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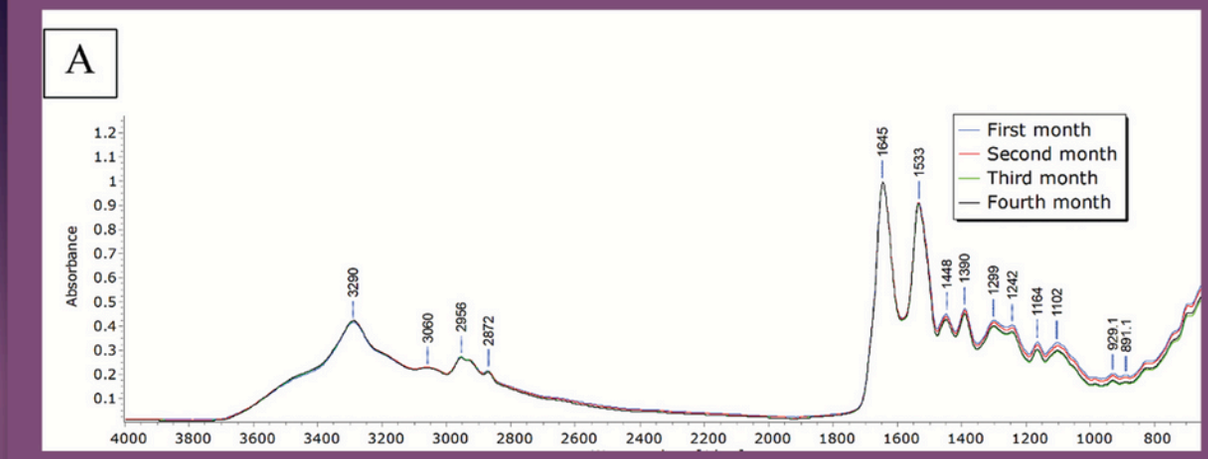

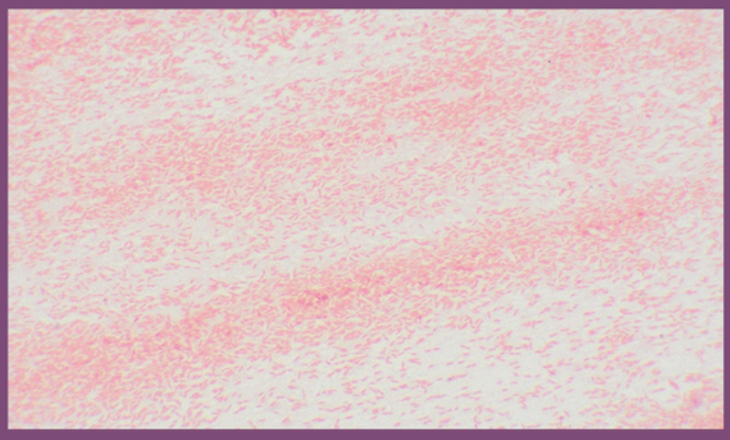

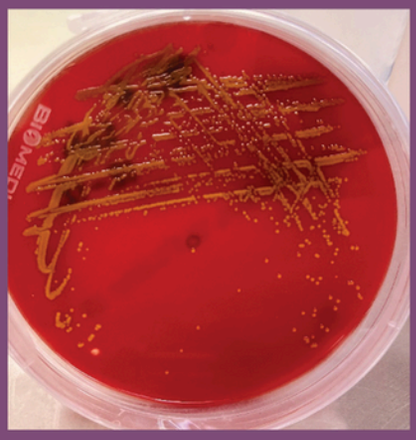

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ORIGINAL ARTICLE

Archives of Allied Health Sciences 2025; 37(2): 58-68.

ATR-FTIR detection of secondary structural stability of hemoglobin in hemolysate samples stored at freezing temperature

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KEYWORDS
ATR-FTIR;
HbA1c;
Hemoglobin structure;
Structural stability.

ABSTRACT

As for the importance of HbA1c for the diagnosis and monitoring of diabetes patients, many research studies evaluate HbA1c concentration upon storage. However, there are limited studies concerning the structural compositions during storage. The objective of this study was to examine the stability of secondary structure of hemoglobin in hemolysate samples stored for four months using the attenuated total reflectance-fourier transform infrared (ATR-FTIR) spectrometer. The leftover EDTA samples with known HbA1c values were separated into normal and diabetes groups, prepared in hemolysate form, and stored at -20°C. To evaluate the secondary structure of hemoglobin, FTIR spectra were collected for four months using the Agilent 4500 portable FTIR spectrometer (Agilent Technologies, CA). Qualitative and quantitative comparisons of amide I bands were performed in Spectragryph software and Origin software. The difference between the relative intensity ratios of amide A/B and amide I/II were not significant (p-value > 0.05). In qualitative comparison, the position, pattern, and signal intensity of the second derivative spectra remained identical up to four months. In quantitative comparison graph, alpha helix and beta sheet compositions did not show increasing or decreasing trend. This study demonstrated that the structural firmness of hemoglobin in samples remains unchanged after four months of storage at -20°C.

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Introduction

Diabetes mellitus (DM) is a chronic metabolic disease indicated by consistently high blood glucose, caused by either inadequate secretion of insulin from the pancreas or insulin resistance of the body cells. Statistics indicate a total of 4.2 million deaths by diabetes[1]. Glycosylated hemoglobin (HbA1c) has proven to be a keystone for glycemic control and DM diagnosis because it indicates the risk of diabetic complication development[2]. The American Diabetes Association (ADA) recommends that diabetic patients should maintain their HbA1c level below 7%[3]. The UK Prospective Diabetes Study (UKPDS) publication stated that every 1% reduction of HbA1c levels can lower the risk of diabetes complications along with the usefulness of HbA1c, the reliability of HbA1c results from clinical laboratories has become pivotal[2].

In the literature, many studies have used HbA1c analyzers to evaluate HbA1c concentrations upon storage. Bergmann and Sypniewska et al[4] evaluated the effect of a single freeze/thaw cycle on HbA1c concentrations measured by the HPLC method for 2-12 weeks. Farshad et al[5] studied the effect of different sample storage conditions on HbA1c concentrations using Cobas Integra 400 assays. Vijayachandrika et al[6] validated the stability of glycated hemoglobin measurements in blood samples stored at -20 °C for up to one month. Liotta et al[7] showed the reliability of HbA1c using the IE-HPLC method in frozen blood samples stored for 1.5 years. However, there is still a lack of structural detection in HbA1c samples during storage.

Fourier transform infrared (FTIR) spectroscopy is an analytical technique that provides information about the structure and composition of molecules by obtaining an infrared spectrum of absorption or emission. In FTIR spectroscopy, high-resolution spectral data are obtained over a wide spectral range, 4000 - 400 cm^{-1}. The attenuated total reflectance (ATR) technique uses a crystal with a high refractive index to analyze solid or thin film samples by simplified measurement. Diamond, Zinc selenide (ZnS), and Germanium crystals are commonly used for ATR. ATR-FTIR has many advantages such as rapid, non-destructive samples and requiring a small amount of sample for measurements. All forms of materials-- solid, liquid, and gas--can be identified[8]. ATR-FTIR spectroscopy can provide structural information on biomolecules like proteins, nucleic acid, lipids, and carbohydrates in their specific spectral regions[9,10]. Previous studies used ATR-FTIR to establish structural changes in hemoglobin caused by increased glucose concentration in diabetes samples, temperature and pH change[11], irradiation[12], and magnetic field[13]. However, there are limited studies in the literature regarding the structure of Hb upon storage. This study aimed to illustrate the secondary structural information of hemoglobin in HbA1c samples during four months of storage at freezing temperature.

Materials and methods

Sample collection and preparation

The leftover EDTA samples with known HbA1c results were obtained from the clinical laboratory of Srinagarind Hospital, Khon Kaen, Thailand. Samples were divided into normal and abnormal groups at 6.5% of HbA1c level as a cut-off. This study was approved by Khon Kaen University ethics committee for Human research (HE651015). The red blood cells were separately pooled for normal and abnormal groups. The hemolysate was performed by adding an equal volume of distilled water. Next, cell debris were filtrated using Whatman No.1 filter paper (Whatman, England). The pure hemolysates were aliquoted into 1.5 ml polystyrene capped tubes. After pooling, the HbA1c levels of normal and abnormal HbA1c control were measured in the pure hemolysates by Bio Rad D-10 TM analyzer (Bio Rad Laboratories, CA) based on the ion-exchange HPLC method. Then, the aliquots were immediately stored at -20°C for further measurements over four months.

Ethical Approval

This study was approved by the Khon Kaen University ethics committee for human research (HE651015).

FTIR Spectrum Acquisition

The Agilent 4500 portable FTIR spectrometer (Agilent Technologies, CA) was used to collect spectra. The ATR crystal was cleaned with deionized water and methanol before and after sample measurement. For measurement, 3 µl of sample was pipetted and placed on ATR crystal surface, spreading gently over the area. A low speed of hairdryer was applied to make a dry film. Then, spectrum was collected in spectral range 4000 - 650 cm^{-1}, with 64 scans and a spectral resolution of 4 cm^{-1}. Five replicate measurements were performed for each vial and averaged in Spectragryph software V1.2. The average spectra of each month were normalized to remove variations caused by unequal initial quantity of sample. Relative intensity ratios of amide A/B and amide I/II were compared monthly with the initial month using paired t-test (*p*-value = 0.05). The second derivative spectra of amide I band were produced for qualitative comparison of hemoglobin secondary structures using Spectragryph software V1.2.

For quantitative comparison, the baseline correction of collected raw spectra was performed and a fit was obtained in spectral range 1700 - 1600 cm^{-1} by using Gaussian curve fitting analysis in Origin software. First, the collected spectra of each month were averaged and smoothed with Savitsky-Golay smoothing at 17 smoothing points to avoid interference. Then, peaks within the spectral part 1600-1700 cm^{-1} were normalized. Second, derivative spectra were produced to detect the exact position of secondary structures of hemoglobin. After that, the spectra were put in Origin software, the baseline was corrected and Gaussian curve fitting analysis of the amide I band was performed for peak deconvolution.

Statistical analysis

Using SPSS software 28.0, the mean difference of intensity ratio between the first month and the other months was compared using a paired sample t-test. H_0 (null hypothesis): the mean difference between two groups is equal to zero. H_a (alternate hypothesis): the mean difference between the two groups is different from zero. If *p*-value < 0.05, reject the null hypothesis; if *p*-value > 0.05, accept the null hypothesis.

Results

The infrared spectra of normal and abnormal HbA1c control

The average raw spectral patterns of HbA1c normal level (Figure 1A) and HbA1c abnormal level (Figure 1B) were collected for four months. The spectra exhibited the expected absorption peaks associated to human blood[9]. As shown in figure 1, IR peaks could be described briefly as: 3290 cm^{-1} (amide A band), 3060 cm^{-1} (amide B band), 2956 cm^{-1} (asymmetric vibration of CH_3 stretching of proteins and lipids), 2872 cm^{-1} (symmetric vibration of CH_3 stretching of proteins and lipids), 1645 cm^{-1} and 1534 cm^{-1} (vibration of C=O and N-H stretching of amide I and amide II bands), 1448 cm^{-1} (bending vibration of CH_2 and CH_3 of phospholipid, fatty acids and glycerides), 1390 cm^{-1} (symmetric vibration of COO- of lipids and proteins), 1299 cm^{-1} (amide III band), 1242 cm^{-1} (asymmetric vibration of phospholipids), 1164 cm^{-1} (asymmetric vibration of COOC- of carbohydrates and proteins), 1102 cm^{-1} (symmetric vibration of phospholipids), 929 cm^{-1} (phosphodiester stretching bands region), 891 cm^{-1} (vibration of N-H of thymine).

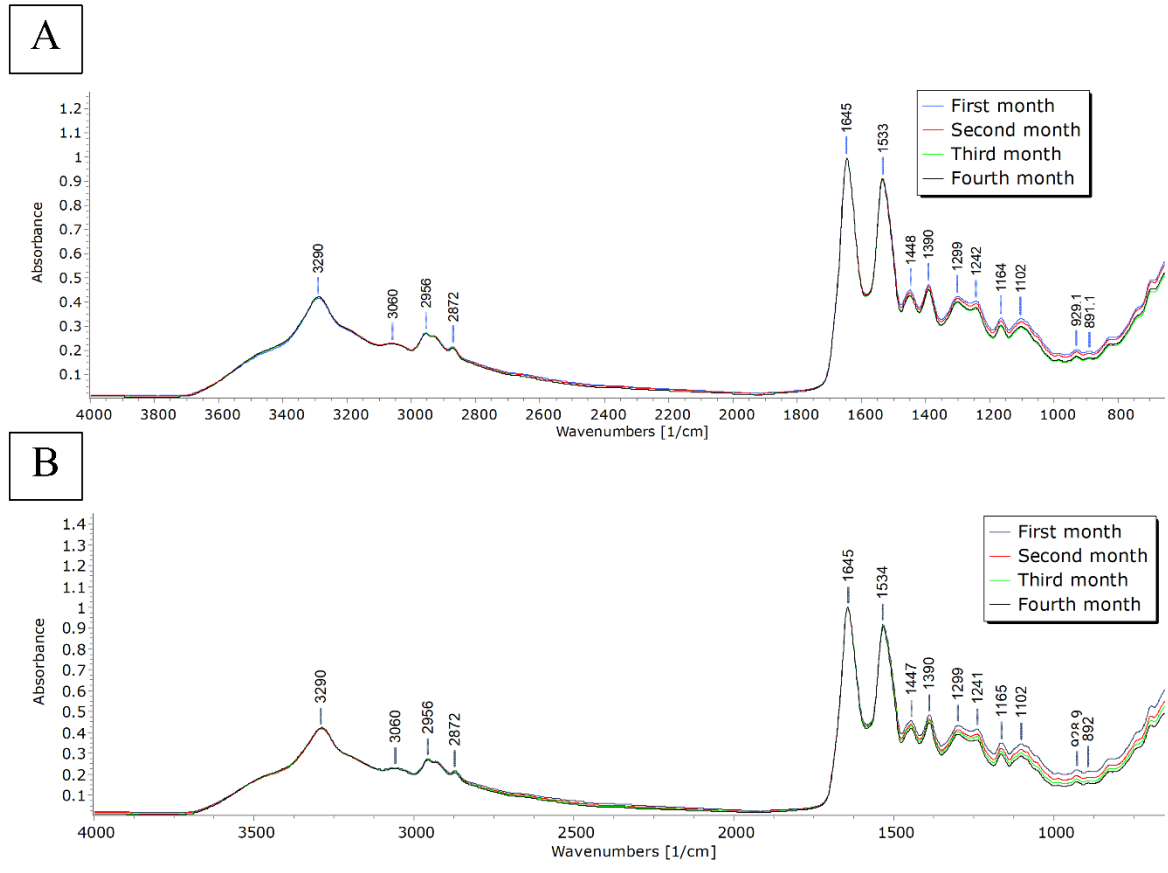

Figure 1 Average raw infrared spectra of hemolysate samples stored at 1st, 2nd, 3rd and 4th month.
(A) Normal HbA1c level
(B) Abnormal HbA1c level

Comparison of the relative intensity ratio of protein bands

FTIR spectroscopy has been proven to be a non-destructive tool for detecting secondary structural changes in proteins. The relative intensity ratio of FTIR spectra at different peaks and wavenumbers could provide information for qualitative changes in specific functional groups. The changes in protein composition were monitored using the relative intensity ratios of amide A/B and amide I/II. The values of I_{3290}/I_{3061} and I_{1650}/I_{1542} were calculated based on the averaged intensity of amide A, amide B, amide I and amide II bands. Then, the relative intensity ratios for each month were analyzed using SPSS software and paired t-tests were performed (p-value = 0.05). As shown in table 1, the difference was not statistically significant (p-value > 0.05) for both ratios, leading to the conclusion that protein functional groups did not change during storage months.

Table 1 Relative intensity ratio of amide bands in normal and abnormal groups

	Months	Normal group		Abnormal group	
		Intensity ratio	*p*-value	Intensity ratio	*p*-value
Amide A/B (I_{3290}/I_{3061})	First month	1.83±0.01		1.82±0.018	
	Second month	1.85±0.013	0.077	1.83±0.017	0.272
	Third month	1.85±0.007	0.049	1.85±0.026	0.230
	Fourth month	1.87±0.022	0.054	1.85±0.02	0.103
Amide I/II (I_{1650}/I_{1542})	First month	1.13±0.006		1.14±0.003	
	Second month	1.14±0.005	0.122	1.13±0.003	0.289
	Third month	1.14±0.003	0.295	1.14±0.007	0.314
	Fourth month	1.14±0.008	0.240	1.14±0.006	0.786

Note: The data are presented by mean ± SD.

Qualitative analysis of amide I band

The second derivative spectra of amide I bands of each month were produced to detect the qualitative composition of secondary structures of hemoglobin. Protein secondary structures such as alpha helices, beta sheets, and beta turns were observed in second derivative spectra at 1650 cm^{-1}, 1685 cm^{-1} and 1627 cm^{-1} respectively. A comparison of the position, pattern, and signal intensity of the second derivative spectra of the first month with those of 2nd, 3rd, and 4th months as shown in figure 2 and figure 3, revealed identical spectra in both groups. According to qualitative comparison, we may suggest that the secondary structure of hemoglobin remained unchanged in both groups after four months of storage at -20°C.

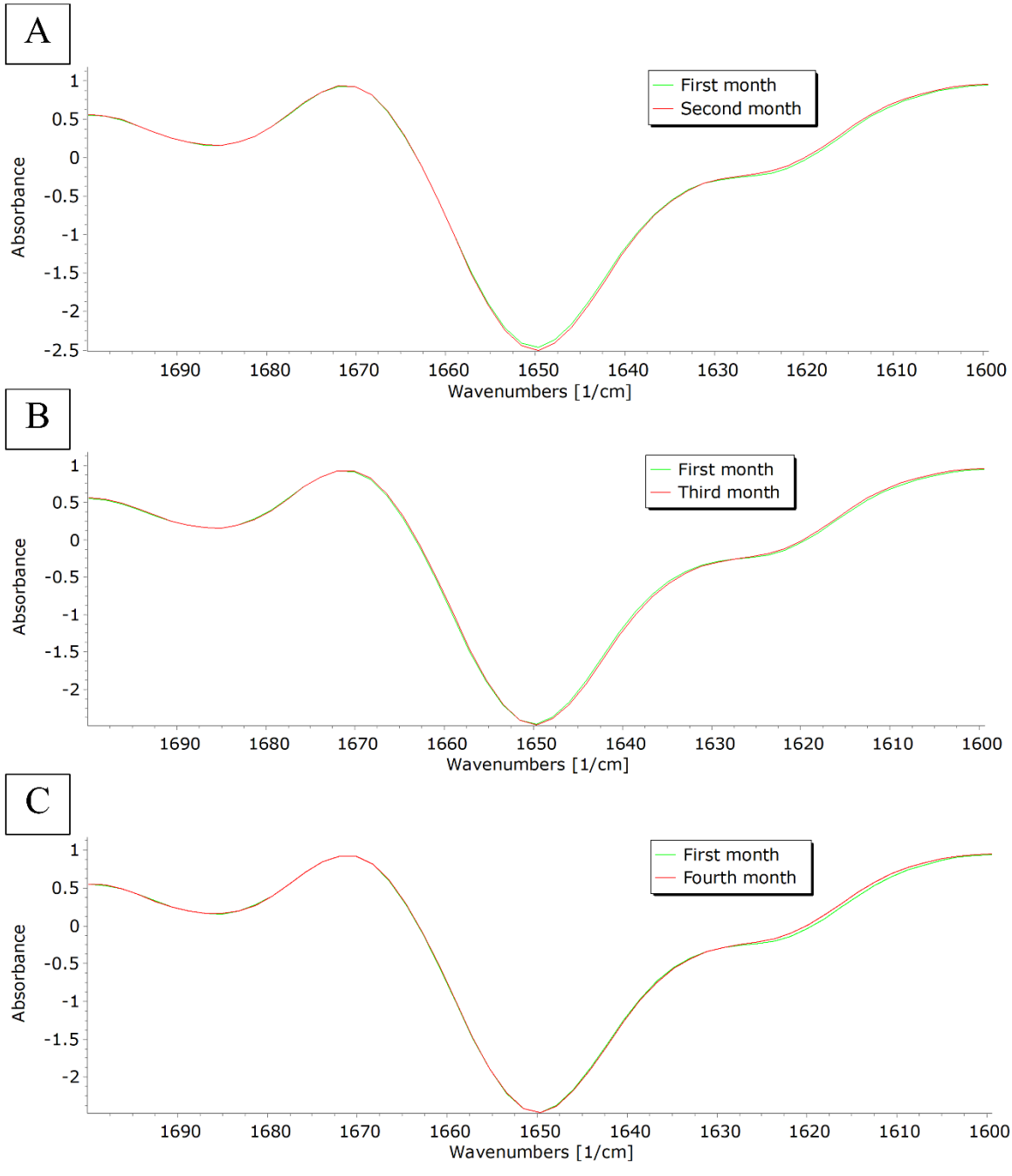

Figure 2 Second derivative spectra of amide I region of normal HbA1c level.
(A) Spectrum of the first month and the second month
(B) Spectrum of the first month and the third month
(C) Spectrum of the first month and the fourth month

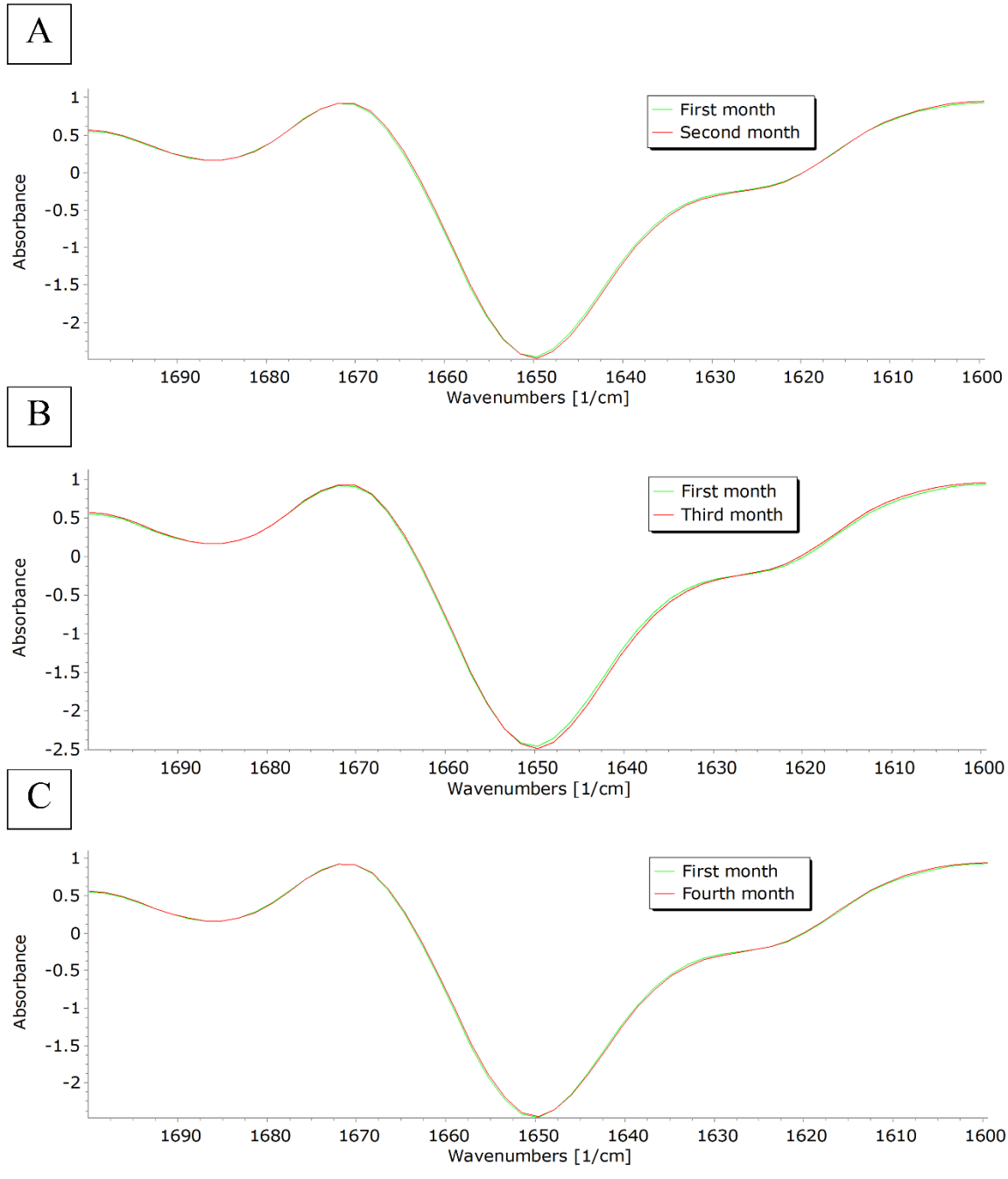

Figure 3 Second derivative spectra of amide I region of abnormal HbA1c level.
(A) Spectrum of the first month and second month
(B) Spectrum of the first month and third month
(C) Spectrum of the first month and fourth month

Quantitative comparison of amide I band

For quantitative determination of secondary structures of hemoglobin, Gaussian curve fitting analysis was performed on the amide I region using Origin software. The percentage compositions of alpha helices and beta sheets were demonstrated in figure 4. There was no change in alpha helix and beta sheet compositions in both groups. According to this finding, the secondary structure of hemoglobin remained unchanged in both groups after four months of storage.

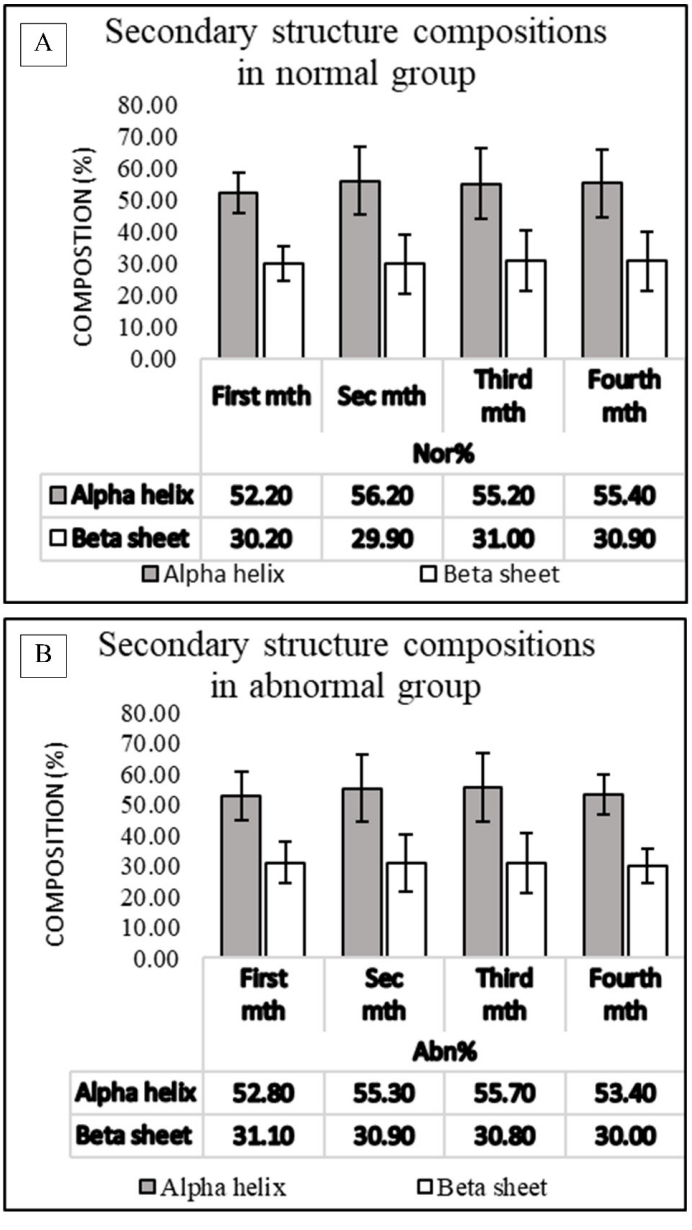

Figure 4 Secondary structure compositions in four months.
(A) Alpha helix and beta sheet compositions of normal group
(B) Alpha helix and beta sheet compositions of abnormal group

Discussion

Hemoglobin molecule is the major protein in red blood cells, and secondary structures include alpha helices, beta sheets, and other elements such as beta turns and unordered structures. In the FTIR spectrum, amide I (1600 - 1700 cm^{-1}) and amide II (1500 - 1600 cm^{-1}) bands, which exhibit the highest absorption peaks in the entire spectrum, mainly reflect protein secondary structures[14]. Andleeb et al[15] used the spectral region of 1600 – 1700 cm^{-1} to detect hemoglobin secondary structure at higher levels of hemoglobin A1c in type 2 diabetes. Their findings indicated a decrease in the intensity ratio of amide I/II in patients with HbA1c > 9% due to a change in Hb structure[15]. Ye et al[16] examined the spectra between 1600 - 1700 cm^{-1} to access the impact of HbA1c levels on the hemoglobin structure in type 2 diabetes patients. They reported that the variations of protein structures were detected by comparing the relative intensity ratio of different peaks between group H (healthy controls), group A (patients with HbA1c < 7%), and group B (patients with HbA1c > 9%). The ratio of amide I/II was slightly lower in group A (p-value > 0.05) and significantly lower in group B (p-value < 0.05) than in group H. They concluded that the Hb structure of group B may have changed[16]. Therefore, this study used the spectra between 1600 - 1700 cm^{-1} region to detect the secondary structure of hemoglobin in HbA1c samples.

Previous studies have reported the structural changes in Hb due to temperature, pH, irradiation, glucose concentration, and magnetic field changes. Increasing temperature leads to the weakening of hydrogen bonds[17]. Calabro and Magazu[13] demonstrated the unfolding of hemoglobin after exposure to a low-frequency electromagnetic field[13]. In the study of Ye et al[16], the secondary structures of hemoglobin changed as the HbA1c level exceeded 9.0%[16]. In the study of Saeed et al[12], the secondary protein structures in rat erythrocytes upon neutron irradiation were detected[12]. In the present study, we studied the secondary structure of hemoglobin at four months of storage at -20 °C. The relative intensity ratio of amide bands reflected the change in the composition of protein portions in samples. In our study, the relative intensity ratios of amide A/B and amide I/II of 2nd, 3rd and 4th months were compared with those of the initial month. There was no statistically significant difference (p-value > 0.05) between the relative intensity ratio of amide bands in both groups, suggesting that protein composition remained the same as the initial month after four months of storage.

Protein secondary structures include alpha helix, beta sheet, and beta turns. The second derivative spectra of the amide I region can indicate the exact position of these structures[18]. In Ye et al[16] study, the second derivative spectra of 1600-1700 cm^{-1} region were compared among three groups. Obvious differences were observed in the number, position, signal intensity, and pattern of the underlying components among the three groups. Andleeb et al[15] also observed that the qualitative comparison of the second derivative spectra clearly differed in pattern, number, and intensity among the three groups. In our study, the qualitative comparison in pattern, number, and intensity of second derivative spectra of both groups did not show any difference after four months of storage. We suggest that the hemoglobin structure did not change in both groups after four months of storage at -20°C. However, a limitation of ATR-FTIR technique is the interference from water vapor; therefore, lyophilized hemolysate may reduce the effects of water vapor. Moreover, the stability of hemoglobin in various temperatures and freeze-thawed conditions should be further investigated. This information can be applied to monitor the stability of hemoglobin as control material in further studies.

Conclusion

In conclusion, our study illustrated the secondary structural composition of hemoglobin in HbA1c samples over four months of storage using ATR-FTIR spectroscopy, along with the relative intensity ratio and peak deconvolution technique. According to the relative intensity ratio of amide bands and secondary structural compositions in both groups, there was no evidence of protein structures unfolding. This study concludes that the structural integrity of hemoglobin in HbA1c samples remains unchanged after four months of storage at -20°C.

Take home messages

The ATR-FTIR spectra showed the stability of the secondary structure of hemoglobin, which was stored at -20°C for four months.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

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Author contributions

Htet Htet Htin Khine: Conceptualization, Design of the experiments, Samples collection, Research methodology, Formal analysis, Writing – Original draft.

Siriporn Proungvitaya: Conceptualization, Design of the experiments, Research methodology validation, Writing – Review & Editing.

Patcharaporn Tippayawat: Conceptualization, Design of the experiments, Research methodology validation, Writing – Review & Editing.

Chirapond Chonanant: Conceptualization, Design of the experiments, Research methodology validation, Writing – Review & Editing.

Molin Wongwattanakul: Conceptualization, Design of the experiments, Research methodology validation, Formal analysis, Resources, Project administration and funding acquisition, Writing – Review & Editing.

Data availability

Data available on request due to privacy/ ethical restrictions

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CASE REPORT

Arch AHS

Archives of Allied Health Sciences 2025; 37(2): 69-74.

The genus *Dyella* spp. bacteremia from hemodialysis blood culture: a case report

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KEYWORDS
Dyella spp.;
Automated blood culture;
Matrix- assisted laser desorption/ ionization;
Mass spectrometry.

ABSTRACT
This study presents a patient identified to have *Dyella* spp., a rare genus of bacteria, bacteremia. *Dyella* spp. cannot be identified through biochemical testing methods. At present, Matrix-assisted laser desorption/ionization is applied to differentiate the types of microbes. However, there is a limitation in distinguishing the specific type of bacteria because of the limitation of library databases. The 16S rRNA sequencing is crucial for differentiating the rare genus of bacteria that are improbable to recognize by mass spectrometry technique. This report highlights the discovery of *Dyella* spp. in patient blood culture, emphasizing the challenges in identification by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometer (MALDI-TOF MS), an ordinary technique. The instruction provided in this report aims to improve the application of this high-technology machine to accurately differentiate the rare genus of bacteria, ultimately enhancing patient care and treatment outcomes.

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Introduction

Dyella spp. are aerobic gram-negative rods, small, yellow, classified under gamma- proteobacteria. In 2005, a novel finding related to the genus *Dyella*, in the family *Xanthomonadaceae*, was reported, with the species *Dyella japonica* mentioned in a previous report[1]. The genus *Dyella* includes six species: *D. japonica*, *D. koreensis*, *D. ginsengisoli*, *D. marensis*, *D. soli* and *D. terrae*[2]. This genus is typically isolated from water, soil, and other environment sources[2, 3]. All *Dyella* spp. are environmental isolates and have not reported to cause human infections. Human infections caused by *Dyella* spp. are extremely rare, and their pathogenicity in humans remains unclear. In Thailand, the first case of infection by *Dyella japonica* in hemodialysis patients was reported. Contaminated hemodialysis may have caused bacteremia. The severity of bacteremia was mild, and the patient responded well to antibiotic therapy[1]. These environmental bacteria are commonly found in hemodialysis patients, who are at high risk for opportunistic infections[1].

The methods for identification of this genus are difficult and challenging for clinical microbiology laboratories. Conventional and automated biochemical identification methods failed to identify these bacteria. The Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometer (MALDI-TOF MS) also misidentified these bacteria due to certain limitations of the technique[4].The spectrum of the *Dyella* spp. in database for MALDI-TOF MS identification includes limited isolates. To further confirm the identification may use 16s rRNA gene sequencing. After confirming by 16s rRNA then generates a reference spectrum in the MALDI-TOF MS database for identification of bacteria next patients.

Herein, we report a patient identified to have *Dyella* spp., a rare genus of bacteria, which cannot be identified through biochemical testing and MALDI-TOF MS.

This report demonstrates the usefulness of molecular methods for identifying uncommon bacteria from clinical specimens.

Case history

A 35-year-old woman presented to the emergency room with fever and chill symptoms for one day during a hemodialysis session. Her underlying diseases were end-stage renal disease (ESRD), diabetes, hypertension, and secondary hyperparathyroidism. The physician sent three blood culture samples drawn from different sites: the A-line, the V-line of the dialysis permanent catheter, and the peripheral line. The samples were placed in BACTEC Plus Aerobic/F bottles and incubated in a BACTEC FX automated blood culture system (Becton Dickinson, USA). All bottles showed positive results after 48 hours of incubation (Table 1).

Table 1 Time to positive for all blood cultures from hemodialysis patient

Blood collection position	Time to positive
A-line (dialysis permanent catheter)	17 hours and 33 minutes
V-line (dialysis permanent catheter)	19 hours and 33 minutes
Peripheral line no.1	3 days, 13 hours, and 38 minutes
Peripheral line no.2	No growth after 3 days
Peripheral line no.3	1 day, 14 hours, and 34 minutes
Peripheral line no.4	1 day, 11 hours, and 34 minutes

Materials and methods

Ethical approval

In this study, *Dyella* spp. isolate was cultured from left-over blood bottle after a routine diagnostic examination. The isolate was then anonymized with no patient's data links to protect patients. This case report was approved by the Ethics Committee for Human Research, Lerdsin Hospital, Department of Medical Services (Certificate of approval number LH671044).

Sample processing

The specimens from the positive bottles were cultured on solid media, including 5% sheep blood agar, MacConkey agar, and chocolate agar as the routine of clinical microbiologic laboratory protocol. The morphology of the bacteria was demonstrated as Gram-negative bacilli (Figure 1). Specific colony characteristics on blood agar were light yellow color and less than one mm. in size (Figure 2). Specimens from the plates consistently demonstrated negative bacilli on Gram staining with a positive oxidase test. Identification by mass spectrometry with the MALDI-TOF MS Sirius (Bruker Daltonics, Germany)[4] could not determine the species, yielding an identification score of < 1.4, which is considered "unreliable" according to the manufacturer's guidelines.

The culture plates were incubated for 16 hours and then retested for species identification and antimicrobial susceptibility for Gram-negative bacilli. The species identification results remained the same, with an identification score of <1.4[4]. Antimicrobial susceptibility testing using the dilution method was performed, with results interpreted as minimum inhibitory concentration (MIC). However, interpreting the MIC results as susceptible, intermediate, or resistant according to The Clinical & Laboratory Standards Institute (CLSI) guidelines was challenging if precise genus and species identification were undetermined[5].

Result

To further identify the species, the laboratory sent the sample to another laboratory for automated biochemical testing with a ready-to-use test kit (Sensititre, United Kingdom). The preliminary result was *Elizabethkingia meningoseptica*, which was reported along with the antimicrobial susceptibility result. Subsequently, the laboratory conducted the additional analysis using another automated biochemical test (Phoenix, USA) and a different MALDI-TOF MS Sirius machine. The automated biochemical test (Phoenix) identified the bacterium as *Spingomonas paucimobilis*, whereas the MALDI-TOF MS Sirius machine insistently unsuccessful to identify the species. According to the manufacturer's and CLSI recommendations[5], an extraction method was used to improve sample preparation, increasing the likelihood of detecting bacterial proteins and obtaining a better spectrum from the MALDI-TOF MS. This preparation directed the identification scores of 1.4-1.5 for *Dyella jiangningensis*, but the score was still categorized under "No reliable identification."

The analyses were inconclusive, so molecular biology techniques were essential to advance the identification. Specifically, 16S rRNA sequencing, defining the bacterial base sequence, was applied[1, 6]. The sequence base of the sample was compared to the reference strains in the National Center for Biotechnology Information (NCBI) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sample's base sequence needed a percent identification score of at least 98.5%, similar to the database's standard strains[6]. The comparison revealed that the patient's sample had percent identification scores of 99.03% and 99.02% for *Dyella* spp. and *Dyella jiangningensis*, respectively. Therefore, it was preliminarily concluded that the patient's sample was the *Dyella* spp.

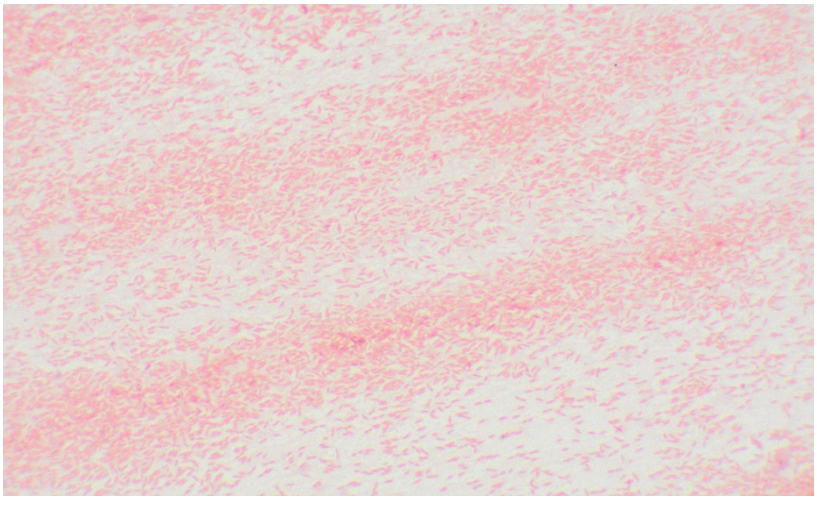

Figure 1 The Gram stain morphology of bacteria from blood culture.

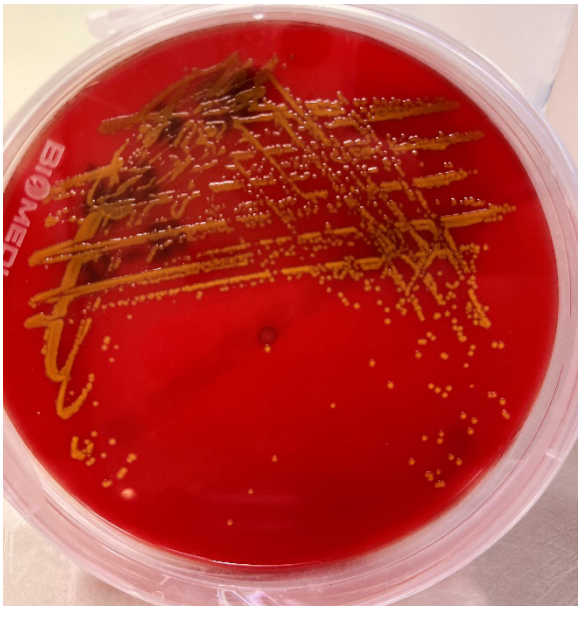

Figure 2 The morphology of bacteria on blood agar plate.

Discussion

The genus *Dyella* spp. was firstly discovered in 2005 in Japan from environmental samples. It was found to be closely related to bacteria in the family *Xanthomonadaceae*. Reports of *Dyella* spp. from clinical specimens have been limited[7,8]. In 2007, Kiratisin P. reported *Dyella japonica* bacteremia in a hemodialysis patient, the first report of *Dyella* spp. human infection[1]. Duus LM reported *Dyella* spp. colonization in sputum from patients with cystic fibrosis[7, 8]. In this study, the bacterium isolated from blood specimens is *Dyella* spp., genetically related to *Dyella japonica*[9].

Conventional and commercial biochemical tests used for routine species identification have proven inaccurate for *Dyella* spp. Previous tests have often misidentified it as *Elizabethkingia meningoseptica*, or *Stenotrophomonas maltophilia*[7, 10].

These discrepancies could be due to variations in the types of culture media used by different manufacturers, leading to inaccurate species identification. In this study, two different commercial biochemical tests produced conflicting results. The MALDI-TOF MS method also reported "No reliable identification," even with additional sample preparation using extraction methods. The lack of reliable identification reports may be because *Dyella* spp. is rare, and the database for the MALDI-TOF MS has few reference strains, resulting in the scores below 1.7[7, 10].

Once routine methods are unsuccessful in identifying the bacterium accurately, more precise methods like 16S rRNA sequencing are necessary. This study used 16S rRNA sequencing, which showed that the sample's sequence was more than 98.5% like the three strains in the NCBI database. Since *Dyella* spp. is uncommon, few researchers studied its sequences, and only some sequences are available in the NCBI database. Consequently, this study could only preliminarily identify the sample as *Dyella* spp. A reference spectrum for this bacterium was created in the MALDI-TOF MS system to aid future species identification[7, 8].

Conclusion

The antibiotic piperacillin-tazobactam was a treatment for this case. The genus *Dyella* spp. is mostly isolated from environmental sources such as soil or air, but there have been increasing reports of its presence in clinical specimens, particularly in the patients at risk for opportunistic infections. Laboratory identification tests have difficulty accurately identifying *Dyella* spp. Conventional biochemical tests cannot distinguish the strain, and even using MALDI-TOF MS has limitations due to the limited reference strains in the database, resulting in unreliable identifications. The authors recommend that once biochemical tests or MALDI-TOF MS cannot identify the bacterium or raise doubts about its identification, it is necessary to confirm the species using 16S rRNA sequencing. The recommendation should be followed for all cases, and the data should be reported to improve future species identification capabilities.

Take home messages

This environmental bacteria genus *Dyella* spp. is a rare case of human infection. However, bacteria can cause bacteremia in patients who are at risk of opportunistic infection.

Conflicts of interest

The authors declare no conflict of interest.

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Author contributions

Monchai Siribamrungwong: Conceptualization, Methodology, Writing – Review & Editing (clinical perspective).

Kwanchon Jearakitiwanich: Methodology, Formal analysis, Writing Original draft, Writing – Review & Editing (laboratory perspective).

Data availability

Data available on request due to privacy/ethical restrictions

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Ability of a single question to discriminate physical and psychological problems relating to the fear of fall in hyperkyphosis older adults

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KEYWORDS
Dowager's hump; Falls Efficacy Scale-International; five times sit-to-stand test; Screening tool; Fear of falling.

ABSTRACT

Fear of falling (FOF) is a significant concern among older adults, especially those with hyperkyphosis that may induce several negative impacts, such as reduced both physical and social interaction, increased risk of future falls and associated mortality, and decreased quality of life. Given these consequences, early and accurate identification of FOF is essential to inform targeted preventive strategies. Various assessment tools are available to evaluate FOF, including single-item question, self-reported scales such as the Falls Efficacy Scale-International (FES-I), and objective assessments, including the five times sit-to-stand test (FTSST). However, a universally accepted gold standard for assessing FOF in older adults with hyperkyphosis has not yet been established. This study aimed to evaluate whether a single-question assessment can effectively identify individuals with and without FOF, using the FES-I and FTSST as reference measures. Sixty-three community-dwelling older adults aged 60 years and above with clinically confirmed hyperkyphosis participated in the study. All participants were assessed for FOF using the single-question method to divide the participants into non-FOF and FOF groups. Then they were assessed for physical and psychological problems relating to FOF using the FES-I and FTSST. The results indicated that participants in the FOF group had significantly higher FES-I scores than those in the non-FOF group (p-value < 0.001). Similarly, participants with FOF required significantly more time to complete the FTSST (p-value < 0.001). These findings suggested that the single-question assessment can effectively distinguish individuals with FOF from those without FOF. In conclusion, the single-question approach appears to be a simple, time-efficient, and practical screening tool for identifying FOF in older adults with hyperkyphosis. Its implementation in clinical and community settings may contribute to early detection and timely intervention, potentially reducing fall risk and enhancing functional outcomes in this population.

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Introduction

Fear of falling (FOF) is a common problem among older adults, defined as a persistent fear or anxiety about falling, regardless of whether they have a prior history of falls[1]. FOF is not merely a temporary worry; it is a psychological condition that often results in physical inactivity, which can subsequently lead to muscle weakness and reduced functional capacity[2,3]. These functional impairments may induce negative impacts on these individuals, including loss of mobility, reduced both physical and social interaction, increased risk of future falls and associated mortality, and decreased quality of life[4].

One of the biomechanical factors contributing to FOF is hyperkyphosis, or excessive thoracic spinal curvature, which commonly occurs in older adults[5]. Hyperkyphosis affects postural alignment by shifting the body's center of gravity forward, impairing the ability to maintain balance and stability[6]. This postural change places demands on the lower limbs, as they must work harder to compensate for the altered alignment[7]. As a result, individuals with hyperkyphosis often experience weakness in the lower limb muscles, which further compromises their ability to perform basic movements, such as standing up, walking, and maintaining balance[8]. The reduced lower limb strength not only exacerbates the risk of falling but also intensifies the fear of falling, creating a cyclical relationship between muscle weakness and psychological distress[9]. The prevalence of FOF among adults aged 60 and older ranges from 20% to 40%, with the rates increasing with age. One study found that approximately 30% of individuals aged 60-64 experience some level of FOF, while this percentage increases significantly in individuals aged 75 and older, reaching as high as 50% or more[10,11]. Research indicates that older adults with hyperkyphosis are at an increased risk of falling compared to those without the condition, with one study reporting that up to 50% of individuals with hyperkyphosis experience frequent falls[12].

In consideration of the complex and the multidimensional aspects of FOF, it is crucial to evaluate both the psychological and physical components of fall risk[13]. A single-question assessment, such as "Are you afraid of falling?" is commonly used for initial screening FOF in older adults due to its simplicity and time-efficiency[13]. This approach has demonstrated acceptable levels of validity and reliability. Previous studies have shown that the single-question assessment has moderate to strong correlations with more comprehensive tools, such as the Falls Efficacy Scale-International (FES-I), indicating good concurrent validity[14]. In terms of reliability, the question has shown moderate test-retest reliability when administered to community-dwelling older adults[14]. Despite these strengths, the single-question assessment may not fully capture the multidimensional nature of FOF, particularly regarding the task-specific concerns and the behavioral consequences associated with the fear. As such, more detailed instruments, including the FES-I, are often utilized to provide a more nuanced understanding of how FOF affects daily functioning and mobility.

In addition to self-reported measures, performance-based assessments are equally important in evaluating physical contributors to fall risk. The five times sit-to-stand test (FTSST) is widely recognized as a reliable indicator of lower limb strength, dynamic balance, and the ability to perform transitional movements[15].Impaired performance on the FTSST has been shown to correlate with heightened levels of FOF, reflecting muscular weakness and postural instability[16]. Accordingly, the present study aimed to examine the discriminative capability of the single-question assessment in identifying older adults with and without FOF, using the FES-I and FTSST as reference measures. Establishing the utility of a brief screening tool in comparison with more comprehensive assessments may provide valuable insights for efficient early detection strategies in both clinical practice and community-based interventions.

Materials and methods

Study design and participants

This cross-sectional study investigated individuals with hyperkyphosis from several communities in Khon Kaen province, Thailand. The sample size was calculated using the formula for comparing the means of two independent groups. The significance level (α) was set at 0.05 and the statistical power (β) was set at 80%. The pooled variance of FES-I being 15.27 and FTSST being 8.33, and the expected difference in group means of FES-I being 16.8 and FTSST being 7.75. All of these were obtained from a pilot study involving 10 older adults with hyperkyphosis. Along with sample size calculation, the number required at least 19 participant per group to cover all parameters.

Participants aged at least 60 years, both male and female, were included if they were diagnosed as hyperkyphosis based on the 7th cervical vertebra wall distance (C7WD) of ≥7.5 cm.[17]. However, the participants were excluded if they had spinal abnormalities of the thoracic region (e.g., scoliosis, cervical hump), uncontrolled or unstable medical conditions (e.g., hypertension, heart disease), musculoskeletal pain > 5 out of 10 on the numeric rating scale, neurological impairments with residual functional deficits (e.g., stroke, Parkinson's disease, and spinal cord injury) and loss of hearing or visual condition. Additionally, individuals with acute cardiovascular or respiratory conditions (e.g., acute myocardial infarction, unstable angina, and severe arrhythmias) were ineligible. Then, all eligible participants were informed about the study's objectives and procedures and provided written informed consent prior to participation. Ethical approval was granted by the Khon Kaen University Ethics Committee for Human Research (HE672234).

Research protocol

The eligible participants were interviewed for their demographics, including age, weight, height, body mass index, underlying disease, gait aid, and history of fall. FOF was assessed using a single-question, "Are you afraid of falling?" to categorize the participants into fear and non-fear persons. Then, all participants were additional interviewed the FES-I and performed the FTSST. Details of the questionnaire and functional test are as follows;

Falls Efficacy Scale-International

This questionnaire contains 16 items, designed to assess fall-related self-efficacy. Each item measures concern about falling across four subscales: not at all concerned (1), somewhat concerned (2), fairly concerned (3), and very concerned (4), yielding a total score ranging from 16 to 64. Higher scores indicate lower self-efficacy and greater concern about falling[18].

Five times sit-to-stand test

This test evaluates lower extremity muscle strength, as well as balance control and mobility during the sit-to-stand movement[19]. The participants were asked to sit on a standard armless chair with their arms by their sides, their back upright against the backrest, and their feet flat on the floor, 10 cm behind their knees. They were then instructed to stand up from the chair with fully straight knees and hips and sit down as quickly and safely as possible for five repetitions. The time was recorded from the command "Go" until the participants completed five repetitions and sat down with their back touching the backrest of the chair [20]. The average score from three trials was recorded in seconds.

Statistical analysis

The statistical analysis was conducted using SPSS for Mac, version 29.0. Descriptive statistics, including mean, standard deviation, and percentage, were used to summarize participants' demographic characteristics. To compare differences between groups (non-FOF and FOF), an independent samples t-test was performed for continuous variables, while a Chi-square test was applied for categorical variables, provided the data met the assumption of normality. However, some variables of

demographic characteristics (sex, age, height, C7WD, underlying diseases, history of fall, and gait aid) were non-normally distributed, the Mann-Whitney U test was used for continuous variable and Fisher's Exact test was used for categorical variable comparisons. A *p*-value of less than 0.05 was considered statistically significant.

Result

Demographic characteristics of the participants

Seventy-seven individuals with hyperkyphosis were interested to participate in the study. However, 14 individuals were excluded because of missing the inclusion criteria (Figure 1). Thus, a total of 63 participants completed the study, and were divided into fear of fall (n = 43, 68.25%) and non-fear of fall (n = 20, 31.75%) groups based on their single question scores. From the participant data, the distribution of sex, age, height, C7WD, underlying diseases, history of fall, and gait aid was non-normally distributed; therefore, the median was reported for these variables. All demographic characteristics of the participants were shown in table 1. A total of 19 participants (30.2%) used gait aid, which included 15 participants (78.94 %) using a cane, 2 participants (10.53 %) using a wheel walker flame, and 2 participants (10.53 %) using a walker frame. A total of 45 participants (71.4%) had underlying disease ranged from 1-4 diseases, that included hypertension (39.06%), diabetes mellitus (25%), hyperlipidemia (12.5%), heart disease (6.25%), kidney disease (1.56%), others (15.63%), such as osteoarthritis, rheumatoid arthritis, prostatic hyperplasia, thyroid, and peptic ulcer. A total of 13 participants (20.6%) had history of fall in the last 6 months, which was ranged from 1-3 times.

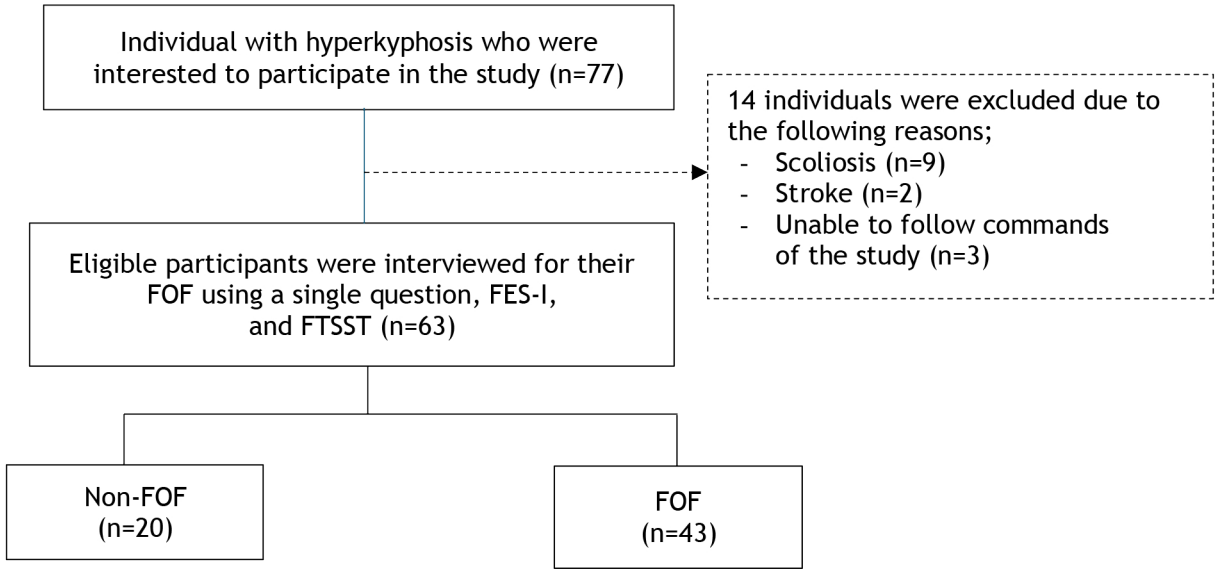

Figure 1 Participation flowchart.
Abbreviations: FOF, fear of fall; FES-I, Falls Efficacy Scale-International; FTSST, five times sit-to-stand test.

Table 1 Demographic characteristics of the participants

Variable	All (n=63)			Non-fear of fall (n=20)			Fear of fall (n=43)			*p*-value
	Mean ± SD (95%CI)	Median	Min - Max	Mean ± SD (95%CI)	Median	Min - Max	Mean ± SD (95%CI)	Median	Min - Max	
Sex: female, n (%)‡		42 (66.70)			10 (50.00)			32 (74.40)		0.056[a]
Age (years)	74.40 ± 6.30 (72.81-75.98)	75	60-85	74.80 ± 6.17 (71.91-77.69)	78	64-83	74.21 ± 6.43 (72.23-76.19)	74	60-85	0.732[c]
Weight (kg)	56.76 ± 13.46 (53.37-60.15)	55	32-95	56.10 ± 11.90 (50.53-61.67)	55.5	37-82	57.67 ± 14.25 (52.67-61.45)	53	32-95	0.794[b]
Height (cm)	154.81 ± 7.92 (152.81-156.81)	154	140-174	153.08 ± 8.69 (153.08-161.21)	155.5	140-172	153.72 ± 7.39 (151.44-155.99)	152	140-174	0.110[c]
BMI (kg/m^2)	23.58 ± 4.81 (22.37-24.79)	23.14	14.81-37.33	22.67 ± 3.81 (20.89-24.45)	22.56	16.65-30.30	24.01 ± 5.20 (22.41-25.61)	23.56	14.81-37.33	0.308[b]
C7WD (cm)	9.75 ± 2.63 (9.08-10.41)	8.50	7.51-15.75	9.53 ± 2.19 (8.50-10.56)	8.4	7.51-15.20	9.85±2.83 (8.98-10.72)	8.70	7.51-15.75	0.656[c]
UD [Yes, n (%)]‡		45 (71.40)			13 (65.00)			32 (74.40)		0.441[a]
History of fall [Yes, n (%)]‡		13 (20.60)			4 (20.00)			9 (20.90)		0.932[a]
Gait aid [Yes, n (%)]‡		19 (30.20)			2 (10.00)			17 (39.50)		0.017[a*]

Note: The data were compared using independent t-test. ‡The data are presented using the number (percent of total participants). [a]The data were compared using Chi-square test, [b]Independent samples t-test, and [c] Mann-Whitney U Test, *Indicated significant differences (*p*-value < 0.05).

Abbreviations: BMI, body mass index; C7WD, 7th cervical vertebra wall distance; UD, underlying disease; SD, standard deviation; CI, confidence interval.

Table 2 Comparison of the FES-I and FTSST between non-FOF and FOF groups

Test	All (n=63)			Non-fear of fall (n=20)			Fear of fall (n=43)			Mean difference (95%CI)	*p*-value
	Mean ± SD (95%CI)	Median	Min - Max	Mean ± SD (95%CI)	Median	Min - Max	Mean ± SD (95%CI)	Median	Min - Max		
FES-I (points)	38.52 ± 14.93 (34.76-42.28)	41	16-63	23.25±13.03 (17.15-29.35)	18	16-62	45.63 ± 9.45 (42.72-48.54)	45	21-63	22.38 (16.01- 28.75)	<0.001*
FTSST (s)	15.10 ± 4.712 (13.91-16.29)	14.75	6.89-36.20	11.67 ± 2.76 (10.37-12.96)	11.50	6.89-17.72	16.69 ± 4.59 (15.28-18.11)	16.30	9.98-36.20	5.02 (3.20- 6.84)	<0.001*

Note: The data were compared using Mann-Whitney U Test, *Indicated significant difference (*p*-value < 0.001).

Abbreviations: FES-I, Falls Efficacy Scale-International; FTSST, five times sit-to-stand test; SD, standard deviation; CI, confidence interval.

The findings of the FES-I and FTSST

Table 2 presents the FES-I and FTSST data. Regarding the FES-I score, the results showed that the FOF group had a significantly higher mean score than the non-FOF group (p-value < 0.001). Similarly, those with the FOF group took the time to complete the test significantly longer than those in the non-FOF group (p-value < 0.001).

Discussion

The study aimed to evaluate the discriminative capability of a single-question assessment in identifying FOF among older adults with hyperkyphosis using FES-I and FTSST as reference measures. The results showed that the participants categorized as having FOF based on the single-question method exhibited significantly higher FES-I scores and required significantly more time to complete the FTSST compared to those without FOF (p-value < 0.001). These results support the hypothesis that the single-question assessment is an effective and efficient screening tool for detecting FOF in this specific population.

FOF is a prevalent concern among older adults, with reported rates ranging from 21% to 85%, particularly higher among those with physical impairments or postural changes such as hyperkyphosis[3,21]. This emphasizes the necessity of implementing appropriate screening strategies for individuals within this population. To the best of our knowledge, there is currently no universally accepted gold standard for the assessment of FOF, particularly among older adults with postural abnormalities, such as hyperkyphosis. A variety of assessment tools have been utilized to evaluate FOF, including single-item question, self-reported questionnaire, and performance-based functional test, each of which captures distinct dimensions of the construct, encompassing psychological concern and physical capability[3,14]. In this study, a single-question assessment was applied as a standard measure to classify people as with or without FOF. The single-question approach presents several practical advantages, such as time-efficient, easy to administer, requires no specialized equipment or training, and is feasible for use in both clinical and community settings[20]. These characteristics render it particularly suitable for use among frail older adults who may experience fatigue or cognitive limitations. Nevertheless, despite its feasibility and demonstrated acceptable levels of reliability and concurrent validity, single-question assessment may not comprehensively reflect some task-specific fears or behavioral consequences associated with FOF, such as avoidance of uneven surfaces or reduced outdoor mobility. To address these limitations and provide a more comprehensive evaluation, this study incorporated the FES-I, a validated, multidimensional instrument that assesses concern about falling across a range of daily activities, as well as the FTSST, an objective, performance-based test that evaluates lower limb strength, balance, and functional mobility—intrinsic factors linked to fall risk, particularly in individuals with hyperkyphosis[3,14].

Prior studies have validated the utility of single-question assessment for FOF. Delbaere et al[14] reported moderate to strong correlations between the single-question assessment and FES-I, demonstrating good concurrent validity. Similarly, research by Scheffer et al[3] established acceptable levels of test-retest reliability for the single-question assessment in community-dwelling older adults. Furthermore, several studies have highlighted the relationship between performance-based measures and FOF, reinforcing the utility of both subjective and objective assessments[20]. In particular, a study by Goldberg et al[22] found that extended completion times on FTSST were associated with higher levels of FOF, suggesting that individuals who experience difficulty with this task are more likely to report FOF. Similarly, other studies, including research by Lord et al[23], showed that slower FTSST times correlate with poorer lower limb strength and balance, both of which are known risk factors for falling and FOF. These findings emphasize the intricate relationship between lower limb weakness, compromised mobility, and psychological distress

in individuals with postural abnormalities, such as hyperkyphosis. These results further support the value of combining performance-based tests, such as FTSST with single-question assessment, offering a more comprehensive evaluation of FOF. While single-question assessment is valuable for quick screening, the FTSST provides objective insights into physical limitations that may contribute to FOF, enhancing overall fall risk assessment.

In conclusion, this study highlights the effectiveness of the single-question assessment as a practical tool for identifying FOF in older adults with hyperkyphosis. The results showed that the single-question assessment successfully distinguishes individuals with FOF, as indicated by higher FES-I scores and longer FTSST time. While the single-question assessment offers advantages, such as ease of use and time efficiency, its limitations in capturing all aspects of FOF are addressed by incorporating the FES-I and FTSST. These combined measures provide a comprehensive evaluation by linking psychological concerns with objective functional assessments. Overall, the single-question assessment, when utilized in conjunction with performance-based tests, proves to be a valuable screening method for early detection of FOF, facilitating prompt interventions that may reduce fall risk and enhance health outcomes in hyperkyphosis older adults

Conclusion

This study demonstrated the effectiveness of the single-question assessment as a practical and efficient tool for identifying FOF in older adults with hyperkyphosis. The results confirmed that the single-question assessment can successfully distinguish individuals with FOF, as evidenced by significantly higher FES-I scores and prolonged completion time on the FTSST compared to those without FOF.These findings supported the validity of the single-question assessment as a rapid and feasible approach for identifying FOF in hyperkyphosis older adults.

Take home messages

The single-question assessment is a simple, quick, and practical tool that can effectively identify FOF in older adults with hyperkyphosis. Clinicians can utilize these measures to screen for FOF in this population, leading to earlier preventions that could reduce fall risks and improve overall well-being.

Conflicts of interest

The authors declare no conflict of interest.

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Author contributions

Sirirut Multakorn: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft.

Wilairat Namwong: Conceptualization, Methodology, Writing – review & editing.

Thiwabhorn Thaweewannakij: Conceptualization, Funding acquisition, Methodology, Project administration, Writing – review & editing.

Data availability

Author elects to not share data

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Validity and reliability of 30-second chair-stand test and modified 30-second chair-stand test in obese older adults

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KEYWORDS
Sit-to-stand; Balance assessment; Functional performance.

ABSTRACT

Obesity in older adults can negatively affect muscle strength and balance, putting them at a greater risk of falling and experiencing mobility limitations. While the 30-second chair-stand test (30sCST) is widely used to assess lower limb strength, its validity and reliability have not been well established in obese older individuals, who may face unique physical challenges. Additionally, a modified version of the test (m30sCST), which involves standing up from a foam surface with eyes closed, may help reveal more subtle balance impairments that are not captured by the standard version. However, this version has also not been properly validated by this population Assessing the reliability and validity of both tests in obese older adults is important to ensure whether these tools can accurately identify individuals at higher risk of falling—allowing for earlier, more targeted interventions to prevent falls and support safe aging. Therefore, this study aimed to examine the concurrent validity and reliability of 30sCST and m30sCST in obese older adults. Twenty-six community-dwelling obese older adults aged 60 years and over participated in this study. All participants completed the 30sCST and m30sCST, along with the Timed Up and Go (TUG) test and the modified Clinical Test of Sensory Interaction on Balance (m-CTSIB). Validity was assessed using Pearson's correlation coefficient to examine the relationship between 30sCST and m30sCST with TUG and m-CTSIB. Reliability was determined by administering the 30sCST and m30sCST twice, five days apart. Intra-rater reliability was assessed using the Intraclass Correlation Coefficient (ICC3,2). The results showed that both the 30sCST and the m30sCST were significantly correlated with the TUG (r = -0.54 and – 0.52) and m-CTSIB test (r = -0.53 and – 0.52). In addition, both 30sCST and m30sCST have good intra-rater reliability with an ICC of 0.98. The results suggest that both the 30sCST and m30sCST are reliable and valid tools for assessing balance in obese older adults. These findings highlight the potential of the 30sCST and m30sCST as practical balance assessment tools in clinical settings.

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Introduction

The aging population not only experiences fall problems, but also has an increase in obesity rates, which are multifaceted problems for healthcare professionals and public health programs[1]. Falls in older adults constitute a major public health issue, frequently leading to severe injuries, decreased quality of life, and heightened healthcare expenses[2]. Obesity, characterized by an excessive accumulation of body fat, is linked to numerous health issues, including cardiovascular diseases, diabetes, and musculoskeletal disorders[3]. Moreover, obesity increases the risk of falls by inducing physical limitations and diminishing functional independence, thus making older adults more susceptible[4]. A recent systematic review and meta-analysis found that older adults with obesity are 16% more likely to experience falls than those without obesity[4]. Several plausible biomechanical and physiological mechanisms may explain the increased risk of falls in obese older adults. Previous research suggests that obesity is associated with factors such as sedentary behavior, chronic health conditions, and polypharmacy[5], all of which can contribute to falling risk. More specifically, excess body weight may lead to biomechanical challenges, including impaired postural control[6], increased foot loading[7], and reduced lower-limb muscle quality[8]. Consequently, early identification of individuals at a greater risk of falling is essential for implementing specific programs to diminish fall incidence within this population.

The sit-to-stand (STS) task, one of the most mechanically demanding daily activities for transferring[9], has been identified as a significant predictor of fall risk in older community dwellers[10]. Previous studies reveal that the STS accounts for 12% of falls among older adults[11], while transfers, such as getting in or out of a bed or chair, contribute a substantial 41% of falls in nursing homes[12]. Since falls frequently occur during transitional movements such as rising from a chair, using performance-based assessments that mimic this movement pattern may enhance the ability to detect fall risk. Tests like the STS, which closely resemble real-life functional tasks, could therefore serve as practical tools not only for assessing fall risk but also for guiding preventive strategies tailored to high-risk individuals, particularly those with obesity.

One commonly used assessment is the 30-second chair-stand test (30sCST), which measures the maximum number of chair stands within 30 seconds[13]. This test has shown good to excellent test-retest reliability (intraclass correlation coefficient, ICC = 0.97) and moderate to good construct validity when compared with the Fullerton Advanced Balance Scale (r = 0.78) in community-dwelling older adults. Additionally, the test's area under the receiver operating characteristic curve is 0.77, indicating moderate accuracy in predicting fall risk in community-dwelling older adults[10]. Nonetheless, prior research[10,14] had been conducted predominantly among older adults with not considered body mass index (BMI). Research has demonstrated that individuals with obesity exhibit distinct movement patterns during the STS task compared to those with normal BMI[15]. Furthermore, when lower-limb strength is normalized to body mass, individuals with obesity demonstrate a reduced capacity relative to non-obese individuals[16]. These findings may suggest that the psychometric properties of the 30sCST in older obese adults may differ from those in their non-obese counterparts from the previous study. However, evidence regarding the psychometric properties of the 30sCST in older obese adults remains limited.

The body's ability to perform movements and maintain stability relies on a complex interaction of sensory inputs from multiple systems, which are cognitively processed to initiate muscular responses and maintain balance through the musculoskeletal system. Degenerative changes in sensory processing among older adults

affect sensory reweighting, leading to postural instability during functional tasks[17]. A previous study found that in a standing position on an unstable surface, older adults with obesity exhibited greater body oscillation than those with normal weight[18]. Additionally, research suggests that obesity is associated with reduced lower-limb sensory function due to pressure exerted by excess body mass[19]. These sensory impairments may compromise the ability to respond effectively to environmental challenges that require rapid postural adjustments. Accordingly, a modified version of the 30sCST (m30sCST) has been developed, incorporating visual and somatosensory perturbations by having participants close their eyes and perform the test on a foam surface. These sensory challenges reflect real-life conditions where visual input may be limited (e.g., poor lighting or night-time mobility) and somatosensory feedback may be compromised (e.g., walking on soft, uneven, or slippery surfaces). This modified test has demonstrated good to excellent test-retest reliability (ICC = 0.96) and moderate to good construct validity with the Fullerton Advanced Balance Scale (r = 0.69) in community-dwelling older adults. Furthermore, the m30CST has shown a higher area under the ROC curve (0.91) compared to the 30sCST, suggesting improved accuracy in predicting fall risk among community-dwelling older adults[10]. Thus, modifying the 30sCST by altering visual and somatosensory conditions may enhance its predictive accuracy for falls in obese older adults.

In both research and clinical settings, it is essential to establish reliable and valid outcome measures for specific populations. However, the validity and reliability of the 30sCST and m30CST have not been examined in obese older adults. By confirming the psychometric properties of both the 30sCST and m30sCST in obese older adults, this study contributes practical tools for clinicians to better assess fall risk and functional status in this specific population. This can lead to more targeted interventions and improved fall prevention strategies tailored to the physical capabilities of individuals with obesity. Therefore, this study aims to assess the concurrent validity and intra-rater reliability of the 30sCST and m30sCST in this population. We hypothesize that (1) both tests demonstrate moderate validity in assessing physical function compared to the Timed Up and Go (TUG) test and the modified Clinical Test for Sensory Interaction on Balance (m-CTSIB), and (2) both tests will exhibit good intra-rater reliability.

Materials and methods

Study design and participants

A cross-sectional study was conducted to assess the concurrent validity and reliability of the 30sCST and the m30sCST in obese older adults. A convenience sample of twenty-six adults aged 60 years or older with obesity was recruited for this study through leaflet distribution at the university faculty and online postings on social media platforms. Participants were included if they 1) had a BMI greater than 25 kg/m^2, in accordance with the World Health Organization's (WHO) criteria for defining obesity in Asian populations[20], 2) had a waist-to-hip ratio of at least 0.90 for men or 0.85 for women, based on WHO guidelines for central obesity[21], 3) were able to independently perform an STS task, 4) had normal visual acuity, and 5) had no uncontrolled or unstable health conditions that affect the ability to stand up from a chair and maintain balance such as arthritis, and hypertension. Exclusion criteria included participants who had an incident impairing STS performance during the data collection period or who were unable to complete the procedures. The University Ethics Review Committee granted Human Projects Research ethical approval. All participants and/or their guardians provided written informed consent prior to the trial. The characteristics of the participants are shown in table 1.

Table 1 Characteristics of study participants (n = 26)

Characteristic	Findings
Gender; n (%)	
- Female	21 (80.8)
- Male	5 (19.2)
Age (years); mean ± SD	71.81 ± 4.83
Body mass index (kg/m^2); mean ± SD	28.96 ± 3.21
Waist-to-Hip ratio; mean ± SD	0.92 ± 0.05
Timed Up and Go test (s); mean ± SD	12.43 ± 3.40
Modified Clinical Test for Sensory Interaction on Balance (stability index); mean ± SD	2.68 ± 0.45
30-second chair-stand test; mean ± SD	9.54 ± 2.65
Modified 30-second chair-stand test; mean ± SD	8.25 ± 3.04
Fall history within one year; n (%)	
- Yes	14 (53.8)
- No	12 (46.2)

The sample size for this study was calculated using Wan nor Arifin's sample size calculator. The calculation was based on the minimum acceptable reliability of 0.7, the expected reliability of 0.9, a 0.05% significance level, and a power (1-β) of 80%. Additionally, a 10% dropout rate was anticipated, and the test would be repeated twice for reliability assessments. After incorporating these factors, the final sample size was determined to be 26 participants.

Procedures

A licensed physiotherapist with clinical experience in geriatric assessment conducted all testing procedures. Prior to data collection, the tester was trained by the senior author, who has expertise in functional assessment and reliability research. The training process included detailed instruction, observation of pilot trials, and supervised practice sessions to ensure consistency and adherence to the standardized testing protocol. Participants underwent four tests on the first day, including 30sCST, m30sCST, TUG, and m-CTSIB tests. For the second day, only the 30sCST and m30sCST were repeated. The interval between two assessments was 5 days. The testing sequence was allocated to the participants by a simple random sampling method.

30-Second Chair-Stand Test

During the 30sCST, the procedure involved getting up and down from a chair as fast as possible with their arms folded across the chest. Each participant began in the same posture, with their feet flat on the floor and their hips and knee joints at the 90-degree angle. The chair surface level with the distance from the lateral knee joint line when the tibia was perpendicular to the floor with the barefoot in standing. The participants then rose from the chair with their arms folded across their chests. The assessor provided the participant with the following instructions: 'Look straight ahead and rise to a full stand, then return to a complete sitting position.' After the 'go' signal, the participant was instructed to repeat this task as many times as safely possible within 30 seconds. The assessor counted down '1, 2, 3, go' to initiate the test[10,13].

Modified 30-Second Chair-Stand Test

For a m30sCST, a foam pad (Airex®), made of polyurethane foam and measuring 16 × 20 × 2.5 inches, was used. A foam was placed on the floor in front of the adjustable chair, and opaque swimming goggles were used as blindfolds[10]. For the starting position and the instruction were the same as the 30sCST[10,13].

For both 30sCST and m30sCST, the number of repetitions was recorded in each condition, with full standing and sitting on a chair. In addition, if the participant's time ran out while they were moving up or down, it was counted as a repetition. Each test was repeated two times, and average performance was used for analysis. After every try, the participant took a minimum of two minutes for rest, or as much time as necessary, to avoid muscle fatigue and motor learning. Throughout the testing, safety was ensured by closely monitoring participants and providing physical support as necessary to prevent falls or injuries.

Timed Up and Go test

In the TUG test, participants were instructed to sit in a chair at the starting location, stand, walk forward 3 meters as swiftly and safely as possible, turn at a traffic cone, walk back, and sit down at the starting position. The task was performed at a self-selected, comfortable walking speed rather than a rapid one[22]. The times were recorded, and two of the times were repeated. The average of these times was used for analysis.

Modified Clinical Test for Sensory Interaction on Balance

For the m-CTSIB test, the Biodex Balance System™ SD (Biodex Medical Systems, Inc.) was used to assess m-CTSIB. Participants were asked to stand at the center of the balance system platform with their feet shoulder-width apart and placed their hands on their iliac crests during the 4 different conditions. The conditions were 1) eyes opened, firm surface, 2) eyes closed, firm surface, 3) eyes opened, foam surface, and 4) eyes closed, foam surface. Each participant's feet were positioned on the platform using default values based on their individual height. The four conditions each lasted for 30 seconds, and all four conditions were performed twice, resulting in a total of eight trials. The overall stability index was calculated and was used for further analysis. A high score in this index, for instance, indicates poor balance.

Statistical analysis

Statistical analysis was conducted using SPSS version 29.0 (SPSS Inc., 233 S Wacker Dr, 11th Fl, Chicago, IL 60606). Pearson's correlation coefficient (r) was used to assess the concurrent validity of the 30sCST and m30sCST to the TUG and m-CTSIB tests. The strength of the correlation was classified as follows: little or none ($r < 0.25$), poor ($r = 0.25–0.50$), moderate ($r = 0.51–0.75$), and good to excellent ($r > 0.75$) [23].

To evaluate the intra-rater (ICC 3,2) reliability of the time to complete the 30sCST and m30sCST, the ICC with a 95% confidence interval was calculated. An ICC greater than 0.75 indicated good reliability, while an ICC between 0.5 and 0.75 suggested moderate reliability[23]. Furthermore, the standard error of measurement (SEM) and minimal detectable change (MDC) were computed to assess absolute reliability, using the following formulas:

SEM = Standard deviation (SD) × $\sqrt{}$ (1 – ICC) and MDC = 1.96 × $\sqrt{}$2 × SEM.

Results

For the concurrent validity analysis, both the 30sCST and the m30sCST were significantly correlated with the TUG and m-CTSIB tests. Analysis using Pearson's correlation coefficient revealed a moderately negative relationship between each test as shown in table 2.

Table 2 Correlation between the 30sCST, m30sCST, and TUG and m-CTSIB tests

Tests	TUG		m-CTSIB	
	r (95% CI)	***p*-value**	**r (95% CI)**	***p*-value**
30sCST	-0.54 (-0.76, -0.18)	0.005*	-0.53 (-0.76, -0.18)	0.006*
m30sCST	-0.52 (-0.75, -0.15)	0.008*	-0.52 (-0.75, -0.16)	0.008*

Abbreviations: 30sCST, 30-second chair-stand test; m30sCST, modified version of the 30-second chair-stand test; TUG, Timed Up and Go; m-CTSIB, modified Clinical Test for Sensory Interaction on Balance; CI, confident interval.

The means and standard deviations (SD) of the repetition to complete the 30sCST and m30sCST, which were used to determine reliability, are reported in table 3. The ICC of both tests exhibited good intra-rater reliability. The SEM and MDC of both tests are also shown in Table 3.

Table 3 The reliability and corresponding minimal detectable change (MDC) and standard error of measurement (SEM) of the 30sCST and m30sCST.

Variable	Repetition (mean ± SD)		$ICC_{3,2}$	95% CI	*p*-value	SEM	MDC
	First day	Second day					
30sCST	9.54 ± 2.65	9.42 ± 2.79	0.98	0.96 - 0.99	< 0.001	0.38	1.05
m30sCST	8.25 ± 3.04	7.98 ± 2.94	0.98	0.96 - 0.99	< 0.001	0.42	1.16

Abbreviations: 30sCST, 30-second chair-stand test; m30sCST, modified version of the 30-second chair-stand test; TUG, Timed Up and Go; m-CTSIB, modified Clinical Test for Sensory Interaction on Balance; CI, confident interval; ICC, Intraclass correlation coefficient.

Discussion

This study aimed to evaluate the concurrent validity and intra-rater reliability of the 30sCST and its modified version (m30sCST) in obese older adults. The findings indicate that both tests possess moderate concurrent validity when compared with the TUG test and the m-CTSIB. Additionally, both the 30sCST and m30sCST demonstrated good intra-rater reliability and low SEM and MDC values when used in obese older adults. In addition, throughout the study, no adverse events or safety concerns were reported during either the 30sCST or the m30sCST.

The 30sCST and m30sCST were moderately and negatively correlated with TUG and m-CTSIB tests when employed in older obese adults. The TUG test is an efficient, rapid, and often utilized instrument for evaluating mobility, balance, and functional ability in elderly individuals[24]. In addition, this test was recommended by the latest falls prevention and management as one of the screening tools in older adults[25]. For the m-CTSIB, this test is used for identifying sensory integration deficits affecting balance by isolating the contributions of visual and somatosensory[26]. The moderate negative correlation found in this study (r = -0.52 to -0.54) suggests that both 30sCST and m30sCST are in partial agreement with the functional balance and sensory integration balance assessment. These results are consistent with previous findings in non-obese older adults, demonstrating that the 30sCST and m30sCST showed a significant, moderate correlation (r = 0.73 and 0.69) with the Fullerton Advanced Balance Scale[10]. This slight reduction may be

attributed to obesity-related factors such as altered movement patterns, impaired sensory processing, and reduced relative lower-limb strength, which are known to influence functional performance[15,16]. However, unlike earlier studies that focused on clinical balance measures, our study aimed to validate the 30sCST and m30sCST against laboratory assessments, providing new insights into their applicability in objective balance evaluations. Although the m30sCST includes additional sensory challenges, the similar validity outcomes with the 30sCST may suggest that both tests primarily capture lower-limb functional capacity rather than uniquely assessing sensory integration. This could indicate a ceiling effect or limited variability in sensory challenge response among the participants. Given these findings, we suggest that the 30sCST and m30sCST be used in combination with other established assessments like the TUG or m-CTSIB rather than as standalone tools.

Importantly, this study's reliability results were notably high, with intraclass correlation coefficients (ICC) of 0.98 for both the 30sCST and m30sCST. These findings are in line with or exceed previously reported values in community-dwelling older adults[10] and support the use of these tests as stable and consistent measures. The provision of practice sessions utilizing methods for the assessors prior to the beginning of data collection may have enhanced the good reliability outcomes shown in the current investigation. Moreover, explicit and standardized directives from the assessor may facilitate the participants' effective execution of the assigned task.

Understanding the measurement error is essential for determining whether a tool is sufficiently reliable for clinical decision-making. Previous studies have reported acceptable SEM values for the 30sCST in older adults (0.71)[10], older adults with osteoarthritis (1.97)[27], and for the m30sCST (0.96) in older adults[10]. In the present study, the SEM values for both the 30sCST and m30sCST were found to be less than 0.5 repetitions, indicating very low variability in performance. This suggests that the measurements are highly consistent, and the error is minimal, making them reliable for use in older adults with obesity. Moreover, this study established MDC values for both the 30sCST and m30sCST, which are straightforward and easy-to-administer assessments. When comparing the MDC values from our study with those reported in previous research[10], we found that the MDC for the 30sCST in our sample of obese older adults was 1.05 repetitions, which is lower than the 1.96 repetitions reported in a prior study involving non-obese older adults. Similarly, the MDC for the m30sCST in our study was 1.16, compared to 2.67 in the previous research. These lower MDC values observed in obese older adults may suggest that even small changes in performance could reflect meaningful improvements, potentially making it easier to detect clinically relevant changes in this population. These MDC values provide a helpful standard for understanding results in different groups and assist in figuring out the smallest change needed to show a real improvement in obese older adults after treatment.

The study's strengths lie in the population it studied, particularly the obese older adults. This population is facing a risk of falling due to compounded effects of age-related and weight-related impairments in balance and mobility. In addition, using both clinical and instrumented balance assessments offers a well-rounded evaluation of balance. Despite these strengths, some limitations remain. First, the participants in this study were a convenience sample of obese older adults, mostly female, from one community, which may not reflect the overall older adult population. Second, this study only examined intra-rater reliability without assessing inter-rater reliability. Evaluating inter-rater reliability is crucial for determining whether the tests can produce consistent results across different evaluators, a key factor for their clinical applicability. Additionally, future studies should include an investigation of the other psychometric properties of the 30sCST and m30sCST, such as

their accuracy in detecting falls in obese older adults.

Conclusion

The 30sCST and m30sCST are reliable and moderately valid functional evaluations for community-dwelling obese older adults. The 30sCST provides a quick and practical measure of lower-limb strength and mobility under normal conditions, while the m30sCST introduces sensory challenges that may better reveal subtle balance impairments related to sensory processing deficits. Clinically, these tests can be used together to gain a fuller picture of an individual's functional status and to help guide targeted interventions for fall prevention but should always complement other assessments rather than replace them.

Take home messages

The 30sCTS and its modified version are reliable and valid instruments for evaluating physical function and balance in obese elderly individuals, endorsing its application in fall risk assessment and rehabilitation strategy formulation.

Conflicts of interest

The authors declare no conflict of interest.

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Author contributions

Tanida Vajaradesa: Conceptualization, Methodology, Formal analysis Writing – Original draft.

Pawan Chaiparinya: Conceptualization, Writing – Review & Editing.

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Data availability

Data available on request from the authors

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Relationships between arterial stiffness and the cluster of cardiovascular disease risk factors

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KEYWORDS
Cardiovascular disease risk factor; Arterial stiffness; Pulse wave velocity; Cluster.

ABSTRACT

Evidence indicated that individuals with more than one cardiovascular disease (CVD) risk factor were more likely to develop CVD events, compared with those with a single risk factor. Arterial stiffness is known as an independent risk factor for CVD. This study aimed to explore the association of clustering of CVD risk factors and arterial stiffness. Arterial stiffness was measured in both male and female adults aged 49.04 ± 15.79 years who visited the vascular screening clinic at the Physical Therapy Center, Mahidol University. Participants with two or more risk factors were assigned to the cluster of CVD risk factors group. This study was part of a routine-to-research (R2R) project; therefore, all participants who were willing to receive the service from September 2021 to April 2023 were recruited into the study with their permission. The results showed that the brachial-ankle pulse wave velocity (baPWV), an arterial stiffness indicator, was highest in the clustering of CVD risk factors group (1594 cm/sec^2), higher in the single CVD risk factor group (1263cm/sec^2) than in none of CVD risk factors participant (1148 cm/sec^2). The overall mean resting mean arterial blood pressure level was 90 mmHg. In conclusion, clustering of CVD risk factors significantly correlated with arterial stiffness. Lifestyle modification should be strongly encouraged for individuals with more than one CVD risk factor to reduce the burden of CVD.

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Introduction

Cardiovascular disease (CVD) is the leading cause of mortality and morbidity globally, affecting over 523 million people[1]. To combat this burden of CVD, it is crucial to focus on primary cardiovascular prevention. Addressing CVD risk factors is one of the fundamental strategies to identify high-risk patients so that management with counselling and medicine can begin as early as possible. Hypertension, type 2 diabetes, dyslipidemia, and overweight are four major risk factors of cardiovascular disease[2,3]. Previous studies have revealed that CVD risk factors tend to be clustered in specific individuals[4,5]. A survey of suburban residents in Beijing, China, demonstrated that the prevalence of ≥1, ≥2, and ≥3 major CVD risk factors was 83.5%, 47.2%, and 17.5%, respectively[6].

Arterial stiffness is well-recognized as an emerging risk factor for CVD [7,8]. The brachial-ankle pulse wave velocity (baPWV) is a simple, accurate, and reproducible method for assessing the stiffness of medium- to large-sized arteries[9]. Based on the current meta-analysis study, Japanese participants who had higher baPWV values also had a higher risk of CVD. These results suggest that the baPWV may predict the future risk of CVD development in clinical practice, independent of traditional risk factors[10]. Moreover, evidence showed that major CVD risk factors, including hypertension[11], type 2 diabetes[12], dyslipidemia[13], and overweight and obesity[14], were associated with an accelerated increase in the brachial-ankle PWV. In the Baltimore Longitudinal Study of Aging (BLSA), it was found that arterial stiffness can predict the onset of hypertension[15]. Another critical CVD risk factor is hyperglycemia, which affects the properties and function of the arterial wall. In pre-diabetes, diabetes, or subjects with metabolic syndrome, these structural changes in the arterial wall can be observed[16]. Findings from a longitudinal study indicated that central obesity is a predictor of arterial stiffness over a 16-year period[17].

Increased arterial stiffness has been documented as an independent risk factor for future cardiovascular events and total mortality[18]. The Faculty of Physical Therapy, Mahidol University, has launched a vascular health screening program for adults to examine arterial stiffness levels as a CVD risk factor. Lifestyle modification strategies have been suggested to all participants, especially individuals with clustered CVD risk factors and high levels of arterial stiffness. Previous studies have been focused on the association of arterial stiffness level and individual risk factors. However, the CVD patients commonly have many comorbid diseases, such as hypertension, diabetes, and dyslipidemia together. To our current understanding, the relationship between arterial stiffness and the aggregation of CVD risk factors remains inadequately explored. Consequently, we conducted a cross-sectional study as part of a routine-to-research (R2R) initiative. This study aimed to compare arterial stiffness, as measured by baPWV, among individuals with clustering, single, and no cardiovascular disease risk factors.

Materials and methods

Participants

Adults who visited the vascular screening clinic at the Physical Therapy Center, Mahidol University, were enrolled in the study. The inclusion criteria were: 1) sedentary participants aged ≥ 18 years; 2) no cardiovascular disease, pulmonary disease, kidney disease, or infection; 3) not pregnant. Sedentary behavior is defined as individuals who engage in less than 60 minutes of moderate-intensity physical activity per week, as determined by a history-taking assessment. The participant who are smoking were excluded from data analyses. The final data analysis includes a total of 148 individuals. The investigation was conducted from September 2021 to April 2023 after approval from the Ethics Committee of the Mahidol University Institutional Review Board (MU-CIRB 2021/324.2806). All participants provided written informed consent prior to data collection.

Participant classifying criteria

We defined the four major traditional CVD risk factors as follows: 1) hypertension, 2) type 2 diabetes, 3) dyslipidemia, and 4) overweight and obesity. Participants with two or more risk factors were assigned to the cluster of CVD risk factors group. All risk factors are based on physician diagnosis, except overweight and obesity. A body mass index (BMI) greater than 25 kg/m^2 was defined as overweight or obesity.

Outcome measurements

Before the testing session, participants abstained from food, alcohol, and caffeine for at least four hours. Premenopausal women were tested during the early follicular phase of the menstrual cycle. All testing was performed 24 to 48 hours after the last exercise bout.

During the on-site screenings, the physical therapist reviewed the medical history, lifestyle behavior (e.g., alcohol use, smoking), and blood biochemistry laboratory. Weight and height were measured using an electronic scale. BMI was calculated as weight in kilograms divided by height in square meters.

An automated vascular testing system (VP-1000 plus, Omron Healthcare, Bannockburn, Illinois) was used to assess the participants' heart rate, brachial and ankle blood pressure, and baPWV after they had been resting quietly in a supine position for at least 15 minutes. Brachial and ankle artery pulse waves were recorded by arterial applanation tonometry in the blood pressure cuff wrapping around four extremities. Time delay was measured automatically with the foot-to-foot method, and pulse wave velocity was subsequently calculated. Ankle–brachial pressure index (ABI) was calculated as ankle systolic blood pressure divided by brachial systolic blood pressure.

Statistical analysis

All variables were expressed as mean ± SD. The significance of differences among groups was determined using one-way ANOVA or Kruskal-Wallis H test, as appropriate. Pearson correlation coefficients were used to examine the association between baPWV, BMI, and mean arterial pressure. A significance level of p-value < 0.05 was used to determine statistical significance.

Results

Table 1 presents participant characteristics based on a number of CVD risk factors. Out of the 95 individuals in the study, 33.1% had clustering of CVD risk factors, and 45.3% were free of any defined CVD risk factors. The participants in the single and clustering of CVD risk factors group were older and had a higher BMI than those in the free of CVD risk factors group. In addition, the participants in the cluster group had significantly higher arterial blood pressure than those in the single and none of the CVD risk groups.

The brachial-ankle pulse wave velocity (baPWV) was significantly highest in the group with a clustering of cardiovascular disease (CVD) risk factors (1594 cm/sec^2). This was followed by the group with a single CVD risk factor (1263 cm/sec^2). Participants with no CVD risk factors had the lowest measurement at 1148 cm/sec^2, as shown in figure 1.

Body mass index (Figure 2) and mean arterial pressure (Figure 3) were positively associated with baPWV in the pooled population (r = 0.26, p-value < 0.05 and r = 0.74, p-value < 0.05, respectively).

Table 1 Selected participant characteristics

Variable	Total (n=148)	None (n=67)	Single (n=32)	Cluster (n=49)
Age, years	49.04 ± 15.79 (52)	43.29 ± 15.89 (37)	50.97 ± 17.80* (56)	65.31 ± 6.66* (64)
Body weight, kg	61.04 ± 12.95 (57)	56.36 ± 9.82 (51)	64.56 ± 15.80* (60)	68.12 ± 12.22* (62)
Height, cm	159.69 ± 7.81 (155)	161.26 ± 8.05 (158)	158.78 ± 7.43 (155)	158.37 ± 6.55 (155)
BMI, kg/m^2	23.84 ± 4.22 (22.45)	21.57 ± 2.66 (21.25)	25.68 ± 4.48* (25.23)	25.85 ± 4.33* (24.2)
Heart rate (beats/min)	65 ± 2 (63)	63 ± 2 (61)	65±3 (63)	68 ± 2 (65)
Systolic BP, mmHg	123.84 ± 17.89 (117)	116.57 ± 16.28 (114)	120.03 ± 12.27 (120)	145.38 ±16.90*† (145)
Diastolic BP, mmHg	74.08 ± 9.59 (73)	70.07 ± 9.32 (69)	73.30 ± 7.64 (75)	81.46 ± 6.79*† (80)
Mean BP, mmHg	93.65 ± 13.74 (90)	88.54 ± 12.89 (86)	91.37 ± 9.46 (91)	110.54 ±12.94*† (109)
Pulse pressure, mmHg	49.23 ± 11.66 (45)	45.98 ± 9.58 (44)	46.73 ± 10.15 (44)	63.92 ± 15.98*† (61)
Ankle-brachial index	1.11 ± 0.08 (1.11)	1.10 ± 0.08 (1.11)	1.14 ± 0.07 (1.15)	1.12 ± 0.08 (1.12)
Hypertension, n (%)	35 (21.25)	-	8 (1.56)	27 (19.57)
Diabetes, n (%)	47 (25.19)	-	12 (4.95)	35 (21.29)
Overweight, n (%)	24 (17.39)	-	11 (9.42)	13 (7.97)
Dyslipidemia, n (%)	47 (34.06)	-	14 (8.70)	28 (25.36)

Note: Values are mean ± SD (median). * *p*-value < 0.05 vs. none, † *p*-value < 0.05 vs. single.

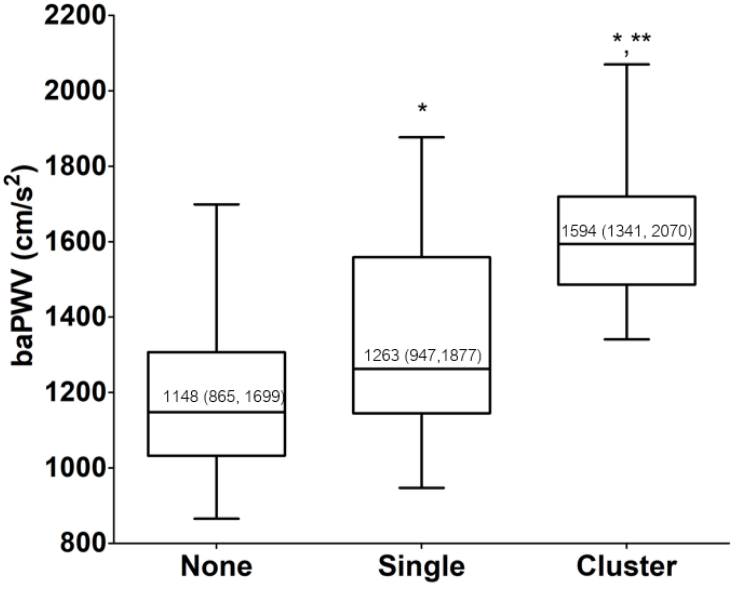

Figure 1 Brachial-ankle pulse wave velocity among none-, single-, and cluster CVD risk factors.
Note: Values are presented using median and interquartile range. * *p*-value < 0.05 vs. none, ***p*-value < 0.05 vs. single

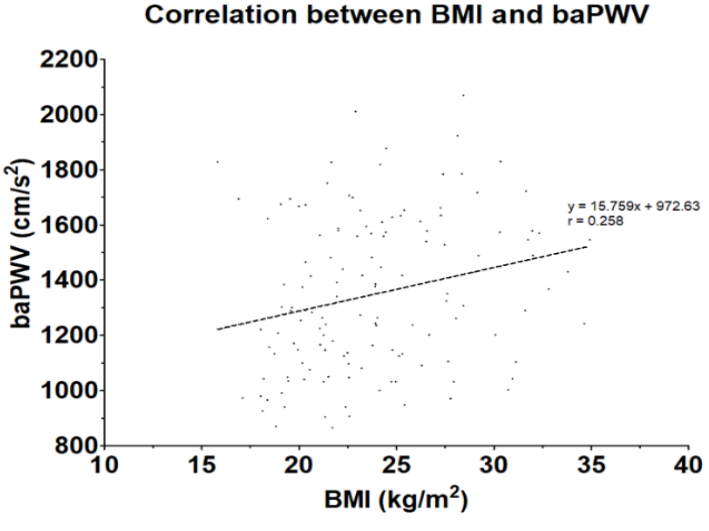

Figure 2 Association between body mass index (BMI) and brachial ankle pulse wave velocity (baPWV).

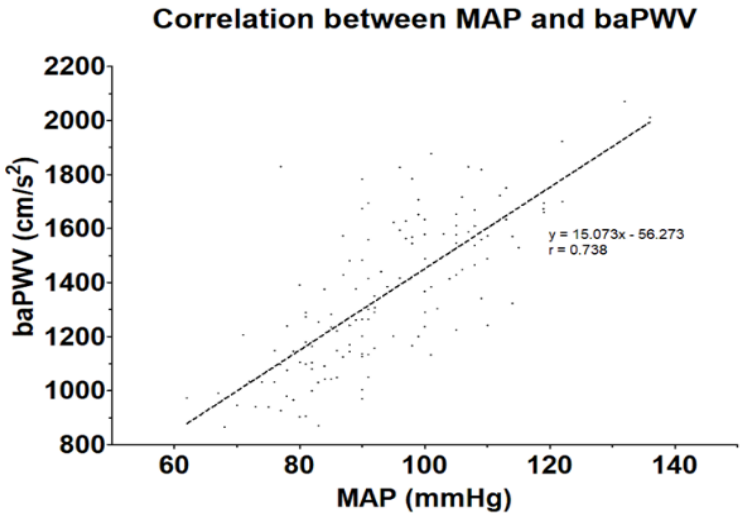

Figure 3 Association between mean arterial pressure (MAP) and brachial ankle pulse wave velocity (baPWV).

Discussion

Our study indicated that the adult population characterized by a cluster of CVD risk factors has the highest arterial stiffness level compared to those with single or no CVD risk factors. The possible mechanisms underlying the relationship between the clustering of CVD risk factors and arterial stiffness remain unclear. Previous studies have reported that diabetes and hypertension contribute additively to increased pulsatility, systolic blood pressure, and aortic pulse wave velocity (PWV)[19,20]. In our study, the leading risk factors in the cluster group were hypertension and diabetes. The explanation may be that high blood pressure and insulin resistance lead to endothelial dysfunction to a greater severity than a single risk factor. Endothelial dysfunction induces structural and functional changes in the arterial wall, resulting in increased arterial stiffness[21].

The cluster risk factor group was older than the single and no-risk factor groups. With advancing age, individuals are more likely to develop multiple health conditions, commonly referred to as multimorbidity[22]. Numerous studies have demonstrated that arterial stiffness increases progressively with age even in healthy individuals. This relationship remains even after adjusting for blood pressure, indicating that aging itself contributes independently to arterial stiffness[23]. This finding was consistent with the results of this study, which showed that baPWV was highest in the group with cluster risk factors.

In addition, the results of this study demonstrated the positive relationship between baPWV and BMI and MAP. This finding aligns with a previous systematic review and meta-analysis study. Overweight and obese adults exhibit significantly greater arterial stiffness compared to healthy-weight controls, even without prevalent cardiovascular disease[24]. Arterial stiffness is associated with hypertension. Baseline blood pressure is associated with a clinically significant progression of arterial stiffness, regardless of age[25].

Arterial stiffness is recognized as a predictor of cardiovascular events and all-cause mortality[26]. Evidence of meta-analysis shows that measuring arterial stiffness provides valuable predictive information for CVD risk, beyond what is indicated by the traditional Framingham Heart Study risk score[10,26]. Aortic PWV and carotid femoral PWV (cfPWV) are considered as the gold standard indices of arterial stiffness[27]. However, it might

be challenging to incorporate measurement of the aortic PWV or cfPWV in routine clinical settings because of the technical difficulties involved in its measurement, special training requirements, and exposure of the inguinal region.

Brachial ankle pulse wave velocity (baPWV) is automatically measured using a separate cuff for each of the four limbs by an oscillometric method[28]. Because of its simplicity and ease of measurement, baPWV may be more easily applied in clinical practice than aortic PWV or cfPWV. A previous study reported that baPWV had closely correlated with the directly measured aortic PWV and cfPWV[29]. The meta-analysis study from 14,673 Japanese participants without a history of CVD reported that measurement of the baPWV could enhance the efficacy of predicting the risk of development of CVD over that of the Framingham risk score, which is based on the traditional cardiovascular risk factors[10]. This study indicated the relationship between baPWV and a cluster of CVD risk factors. This finding highlights the role of arterial stiffness in mediating CVD risk factors, while also providing a valuable reference for informing the design of clinical trials. Thus, the measurement of the baPWV should be encouraged to be applied more broadly in general clinical settings as a tool for CVD risk prediction.

The Physical Therapy Center at Mahidol University has launched a service, focusing on screening for CVD risk using baPWV. We encourage exercise as a lifestyle modification in the population with a high level of baPWV and who have other CVD risks. Aerobic exercise has been proven to be an effective lifestyle modification strategy to improve arterial stiffness and endothelial function[30]. We educate our clients on how to exercise effectively and lifestyle modification strategies to control CVD risk factors and reduce the burden of CVD.

Our study has some limitations that must be monitored. This study used a convenience sample, which could introduce bias. It is a routine-to-research study (R2R) launched between September 2021 and April 2023. All clients who met the inclusion criteria were recruited into the study. We had not calculated the sample size. We were unable to collect the medication information that the participant received. Some medications may affect arterial stiffness. Finally, our study employed a cross-sectional design, which cannot provide insight into the mechanisms underlying the observed association.

Conclusion

The clustering of CVD risk factors, including high blood pressure, elevated cholesterol levels, obesity, and diabetes, has been significantly correlated with increased arterial stiffness.

Take home messages

The arterial stiffness is associated with adverse cardiovascular outcomes, highlighting the importance of effectively addressing these risk factors. Consequently, lifestyle modifications are crucial in mitigating this risk. Individuals exhibiting multiple CVD risk factors should be strongly encouraged to adopt healthier lifestyle changes.

Conflicts of interest

The authors declare no conflict of interest.

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Author contribution

Sarinda Sataman: Conceptualization, Project administration Data Collection, Writing.

Pornsiri Pipatkasira: Conceptualization, Project administration, Data Collection.

Pornpiroon Phuegsilp: Data Collection, Resources.

Benjawan Saelao: Data Collection, Resources.

Thanwalai Pisalayon: Formal analysis.

Nantinee Nualnim: Conceptualization, Formal analysis, Writing – Review & Editing.

Data availability

Data available on request due to privacy/ ethical restrictions

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