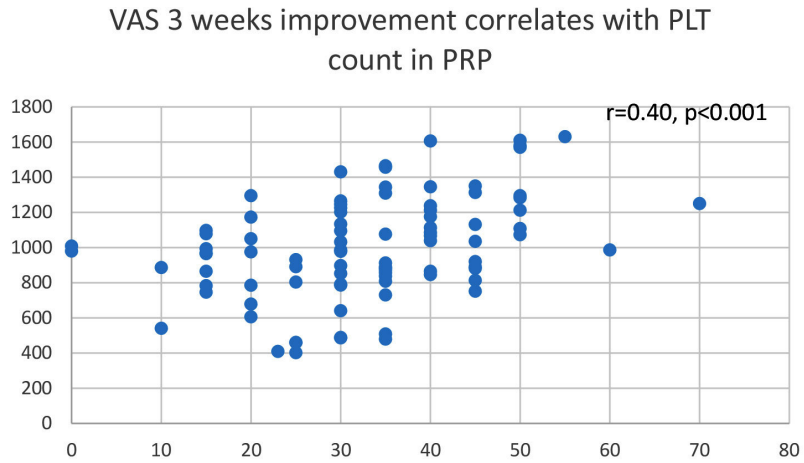


Figure 6. Correlation between improvement in VAS at 3 weeks and platelet concentration, VAS improvement on the X-axis and concentration of the platelet was plotted on the Y-axis.

Table 4. Clinical improvement scores and platelet concentration

	PLT<837 x10^3/mL	PLT>1212 x10^3/mL	*p*- value
K&L grade 1	10	9	
K&L grade 2	6	7	
K&L grade 3	5	5	
3 weeks WOMAC (Mean±SD) improvement	22.38±10.87	30.57±8.71	0.01
12 months WOMAC (Mean±SD) improvement	22.5±12.13	23.69±9.82	0.7
3 weeks VAS (Mean±SD) improvement	27.76±9.06	41.52±10.91	0.001
12 months VAS (Mean±SD) improvement	30.9±19.49	40.65±20.90	0.1

WOMAC; Western Ontario and McMaster Universities Arthritis Index, VAS; visual analog scale

According to platelet concentration, patients were divided in two groups: patients with a platelet count within the first quartile (platelet count <837 x10^3/mL) and those with a platelet count of more than the third quartile (platelet count >1212 x10^3/mL) (**Table 4**). Each group consisted of 21 patients. Mean improvement score of WOMAC at 3 weeks in the lower platelet group was 22.85 ± 10.87 and that in the higher group was 30.26 ± 8.56 showing a statistically significant difference (p=0.01) whereas mean WOMAC improvement score at 12 months in the lower platelet group was 22.5± 12.13 and that in the higher group was 23.69± 9.82 showing no difference (p=0.7). Similar results were observed in VAS pain score. Mean improvement score of the VAS at 3 weeks in the lower platelet group was 28.9 ± 8.42 and that in the higher group was 41.52 ± 10.91 showing a statistically significant difference (p=0.001). However, mean VAS improvement score at 12 months in the lower platelet group was 30.9± 19.49 and that in the higher group was 40.65± 20.90 showing no difference (p=0.1). The outcome scores in patients receiving PRP with a higher concentration of platelet were better than those at lower concentrations especially at 3 weeks. Nevertheless, this relationship was negligible in the later follow-up visits irrespective of osteoarthritis severity.

Discussion

This study aimed to determine whether the platelet concentration in PRP influenced the outcome of primary OA knee. Several studies had proved that platelet rich plasma had positive effects on ligamentous injury, tendinopathy and cartilage lesions and knee OA.[22-26] Various factors may impact the clinical outcome of PRP injection in primary knee OA such as the platelet concentration in PRP, the doses and frequency of injection as well as the preparation method of PRP.

Theoretically, the platelet concentration may affect the outcome of the disease process. To our knowledge, limited scientific evidence is available regarding optimal platelet concentration to treat knee OA. Patel et al. reported that a single injection of PRP containing 10 times the baseline platelet count was as effective as injecting twice at platelet concentration of more than 4 times baseline. [6] However, the optimal concentration of platelet in PRP to obtain the maximal result was unreported. However, the study of Bahar et al. stated that the concentration of 1,000 x10^3 platelet/mL was sufficient for pain relief and functional recovery in treating early OA. [21] Whether the concentration of less than 1,000 x10^3 platelet/mL influenced the outcomes remained unconfirmed. In the present study, the maximal relieving effect of PRP yields at 3 weeks postintervention irrespective of platelet concentration and the relieving effect seemed to be sustained until 12 months comparable to the study of Kon et al. [7] Moreover, the positive correlation between the improvement scores of WOMAC and VAS and the platelet concentration in the PRP, was found regardless of the severity of OA of the patients. The highest improvement outcome scores were observed only at 3 weeks after intervention and the improvement scores gradually declined throughout the later follow-ups. These might

have been the reasons for the life span of platelets and the gradual increase in the release of growth factors up until the 19th day and the consecutively delivered slow and constant release until the 23th day. [27]

Many studies have suggested the clinical application of PRP via multiple injections to favor knee OA healing at 3 weeks, once monthly or 3 months.[6,13] In our study, we used single injection of PRP in OA knee and observed that PRP injection produced favorable outcomes. This effect could be sustained over one year whereas in the study of Filardo et al., the mean beneficial effect of lasted ten months with sustained action up to 24 months.[24] In the study of Patel et al., single injection of PRP also produced comparable results as injecting twice.[6]

Different PRP separation systems and devices were used (Arthrex ACP Double Syringe System and Biomet Biologics GPS System). Briefly, they relied on single centrifugation, double centrifugation and on manual or automatic systems operated in open or closed circuits with platelet concentrations varying from 1.99 to 9.3 fold over baseline. [6, 28-30] However, no standardized preparation methods and optimal concentration of platelets in PRP that induce maximal pain relief remain unknown.[30-32]

Diverse PRP preparation and application techniques of PRP have been used for platelet activation and inactivation. Both PRP formulations provide pain relief and none is more efficacious than the other.[33] In this study, we used an inactivated technique which also produced quite relieving effects on OA knee pain.

In the present study, centrifugation procedures to prepare PRP increased the platelet count as well as white blood cell concentration. However, related studies have already pointed out the key role of leucocytes in PRP for their anti-infectious action, immune regulation and potential regeneration effects. [34, 35] The leucocyte content did not seem to induce negative effects or impair the potential beneficial effects of PRP, even when used in the joints. However, the study of Milants et al. recommended using a single spinning technique, a platelet concentration of lower than 5 times baseline and avoidance of leukocytes. [36] The present study showed that increased white blood cells in PRP produced no negative effects on the knee joint.

In this study, no major adverse events related to the injections were observed during the treatment and follow-up periods. Some authors have reported some injection pain, local inflammation of short duration, and re-accumulation of effusion, but these symptoms resolved spontaneously.[6, 24, 26] In the present study, all the functional and pain scores improved and were maintained until 12 months compared with baseline scores irrespective of platelet count in PRP. Although PRP has affected both functional and pain scores of patients up to one year, the influence of platelet concentration was observed only at early postintervention.

This study constitutes one of the first *in vivo* studies in our hospital to treat knee OA with PRP injection. However, it encountered some limitations. We evaluated the platelet count in PRP but did not include red and white blood cells. We didn`t use commercial platelet concentration systems, the platelet concentration was not homogeneous and revealed high variability in this study. Moreover, the drop-out rate in this study was very high because the patients from the outreach area were challenged to complete the one year follow-up visit. Despite these limitations, this study demonstrated that PRP is a potentially safe, simple and low cost method to improve articular joint healing, with promising results in treating early stage primary knee OA.

Conclusion

A significant positive correlation was observed between the improvement of clinical outcomes and platelet concentration in PRP especially at 3 weeks postintervention suggesting repeating injection might be beneficial. Further study would also be required to compare different PRP formulations and preparation methods either manually or via the use of commercial kits.

Conflict of interest

The authors declare they have no conflict of interest.

Acknowledgement

We thank Associate Professor Dr. Thidar Win, Department of Preventive and Social Medicine, Mandalay, Myanmar for statistical support in this study.

REFERENCES

1. Laudy AB, Bakker EW, Rekers M, Moen MH. Efficacy of platelet-rich plasma injections in osteoarthritis of the knee: a systematic review and meta- analysis. Br J Sports Med 2015; 49: 657–72.
2. Anitua E, Sanchez M, Javier Aguirre JJ, Prado R, Padilla S, Orive G. Efficacy and safety of plasma rich in growth factors intra-articular infiltrations in the treatment of knee osteoarthritis. Arthroscopy 2014; 30: 1006–17.
3. Shen L, Yuan T, Chen S, Xie X, Zhang C. The temporal effect of platelet-rich plasma on pain and physical function in the treatment of knee osteoarthritis: systematic review and meta-analysis of randomized controlled trials. J Orthop Surg Res 2017; 12: 16.
4. Meheux CJ, McCulloch PC, Lintner DM, Varner KE, Harris JD. Efficacy of intra-articular platelet-rich plasma injections in knee osteoarthritis: a systematic review. Arthroscopy 2016; 32: 495–505.
5. Chang KV, Hung CY, Aliwarga F, Wang TG, Han DS, Chen WS. Comparative effectiveness of platelet-rich plasma injections for treating knee joint cartilage degenerative pathology: a systematic review and meta-analysis. Arch Phys Med Rehabil 2014; 95: 562–75.
6. Patel S, Dhillon M. S, Aggarwal S, Marwaha N, Jain A. Treatment of platelet rich plasma is more effective than placebo for knee osteoarthritis. Am J Sports Med. 2013; 41: 356-64.
7. Kon E, Buda R, Filardo G, Martino AD, Timoncini A, Cenacchi A, et al. Platelet-rich plasma intraarticular knee injections produced favourable results on degenerative cartilage lesions. Knee Surg Sports Traumatol Arthrosc 2010; 18: 472-9.
8. Sampson S, Gerhardt M, Mandelbaum B. Platelet rich plasma injection grafts for musculoskeletal injuries. Curr Rev Musculoskelet Med 2008; 1:165-74.
9. Nurden AT. Platelets, inflammation and tissue regeneration: Thrombosis & Haemostasis Supplement. Schattauer 2011; S13-S33.
10. Dhillon RS, Schwarz EM, Maloney MD. Platelet-rich plasma therapy-future or trend? Arthritis Res Ther 2012; 14: 219.
11. Hickey DG, Frenkel SR, Di Cesare PE. Clinical applications of growth factors for articular cartilage repair. Am J Orthop 2003; 32: 70-6.
12. Dhurat R, Sukesh M. Principles and methods of preparation of platelet- rich plasma: A review and author's perspective. J Cutan Aesthet Surg 2014; 7: 189-97.
13. Spakova T, Rosocha J, Lacko M, Harvanova D, Gharaibeh A. Treatment of knee joint osteoarthritis with autologous platelet rich plasma in comparison with hyaluronic acid. Am J Phys Med Rehabi 2012; 91: 1-7.
14. Magalon J, Bausset O, Serratrice N, Giraudo L, Aboudou H, Veran J, et al. Characterization and comparison of 5 platelet- rich plasma preparations in a single-donor model. Arthroscopy 2014; 30: 629–38.
15. Sundman EA, Cole BJ, Fortier LA. Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. Am J Sports Med 2011; 39: 2135–140.
16. Magalon J, Bausset O, Serratrice N, Giraudo L, Adoudou H, Veran J, et al. Characterization and comparison of 5 platelet- rich plasma preparations in a single-donor model. Arthroscopy 2014; 30: 629–38.
17. Sundman EA, Cole BJ, Fortier LA. Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. Am J Sports Med 2011; 39: 2135–40.
18. Castillo TN, Pouliot MA, Kim HJ, Dragoo JL. Comparison of growth factor and platelet concentration from commercial platelet- rich plasma separation systems. Am J Sports Med 2011; 39: 266.
19. Eppley BL, Woodell JE, Higgins J. Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. Plast Reconstr Surg 2004; 114: 1502-8.

20. Weibrich G, Hansen T, Kleis W, Buch R, Hitzler WE. Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. Bone 2004; 34: 665-71.
21. Dernek B, Kesiktas FM, Duymus TM, Aydin T, Isiksacan N, Diracoglu D, et al. Effect of platelet concentration on clinical improvement in treatment of early stage- knee osteoarthritis with platelet-rich plasma concentrations. J Phys Ther Sci 2017; 29: 896-901.
22. Aqil A, Jacobs L. Physical therapy or platelet rich plasma injections in the treatment of tennis elbow: A ramdomised clinical trial. Webmed Central ORTHOPAEDICS 2010; 1: WMC00701
23. Engebretsen L, Schamasch P. The use of platelet-rich plasma in sports medicine-The international olympic committee opinion. Operat Tech Orthop 2012; 22: 43-8.
24. Filardo G, Kon E, Buda R, Timoncini A, Martino AD, Cenacchi A, et al. Platelet-rich plasma intra articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis. Knee Surg Sports Traumatol Arthrosc 2011; 19: 528-35.
25. Andia I, Sanchez M, Maffulli N. Joint pathology & platelet rich plasma therapies. Expert Opin Biol Ther 2012; 12: 7-22.
26. Sánchez M, Fiz N, Azofra J, Usabiaga J, Recalde EA, Gutierrez AG, et al. A randomized clinical trial evaluating plasma rich in growth factors (PRGF Endoret) versus hyaluronic acid in the ahort-term treatment of symptomatic knee osteoarthritis. Arthroscopy 2012; 28: 1070-8.
27. Chatterjee A, Debnath K. Comparative evaluation of growth factors from platelet concentrates: An in vitro study. J Indian Soc Periodontol 2019; 23: 322-8.
28. Gato-Calvo L, Magalhaes J, Ruiz- Romero C, Blanco FJ, Burguera EF. Platelet- rich plasma in osteoarthritis treatment: review of current evidence. Ther Adv Chronic Dis 2019; 10: 1-18.
29. Machado ES, Leite R, Santos CCd. Artuso GL, Gluszczak F, Jesus LGD, et.al. Turn down-turn up: a simple and low- cost protocol for preparing platelet-rich plasma. CLINICS 2019; 74: e1132
30. Everts PAM, Mahoney CB Hoffmann JJML, Schönberger JPAM, Box HAM, Zundert AV, et al. Platelet-rich plasma preparation using three devices: implications for platelet activation and platelet growth factor release. Growth Factors 2006; 24: 165-71.
31. Kevy SV, Jacobson MS. Comparison of methods for point of care preparation of autologous platelet gel. J Extra Corpor Technol 2004; 36: 28-35.
32. Tamimi FM, Montalvo S, Tresguerres I, Blanco Jerez L. A comparative study of 2 methods for obtaining platelet-rich plasma. J Oral Maxillofac Surg 2007; 65:1084-93.
33. Kuffler DP. Variables affecting the potential efficacy of PRP in providing chronic pain relief. J Pain Res 2019; 12: 109-16.
34. Bielecka AC, Ehrenfest DMD, Lubkowska A, Bielecki T. Microbicidal properties of leukocyte-and platelet-rich plasma/fibrin (L-PRP/L-PRF): New perspectives. J Biol Regul Homeost Agents 2012; 26 (2 Suppl 1): 43S-52S.
35. Lana JF, Macedo A, Ingrao ILG, Huber SC, Santos GS, Santana MHA. Leukocyte-rich PRP for knee osteoarthritis: Current concepts. J Clin Orthop Trauma 2019: 10 (Suppl 1): S179-S82.
36. Milants C, Bruyere O, Kaux JF. Responders to platelet-rich plasma in osteoarthritis: A technical analysis. Biomed Res Int 2017: 7538604.

INCIDENCE AND RISK FACTORS OF DIABETIC RETINOPATHY AMONG PATIENTS WITH TYPE 2 DIABETES IN A COMMUNITY HOSPITAL, CENTRAL THAILAND

Patcharapol Wittayatechakul, *Paniti Hanyos*, *Ram Rangsin*, *Boonsub Sakboonyarat*

Department of Military and Community Medicine, Phramongkutklao College of Medicine, Bangkok, Thailand

Abstract

Background: Diabetic retinopathy (DR) is one of the ocular complications among patients with type 2 diabetes (T2D) in both developed and developing countries. At present, epidemiological data of DR and the risk factors among patients with T2D especially in Thai community hospitals have been limited.

Methods: A retrospective cohort study was conducted between January 1, 2013 and December 31, 2020 to determine the incidence and risk factors of DR among patients with T2D visiting Tha Wung Hospital, Lop Buri Province, central Thailand. DR was determined according to the International Classification of Diseases, Tenth Revision codes in E113 presented in medical records. Multivariate Cox regression analysis was performed to obtain the adjusted hazard ratios (HR) and 95% confidence interval (CI) of the factors related to DR.

Results: A total of 2007 patients with T2D were enrolled in the present study. During the study period, participants (5.3%) had a diagnosis of DR; the incidence rate was 0.9 per 100 person-years (95% CI; 0.7-1.1). The independent risk factors for DR included HbA1c ≥8% (adjusted hazard ratio (AHR) = 4.7, 95% CI; 2.5-8.7), urine albumin 3+ (AHR = 2.4, 95% CI; 1.1-5.3), urine albumin 4+ (AHR = 20.3, 95% CI; 2.7-150.9), and a longer distance between residential area (AHR= 1.3, 95% CI; 1.2-1.4).

Conclusion: Patients with T2D should be encouraged to reach their glycemic control indicated by HbA1c level. Additionally, effective health interventions should be conducted to contribute appropriate access to diabetic care for patients residing in remote areas.

Keywords: Diabetic retinopathy, Community hospital, Thailand, Incidence, Risk factors

J Southeast Asian Med Res 2020; 5(1): 11-20
http://www.jseamed.org

Correspondence to:
Sakboonyarat B, Department of Military and Community Medicine, Phramongkutklao College of Medicine, Bangkok, Thailand
E-mail: boonsub1991@pcm.ac.th

Received: 10 May 2021
Revised: 25 May 2021
Accepted: 7 June 2021

Introduction

Diabetic retinopathy (DR) not only affects health and quality of life, but also presents a costly burden to patients with type 2 diabetes (T2D), their family members, communities and public health systems. Estimated DR prevalence was 18.45 to 36.2%, globally; additionally, DR prevalence has increased over the past decade.[1-4] Furthermore, the annual incidence of DR among patients with T2D were 4.0%, 5.0%, 10.4% and 12.7% in Europe, Sweden, the US, and China, respectively.[5-8] Related studies have reported that essential risk factors for DR included duration of T2D diagnosis, history of insulin used[1,2,4], hemoglobin A1C level[1,4,9], high blood pressure[4, 9], pulse pressure[10, 11] and lengthy distance from home to obtain care.[12] In many countries such as Germany, the cost of DR increased with the severity of DR accounting for 1.5% of the cost to the public health system in 2002.[13] In the US, people with financial burden were poorly followed up for treatment.[14] Moreover, a rapidly growing rate of diabetes prevalence and complications in India has been observed, leading to an increase in public health issues.[15]

However, epidemiological data of DR and the risk factors among patients with T2D especially in Thai community hospitals were limited. Thus, this study collected data from Tha Wung Hospital, Lop Buri Province central Thailand to determine the incidence and risk factors of DR among patients with T2D over the past decade.

Methods

Study designs and subjects

A retrospective cohort study was conducted between January 1, 2013 and December 31, 2020 to determine the incidence and risk factors of DR among patients with T2D. The data were retrieved from the electronic medical records of patients with T2D visiting Tha Wung Hospital. Inclusion criteria for this study comprised patients with T2D aged at least 18 years receiving medical treatment at the noncommunicable diseases (NCDs) clinic of Tha Wung Hospital. Any patient presenting a history of retinal complication or receiving a diagnosis of DR by the ophthalmologists before 2013 was excluded. The study was reviewed and approved by the Royal Thai Army Medical Department Institutional Reviewed Board (approval number R197h/63).

Data collection

A standardized case report form was used to collect data from the electronic medical records, including demographic characteristics, comorbidities and laboratory test results. Collected data included age, sex, distance between residence area and hospital, risk behaviors including smoking and alcohol consumption, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), low density lipoprotein cholesterol (LDL), diagnosed DR and comorbidities including hypertension (HT) and dyslipidemia (DLP). T2D was defined by Diabetes Care, 2013 as FPG $\geq$126 mg/dL and confirmed by repeat testing at a second visit, or HbA1c $\geq$6.5%.[16] DR was determined according to the International Classification of Diseases, Tenth Revision Codes in E113, presented in medical records.[17] BMI was calculated as body weight in kilograms divided by height in meters squared. The pulse pressure (PP) was calculated as the difference of SBP and DBP levels.

Statistical analysis

Data were analyzed using StataCorp, 2021. *Stata Statistical Software: Release 17*. College Station, TX, USA: StataCorp LLC. Demographic characteristics were presented using descriptive statistics. Categorical data were illustrated as number and percentage while continuous data were illustrated as mean and standard deviation (SD). The incidence rates per 100 person-months of observation were calculated for DR. The person -times of observation of those participants with diagnosed DR were censored at the date of the disease recorded. Multivariate Cox regression analysis was performed to obtain the adjusted hazard ratios (HR) and 95% confidence interval (CI) of the factors related to DR, and statistical significance was set at p-value <0.05.

Results

A total of 2007 patients with T2D was enrolled in the present study. The enrolled participants were aged 59.0±11.0 years. The majority of participants were female (68.7%), and one half of enrolled participants had DLP and HT comorbidities. At baseline, almost 20% of the subjects were current smokers. The average BMI of participants was 26.8±4.7 kg/m^2. The average of HbA1c level of participants at baseline was 7.4±1.8% while one half of those had HbA1c ≥7%. The average distance between the residential area of participants and hospital was 4.8±4.2 km. The demographic data are presented in **Table 1**.

During the study period, for participants (5.3%) with diagnosed DR; the incidence rate was 0.9 per 100 person-years. (95% CI; 0.7-1.1). **Figure 1** illustrates the cumulative incidence of DR among patients with T2D in relation to HbA1c level (p<0.001). Univariate and multivariate cox regression analyses identifying risk factors for DR are shown in **Tables 2 and 3**. The independent risk factors for DR included HbA1c ≥8% (adjusted hazard ratio (AHR) = 4.7, 95% CI; 2.5-8.7), urine albumin 3+ (AHR = 2.4, 95% CI; 1.1-5.3), urine albumin 4+ (AHR = 20.3, 95% CI; 2.7-150.9), and a longer distance between residential area (AHR= 1.3, 95% CI; 1.2-1.4).

Table 1. Demographic characteristic of participants (n=2007)

Characteristics	n (%)
Gender	
Male	628 (31.29)
Female	1379 (68.71)
Age (year)	59.0±11.0
<40	88 (4.38)
40-59	948 (47.23)
≥60	971 (48.38)
Comorbidities	
Type 2 diabetes (T2D) only	368 (18.34)
T2D with dyalipidemia (DLP)	370 (18.44)
T2D with hypertension (HT)	275 (13.70)
T2D with DLP and HT	994 (49.53)
Current alcohol consumption	
No	1529 (76.34)
Yes	474 (23.66)

Table 1. Demographic characteristic of participants (n=2007) (continue)

Characteristics	n (%)
Current smoker	
No	1604 (80.04)
Yes	400 (19.96)
Body Mass Index (kg/m²)	26.79±4.74
<18.5	43 (2.18)
18.5-22.99	355 (18.04)
23.0-24.99	358 (18.19)
25.0-29.99	791 (40.19)
≥30.0	421 (21.39)
Fasting blood sugar (mg/dL)	153.69±39.54
HbA1c levels (mg%)	7.41±1.77
<7.0	882 (50.26)
7.0-7.9	402 (22.91)
≥8.0	471 (26.84)
Distance from residential area (km)	2.28±1.82

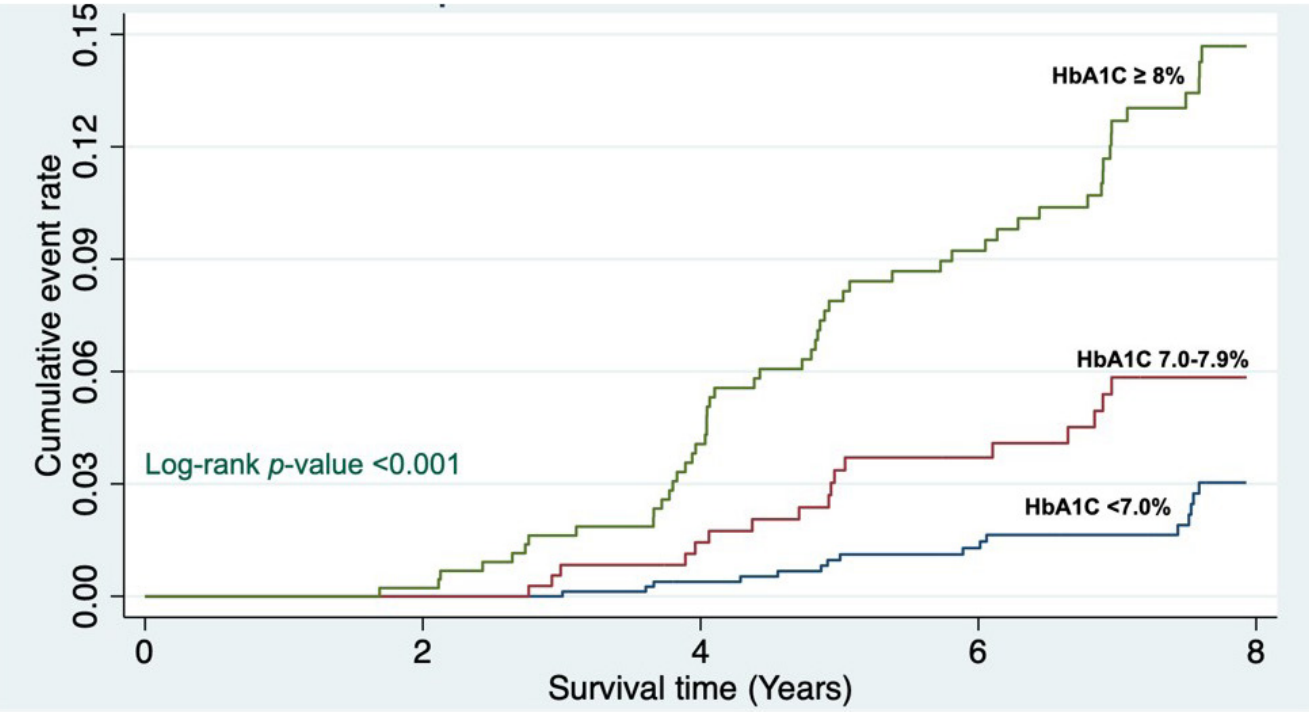


Figure 1. Cumulative incidence of diabetic retinopathy in patients with type 2 diabetes in relation to HbA1c level.

Table2. Univariate analysis for risk factors of DR among patients with T2D in community hospital, central, Thailand

Factors	Total participants	No. of DR	Person-Years	Incidence Rate (/100 person-years)	Hazard Ratio	95% CI	*p*-value
Genders	2007	107	11920.36	0.90			
Female	1379	75	8358.37	0.90	1.00		
Male	628	32	3561.99	0.90	1.03	0.68-1.56	0.875
Age (years)	2007	107	11920.36	0.90	0.97	0.95-0.98	<0.001
Current alcohol consumption	2003	107	11920.36				
No	1529	83	8800.95	0.94	1.00		
Yes	474	24	3098.76	0.78	0.78	0.50-1.23	0.289
Current smoker	2004	107	11902.02				
No	1604	84	9256.90	0.91	1.00		
Yes	400	23	2645.12	0.87	0.91	0.57-1.44	0.675
Comorbidities	2007	107	11920.37				
DM only	368	28	1703.69	1.64	1.00		
DM with DLP	370	21	2206.31	0.95	0.51	0.29-0.90	0.020
DM with HT	275	8	1370.12	0.58	0.34	0.15-0.74	0.007
DM with DLP and HT	994	50	6640.25	0.75	0.38	0.24-0.61	<0.001
Duration of DM (months)	1064	65	6666.31	0.98	0.99	0.99-1.00	0.383

Table2. Univariate analysis for risk factors of DR among patients with T2D in community hospital, central, Thailand (continue)

Factors	Total participants	No. of DR	Person-Years	Incidence Rate (/100 person-years)	Hazard Ratio	95% CI	*p*-value
Body Mass Index (kg/m^2)	1968	106	11796.23	0.90	0.96	0.92-1.01	0.093
<18.5	43	4	259.82	1.54	1.00		
18.5-22.9	355	25	2146.89	1.16	0.75	0.26-2.15	0.593
23.0-24.9	358	21	2145.91	0.98	0.64	0.22-1.86	0.411
25.0-29.9	791	39	4763.60	0.82	0.53	0.19-1.49	0.228
≥30.0	421	17	2480.01	0.69	0.45	0.15-1.33	0.147
Systolic blood pressure(mmHg)	1963	106	11778.31	0.90	1.00	0.99-1.02	0.717
<140	1,536	78	8922.51	0.87	1.00		
≥140	427	28	2855.80	0.98	1.06	0.69-1.63	0.797
Diastolic blood pressure (mmHg)	1963	106	11778.31	0.90	1.01	0.99-1.04	0.318
<90	1868	100	11163.19	0.90	1.00		
≥90	95	6	615.12	0.98	1.06	0.46-2.42	0.890
Fasting plasma glucose (mg/dL)	1893	104	1148.84	0.91	1.02	1.01-1.02	<0.001
HbA1c level (mg%)	1755	86	10912.20	0.79	1.37	1.27-1.48	<0.001
<7.0	882	16	5496.35	0.29	1.00		
7.0-7.9	402	17	2467.50	0.69	2.37	1.20-4.68	0.013
≥8	471	53	2948.35	1.80	6.08	3.48-10.64	<0.001

Table 2. Univariate analysis for risk factors of DR among patients with T2D in community hospital, central, Thailand (continue)

Factors	Total participants	No. of DR	Person-Years	Incidence Rate (/100 person-years)	Hazard Ratio	95% CI	*p*-value
HDL cholesterol level (mg/dL)	1864	104	11348.15	0.92	1.00	0.98-1.02	0.942
<40 in male, <50 in female	882	56	5708.62	0.98	1.00		
≥40 in male, ≥50 in female	982	48	5639.53	0.85	0.91	0.62-1.34	0.641
LDL cholesterol level (mg/dL)	1861	103	11333.40	0.91	1.00	1.00-1.01	0.113
<100	675	38	4136.35	0.92	1.00		
≥100	1186	65	7197.05	0.90	1.00	0.67-1.48	0.972
Total cholesterol level (mg/dL)	1659	38	10197.38	0.37	1.00	1.00-1.01	0.460
<200	1121	23	6929.71	0.33	1.00		
≥200	538	15	3267.67	0.46	1.34	0.70-2.56	0.383
Triglyceride level (mg/dL)	1866	104	11362.55	0.92	1.00	1.00-1.00	0.036
<150	1004	46	6037.94	0.76	1.00		
≥150	862	58	5324.61	1.09	1.42	0.97-2.10	0.074
Urine albumin	1682	86	10338.63				
Negative/Trace	1465	70	9101.81	0.77	1.00		
1+	83	3	462.23	0.65	0.85	0.27-2.70	0.785
2+	74	4	433.82	0.92	1.22	0.45-3.34	0.698
3+	57	8	328.34	2.44	3.23	1.56-6.72	0.002
4+	3	1	12.43	8.04	11.55	1.60-83.21	0.015
Distance from residential area (km)	2007	107	11920.36	0.89	1.30	1.22-1.37	<0.001

Table 3. Multivariate analysis for risk factors of DR among patients with T2D in community hospital, central, Thailand

Factors	Adjusted Hazard Ratio	95% CI	*p*-value
Age (years)	0.98	0.96-1.00	0.116
Male vs Female	1.07	0.65-1.76	0.785
Insulin used	1.25	0.68-2.29	0.467
HbA1c level (%)			
<7.0	1		
7.0-7.9	1.70	0.81-3.59	0.154
≥8	4.70	2.54-8.75	<0.001
Urine albumin			
Negative/Trace	1		
1+	0.71	0.22-2.30	0.581
2+	0.92	0.27-3.06	0.879
3+	2.42	1.11- 5.32	0.026
4+	20.30	2.73-150.92	0.003
Distance from residential area (km)	1.32	1.23-1.42	<0.001

Discussion

The present study illustrated that the cumulative incidence of DR among patients with T2D was 5.33% which was comparable with one related report in Thailand.[10] However, compared with the incidences of DR in T2D in other countries, the incidence of those in this study were relatively low.[5-8] Notably, the cumulative incidences of DR stratified by HbA1c level significantly differed. We found that HbA1c level ≥8% at the time of enrollment was a potential risk for DR. A related nationwide study in Thailand also indicated that the insulin used was associated with DR.[10] This phenomenon could be explained by prolongated elevated blood sugar level and poorly controlled HbA1c leading to small vessels injury and occlusion that accompanied abnormal neovascularization resulting in vascular rupture.[18] Our data suggested that effective interventions such as glycemic controlled should be implemented among patients with T2D to alleviate diabetic complications including DR and other cardiovascular complications. The present study reported that insulin used held a positive relationship to the incidence of DR; nevertheless, this was not statistically significant in the final model.

Our finding reported that higher level of urine albumin screened at baseline was an independent risk for DR among patients with T2D. Similarly, related evidence in Brazil demonstrated that proliferative DR was associated with microalbuminuria among patients with T2D.[19] Furthermore, one related study indicated that an increase in albumin creatinine ratio predicted the risk for DR.[20] The laboratory results of diabetic nephropathy could provide information regarding the severity of current diabetes as shown in our analysis. Albuminuria might represent a state of generalized vascular dysfunction that described a higher risk among patients with high urine albuminuria.[21]

Our study site represented a community hospital and implied the limited setting of public health care resources. Our study presented that a longer distance between residential area and the hospital was related to risk for DR. One related qualitative study in China indicated that lengthy travel times and transportation barriers may affect the quality of care of patients with T2D, leading to the occurrence of complications.[12] Our data suggested that effective interventions such as fundoscopic exam and teleophthalmology for remote grading should be provided at the community level including primary care units. Further, patients with T2D should be encouraged to receive appropriate care.[22]

According to secondary data used for analysis, some variables were incomplete. Another limitation was the small sample size in the study; therefore, the association between well-known risk factors such as elevated blood pressure, cholesterol level and outcome could not be presented. The result of our study may not be generalized to the whole country but may reflect the real situation of patients with T2D visiting a Thai community hospital.

Conclusion

We reported the incidence and risk factors of DR among patients with T2D visiting a community hospital. Patients with T2D should be encouraged to reach their glycemic control indicated by HbA1c level. Additionally, effective health interventions should be conducted to contribute to appropriate access to diabetic care for patients residing in remote areas.

Conflict of interest

The authors declare they have no conflict of interest.

References

1. Du ZD, Hu LT, Zhao GQ, Ma Y, Zhou ZY, Jiang -T. Epidemiological characteristics and risk factors of diabetic retinopathy in type 2 diabetes mellitus in Shandong Peninsula of China. Inter J Ophthalmol 2011; 4: 202.
2. Giloyan A, Harutyunyan T, Petrosyan V. The prevalence of and major risk factors associated with diabetic retinopathy in Gegharkunik province of Armenia: cross-sectional study. BMC Ophthalmol 2015; 15: 1-7.
3. Willis JR, Doan QV, Gleeson M, Haskova Z, Ramulu P, Morse L, et al. Vision-related functional burden of diabetic retinopathy across severity levels in the United States. JAMA Ophthalmol 2017; 135: 926-32.
4. Song P, Yu J, Chan KY, Theodoratou E, Rudan I. Prevalence, risk factors and burden of diabetic retinopathy in China: a systematic review and meta-analysis. J Glob Health 2018; 8: 010803.
5. Li JQ, Welchowski T, Schmid M, Letow J, Wolpers C, Pascual-Camps I, et al. Prevalence, incidence and future projection of diabetic eye disease in Europe: a systematic review and meta-analysis. Eur J Epi 2020; 35: 11-23.
6. Henricsson M, Nystrom L, Blohme G, Ostman J, Kullberg C, Svensson M, et al. The incidence of retinopathy 10 years after diagnosis in young adult people with diabetes: results from the nationwide population based diabetes incidence study in Sweden (DISS). Diabetes Care 2003; 26: 349–54.
7. Jin P, Peng J, Zou H, Li M, Li J, Xie SY, et al. The 5 year onset and regression of diabetic retinopathy in Chinese type 2 diabetes patients. PLoS One 2014; 9: e113359.
8. Varma R, Choudhury F, Klein R, Chung J, Torres M, Azen SP, et al. Four year incidence and progression of diabetic retinopathy and macular edema: the Los Angeles Latino Eye Study. Am J Ophthalmol 2010; 149: 752–61. e1–3.
9. Khan R, Singh S, Surya J, Sharma T, Kulothunga V, Raman R. Age of onset of diabetes and its comparison with prevalence and risk factors for diabetic retinopathy in a rural population of India. Ophthalmic Res 2019; 61: 236-42.
10. Euswas N, Phonnopparat N, Morasert K, Thakhampaeng P, Kaewsanit A, Mungthin M, et al. National trends in the prevalence of diabetic retinopathy among Thai patients with type 2 diabetes and its associated factors from 2014 to 2018. Plos One 2021; 16: e0245801.
11. Reutrakul S, Deerochanawong C. Diabetes in Thailand: status and policy. Curr Diab Rep 2016; 16: 28.
12. Liu Y, Zupan NJ, Shiyanbola OO, Swearingen R, Carlson JN, Jacobson NA, et al. Factors influencing patient adherence with diabetic eye screening in rural communities: A qualitative study. PLoS One 2018; 13: e0206742.
13. Happich M, Reitberger U, Breitscheidel L, Ulbig M, Watkins J. The economic burden of diabetic retinopathy in Germany in 2002. Graefe's Arch Clin Exp Ophthalmol 2008; 246: 151-9.
14. Peavey JJ, D'Amico SL, Kim BY, Higgins ST, Friedman DS, Brady CJ. Impact of socio economic disadvantage and diabetic retinopathy

severity on poor ophthalmic follow-up in a rural Vermont and New York population. Clin Ophthalmol 2020 ; 14: 2397-403.
15. Pradeepa R, Mohan V. Prevalence of type 2 diabetes and its complications in India and economic costs to the nation. Eur J Clin Nutr 2017; 71: 816-24.
16. Association AD. Standards of medical care in diabetes-2013. Diabetes Care 2013; 36 (Suppl 1): S11–66.
17. Organization WH. ICD-10: international statistical classification of diseases and related health problems. 10th revision, 5th edition, 2016.
18. Cai J, Boulton M. The pathogenesis of diabetic retinopathy: old concepts and new questions. Eye 2002; 16: 242-60.
19. Boelter MC, Gross JL, Canani LH, Costa LA, Lisboa HR, Três GS, et al. Proliferative diabetic retinopathy is associated with microalbuminuria in patients with type 2 diabetes. Braz J Med Biol Res 2006; 39: 1033-9.
20. Lee MK, Han KD, Lee JH, Sohn SY, Hong OK, Jeong JS, et al. Normal-to-mildly increased albuminuria predicts the risk for diabetic retinopathy in patients with type 2 diabetes. Sci Rep 2017; 7: 1-8.
21. Rani PK, Raman R, Gupta A, Pal SS, Kulothungan V, Sharma T. Albuminuria and diabetic retinopathy in type 2 diabetes mellitus Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetic Study (SN-DREAMS, report 12). Diabetol Metab Syndr 2011; 3: 1-8.
22. Vujosevic S, Aldington SJ, Silva P, Hernández C, Scanlon P, Peto T, et al. Screening for diabetic retinopathy: new perspectives and challenges. Lancet Diabetes Endocrinol 2020; 8: 337-47.

REAL TIME RT-PCR ASSAY TO DETECT SARS-COV-2 IN THAILAND

Sutchana Tabprasit, Krongkan Saipin, Kamonwan Siriwatthanakul, Min Kramyoo, Watcharee Yokanit, Wuttikon Rodkvamtook, Kunakorn Kana, Pramote Imwattana, Thanainit Chotanaphuti

The Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Abstract

The Armed Forces Research Institute of Medical Sciences (AFRIMS) conducts medical research and disease surveillance to develop and evaluate medical products, vaccines and diagnostics to protect Royal Thai Army personnel from infectious diseases. Currently regarding globalized travel, infectious diseases pose a constantly evolving threat, indiscriminately transcending national, regional and even intercontinental boundaries. Since the COVID-19 outbreak, AFRIMS has gained knowledge from diagnostic tests for SARS-CoV-2 using the Centers for Disease Control and Prevention (CDC) as a reference protocol. We set up and developed the molecular diagnosis detection for SARS-CoV-2, Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR), to analyze the nucleocapsid (N) genome of SARS-CoV-2 which is a standardized method in the laboratory. AFRIMS is certified by the Department of Medical Science, Ministry of Public Health (MOPH) as a COVID-19 laboratory network. We provided COVID-19 screening services to government units and private hospitals in late February 2020. It could be stated that AFRIMS is the first military unit to be certified by the MOPH. Since the COVID-19 pandemic started in Bangkok, 3,172 samples have been tested and 96 samples have been confirmed. Detecting viral RNA not only aids in the diagnosis of illness but also provides epidemiological and surveillance information.

Keywords: Real time RT-PCR, COVID-19, SARS-CoV-2

J Southeast Asian Med Res 2021: 5 (1): 21-25
http://www.jseamed.org

Correspondence to: Tabprasit S, Chief of Microbiology Section, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand
E-mail: Sutchanat@afrims.org, Suttab@yahoo.com

Received: 7 October 2020
Revised: 23 November 2020
Accepted: 03 December 2020

Introduction

Emerging and reemerging pathogens constitute global problems for public health.[1] Coronaviruses are enveloped RNA viruses that cause diseases broadly among humans and other mammals such as respiratory, enteric, hepatic and neurologic diseases.[2,3] Six coronavirus species are known to cause human disease.[4] Four viruses, 229E, OC43, NL63 and HKU1, are prevalent and typically cause common cold symptoms among immunocompetent individuals.[4] The two other strains cause severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). They are zoonotic in origin and during 2002 and 2003; SARS-CoV was the causal agent of severe acute respiratory syndrome outbreaks in Guangdong Province, China.[5-8] Later, MERS-CoV was the pathogen responsible for severe respiratory disease outbreaks in 2012 in the Middle East.[9] In 2020, the emergence of Coronavirus disease (COVID-19) outbreak, caused by a novel coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has spread globally. The outbreak of pneumonia caused by the SARS-CoV-2 continues to pose a serious threat to people's health. A better understanding of this new virus and developing ways to control its spread are needed imminently. However early detection and treatment is necessary for to prevent and control the outbreak. The SARS-CoV-2 was identified by RT-PCR[10] Specimens of the upper and lower respiratory tracts, such as bronchi or alveolar lavage and deep cough sputum were collected from each case, as well as serum from the onset and 14 days after onset.[11] At that time, testing in Thailand was limited to laboratories certified under the Department of Medical Sciences, MOPH so we attempted to set up Real time RT-PCR to diagnostically detect SARS-CoV-2 (COVID-19) to cope with outbreaks.

Methods

We have used Real time RT-PCR assays for in vitro qualitative detection of SARS-CoV-2 in respiratory specimens.[12] (**Table 1**) A variety of RNA gene targets are used by different protocols, with most tests targeting 1 or more of the envelope (*env*), nucleocapsid (*N*), spike (*S*), RNA polymerase and *ORF1* genes.[12] The primer and probe sets are designed to specifically detect SARS-CoV-2. This protocol is used to detect three points of the N gene of SARS-CoV-2 (**Figure1**). In addition, respiratory specimens include nasopharyngeal or oropharyngeal aspirates or washes, nasopharyngeal or oropharyngeal swabs, bronchoalveolar lavage, tracheal aspirates and sputum. Moreover, swab specimens should be collected only on swabs with a synthetic tip (such as polyester or Dacron®) with aluminum or plastic shafts. Swabs with calcium alginate or cotton tips with wooden shafts are unacceptable. Performing Real time RT-PCR amplification-based assays depends on the amount and quality of sample template RNA. RNA extraction procedures should be qualified and validated for recovery and purity before testing specimens. Commercially available extraction procedures have been shown to generate highly purified RNA when following manufacturer's recommended procedures for sample extraction. Retained residual specimens and nucleic extracts should be stored immediately at -70°C. We did not freeze/thaw extracts and specimens more than once before testing. Due to the sensitivity of Real time RT-PCR, these assays should be conducted using strict quality control and quality assurance procedures. A false negative result may occur when inadequate numbers of organisms are present in the specimen due to improper collection, transport or handling. The cycle threshold (Ct) is the number of replication cycles required to produce a fluorescent signal, with lower Ct values representing higher viral RNA loads. A Ct value less than 40 is clinically reported as PCR positive. The interpreted results are described in **Table 2**.

Table 1. Real time RT-PCR protocol
Protocol preparation for real time RT-PCR

Step	Cycles	Temp	Time
RT incubation	1	95°C	3 min
Enzyme activation	1	50°C	30 sec
Amplification	45	95°C	10 sec
		55°C	30 sec

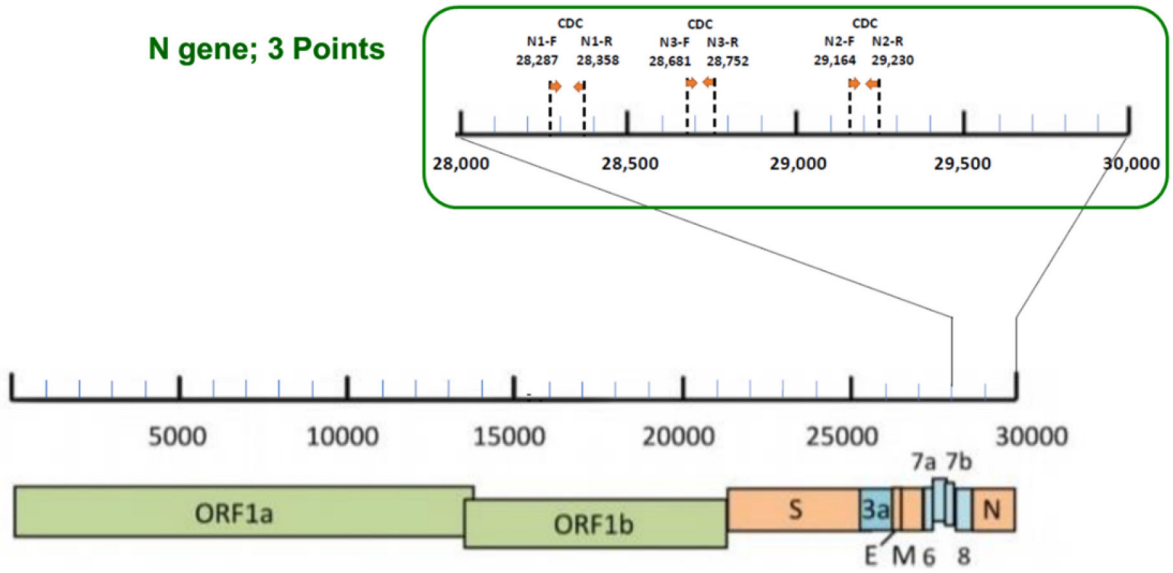


Figure 1. Detecting three points of the N gene of SARS-CoV-2 (N1, N2 and N3)

Table 2. SARS-CoV-2 real time RT-PCR diagnosis panel result interpretation

2019 nCOV_N1	2019 nCOV_N2	2019 nCOV_N3	RP	Result Interpretation[a]
+	+	+	±	2019-nCOV detected
If only one, or two, of three targets is positive			±	Inconclusive Result
-	-	-	+	2019-nCOV not detected
-	-	-	-	Invalid Result

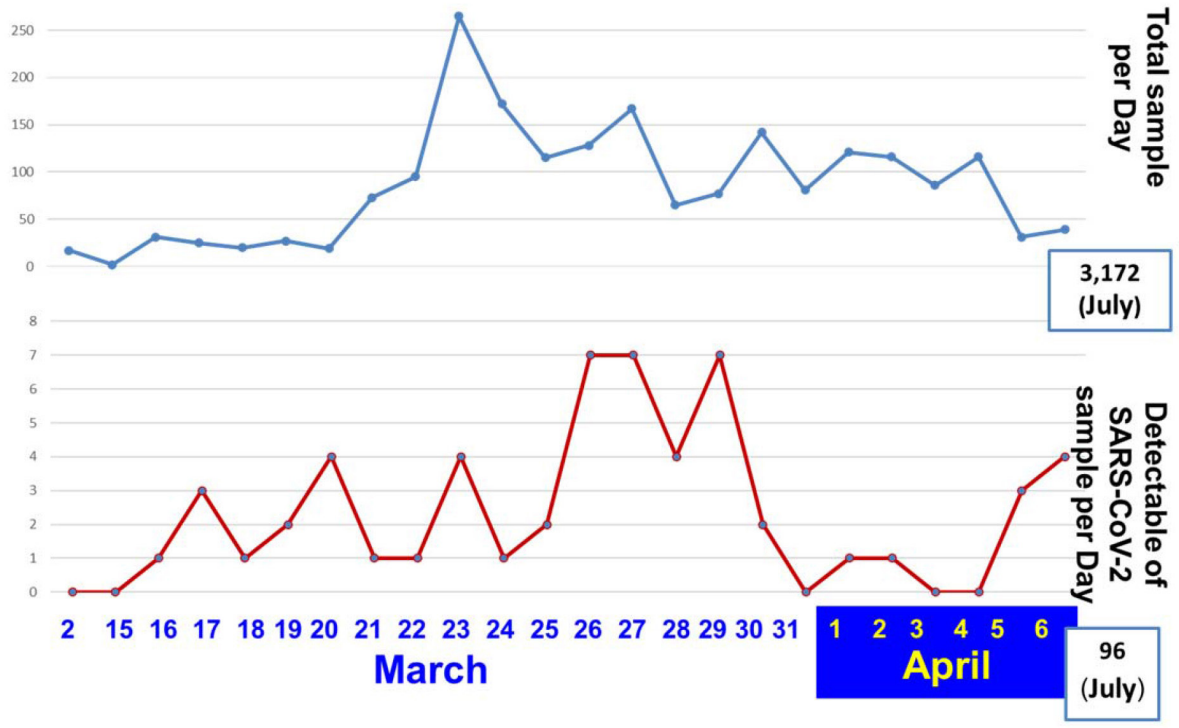


Figure 2. Number of total samples and detectable SARS-CoV-2 samples daily

Results

Timely and accurate laboratory testing of cases under investigation is an essential part of managing COVID-19 outbreaks. We should set up reliable SARS-CoV-2 testing laboratories to perform primary detection or confirmatory testing. AFRIMS is currently working closely with researchers so the diagnostics test will be set and validated promptly. As sequence information from the SARS-CoV-2 has recently been made available, PCR assays can be designed to detect these sequences. Moreover, sequence data can providevaluable information to understand the origin of a virus and how it spreads. After publishing the sequence of SARS-CoV-2, we set and validated the protocol to diagnostically detect SARS-CoV-2 (COVID-19). In February, all reagents, specific primers and specific probes were ready to perform Real time RT-PCR for COVID-19. After that, the virology laboratory was certified by the Department of Medical Sciences, MOPH 9 March 2020. However, Phramongkutklao Hospital sent the first specimen to AFRIMS in late February. In the middle of March, private hospitals sent COVID-19 suspected cases to diagnostically detect SARS-CoV-2.

Patients meeting the case definition for suspected SARS-CoV-2 infection should be screened for the virus using Real time RT-PCR. Since the COVID-19 outbreak, we obtained specimens to detect more than 3000 SARS-CoV-2cases. Most cases came from the PMK Hospital and private hospitals. Ninety-six samples (3%) had the SARS-CoV-2 *N* genome, while 3,076 samples (97%) did not reveal the SARS-CoV-2 *N* genome as shown in **Figure 2**. In the beginning, detectable cases of SARS-CoV-2 or inconclusive results with external assistance were confirmed by the reference laboratory (Department of Medical Sciences, MOPH) that deployed the additional or confirmatory assays. To report detectable SARS-CoV-2 *N* genome results, all laboratories should follow the national reporting requirements, but in general, suspected cases should be reported to relevant public health authorities as soon as the laboratory receives a specimen. All test results, whether positive or negative, should likewise be immediately reported to authorities. Laboratories should also periodically report the number of test results to the MOPH weekly. The first wave of the COVID-19 outbreak in Thailand occurred in

the middle of March. At that time, we performed Real time RT-PCR to detect SARS-CoV-2 more than fifty samples daily. The highest number COVID-19 suspected cases totaled 262 samples from PMK and private hospitals 23 March 2020. COVID-19 infection has slightly decreased since April.

Discussion

All users, analysts and any individuals reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results before performing the assay independently. Moreover, collecting multiple specimens (types and time points) from the same patient may be necessary to detect the virus. A false-negative result may occur when a specimen is improperly collected, transported or handled and may also occur when amplification inhibitors are present in the specimen or when inadequate numbers of organisms are present in the specimen. If the virus mutates in the Real Time RT-PCR target region, SARS-CoV-2 may be undetected or may be detected less predictably.

In every individual with COVID-19 infection, the Real time RT-PCR showed three point of the *N* gene and the PCR positive cases exhibited the same results with the reference laboratory (Department of Medical Sciences, MOPH). The number of suspected cases slightly increased in midMarch which was the same time as the first wave outbreak of SARS-CoV-2 in Thailand. Thus, we can use these principles as the model to detect unknown diseases in the future.

Acknowledgements

The following people contributed to diagnostically detecting SARS-CoV-2 (COVID-19) using real time RT-PCR: Krongkan Saipin, Kamonwan Siriwatthanakul, Min Kramyoo, Watcharee Yokanit, Wuttikon Rodkvamtook, Kunakorn Kana, Pramote Imwattana and Thanainit Chotanaphuti.

References

1. Gao GF. From “A” IV to “Z” IKV: attacks from emerging and re-emerging pathogens. Cell 2018; 172: 1157-9.
2. Weiss SR, Leibowitz JL. Coronavirus pathogenesis. Adv Virus Res 2011; 81: 85-164.
3. Masters PS, Perlman S. Coronaviridae. In: Knipe DM, Howley PM, eds. Fields virology. 6th ed. Lippincott Williams & Wilkins, 2013: 825-58.
4. Su S, Wong G, Shi W, Liu J, Lai ACK, Zhou J, et al. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol 2016; 24: 490-502.
5. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019; 17: 181-92.
6. Zhong NS, Zheng BJ, Li YM, Poon LLM, Xie ZH, Chan KH, et al. Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People’s Republic of China, in February 2003. Lancet 2003; 362: 1353-8.
7. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 2003; 348: 1953-66.
8. Drosten C, Günther S, Preiser W, Werf SVD, Brodt HR, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 2003; 348: 1967- 76.
9. Zaki AM, Van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 2012; 367: 1814-20.
10. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW et al, Diagnostic detection of 2019-nCoV by real-time RT-PCR. Eurosurveillance 25(3), DOI: 10.2807/1560-7917.ES.2020.25.3.2000045
11. Zhou Wang, Wang Qiang and Hu Ke. A Handbook of 2019 nCoV Pneumonia Control and Prevention. Hubei Science and Technology Press
12. Real-Time RT-PCR Panel for Detection 2019-Novel Coronavirus by Centers for Disease Control and Prevention (CDC) Effective: 03/30/2020

DURAL REPAIR: EFFICACY ASSESSMENT OF DIFFERENT TECHNIQUES, A CADAVERIC STUDY COMPARING THE NAKED EYE AND SURGICAL LOUPES

Roongrath Chitragran, Sakpan Panyaporn**, Sompob Poopitaya**

***Spine Surgery Unit, Department of Orthopaedics, Phramongkutklao Hospital and Phramongkutklao College of Medicine, Bangkok, Thailand**
****Department of Orthopaedic Surgery, Ananda Mahidol Army Hospital, Lopburi, Thailand**

Abstract

Background: Watertight dural repair is crucial to achieve successful dural tear sutures. Microscopic or surgical loupes are recommended to use to magnify and assist repairing the dura. However, many spine surgeons repair dural tears under the naked eye. The efficacy of repairing dural tears by the naked eye compared with microscopic or surgical loupes has never been studied.

Objective: This study aimed to compare the efficacy of dural repairing techniques using the naked eye or surgical loupes.

Methods: A cadaveric experimental study was conducted. Four fresh human cadaveric specimens were used to harvest the spinal cord. Dural tear and CSF leakage were simulated with a water pressure control system (Arthrex AR-6475 arthroscopic pump). We compared surgical repair using the naked eye and surgical loupes. Surgical closure was achieved using Prolene 6-0 and Durepair®. A total of 32 experimental dural tears were subdivided to four groups. The 4 groups were Prolene6-0 with the naked eye (n=8), Prolene 6-0 with surgical loupe (n = 8), Durepair® with the naked eye (n=8) and Durepair® with surgical loupe (n=8). The total time used for sutures and postsuture CSF water leakage pressure were recorded and compared among the subgroups.

Results: Our results showed that surgical loupe assisted dural closure and sutures were significantly faster than the naked eye in both Prolene 6-0 (surgical loupe = 4.87±0.19 min, naked eye = 7.18±0.36 min, p <0.001) and Durepair® groups (surgical loupe = 9.84±0.21 min naked eye = 13.27±0.42 min, p <0.001). CSF Leakage pressure in the surgical loupe groups were higher than in the naked eye groups in both Prolene 6-0 (surgical loupe = 100.00±5.35 mmHg, naked eye = 96.88±7.99 mmHg, p = 0.373) and Durepair® (surgical loupe = 96.88±4.58 mmHg, naked eye = 95.63±4.17 mmHg, p = 0.577) but without significant difference. Prolene 6-0 was significantly faster to use for sutures than Durepair® in both sutures by the naked eye and surgical loupe assisted (p <0.001). Prolene 6-0 showed a higher leakage pressure than Durepair® in both the naked eye and surgical loupe assisted sutures but without significant difference (naked eye, p = 0.701, surgical loupe, p = 0.230)

CONCLUSION: Repairing a dural tear without using surgical loupes consumed more time and did not achieve similar maximum leak pressure compared with using surgical loupes. However, no statistically significant difference was observed in terms of CSF leakage pressure. Durepair® consumed more time than Prolene 6-0 while leakage pressure was similar. We recommended the use of surgical loupes when performing dural repair. Durepair® is suitable to repair larger dural defects that cannot be closed using a simple suture technique.

Keywords: Dural tear, Dural repair, CSF leakage

J Southeast Asian Med Res 2021: 5(1):26-34
http://www.jseamed.org
Correspondence to:
Chitragran R, Department of Orthopaedics, Phramongkutklao Hospital and Phramongkutklao College of Medicine, Bangkok, Thailand
Email: roongrathc@hotmail.com

Received: 09 February 2021
Revised: 26 May 2021
Accepted: 07 June 2021

Introduction

Incidental durotomy is a frequently encountered complication during spinal surgery. The incidence ranges from 1-17% in lumbar spine surgeries and 1% in cervical spine surgeries.[1,2] Several studies have reported no changes in long term outcomes after incidental durotomy.[3-5] However it can cause significant morbidity due to postural headaches, meningitis, nerve root entrapment, meningeal pseudocyst, dura-cutaneous fistula, arachnoiditis, delayed wound healing or wound infections. Indirect consequences associated with the prolonged flat bed rest that is often prescribed, include pneumonia, pressure ulcers, deep venous thrombosis, pulmonary embolism and aspiration. Incidental durotomy usually produces benign outcomes but as Goodkin and Laska reported, medicolegal implications often result from this complication.[6] Therefore, incidental durotomy is generally accepted to be primarily repaired intraoperatively.

The primary goals of a dural repair include a watertight closure and containment of nerve fascicles. The actual treatment for an accidental durotomy depends on the size and location of the tear. Primary repair is recommended in the lumbar and cervical spine. When amenable to surgical repair, the dural tear should be addressed in an expedient manner; however, when not readily accessible, careful observation, glue or a cerebrospinal fluid shunt can be employed.[7]

Many dural repair techniques are available, ranging from simple interrupted or continued sutures, glue, bioabsorbable staples and many other types of grafts and patches.[8] The suture technique employed often depends on surgeon's preference. The gold standard of microsurgical anastomosis is a simple interrupted suture technique.[8,9] Cain et al.[10] reported that no significant difference in leak pressure using interrupted versus running locked suture in a dural repair model. Erica et al.[11] showed that 6-0 Prolene, using either interrupted or locked techniques was the best at creating a watertight closure of an incidental durotomy. When a watertight seal cannot be obtained, a hydrogel of fibril glue sealant will improve the strength of repair. In the situation of a large defect that cannot be primarily repaired, a synthetic dural patch such as Duragen (Dural Graft Matrix-Integra Lifesciences Corporation) and Durepair® or a fascial graft may be used.[12]

Microsurgery has traditionally required the use of a surgical microscope. Jacobsen and Suarez showed a 100% patency of 1-mm blood vessel anastomoses performed under a surgical microscope, which helped establish the method as a reference standard.[13] Since then, microsurgery has grown to include anastomoses of different vessels, nerves and other structures. While the surgical microscope permits powerful magnification and illumination, it includes the costs of lengthier surgical setup time, greater initial expense, increased maintenance, less intraoperative positioning flexibility and the need for better coordination among surgical teams. With increased focus on occupational health and ergonomics; however, the microscope may decrease the risk of cervical spine pathology to the surgeon.[14] Surgical loupes offer widely acknowledged portability, flexibility and cost benefits compared with surgical microscopes. These benefits have led to their routine use in hand surgical procedures. Comfort and ease of use with surgical loupes in the presence of microscope-honed technical experience have led some authors to increase the use of surgical loupes while performing microsurgical procedures.[15-17] Compared with microscopes, loupes have many benefits: cost, flexibility, portability and time saving. Loupes allow closer access to the surgical field, wider orientation and rapid changes in viewing angle, depth of field and adjustment of gaze location within the surgical field through postural changes of the head and neck.[15] Luca et al.[12] recommended using a microscope and microsurgical instruments to repair incidental durotomy while Erica et al.[11] used surgical loupe magnification for dural tear repair. Many spine surgeons do not use either a microscope or surgical loupes for dural repair. Usually, dura tears are repaired using the naked eye.

In Thailand, microscopes and surgical loupes are not readily available in many hospitals due to high cost. Many studies compared the efficacy of many materials to repair tear dura; however no

study has investigated the efficacy of repairing tear dura using the naked eye. This study aimed to compare the efficacy of different dural repairing techniques, the naked eye versus surgical loupes, in terms of time spent for suturing and maximum sustainable CSF pressure. The efficacy of Prolene 6-0 vs. Durepair for dural tears was also compared.

Methods

This study was approved by the Institutional Review Board, Medical Department, Royal Thai Army. The four fresh human cadavers were obtained according to standard procedure of a cadaver laboratory. Cadavers were obtained within 7-14 days of death and stored in crypts maintained at 5°C until 2 hours before use. The cadavers were dissected, and a laminectomy was performed along the spine **(Figure 1).** A spinal cord length of 20 cm per cadaver was removed and brought to the operation room for further testing **(Figure 2).**

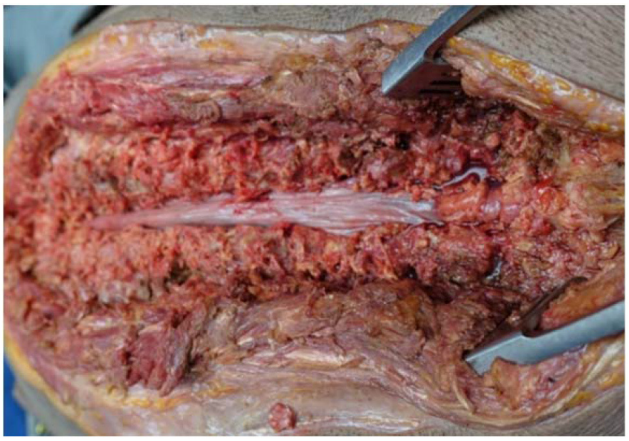


Figure1. Laminectomy along the cadaver spines before removing the spinal cord

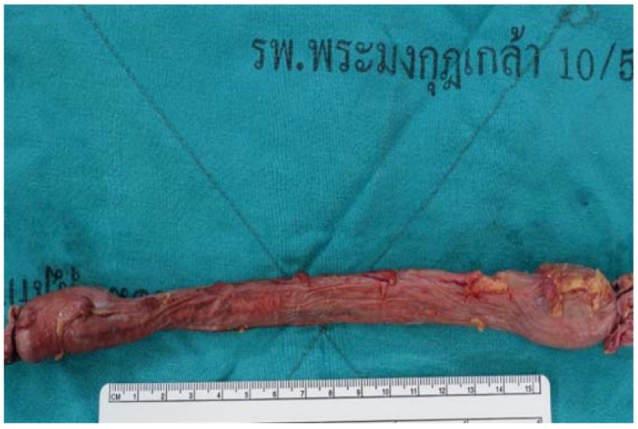


Figure 2. A length of spinal cord recovered from a cadaver for the experiment

Model

The 20 cm intact spinal cord and dura from the cadavers was brought to an operation room. Two 14 French 2-way Foley catheters were placed in the dural space at the cranial and caudal end of the specimen. These were advanced until they were positioned above and below the dura to be tested. The most caudal Foley catheter was clamped with a hemostat. The cranial Foley catheter was connected to a reservoir of normal saline attached to an Arthrex AR-6475 arthroscopic pump for continuous saline flow at adjustable difference pressure **(Figure 3).** The inflatable balloons in each catheter were inflated to isolate the spinal segment to be tested. Between the Foley catheter and the reservoir, a pressure transducer, connected to a monitor, was used to determine the pressure within the system at the level of the spinal canal **(Figure 4)**.

With the Foley catheters positioned, the clamp was opened from the reservoir to the cranial Foley catheter and saline was infused in the dural space. The pressure in the system was able to be adjusted and controlled by the arthroscopic water pump and was subjected to a wide range of pressures to simulate both bed rest and upright active positioning of the spinal column. Previous dural repair models have investigated hydrostatic pressures ranging from 14 mmHg to 80 mmHg for continued CSF leaks.[11,13] Given that leaks were previously observed at a pressure of 35 mmHg when using sutures alone and at 80 mmHg with the use of sealants, the decision was arbitrarily made to report on CSF leaks when present at 40 mmHg or until a leak was identified.

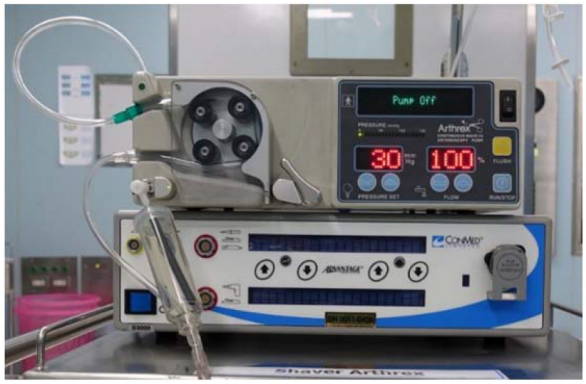


Figure 3. An arthroscopic water pump for pressure control

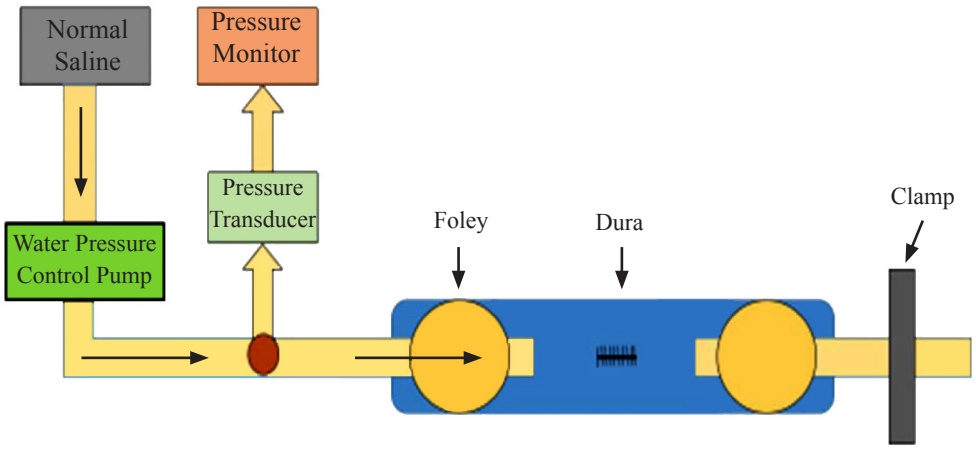
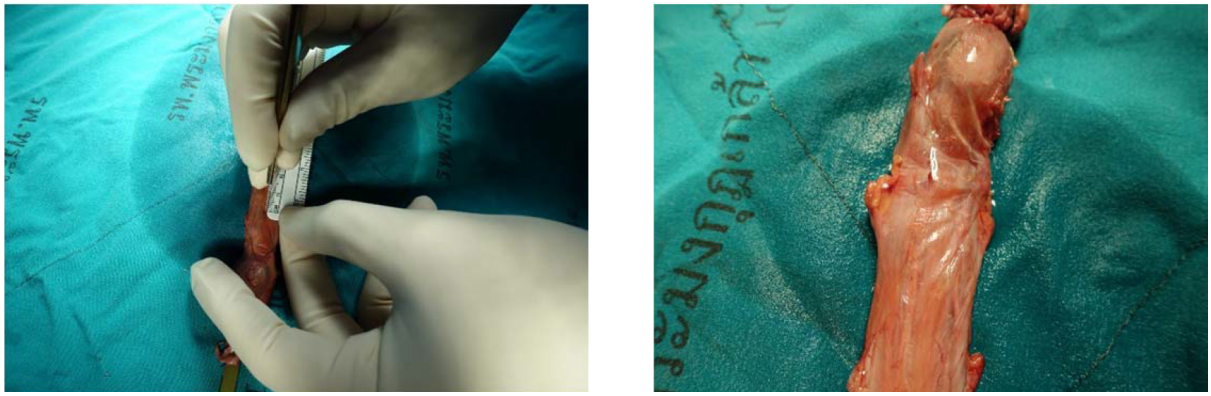


Figure 4. Tear dural experimental model

A midline 1 cm durotomy was made using a ruler and no.15 scalpel blade. The tubing was opened to allow leakage from the durotomy site to ensure flow in the system **(Figure 5)**. The dura was then repaired using Prolene 6-0 or Durepair®. All suture repairs were performed using microsurgical instruments (Castroviejo needle holders, fine tip tissue forceps etc.) and repair was performed under the naked eye or using surgical loupes. For simple repairs, a simple suture technique was used with approximately 1 mm of space between the sutures **(Figure 6).** For Durepair®, a Durepair® patch, formed as a square piece measuring 1x1 cm, was placed under the dural tear and then sutured with Prolene 6-0 in the previously described simple suture technique **(Figure 7).** Time used for sutures in each group was recorded using a digital stopwatch. Once the durotomy was repaired, the pressure was determined from 40 mmHg until a leak was seen and the breakthrough pressure was recorded. The Foley balloons were repositioned on a new dural segment that had not been previously tested, and another dural repair was performed. Dural tears repaired using Prolene 6-0 or Durepair® under the naked eye and surgical loups were tested 8 times in each group.



a **b**

Figure 5 a, b. A standard 1 cm durotomy in spinal dura

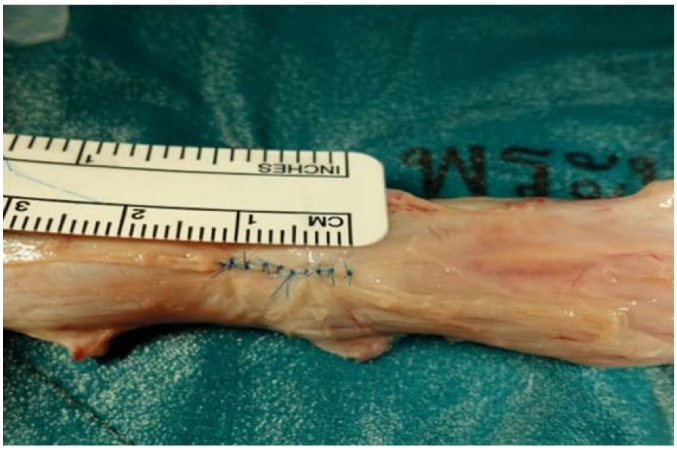
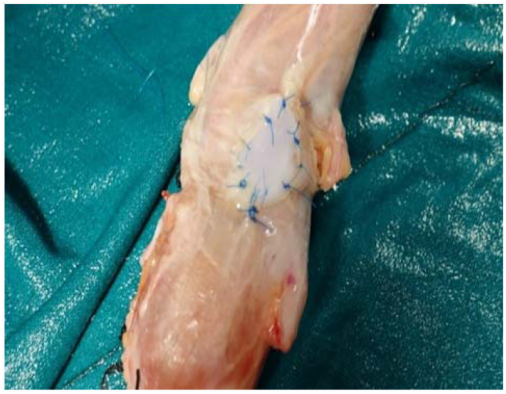


Figure 6. Dural tear repaired by Prolene 6-0 simple suture

a.



b.

Figure 7a. Durepair® cut to square 1 x 1 cm **and 7b** Dural tear repaired by Durepair®

Table 1. Mean suture time and mean leak pressure of dural repairs using Prolene 6-0 and Durepair with the naked eye and using surgical loupes

Material	Mean Suture Time (min)		*p*-value	Mean Leak Pressure (mmHg)		*p*-value
	Naked eye	Surgical Loupe		Naked eye	Surgical Loupe	
Prolene 6-0	7.18±0.36	4.87±0.19	**<0.001***	96.88±7.99	100.00±5.35	0.373
Durepair®	13.27±0.42	9.84±0.21	**<0.001***	95.63±4.17	96.88±4.58	0.577

Suture material

Routinely used sutures were tested clinically. The monofilament Prolene 6-0 suture is a nonabsorbable sterile surgical suture that comprises an isotactic crystalline stereoisomer of polypropylene, a synthetic linear polyolefin. The suture is pigmented blue to enhance visibility in the surgical field.

Dura substitute

Durepair® Dura Regeneration Matrix is a dura substitute for the repair of the dura mater, manufactured by Medtronic. It consists of a collagen implant to repair large defects in the dura mater. Sterile Durepair is supplied in sheet form, in a variety of sizes to be trimmed and sutured by the surgeon to meet the individual patient's needs.

Statistical analysis

The leakage pressure and time using the same material for sutures were compared using the independent t-test. One-way ANOVA was used to compare among all groups. A *p*-value less than 0.05 was considered statistically significant.

Results

Comparison of Prolene 6-0 under the naked eye vs. using surgical loupes

The time used for sutures using surgical loupes of Prolene 6-0 was significantly less than sutures under the naked eye (p <0.001). Mean time for sutures using surgical loupes was 4.87±0.19 min while mean time for sutures under the naked eye was 7.18±0.36 min. All dural repairs by Prolene 6-0 both under the naked eye and surgical loupes did not leak at a pressure of 40 mmHg. The mean leak pressure using Prolene 6-0 sutures under surgical loupes was 100.00±5.35 mmHg, while the leak pressure using Prolene 6-0 sutures under the naked eye was 96.88±7.99 mmHg. For the naked eye group, the leak pressure was less than that of the surgical loupes group without significant difference (p = 0.373) **(Table 1)**

Comparing Prolene 6-0 vs. Durepair®

Results of the mean time to close dural tears using Prolene 6-0 both under surgical loupes and

Table 2. Comparing of mean suture time and mean leak pressure of Prolene6-0 VS Durepair with the naked eye and using surgical loupes

Group	Mean Suture Time (min)		*p*-value	Mean Leak Pressure (mmHg)		*p*-value
	Prolene 6-0	Durepair		Prolene 6-0	Durepair	
Naked eye	7.18±0.36	13.27±0.42	<0.001	96.88±7.99	95.63±4.17	0.701
Surgical loupe	4.87±0.19	9.84±0.21	<0.001	100.00±5.35	96.88±4.58	0.230

the naked eye were significantly less than those repaired using Durepair® under surgical loupes and the naked eye (p <0.001). The leak pressure of Durepair® in both the surgical loupes and the naked eye groups was less than that of repairs made by Prolene 6-0 only, in both surgical loupes and the naked eye groups but without significant difference (p=0.23 in the surgical loupe, p=0.701 in the naked eye group) **(Table 2).**

Discussion

Primary repairs of dural tears are commonly employed to prevent potential postoperative complications. The goals of dura repair include a watertight closure and containment of fascicles. Many research studies have investigated the proper materials to repair dural tears. Although microscopes or surgical loupes are recommended to assist with dural tear sutures, many spine surgeons still repair dural tears using the naked eye only. Our study showed that surgical loupes were significantly faster than naked eye dural sutures. A study by Andrades et al. used loupes (x2.5) and microscopes (x10) to repair rodent vessels that were grouped as large (>2.5 mm), medium (1.5-2.5 mm) and small (<1.5 mm).[18] For vessels in the small group, both surgical time (31 minutes for microscope vs. 52 minutes for loupes) and 24-hour patency (80% microscope vs. 10% loupes) strongly favored the microscope. However, in medium and large group vessels, no statistically significant differences were observed in procedure time or 24-hour patency. The results of the large vessel group revealed the microscope repairs to be quicker than loupe repairs (48 vs. 52 min, respectively) and boasted greater patency (90% vs. 60%) despite the lack of statistical significance. Rooks et al. measured the precision of suture placement in grafts with loupes (x3.5 to x4) and microscopes (x8 to x30).[19] They found that microscope suture placement was 0.03 mm closer to the target (edge of the graft) and that the variability from the mean was 0.01 mm less, both statistically significant differences in favor of the microscope. In a review of 251 free tissue transfers performed with only loupe magnification, Shenaq et al. found an overall success rate of 97.2% with a 1.2% partial flap necrosis rate and an 8.3% revision rate for anastomoses during the initial procedure.[20] The overall loupe-only success rate in that study for free tissue transfers was 98.5%, with 96.4% success with toe-to-hand transfers and 79.2% for digital replantation. In a retrospective review of 151 consecutive microvascular free tissue transfers in the head and neck performed with either loupes or microscope, Ross et al. found no significant difference in complication rates.[21] The only significant difference was decreased surgical time in the loupe group. This was the reason surgical loupes significantly decreased suture time than that of the naked eye. The magnification might relate to the time for surgery. Our study showed that water leakage pressure was slightly higher in the group using surgical loupes than in the group of repairs simply using the naked eye; however, this finding did not differ significantly. Magnifying surgical loupes may play a role in watertight sutures. We observed that in the surgical loupes group, the number of suture stitches was greater than that performed in the naked eye group *(10 stitches vs. 6 stitches)*. Good magnification provided meticulous sutures and helped the surgeon achieve goals of dural repair.

Comparing Prolene 6-0 vs. Durepair® in both surgical loupe and the naked eye groups assisted dural closure regarding that Prolene 6-0 sutures could tolerate more pressure than Durepair® but without significant difference. Durepair® took a significantly longer suture time than Durepair® in both surgical loupe and the naked eye groups. Durepair® is used for large defects that cannot be repaired by simple techniques, explaining why Durepair® takes a longer suture time than using Prolene alone.

Conclusion

Repairing dural tears under the naked eye consumed more time than surgical loupe assisted repairs and did not achieve a similar maximum leak pressure compared with closures using surgical loupes. However, no statistically significant difference was found in terms of CSF leakage pressure. Durepair® consumed more time than using Prolene 6-0 while leakage pressure did not differ. We recommended using surgical loupes when performing dural repair due to providing a better visualization, increasing the number of stitches with higher leakage pressure. Durepair® was suitable to repair large dural defects that cannot use simple suture techniques to repair.

Conflict of interest

The authors declare they have no conflict of interest.

References

1. Hannallah D, Lee J, Khan M, Donaldson WF, Kang JD. Cerebrospinal fluid leaks following cervical spine surgery. J Bone Joint Surg Am 2008; 90A: 1101-5.
2. Saxler G, Kramer J, Barden B, Kurt A, Pförtner J, Bernsmann K The long-term clinical sequelae of incidental durotomy in lumbar disc surgery. Spine 2005; 30: 2298-302.
3. Jones AA, Stambough JL, Balderston RA, Rothman RH, Booth Jr RE. Long-term results of lumbar spine surgery complicated by unintended incidental durotomy. Spine 1989; 14: 443–6.
4. Cammisa FP Jr, Girardi FP, Sangani PK, Parvataneni HK, Cadag S, Sandhu HS. Incidental durotomy in spine surgery. Spine 2000; 25: 2663-7.
5. Wang JC, Bohlman HH, Riew KD. Dural tears secondary to operations on the lumbar spine. Management and results after a two year- minimum follow-up of eighty-eight patients. J Bone Joint Surg Am 1998; 80: 1728-32.
6. Goodkin R, Laska LL. Unintended "incidental" durotomy during surgery of the lumbar spine: medicolegal implications. Surg Neurol 1995; 43: 4-12.
7. Kitchel SH, Eismont FJ, Green BA. Closed subarachnoid drainage for management of cerebrospinal fluid leakage after an operation on the spine. J Bone Joint Surg Am 1989; 71A: 984-7.
8. Eismont FJ, Wiesel SW, Rothman RH. Treatment of dural tears associated with spinal surgery. J Bone Joint Surg Am 1981; 63A: 1132-6.
9. Chen YX, Chen LE, Seaber AV, Urbaniak JR. Comparison of continuous and interrupted suture techniques in microvascular anastomosis. J Hand Surg Am 2001; 26: 530-9.
10. Cain JE Jr, Dryer RF, Barton BR. Evaluation of dural closure techniques. Suture methods, fibrin adhesive sealant, and cyanoacrylate polymer. Spine 1988; 13: 720-5.
11. Erica E. Dafford, Paul A. Anderson. Comparison of dural repair techniques. Spine 2015; 15: 1099-105.
12. Luca Papavero, Nils Engler, Ralph Kothe. Incidental durotomy in spine surgery: first aid in ten steps. Eur Spine J 2015; 24: 2077-84.
13. Lee S, Frank DH, Chou SY. Historical review of small and microvascular vessel surgery. Ann Plast Surg 1983; 11: 53-62.
14. Davidson BJ, Guardiani E, Wang A. Adopting the operating microscope in thyroid surgery: safety, efficiency, and ergonomics. Head Neck 2010; 32154-59.
15. Serletti JM, Deuber MA, Guidera PM, Reading G, Herrera HR, Reale VF, et al. Comparison of the operating microscope and loupes for free microvascular tissue transfer. Plast Reconstr Surg 1995; 95: 270-76.
16. Pieptu D, Luchian S. Loupes-only microsurgery. Microsurgery 2003; 23: 181-88.

17. Shenaq SM, Klebuc MJ, Vargo D. Free-tissue transfer with the aid of loupe magnification: experience with 251 procedures. Plast Reconstr Surg 1995; 95: 261-69.
18. Andrades P, Benitez S, Danilla S, Erazo C, Hasbun A, Fix J. Vascular diameter determining the magnification for a microvascular anastomosis. Reconstr Microsurg 2008; 24: 174-81.
19. Andrades P, Benitez S, Danilla S, Erazo C, Hasbun A, Fix J. Vascular diameter determining the magnification for a microvascular anastomosis. Reconstr Microsurg 2008; 24: 174-81.
20. Shenaq SM, Klebuc MJ, Vargo D. Free-tissue transfer with the aid of loupe magnification: experience with 251 procedures. Plast Reconstr Surg 1995; 95:261-69.
21. Ross DA, Ariyan S, Restifo R, Sasaki CT. Use of the operating microscope and loupes for head and neck free microvascular tissue transfer. Arch Otolaryngol Head Neck Surg 2003; 129: 189 -93.

PREVALENCE OF VITAMIN D INADEQUACY AMONG ORTHOPEDIC SURGEONS

Ongart Phruetthiphat, Nopphadon Kusuwannakul, Saradej Khuangsirikul, Teerapat Tutaworn, Thawee Songpatanasilp, Thanainit Chotanaphuti

Department of Orthopaedics, Phramongkutklao Hospital and Phramongkutklao College of Medicine, Bangkok, Thailand

Abstract

Background: Indoor workers including healthcare professionals are at high risk to develop vitamin D insufficiency and deficiency, due to lifestyle and limited sunlight exposure. Additionally, common health problems in aging men are vitamin D inadequacy, hypogonadism and prostate cancer.

Objective: The study aimed to determine the prevalence of vitamin D inadequacy, testosterone and prostate specific antigen (PSA) among orthopedic surgeons with different ages and regions of residence.

Methods: This cross-sectional study was conducted at the annual meeting of the Royal College of Orthopedic Surgeons of Thailand (RCOST) in October 2017. All participants were orthopedic surgeons working in different regions in Thailand. They received blood examination for vitamin D (25-hydroxyvitamin D) levels, total testosterone and total PSA serum levels.

Results: A total of 257 orthopedic surgeons participated in the study. The prevalence of vitamin D inadequacy was 71.98%. Old age group (≥60 years old) had significantly higher total vitamin D than young age group (<60 years old) (p=0.014). Participants from eastern region had significantly higher total vitamin D than Bangkok and southern regions (p=0.015). Interestingly, the prevalence of low testosterone levels was 13% which occurred significantly in the age group ≥40 year (p=0.004). Additionally, the prevalence of high PSA levels was 4.67%. Even though the old age group had significantly higher PSA than the young age group (p< 0.001), no correlation was found between region and total PSA level.

Conclusion: The prevalence of vitamin D inadequacy among Thai orthopedic surgeons was surprisingly high. This data provide important information to promote greater self-awareness to reduce the possible consequences associated with vitamin D inadequacy. Vitamin D supplementation including prevention strategies need to be promoted among healthcare professionals.

Keywords: Vitamin D inadequacy, Orthopedic surgeons, Prevalence, Elderly patients, Hypogonadism, Prostate cancer

J Southeast Asian Med Res 2021: 5(1):35-41
http://www.jseamed.org

Correspondence to:
Phruetthiphat O, Phramongkutklao Hospital and Phramongkutklao College of Medicine, Bangkok, Thailand
Email: ongart-phr1@hotmail.com, ophruetthiphat@gmail.com,

Received: 04 April 2021
Revised: 01 June 2021
Accepted: 09 June 2021

Introduction

Vitamin D plays an important role not only in bone metabolism but also in a variety of nonskeletal diseases such as diabetes mellitus, autoimmune diseases, infectious diseases, cardiovascular diseases and cancer.[1] Vitamin D inadequacy is now well documented as a contributing factor to muscle weakness including falls and fractures.[2] High prevalence of vitamin D deficiency has been described in more than 1 billion people around the world, especially in the Middle East and Asia[3] and it has been seen in all races, age groups and ethnic background.[4] In Thailand, several studies have demonstrated the prevalence of vitamin D deficiency. Soontrapa et al.[5] found that 66.3% of elderly Thai women, residing in Khon Kaen Province, had vitamin D deficiency [25(OH)D level <35 ng/mL] while the largest study of vitamin D status in a normal population (N=2641) identified overall 45.2% of vitamin D inadequacy.[6] However, none of these studies focused on vitamin D inadequacy among Thai orthopedic surgeons. As we know, Thai orthopedic surgeons are predominantly male. Most are indoor workers at high risk to develop vitamin D inadequacy because their lifestyles and regular work at the hospital usually limited time to sunlight expose. Additionally, hypogonadism, a syndrome characterized by low serum testosterone levels, is a common disorder among aging men. Orthopedic surgeons who are at old age group could have a problem with hypogonadism and prostatic hypertrophy. Thus, this study aimed to define the prevalence of vitamin D inadequacy, hypogonadism and prostatic hypertrophy including prostate cancer among Thai orthopedic surgeons. Secondly, the study determined the relation of age and region of residence with total vitamin D, total testosterone and total prostate surface antigen (PSA).

Methods

After obtaining Institution Research Board approval, this cross-sectional study was conducted at the annual meeting of the Royal College of Orthopedic Surgeons of Thailand (RCOST) October 2017. All Thai orthopedic surgeons including residents were asked to take blood examination of 25-hydroxyvitamin D (25-OH) level, total testosterone and total PSA serum levels. The data of ages and region of residence were collected. Participants were excluded when they presented history of hypovitaminosis D, late onset of hypogonadism, or prostate cancer.

Total 25-OH vitamin D was evaluated and were classified in three categories; normal level (30-100 nanograms per milliliter; ng/mL), vitamin D insufficiency (21-29 ng/mL), and vitamin D deficiency (<20 ng/mL).[4] Considering the wide range of cut off values used for defining vitamin D inadequacy, less than 30 ng/ml was used as a threshold concentration of 25(OH)D for "vitamin D inadequacy", covering the definition of vitamin D deficiency and insufficiency together. Total testosterone levels were divided in two groups; normal level (≥8 nanomole per liter; nmol/L) and low level (<8 nmol/L). Total prostate surface antigen (PSA) was categorized in two types: normal level (0-4 ng/mL) and high level (>4 ng/mL).

Statistical analysis

Data description was based on means and standard deviation for continuous variables and absolute and relative frequencies for categorical variables. A standard student's t-test was used for continuous variables while Chi-squared test and Fisher's exact test were applied for categorical variables. One-way ANOVA was used for continuous data and normal distribution while the Mann-Whitney U-test was applied for continuous data and uneven distribution. Statistical analysis was performed using STATA/MP 12 with statistical significance set at p <0.05.

Results

A total of 257 participants were recruited in this study. Participants' age and region of residence are demonstrated in **Table 1**; orthopedists with ages less than 40 years old totaled 53% (n=136), ages between 40 and 60 years old comprised 34% (n=87) and participants with ages more than 60 years old totaled 13% (n=34). In addition, the most common participants (54.1%) came from Bangkok, the second most common (17.9%) came from the northeastern region, followed by central region (8.56%), eastern region (7.4%), southern region (6.2%), and northern region (5.8%).

Table 1. Baseline characteristics of the study population

Characteristic	N (%)
Age	
< 40 years	136 (53)
40-60 years	87 (34)
> 60 years	34 (13)
Mean ± SD	42.23 ± 13.45
Median (Minimum-Maximum)	37 (24-84)
Region of residence	
Bangkok	139 (54.1%)
Central	22 (8.6%)
Eastern	19 (7.4%)
Northern	15 (5.8%)
Northeastern	46 (17.9%)
Southern	16 (6.2%)

Table 2. Level of 25-OH total vitamin D

25-OH total vitamin D	N (%)
Deficiency (<20)	22 (8.6%)
Insufficiency (20-29)	163 (63.4%)
Normal (≥30)	72 (28.0%)
Mean ± SD	27.4 ± 6.7
Median (Minimum-Max)	26.6 (11.2-58.2)

Overall, the prevalence of vitamin D inadequacy among Thai orthopedic surgeons was 72%. The prevalence of vitamin D insufficiency was 63.4%, vitamin D deficiency was 8.6% while normal vitamin D level was 28.0% as shown in **Table 2**.

Vitamin D levels significantly differed among age groups (One-way ANOVA, p= 0.014). The participants aged >60 years exhibited the highest vitamin D levels as shown in **Table 3**. Additionally, participants from different regions of Thailand showed significantly different Vitamin D levels (One-way ANOVA, p=0.015). Participants from the eastern area had the highest vitamin D levels while those from Bangkok revealed the lowest vitamin D.

Overall, the prevelence of low level testosterone was 13.2% (n=34) while normal testosterone level was 86.8% (n=223) as shown in **Table 4**. Testosterone level significantly differed among age groups (One-way ANOVA,p=0.004) as shown in **Table 4**. Additionally, age group≥40 years had a higher prevalence of low level testosterone than age group <40 years (20.7% vs. 6.6%, respectively). The participants from different regions of Thailand showed significantly different testosterone levels (One-way ANOVA, p=0.04). Those residing in Northern showed the highest testosterone levels while those residing in the Northeast showed the lowest levels. The prevelence of high level PSA was 4.7% (n=12) as shown in **Table 5**. PSA level significant differed among

Table 3. Total vitamin D (25-OH) level classified by age and region of residence

Age group	Vitamin D level (ng/mL)				
	Deficiency (<20)	**Insufficiency (20-29.9)**	**Normal (≥30)**	**Mean±SD**	**Median (Min-Max)**
	(n=22)(8.6%)	(n=163)(63.4%)	(n=72)(28.0%)		
< 40 years	9 (6.6%)	91 (66.9%)	36 (26.5%)	27.1± 5.9	26.5 (14.4-46.5)
40-60 years	10 (11.8%)	54 (63.5%)	21 (24.7%)	26.8±6.0	26.5 (11.2-47.5)
> 60 years	3 (8.3%)	18 (50.0%)	15 (41.7%)	30.4±9.8	28.5 (14.6-58.2)
p-value				0.014	
Region of residence	Deficiency (<20)	Insufficiency (20-29.9)	Normal (≥30)	Mean±SD	Median (Min-Max)
Bangkok	20 (14.60%)	83 (60.58%)	34 (24.82%)	26.6±7.3	25.6 (11.2-58.2)
Central	1 (5%)	11 (55%)	8 (40%)	29.2±7.3	27.9 (14.4-45.6)
Eastern	0 (0%)	9 (50%)	9 (50%)	30.5 ±5.6	29.1 (23.8-46.8)
Northern	0 (0%)	7 (53.9%)	6 (46.2%)	28.7±6.2	29.4 (20.1-42.8)
Northeastern	0 (0%)	28 (71.8%)	11 (28.2%)	27.8±4.6	26.9 (20.8-37.6)
Southern	1 (6.3%)	11 (68.8%)	4 (25%)	26.8 ±6.4	25.6 (18.5-37.7)
p-value				0.015	

Total vitamin D (25-OH) level classified by age groups (<40, 40-60 and >60 years old) was mainly divided into 3 types (vitamin D deficiency, insufficiency and normal levels). The prevalence of vitamin D inadequacy among Thai orthopedic surgeons was 72.0 % , vitamin D insufficiency was 63.4% and vitamin D deficiency was 8.6% while normal vitamin D level was 28.0%.

Table 4. Testosterone level classified by age and living region

Age group	Testosterone level			
	Low (<8 nmol/L) (n=34)(13.2%)	**Normal (≥8 nmol/L) (n=223)(86.8%)**	**Mean±SD**	**Median (Min-Max)**
< 40 years	9(6.62%)	127(93.38%)	14.82±5.79	13.30 (5.08-40.50)
40-60 years	18(21.18%)	67(78.82%)	12.41±5.97	11.40 (3.83-35.90)
>60 years	7(19.44%)	29(80.56%)	12.54±4.64	12.15 (5.19-22.20)
p-value			**0.004**	
Bangkok	23(16.55%)	116(83.45%)	13.44±6.40	12.20 (3.8-40.50)

Table 4. Testosterone level classified by age and living region (Continue)

Age group	Testosterone level			
	Low (<8 nmol/L) (n=34)(13.2%)	**Normal (≥8 nmol/L) (n=223)(86.8%)**	**Mean±SD**	**Median (Min-Max)**
Central	2(9.09%)	20(90.91%)	14.05±5.76	13.35 (4.7-30.30)
Eastern	1(5.26%)	18(94.74%)	14.16±3.11	14.30 (7.50-22.40)
Northern	0 (0%)	15(100%)	17.02±4.34	16.90 (10.10-23.10)
Northeastern	7(15.22%)	39(84.78%)	12.88±5.06	12.80 (4.06-25.50)
Southern	1(6.25%)	15(93.75%)	14.30±5.56	12.60 (6.39-29.90)
p-value			0.04	

Testosterone classified by age groups (<40, 40-60 and >60 years old) was mainly divided into 2 types (low and normal levels). The prevalence of low testosterone level was 6.6% (n=9) among Thai orthopedic surgeans aged < 40 years old (n=136) and 20.7% (n=25) aged > 40 years old (n=121).

Table 5. PSA level classified by age and region of residence

Age group	Testosterone level			
	High (>4 ng/ml) (n=12)(4.7%)	**Normal (0-4 ng/ml) (n=245)(95.3%)**	**Mean±SD**	**Median (Min-Max)**
< 40 years	1(0.45%)	135(61.08%)	0.83±0.83	0.66 (0.17-8.31)
40-60 years	4(1.81%)	81(36.65%)	1.41±3.34	0.80 (0.20-30.00)
>60 years	7(19.44%)	29(80.60%)	2.40±2.53	1.47 (0.35-12.50)
p-value			<0.001	
Bangkok	9 (6.47%)	130 (93.53%)	1.46±2.93	0.79 (0.17-30.00)
Central	0 (0%)	22 (100%)	0.87±0.60	0.69 (0.19-2.59)
Eastern	1(5.26%)	18(94.74%)	1.22±1.09	0.82 (0.38-4.55)
Northern	1 (6.67%)	14(93.33%)	1.13±2.01	0.58 (0.24-8.31)
Northeastern	1 (2.17%)	45(97.83%)	0.99±0.95	0.74 (0.23-4.26)
Southern	0 (0%)	16(100%)	0.73±0.24	0.66 (0.34-1.30)
p-value			0.43	

PSA level classified by age groups (<40, 40-60 and >60 years old) was divided into 2 types (high and normal levels).The prevalence of high PSA level among Thai orthopedic surgeans was 2.3% (n=5) aged < 60 years old (n=221) and 19.4% (n=7) aged > 60 years old (n=36).

different age groups (One-way ANOVA, $p < 0.001$). The prevalence of high PSA level was highest in the age group of >60 years old. However, no significant association was found between region of residence and total PSA.

Discussion

Several studies in Thailand demonstrated the prevalence of vitamin D deficiency. Soontrapa, et al.[5] found that 66.3% of elderly Thai women residing in Khon Kaen Province had vitamin D deficiency [25(OH)D level <35 ng/mL] while the largest study of vitamin D status in a normal population (N=2641) identified overall 45.2 % of vitamin D inadequacy.[6] However, none of these studies focused on vitamin D inadequacy among Thai orthopedic surgeons. Most are indoor workers at high risk to develop vitamin D insufficiency because their lifestyles and regular work at the hospital and usually limited their exposure to sunlight. Additionally, some orthopedic surgeons are aging and could develop hypogonadism and prostatic hypertrophy.

The review of vitamin D status showed that 88.1 % of subjects presented in populations worldwide had mean 25(OH)D values below 30 ng/mL.[7] In addition. A systematic review in 2017 revealed that the prevalence of vitamin D inadequacy (<30 ng/mL) among physicians was as high as 95.7%.[8] One study about vitamin D status among Thai physicians revealed that none of the Thai dermatologists had serum 25(OH) D sufficiency, 38 (38.78%) had vitamin D insufficiency (20-30 ng/mL) and 60 (61.22%) had vitamin D deficiency (<20 ng/mL). [9] In this study, the prevalence of vitamin D inadequacy among Thai orthopedic surgeons (72.0%) was significantly higher than that of normal Thai populations (45.2%) [6] and elderly Thai women (66.3%).[5] The differences in latitude (geographical location), weather, pollution, degree of expose to UVB, using sunscreen, skin color, cultural traditions (clothing), diets and sex could constitute the reasons. Age could be another factor because vitamin D production in the skin declines with increasing age. The results from this study demonstrated that vitamin D inadequacy was not only a major problem in Thai populations, but also common among Thai orthopedic surgeons. Vitamin D inadequacy among Thai orthopedists may represent the overall vitamin D inadequacy in Asian orthopedic surgeons. This data comprises important information to promote self-awareness because the orthopedic field always requires powerful muscle and bone strength. Moreover, further studies need to determine the correlation of any clinical risk factors and vitamin D levels among Thai orthopedic surgeons.

The differences of testosterone and PSA levels in different age group were significant as we expected; male testosterone normally tends to decline but PSA tends to become higher with increasing age. However, our results illustrated that mean testosterone level was not higher among those patients with age more than 60 years compared with those 40-60 years old (12.54 vs. 12.41, respectively). A relatively small number of patients in each age group constituted one of limitations in this study. Moreover, information of underlying diseases, medications and other activities, were not recorded from enrolled participants.

Even though several studies have demonstrated low testosterone associated with metabolic syndrome and cardiovascular events among Japanese men [11,12], we still lacked data identifying low testosterone level and any clinical diseases in Thai populations. Our study comprised a preliminary study among Thai orthopedic surgeons and the results may be helpful to further investigate the association of testosterone level and clinical diseases in Thai populations.

Conclusion

The prevalence of vitamin D inadequacy among Thai orthopedic surgeons was surprisingly high. Greater self-awareness to reduce possible consequences associated with vitamin D inadequacy is important. Vitamin D supplementation including prevention strategies need to be promoted among healthcare professionals.

Declaration of competing interest

The authors declare they have no competing interests.

References

1. ACOG Committee on Obstetric Practice. ACOG Committee Opinion No. 495: Vitamin D: Screening and supplementation during pregnancy. Obstet Gynecol 2011; 118: 197-8.
2. Sprague S, Petrisor B, Scott T, Devji T, Phillips m, Spurr H, et al. What is the role of D supplementation in acute fracture patients? A systematic review and meta-analysis of the prevalence of hypovitaminosis D and supplementation efficacy. J OrthopTrauma 2015; 30: 53-63.
3. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96: 1911-30.
4. Soontrapa S, Soontrapa S, Chailurkit L. The prevalence and the calcidiol levels of vitamin D deficiency in the elderly Thai women in municipality of Khon Kaen province, Thailand. Srinagarind Med J 2002; 17: 231-8.
5. Chailurkit L, Aekplakorn W, Ongphiphad-hanakul B. Regional variation and determinants of vitamin D status in sunshine-abundant Thailand. BMC Public Health 2011; 11: 853.
6. TutawornT,PhruetthiphatO,SongpatanasilpT. Prevalence and Clinical Correlation of Vitamin D Inadequacy in Traumatic Hip Fracture among Elderly Patients. J Ortho Bone Disord 2019, 3: 000191.
7. AkishitaM,FukaiS,HashimotoM,Kameyama Y, Nomura K, Nakamura T, et al. Association of low testosterone with metabolic syndrome and its components in middle-aged Japanese men. Hypertens Res 2010; 33: 587-91.
8. Akishita M, Hashimoto M, Ohike Y, Ogawa S, Iijima K, Eto M, et al. Low testosterone level as a predictor of cardiovascular events in Japanese men with coronary risk factors. Atherosclerosis 2010; 210: 232-6.

CORRELATION OF HEMOGLOBIN A1c AND DIABETES RISK USING THE THAI DIABETES RISK SCORE

Wyn Parksook, Preamrudee Chaisuwirat **, Apussanee Boonyavarakul**

***Division of Endocrinology, Department of Internal Medicine, Phramongkutklao Hospital, Bangkok, Thailand**
**** Blood Bank, Army Institute of Pathology, Phramongkutklao Medical Center, Bangkok, Thailand**

Abstract

Background: Early detection of diabetes allows prompt access to interventions that can improve microvascular and macrovascular disease outcomes. Multiple strategies have been employed, i.e., the use of diabetes risk scores including blood testing.

Objective: The study aimed to evaluate the correlation between point-of-care hemoglobin A1c (POC HbA1c) and Thai diabetes risk score.

Methods: A cross-sectional study was conducted consisting of 252 individuals without diabetes over the age of 35. Demographic data and anthropometric measures were recorded and the blood test for POC HbA1c including plasma glucose were performed.

Results: Of 252 participants, the mean HbA1c was 5.56 ± 0.73%, the median Thai diabetes risk score was 7 [5-10] and American Diabetes Association (ADA) risk score was 3 [2.3-4]. Males had higher risk scores than females. Weak positive correlations were observed between POC HbA1c and both Thai and ADA risk score (r = 0.226 and 0.279, respectively, p<0.001). The predictors of higher HbA1c among males were high BMI and waist circumference.

Conclusion: A weak correlation of POC HbA1c and Thai diabetes risk score suggested that POC HbA1c may not be beneficial in screening diabetes in out-of-clinic situations; however, male participants with WC >100 cm and BMI >27.5 kg/m^2 were associated with highest HbA1c.

Keywords: Type 2 diabetes, Diabetes risk score, HbA1c, Point-of-care, Screening

J Southeast Asian Med Res 2021: 5(1):42-50
http://www.jseamed.org

Correspondence to:
Parksook W, Department of Internal Medicine, Phramongkutklao Hospital, Bangkok, Thailand
E-mail: wynparksook@hotmail.com

Received: 09 February 2021
Revised: 29 May 2021
Accepted: 09 June 2021

Introduction

Type 2 diabetes mellitus is one of more prevalent noncommunicable diseases with increasing prevalence yearly. The International Diabetes Federation (IDF) estimated that diabetes affected 463 million individuals globally.[1] In Thailand, the prevalence of diabetes among adults is 7.0% and is estimated to increase to 8.0% by 2045.[2] Of those affected, 43.6% remain unaware of their diabetic conditions.

Due to the silent nature of the disease, diabetes often goes undetected. The lack of adequate interventions and monitoring until late into the disease progression results in microvascular and macrovascular complications.[3] These include diabetic retinopathy, neuropathy, nephropathy and cardiovascular atherosclerosis. The chronic and additive clinical progression of diabetes means that cost of care increases over time, as demonstrated in a recent UK Prospective Diabetes Study (UKPDS) 84.[4] It would be prudent; therefore, to detect diabetes at the earliest phase so intervention result could reduce complications and mortality.

High risk screening for diabetes has been the mainstay strategy for early detection and has been adopted in a number of guidelines. The Thai Clinical Practice Guidelines for Diabetes 2017 has endorsed a risk score system devised by Aekplakorn et al.[5] This cohort study has good accuracy in predicting 12-year risk of new-onset diabetes (**Table 1**).[6] The maximum score is 17. When the score is 6 or more, further diabetes evaluation is warranted. Waist circumference and BMI are classified according to WHO definitions.

In the absence of risk factors, Thai guidelines also suggested blood tests for diabetes among individuals over the age of 35 with these following conditions: BMI ≥25 kg/m^2, increased waist circumference, first degree relatives having diabetes, hypertension, dyslipidemia, prior gestational diabetes (GDM) or when delivering a child >4 kg, cardiovascular disease or polycystic ovarian syndrome. [5] Studies in other countries validated diabetes risk scores that correlate to their specific populations. The American Diabetes Association (ADA) is one of the major guidelines in the US having its own risk score.[7]

Table 1. Type 2 diabetes risk score

Risk Factor	Diabetes Risk Score
Age	
34-39 years	0
40-44 years	0
45-49 years	1
≥50 years	2
Gender	
Female	0
Male	2
Body mass index (BMI)	
< 23 kg/m^2	0
23-27.5 kg/m^2	3
> 27.5 kg/m^2	5
Waist circumference	
Males<90 cm, Females <80 cm	0
Males ≥90 cm, Females ≥80 cm	2
Hypertension	
No	0
Yes	2
Family history DM	
No	0
Yes	4

Screening of diabetes requires elevated fasting plasma glucose (FPG) or fasting capillary blood glucose (FCBG) ≥126 mg/dL, which has to be repeated once.[7] However, hemoglobin A1c (HbA1c) has not yet been implemented in the Thai guidelines.[5] Alternatively, 75 g oral glucose tolerance test (OGTT) can be used which has higher sensitivity than fasting glucose.[8] However, due to its invasive and time-consuming nature, OGTT is not routinely practiced.

Diagnostic cut off points are similar in all guidelines, requiring two abnormal values for diagnosis. However, the accuracy of HbA1c can vary as over 300 different protocols are available to measure HbA1c.[9,10] The heterogeneity of HbA1c has been addressed and a number of organizations have endeavored to standardize HbA1c. This includes the National Glycohemoglobin Standardization Program (NGSP), International Federation of Clinical Chemistry (IFCC), Mono-S, and Japanese Society of Clinical Chemistry/Japanese Diabetes Society (JSCC/JDS). The IFCC-NGSP master equation is the current accepted standards for HbA1c.[11, 12] The limitation of diabetes screening and risk score use is they are usually evaluated at a doctor's clinic. Even when individuals have routine check-ups, not evaluating other risks and lifestyle, diabetes diagnosis can be missed.

The aim of this study was to evaluate the correlation between POC HbA1c and diabetes risk score, endorsed by the Thai diabetes clinical practice guidelines among blood donors. Additionally, the correlation between ADA diabetes risk score and the measured POC HbA1c was also investigated.

Methods

This study was approved by the Institutional Review Board, Medical Department, Royal Thai Army. Informed consent was signed by all study participants. Individuals, donating blood at the Blood Bank, Pathology Institute, Phramongkutklao Medical Center, were asked to participate in this study. According to the published IDF Diabetes, the prevalence of impaired glucose tolerance (IGT) was 15.5.[13] To calculate the sample size powered to include diabetes and IGT, a minimum of 202 individuals were necessary to provide a 95% confidence interval at the margin of error of 5%. The inclusion criteria were individual blood donors without type 2 diabetes, and aged 35 years or older who had given inform consent. Those who had prior diabetes, hemoglobinopathy, untreated hypothyroidism, chronic liver, chronic kidney diseases, prior splenectomy, received blood transfusion in the past four months, or routinely took supplements of iron, folic acid, vitamin B12 or vitamin E in the past three months were excluded.

All participants were asked to fill in a questionnaire designed to assess individual demographics, anthropometric and lifestyle measures. Blood collection was taken at the time of blood donor screening. Aside from the blood tests required for routine blood donation, hemoglobin, serum creatinine and POC HbA1c (cobas b 101, Roche Diagnostics) were also collected. The Thai diabetes risk score was derived from the study using Aekplakorn et al.[6] In addition, the ADA diabetes risk score was also used for comparison.

Definitions

Diabetes is defined as FPG ≥126 mg/dL,75 g OGTT ≥200 mg/dL, and HbA1c ≥6.5%. Prediabetes is defined as FPG 100-125 mg/dL, 75 g OGTT 140-199 mg/dL, and HbA1c 5.7-6.4%. Dietary control is defined as the self-perceived attitude in an individual's diet in glycemic and hypertensive control. Regular exercise is defined as regular physical activity for at least 30 minutes daily for three to five days weekly.

Statistical analysis

Statistical analysis was performed using SPSS Software, Version 23.0 (SPSS Inc, Chicago, USA) with significance at $p<0.05$. Normality of data was assessed using the one-sample Kolmogorov -Smirnov test. Normally distributed data were expressed as mean ± standard deviation (SD) and nonnormal data were expressed as median (interquartile range). Correlations were evaluated using two-tailed Pearson's tests. The Mann -Whitney test was used to analyze the differences between risk categories of the diabetes risk scores.

Results

A total of 273 individuals agreed to participate in the study. After excluding 21 participants having type 2 diabetes, 252 people were recruited in the study. Of these, 137 (54.4%) were male. The majority of participants (86.1%) did not fast before blood collection. None reported having chronic kidney disease, cardiovascular disease or stroke. No significant differences between sexes were found; however, male participants had higher diabetes risk according to both Thai and ADA scores. Baseline characteristics of participants are shown in **Table 2**.

Table 2. Baseline clinical characteristics of enrolled participants

	All Individuals (n = 252)	Female Individuals (n = 115)	Male Individuals (n = 137)
Clinical			
Age (years, mean ±SD)	44.2 ±7.28	44.23 ±7.10	44.1 ±7.45
≤ 44 years	151	69	82
45 - 49 years	44	18	26
≥ 50 years	57	28	29
Regular check up (N,%)	220 (87.3%)	98 (85.2%)	122 (89.1%)
Underlying disease (N,%)	16 (6.3%)	7 (6.1%)	10 (7.3%)
Hypertension	11	5	6
Dyslipidemia	7	3	4
Family history DM (N,%)	101 (40.1%)	48 (41.7%)	53 (38.7%)
Dietary control (N,%)	157 (62.3%)	73 (63.5%)	84 (61.3%)
Regular exercise (N,%)	106 (42.1%)	47 (40.9%)	59 (43.1%)
Current smoker (N,%)	44 (17.5%)	7 (6.1%)	37 (27.0%)
Current alcoholic (N,%)	89 (35.3%)	23 (20%)	66 (48.2%)
Pregnancy (N,%)		47 (40.9%)	
Prior GDM		5 (10.6%)	
Child >4 kg		2 (4.3%)	
BMI (kg/m^2, mean ±SD)	24.7 ±5.91	23.9 ± 6.31	25.4 ± 5.48
SBP (mmHg, mean ±SD)	133.6± 20.43	129.9 ± 21.5	136.6 ± 19.02
DBP (mmHg, mean ± SD)	78.9 ± 14.10	77.2 ± 14.3	80.35 ± 13.81
Laboratory Investigation			
Glucose (mg/dL, median [IQR])	93.0 [84-108]	93.0 [85-108]	94.0 [82.5-107.5]
HbA1c (%, mean ±SD)	5.56 ± 0.73	5.40 ± 0.70	5.57 ± 0.75
Hb (g/L, median [IQR])	14.5 [13.5-15.5]	13.7 ± 1.61	15.2 [14.5-15.9]
Creatinine (mg/dL, median [IQR])	0.88 [0.73-1.01]	0.73 [0.65-0.81]	0.98 [0.89-1.07]
eGFR (mL/min, median [IQR])	95.2 [83.7-105.9]	99.7 [84.0-108.5]	92.1 ± 15.3
Diabetes Risk Score			
Thai (median [IQR])	7.0 [5-10]	6.0 [4.0-9.0]	9.0 [5-11]
ADA (median [IQR])	3.0 [2.3-4.0]	3.0 [2.0-4.0]	4.0 [3.0-5.0]

DM, diabetes; GDM, gestational diabetes; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Hb, hemoglobin

Data normally distributed expressed as means ± standard deviation; non-normally distributed expressed as median [interquartile range]

Table 3. Correlation between POC HbA1c and diabetes risk scores

HbA1c vs	Correlation	*p*-value
All individuals		
Thai	0.226	<0.001*
ADA	0.279	<0.001*
Female individuals		
Thai	0.228	0.014*
ADA	0.249	0.007*
Male individuals		
Thai	0.233	0.006*
ADA	0.318	<0.001*

Correlation using two-tailed Pearson's test
* Statistically significance at $p<0.05$

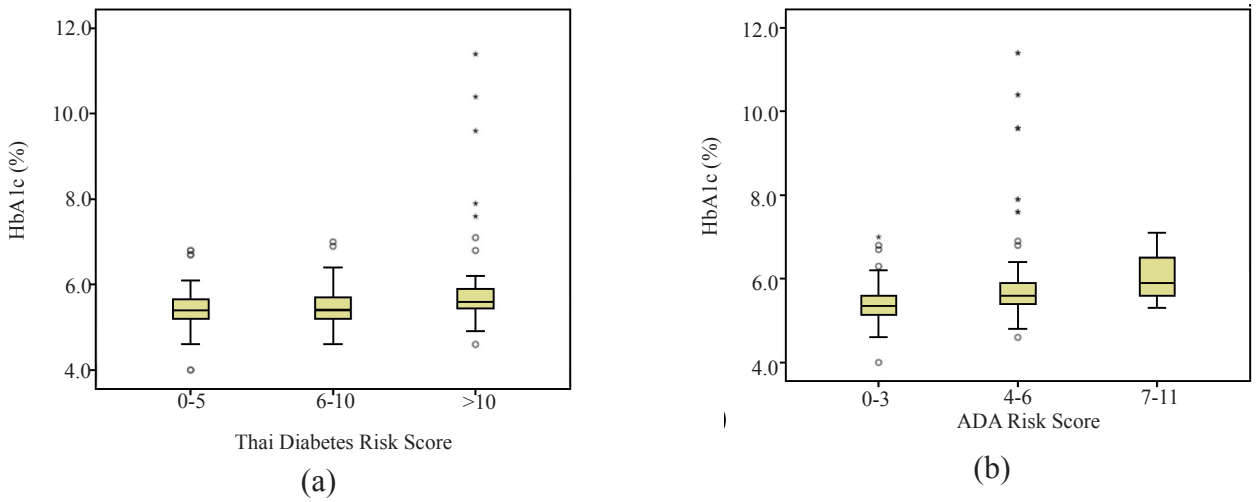


Figure 1. Changes in median, interquartile range, minimum and maximum HbA1c values of all participants in the associated risk categories of the (a) Thai diabetes risk score, and (b) ADA diabetes risk score

Correlation between POC HbA1c and diabetes risk scores

Weak positive correlations between POC HbA1c and both Thai and ADA diabetes risk scores were observed (**Table 3**). Subset analysis showed that POC HbA1c correlated with both male and female individuals. Higher correlation was observed when using the ADA risk score.

POC HbA1c values and risk prediction categories

Increasing diabetes risk scores were associated with higher POC HbA1c. Among all individuals, lower Thai risk scores were associated with lower HbA1c while higher scores were associated with higher HbA1c, many of which appeared outside the 1SD, identified as outliers (**Figure 1**). No significant differences were identified between lower and moderate scores using the Thai score. For the ADA risk score, however, individuals with moderate scores (4-6 points) showed the greatest variability in HbA1c with the highest HbA1c identified in this subgroup.

Male individuals showed similar findings concerning the analysis among all participants. The median HbA1c for high risk Thai and high risk ADA scores were 5.6% and 5.9%, respectively (**Figure 2**). High risk categories other than median HbA1c were highest for both Thai and ADA scores. For females, high risk category was associated with the highest HbA1c. However, no subjects were categorized as high risk. The median HbA1c for high-risk Thai and moderate-risk ADA scores were 5.75% and 5.7%, respectively (**Figure 3**).

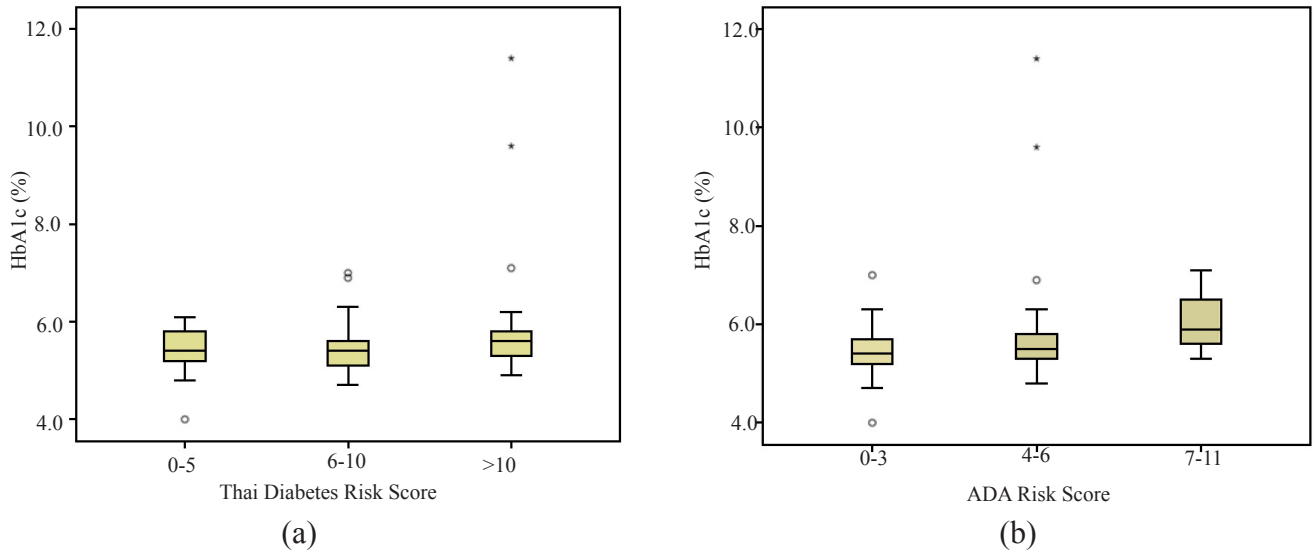


Figure 2. Changes in median, interquartile range, minimum and maximum HbA1c values of male participants in the associated risk categories of the (a) Thai diabetes risk score, (b) ADA diabetes risk score

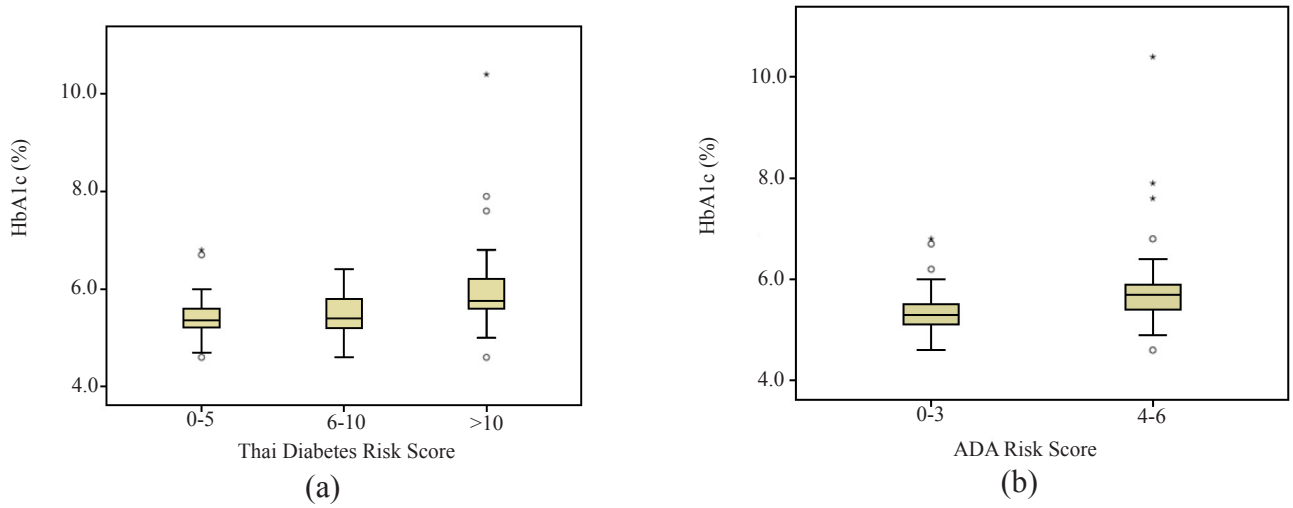


Figure 3. Changes in median, interquartile range, minimum and maximum HbA1c values of females in the associated risk categories of the (a) Thai diabetes risk score, (b) ADA diabetes risk score

Further analysis of the associations between HbA1c and other categorical factors were analyzed. Of all the anthropometric factors, waist circumference >90 cm among males ($p = 0.016$), BMI ≥27.5 ($p = 0.002$), and Thai diabetes risk score >10 ($p = 0.026$) were associated with the highest HbA1c. In multivariate analysis, waist circumference and BMI remained associated with high HbA1c.

Discussion

Early case detection of diabetes has been a key challenge in clinical practices. This study aimed to screen for diabetes in the out-of-clinic setting during blood donation, using diabetes risk scores, POC HbA1c and plasma glucose. The primary outcome showed a weak positive correlation between POC HbA1c and the Thai diabetes risk score. However, the association between HbA1c and diabetes risk has long-been established in related studies. [14, 15] This constituted the first time that POC HbA1c was used to study the correlation to Thai risk score.

POC HbA1c is a relatively recent development in testing glycated hemoglobin, using charge and structural differences of red blood cells to differentiate HbA1c values.[9] Although accuracy of POC systems is inferior to traditional blood samples[16-18], they offered greater access to immediate test results. POC HbA1c has been used

in a number of countries and has shown benefits in glycemic control. It facilitates treatment intensification by allowing immediate HbA1c results.[19] This has led to improved HbA1c by 0.5% over three months. However, paucity of data was found using POC HbA1c diabetes screening.[20] At the present time, the only US FDA-approved POC HbA1c is the Afinion HbA1c Dx Assay while many more are in development. Currently, the ADA and Thai guidelines do not recommend using POC HbA1c instead of HbA1c. Interestingly, a Thai study in testing POC HbA1c among individuals undergoing a dental procedure showed up to 33.8% of individuals presented POC HbA1c ≥5.7%.[21] In addition, a recent study in Indonesia revealed that the use of POC HbA1c showed potential in diagnosing diabetes with a sensitivity of 97% and specificity of 77%.[22]

One notable strength of this study was comparing Thai and ADA guidelines. The correlations demonstrated the validity of the two risk scores. However, the correlation appeared weak and may reflect on the POC system not being as accurate as the standard HbA1c. The other possibility would be the validity of risk scores themselves as demographics may change over the years, leading to a shift in risk score associations. For this study, comparison of the POC HbA1c and serum HbA1c was not available and thus might have reflected on the reliability of the POC HbA1c.

The other main difference in the study was using the out-of-clinic design. The majority of evidence in published literature is limited to in-clinic settings. However, the difference should not affect the HbA1c used as the main measure of this study.

Despite the weak correlation observed, one of the notable associations observed in this study were BMI and waist circumference. Obesity is a disease with rising prevalence and has close links with type 2 diabetes and metabolic syndrome. The Study to Help Improve Early evaluation and management of risk factors Leading to Diabetes (SHIELD) and the National Health and Nutrition Examination Surveys (NHANES) showed that increased BMI was associated with increased prevalence of type 2 diabetes, hypertension, and dyslipidemia.[23] For this study, the association of higher HbA1c was likely to be driven by obesity.

Furthermore, the correlations were shown to be more significant in the higher risk group, while the lower risk group showed little difference. Individuals prone to develop diabetes often have multiple factors at play, as well as having IFG, IGT or both as risk enhancers. However, this study was unable to measure fasting glucose or OGTT and hence was unable to confirm this hypothesis. Interestingly, the ADA diabetes risk score showed significant heterogeneity in HbA1c results while Thai scores did not. This was likely due to fundamentals in the design of the scoring system itself to correlate with a specific population and would suggest that ADA may not be a validated tool for practical use in Thai populations.

Limitations in this study included firstly, the majority of participants did not have fasting glucose levels. General recommendations from the Blood Bank suggest having food before blood donation. Therefore, correlating HbA1c with FPG was not possible, and subsequently not possible to definitely diagnose diabetes according to the new recommendations from ADA. Secondly, the nature of this cross-sectional design was the lack of causality. Also, unlike related cohorts that could predict future risk of diabetes, this study was not designed to evaluate future risk. Thirdly, the use of POC HbA1c did not have a standard serum HbA1c to correlate the findings. Related studies have shown that POC HbA1c was comparable to standard HbA1c, albeit with slightly lower accuracy. Adding the standard HbA1c to the protocol would improve the credibility of the data in this study.

In summary, the weak correlations observed in this study may have suggested that the use of POC HbA1c as a screening tool for diabetes in the out-of-clinic setting is less likely to be of benefit.

Conclusion

Higher Thai diabetes risk score was associated with higher HbA1c. This association was valid for both HbA1c and POC HbA1c. Because of the more rapid test results, POC HbA1c may be more suitable for use in-clinic than out-of-clinic

situations especially among individuals with risk scores >10. Higher BMI and waist circumference are predictors of higher POC HbA1c and may warrant earlier and more comprehensive testing.

Disclosures

The authors declare they have no conflicts of interest.

References

1. IDF Diabetes Atlas 9th Edition [Internet]. IDF. 2019. Available from: https://www.diabetesatlas.org/en/sections/worldwide-toll-of-diabetes.html.
2. IDF Diabetes Atlas [Internet]. IDF. 2019 [cited December 1, 2019]. Available from: https://diabetesatlas.org/data/en/country/196/th.html.
3. Beckman JA, Creager MA. Vascular complications of diabetes. Circulation Res 2016; 118: 1771-85.
4. Alva ML, Gray A, Mihaylova B, Leal J, Holman RR. The impact of diabetes-related complications onhealthcare costs: new results from the UKPDS (UKPDS 84). Diabet Med 2015; 32: 459-66.
5. Clinical Practice Guideline for Diabetes 2017 by Royal College of Physicians of Thailand, 3rd edition 2017.
6. Aekplakorn W, Bunnag P, Woodward M, Sritara P, Cheepudomwit S, Yamwong S, et al. A risk score for predicting incident diabetes in the Thai population. Diabetes Care 2006; 29: 1872-7.
7. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2020. Diabetes Care 2020; 43 (Suppl 1): S14-s31.
8. Aekplakorn W, Tantayothai V, Numsangkul S, Sripho W, Tatsato N, Burapasiriwat T, et al. Detecting prediabetes and diabetes: Agreement between fasting plasma glucose and oral glucose tolerance test in Thai adults. J Diabetes Res 2015; 2015: 396505.
9. Kaur J, Jiang C, Liu G. Different strategies for detection of HbA1c emphasizing on biosensors and point-of-care analyzers. Biosens Bio electron 2019; 123: 85-100.
10. Campbell L, Pepper T, Shipman K. HbA1c: a review of non-glycaemic variables. J Clin Path 2019; 72: 12-9.
11. Penttila I, Penttila K, Holm P, Laitinen H, Ranta P, Torronen J, et al. Methods, units and quality requirements for the analysis of haemoglobin A1c in diabetes mellitus. World J Method 2016; 6: 133-42.
12. Weykamp C. HbA1c: a review of analytical and clinical aspects. Annals Lab Med 2013 ; 33: 393-400.
13. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Practice 2019; 157: 107843.
14. Thewjitcharoen Y, Jones EA, Butadej S, Nakasatien S, Chotwanvirat P, Wanothayaroj E, et al. Performanceof HbA1c versus oral glucose tolerance test (OGTT) as a screening tool to diagnose dysglycemic status in high-risk Thai patients. BMC Endocr Disord 2019; 19: 23.
15. Gray BJ, Bracken RM, Turner D, Morgan K, Thomas M, Williams SP, et al. Examining the relationship between HbA1c and diabetes risk models in a European population indicates a lower threshold to identify 'high risk' is required. Diabetes Vasc Dis Res 2016; 13: 228-35.
16. Marley JV, Oh MS, Hadgraft N, Singleton S, Isaacs K, Atkinson D. Cross-sectional comparison of point-of-care with laboratory HbA(1)c in detecting diabetes in real-world remote Aboriginal settings. BMJ Open 2015: 5: e006277.
17. Sobolesky PM, Smith BE, Saenger AK, Schulz K, Apple FS, Scott MG, et al. Multicenter assessment of a hemoglobin A1c point-of-care device for diagnosis of diabetes mellitus. Clin Biochem 2018; 61: 18-22.
18. Fellows SE, Cipriano GC. Variation between point-of-care and laboratory HbA1c testing in clinical practice. P R Health Sci J 2019; 38: 189-191.

19. Schnell O, Crocker JB, Weng J. Impact of HbA1c testing at point of care on diabetes management. J Diabetes Sci Tech 2017; 11: 611-7.
20. Whitley HP, Hanson C, Parton JM. Systematic diabetes screening using point-of-care HbA1c testing facilitates identification of prediabetes. Annals Fam Med 2017; 15: 162-4.
21. Tantipoj C, Sakoolnamarka SS, Supa-amornkul S, Lohsoonthorn V, Deerochanawong C, Khovidhunkit SP, et al. Screening for type 2 diabetes using point-of-care testing for HbA1c among Thai dental patients. Southeast Asian J Trop Med Pub Health 2017; 48: 455-65.
22. Berbudi A, Rahmadika N, Tjahjadi AI, Ruslami R. Preformance of point-of-care testing compared with the standard laboratory diagnostic test in the measurement of HbA1c in Indonesian diabetic and nondiabetic subjects. J Diabetes Res 2020; 2020: 2037565.
23. Bays HE, Chapman RH, Grandy S. The relationship of body mass index to diabetes mellitus, hypertensionand dyslipidaemia: comparison of data from two national surveys. Inter J Clin Prac 2007; 61: 737-47.

JOURNAL OF SOUTHEAST ASIAN MEDICAL RESEARCH

J Southeast Asian Med Res; 2021

www.jseamed.org

ISSN 2586-8241