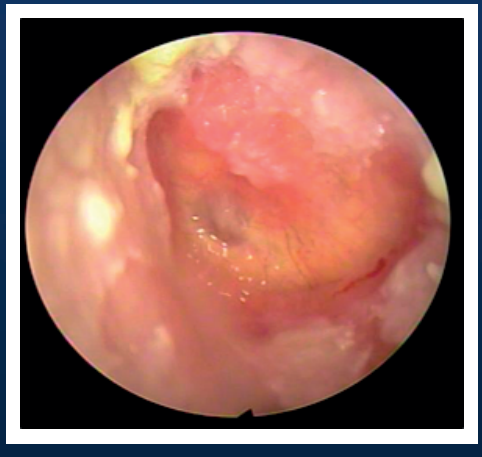
E-ISSN 2228-8082 ISSN 2629-995X
Volume 72, Number 6, November-December 2020

SMJ
Siriraj Medical Journal



By Kanokrat Suvarnsit, et al.

Indexed by Scopus®

Thai Association for Gastrointestinal Endoscopy

International Association of Surgeons Gastroenterologists & Oncologists Thailand Chapter

https://he02.tci-thaijo.org/index.php/sirirajmedj/index
E-mail: sijournal@mahidol.ac.th

SIRIRAJ MEDICAL JOURNAL

www.smj.si.mahidol.ac.th

SIRIRAJ MEDICAL JOURNAL is published bimonthly, 6 issues a year (Jan-Feb, Mar-Apr, May-Jun, Jul-Aug, Sep-Oct and Nov-Dec) and distributed by the end of the last month of that issue.
SIRIRAJ MEDICAL JOURNAL is listed as a journal following the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (URM) by the International Committee of Medical Journal Editors (ICMJE) since 9 July 2010 [http://www.icmje.org/journals.html].

Albumin Versus Gelatin Solution for the Treatment of Refractory Septic Shock: A Patient Baseline-Matched-Cohort Study

Surat Tongyoo, M.D., Chawanee Chayakul, M.D., Siwanat Kanoknatsiwattana, M.D., Chairat Permpikul, M.D.

Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

ABSTRACT

Objective: Although albumin solution is the colloid of choice to resuscitate septic shock patients who do not respond to crystalloid solutions, its usage is limited by its cost. Gelatin solution, is less expensive, but its efficacy has not yet been identified. This study aimed to compare the outcomes of gelatin and albumin solutions for septic shock resuscitation.

Methods: This baseline-matched-cohort study, enrolled septic shock patients who had a mean arterial blood pressure (MAP) below 65 mmHg after receiving at least 30 mL per kilogram of crystalloid resuscitation fluid, and who required either an albumin or gelatin solution as fluid therapy. The primary outcome was the 28-day mortality.

Results: In all, 224 patients who were administered either an albumin or gelatin solution were examined. After adjusting for differences in their baseline characteristics, 206 patients were included (104 receiving albumin, and 102 given gelatin). A comparison of the albumin and gelatin groups revealed no significant baseline differences in their respective mean APACHE II scores (22.8±8.5 vs. 23.2±8.1), MAPs (55.1±8.0 vs. 54.6±9.1 mmHg), and lactate levels (5.6±4.7 vs. 6.3±4.9 mmol/L). The 28-day mortality rates were 27.9% and 38.2% for the albumin and gelatin groups, respectively, with adjusted p=0.02. Moreover, the accumulation of fluid intake over output at 72 hours was significantly lower for the albumin than the gelatin group (5,964.5±4,959.7 vs. 8,133.2±3,743.2 ml; p=0.01). The RRT rate was higher for the albumin group (30.8% vs. 15.7%; p=0.01).

Conclusion: Albumin resuscitation associated with lower 28-day mortality than gelatin resuscitation. The patients in the albumin group had a higher RRT rate and a lower fluid accumulation as at 72 hours.

Keywords: Septic shock; colloid solution; albumin solution; gelatin solution; crystalloid solution; fluid resuscitation (Siriraj Med J 2020; 72: 451-461)

INTRODUCTION

According to the Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock 2016, crystalloid solutions are the fluid of choice for the initial resuscitation of septic shock patients.[1] In the case of patients who do not respond to a certain volume of crystalloid resuscitation (usually at least 30 mL per kilogram of body weight), colloid solutions should be used. Albumin is recommended as the first-choice colloid, based on the evidence of two large randomized controlled trials[2,3] and two meta-analyses.[4,5] Unfortunately, albumin usage in resource-limited countries

Corresponding author: Surat Tongyoo
E-mail: surat.ton@mahidol.ac.th
Received 15 April 2020 Revised 20 May 2020 Accepted 26 May 2020
ORCID ID: http://orcid.org/0000-0003-3772-2990
http://dx.doi.org/10.33192/Smj.2020.61

is restricted by its cost, with many patients unable to afford albumin-based resuscitation. In our hospital, when the cost of albumin therapy would prove to be prohibitive for a patient, the alternative colloid utilized for the resuscitation of refractory septic shock is gelatin solution. Gelatin is a small fragment of collagen, and it is one of a number of synthetic colloids that have long been used for volume expansion in certain situations.[6] A previous study demonstrated that resuscitation with a gelatin solution expanded the intravascular volume by about 1.4 times that gained through resuscitation with a crystalloid solution.[7] A similar ratio was achieved with a 5% albumin solution. In addition, data from a meta-analysis showed that resuscitation with a gelatin solution was not only associated with better hemodynamic stabilization than resuscitation with a crystalloid alone, but may also be associated with lower mortality.[8] Although there has been evidence from a few randomized controlled trials relating to the efficacy of gelatin solution relative to that of other colloid solutions, those clinical trials employed small sample sizes and were not performed on septic shock patients.[9,10] Moreover, there is limited information concerning the comparative outcomes of albumin versus (vs.) gelatin solutions for septic shock patient resuscitation.[11]

Septic shock resuscitation typically requires a high fluid volume.[12,13] This raises concerns about the extravasation of colloid molecules, which may be associated with organ dysfunction. Acute kidney injury is associated with hydroxyethyl starch administration among critically ill patients.[14] Although data in a meta-analysis suggested that gelatin solution resuscitation might be associated with subsequent acute kidney injury, the findings were not statistically significant.[7] Even though the lower-priced gelatin solution is used as an alternative colloid to albumin for septic shock resuscitation, its efficacy and safety outcomes have not yet been identified. The aims of this study were to compare the efficacies and safety outcomes of gelatin and albumin solution usage for septic shock resuscitation.

MATERIALS AND METHODS

Study design and ethical considerations

This patient baseline-matched-cohort, retrospective study enrolled septic shock patients who had been admitted to the Internal Medicine Ward, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, 15 December 2014 - 31 October 2018. We used database of a study protocol number: 100/2018(EC1), which was approved by the Siriraj Institutional Review Board (Si 421/2018).

Participants

We screened for patients who were aged over 18 years and had been diagnosed with septic shock, as defined in the Surviving Sepsis Campaign guidelines of 2012.[15] The septic shock patients had been diagnosed and treated by attending physicians at the emergency room, internal medicine ward, or the medical intensive care unit. The treatments followed the Surviving Sepsis Campaign guidelines of 2012 and 2016, which required the initiation of antibiotics coupled with the administration of fluid resuscitation with a crystalloid solution (30 mL/kg) and norepinephrine to reach a target mean arterial blood pressure (MAP) ≥ 65 mmHg.[1] In the event that a patient's hemodynamic state subsequently remained unstable, the use of a colloid solution was considered. The decision to initiate the colloid infusion as well as the type and dose to be used were dependent on each attending physician's clinical judgment. We enrolled those patients who, despite having received at least 30 mL/kg of crystalloid solution resuscitation, still had either a MAP < lower than 65 mmHg or evidence of inadequate tissue perfusion requiring additional fluid resuscitation. The inadequate tissue perfusion was defined as a urine output of < 0.5 mL/kg of body weight or a serum lactate concentration of ≥ 4 mmol/dL.[16] The requirement for additional fluid resuscitation was indicated by the following criteria: a central venous pressure of < 12 mmHg or 15 cmH_2O; a pulmonary wedge pressure of < 18 mmHg; or evidence of fluid responsiveness from one or more non-invasive tests, specifically, an inferior vena cava diameter variation of > 15%, a pulse pressure variation of > 15%, or a positive passive-leg-raising test. Patients receiving a 5% albumin solution but no gelatin solution were classified as members of the albumin group, while those administered a gelatin solution without an albumin solution were enrolled in the gelatin group. We excluded all patients who received both the albumin and gelatin solutions for septic shock resuscitation, as well as any patient who was given a colloid other than an albumin or gelatin solution. Also excluded were patients who had prolonged shock exceeding 24 hours; were pregnant; had suspected cardiogenic shock (demonstrated by an echocardiogram showing a left ventricular ejection fraction of < 35%); had a chest X-ray revealing cardiogenic pulmonary edema; or had a history of an allergy to colloid solutions causing anaphylaxis.

We performed the patient-matching process following the STROBE recommendations.[17] The patients who received albumin were matched with those administered gelatin in a 1:1 ratio. The matchings were based on each patient's age (± 5 years), Acute Physiology and Chronic

Health Evaluation (APACHE) II severity score (± 3 score), MAP (± 5 mmHg), and baseline lactate level (± 1 mmol/L).

Data collections and outcomes

We compiled the patients' baseline information and hemodynamic parameters (age, gender, APACHE II score, comorbidities, baseline serum albumin, source of infection, pathogenic details, MAP, and initial serum lactate). In addition, details of the treatment strategies employed (fluid resuscitation volume, and vasopressor type and dosage) were collected. Also recorded was information on the crystalloid and colloid solution volumes received during the first 3 days after each septic shock diagnosis, and the fluid balance volumes on Days 1, 2 and 3. The fluid balance was determined from the difference between the fluid resuscitation volume (consisting of the crystalloid and colloid solutions) that a patient received minus all fluid output, which was comprised of the urine output and, in cases where a patient underwent renal replacement therapy, the ultrafiltration volume.

The primary outcome of this study was the all-cause mortality at 28 days. The secondary outcomes were the hospital mortality; the cumulative fluid balance 72 hours after the septic shock diagnosis; and the days alive and free of organ support, ventilator support, vasopressor usage, and renal replacement therapy to Day 28 after the diagnosis.[18]

Statistical analyses

The categorical variables were expressed as number and percentage per group, and the group results were compared using Fisher's exact test or Chi-squared test, as appropriate. The continuous variables were described as mean ± standard deviation and compared using Student's t-test. A Kaplan–Meier curve analysis was performed to reveal the 28-day mortality differences between the albumin- and gelatin-receiving groups. We used PASW Statistics for Windows, version 18 (SPSS Inc., Chicago, Ill., USA) to analyze the data.

RESULTS

In all, 555 patients were diagnosed with septic shock and screened during the study period. Of those, 219 saw their hemodynamic state restored after receiving a crystalloid solution and vasopressor, and they were thus excluded from the study. Another 112 patients were not enrolled as they were given a mix of colloid solutions. The remaining 224 patients received either the albumin solution or the gelatin solution; after their baseline ages, severity scores, MAPs, and lactate levels were matched, 18 of those patients were eliminated from the study. That left a total of 206 enrolled patients: 104 in the albumin group, and 102 in the gelatin group (Fig 1).

The patients' baseline characteristics are summarized in Table 1. There were no significant differences in the mean ages, gender profiles, APACHE II scores, sites

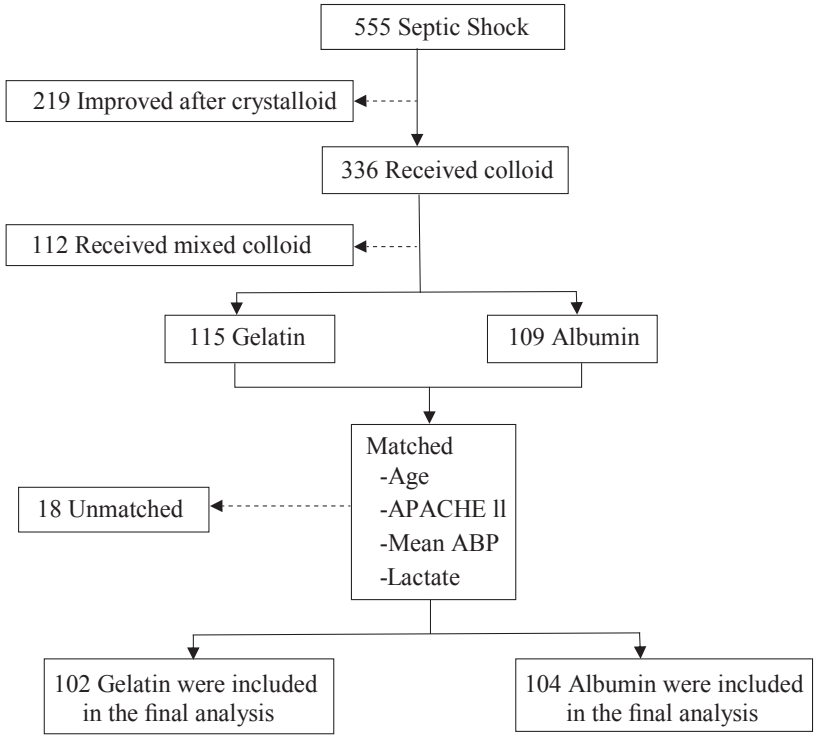


Fig 1. Flow diagram illustrating screening, enrollment, and baseline matching of patients

of infection, and hemodynamic parameters of the 2 groups. However, the patients in the albumin group had a higher proportion of the underlying conditions of hypertension and diabetes mellitus than the patients in the gelatin group. The most common infection source was pneumonia, followed by intra-abdominal and urinary tract infections. As to the respective degrees of septic shock severity at baseline enrollment of the albumin- and gelatin-receiving groups, there were no significant differences between the data of the 2 groups. The mean APACHE II scores were 22.8 ± 8.5 vs. 23.2 ± 8.1; the mean Sequential Organ Failure Assessment scores were 9.7 ± 3.4 vs. 9.7 ± 3.8; the average MAPs were 55.1 ± 8.0 mmHg vs. 54.6 ± 9.1 mmHg; and the average initial serum lactate levels were 5.6 ± 4.7 mmol/L vs. 6.3 ± 4.9 mmol/L. Finally, the average initial serum albumin levels were not statistically different (2.5 ± 0.6 g/dl vs. 2.4 ± 0.7 g/dl; $p = 0.16$).

The septic shock treatments that the patients were provided are detailed in Table 2. The total fluid volume and total crystalloid volume received on the first, second, and third days of resuscitation did not differ. However, the albumin group was given a significantly lower colloid volume on the first day than the gelatin group (1,068 ± 1,002 mL vs. 1,366 ± 1,020 mL; $p = 0.04$). The cumulative fluid balance at 72 hours was also significantly lower for the albumin group than the gelatin group (5,964 ± 4,960 mL vs. 8,133 ± 3,743 mL; $p = 0.01$). On the other hand, there were no significant differences in the two groups' vasopressor requirements, maximum vasopressor doses, hydrocortisone doses, or levels of mechanical ventilator support. The most common vasopressor used in this study was norepinephrine. In addition, a higher proportion of patients in the albumin group received renal replacement therapy than in the gelatin group (30.8% vs. 15.7%; $p = 0.01$).

The 28-day mortality was 27.9% for the albumin group, compared with 38.2% for the gelatin group; nevertheless, the difference was not statistically significant ($p = 0.11$; Table 3). The Kaplan–Meier curves for 28-day mortality are illustrated in Fig 2. The hospital mortalities (39.4% vs. 44.1%; $p = 0.50$) was were also not significantly different. The days alive and free of organ support to Day 28 were not different (11.2 ± 12.0 days vs. 11.9 ± 12.0 days; $p = 0.56$). There was also no significant difference in the rates of long-term renal replacement therapy for the septic shock patients who survived until hospital discharge (14.3% vs. 7.0%; $p = 0.2$).

To identify conditions that may be beneficially associated with the use of the albumin solution over the gelatin solution for septic shock resuscitation, a subgroup analysis was performed based on the patients' baseline APACHE II scores, serum albumin and serum lactate levels, epinephrine requirements, maximum vasopressor doses, need for ventilator support, and requirements for renal replacement therapy. The septic shock patients in the albumin group who had a baseline APACHE II score ≥ 20 or had a baseline serum albumin < 3 g/dL were associated with a significantly lower 28-day mortality rate than the patients in the gelatin group (Table 4).

DISCUSSION

In this retrospective, baseline-matched-cohort study, the resuscitation with an albumin solution for septic shock patients who were refractory to crystalloid solution was not statistically associated with a difference in the 28-day mortality rate, compared with the patients who were resuscitated with a gelatin solution. Nonetheless, among the patients who had either a baseline APACHE II score of ≥ 20 or a baseline serum albumin level of < 3 g/dL, resuscitation with the albumin solution was associated with a lower 28-day mortality than that for the gelatin solution. The occurrence of renal failure requiring renal replacement therapy was higher among patients who received the albumin than the gelatin solution. Still, there was no significant difference in the long-term renal replacement therapy requirements of the two groups of septic shock hospital survivors.

To date, no study has directly compared the efficacies of albumin and gelatin solutions for septic shock resuscitation. One meta-analysis, which compared albumin with other fluids for the treatment of sepsis patients, revealed that there was no mortality benefit in using albumin in preference to a hydroxyethyl starch and gelatin solution.[5] That result corresponds with the finding of our study. Moreover, the 28-day mortality for the albumin therapy of the septic shock patients in the current study was 27.9%, which was similar to the rate of 31.8% for the albumin-treated group reported by the Albumin Italian Outcome Sepsis (ALBIOS) trial. Furthermore, patients in the present study who received albumin resuscitation were associated with acute kidney injury, for which 30.8% of cases required renal replacement therapy; this is comparable with the corresponding figure of 24.6% determined by the ALBIOS trial.[3] In terms of the hospital mortality of the gelatin-solution users, a previous prospective study on shock patients revealed that the mortality rate of patients who received gelatin solutions was 30%[11], which was lower than the proportion of 44.1% found by our study.

TABLE 1. Patients' baseline characteristics.

Patient characteristic	Albumin (n = 104)	Gelatin (n = 102)	*P*-value
Age (years)	67.9 ± 14.8	64.9 ± 14.9	0.15
Male gender, n (%)	47 (45.2)	54 (52.9)	0.27
Body weight (kilogram)	61.0 ± 14.2	59.3 ± 13.6	0.38
APACHE II score†	22.8 ± 8.5	23.2 ± 8.1	0.73
SOFA score*	9.7 ± 3.4	9.7 ± 3.8	0.87
Underlying conditions, n (%)			
Hypertension	74 (71.2)	37 (36.3)	0.002
Diabetes mellitus	53 (51.0)	37 (36.3)	0.03
Chronic kidney disease	32 (30.8)	22 (22.6)	0.13
Cirrhosis	25 (24.0)	19 (18.6)	0.34
Malignancy	18 (17.3)	23 (22.5)	0.35
Ischemic heart disease	24 (23.1)	15 (14.7)	0.13
Site of infection, n (%)			
Pneumonia	37 (35.6)	36 (35.3)	0.97
Intra-abdominal infection	28 (26.9)	17 (16.7)	0.08
Urinary tract infection	18 (17.3)	29 (28.4)	0.06
Skin and soft tissue infection	7 (6.7)	7 (6.9)	0.97
Primary bacteremia	15 (14.4)	13 (12.7)	073
Other	2 (1.9)	8 (7.8)	0.05
Initial vital signs and investigations			
Temperature (°C)	37.4 ± 3.6	37.6 ± 1.4	0.57
Heart rate (per minute)	103.3 ± 23.0	108.0 ± 24.2	0.15
Respiratory rate (per minute)	27.9 ± 7.7	29.4 ± 7.1	0.16
Mean arterial blood pressure (mmHg)	55.1 ± 8.0	54.6 ± 9.1	0.72
Serum lactate (mmol/L)	5.6 ± 4.7	6.3 ± 4.9	0.26
Serum albumin (g/dL)	2.5 ± 0.6	2.4 ± 0.7	0.16
Fluid responsive test			
Central venous pressure guide	58 (55.8)	43 (42.2)	0.07
Inferior vena cava diameter variation	37 (35.6)	37 (36.3)	0.92
Pulse pressure variation	9 (8.7)	22 (21.6)	0.16
Fluid responsiveness	91 (87.5)	95 (93.1)	0.26

†The APACHE II (Acute Physiology and Chronic Health Evaluation) score, a severity-determining score, ranges from 0 to 71. Higher scores indicate a more severe disease.

*The SOFA (Sequential Organ Failure Assessment) score, a severity-determining score, ranges from 0–24. Higher scores indicate greater organ failure and disease severity.

TABLE 2. Detailed treatment strategies.

Clinical parameters	Albumin (n = 104)	Gelatin (n = 102)	*P*-value
Total fluid resuscitation (mL)			
Total fluid received on the first day	6,375 ± 2,227	6,203 ± 2,040	0.63
Total fluid received on the second day	3,037 ± 1,470	2,944 ± 2,056	0.71
Total fluid received on the third day	2,228 ± 1,637	2,232 ± 1,890	0.99
Crystalloid resuscitation (mL)*			
Total crystalloid received before colloid administration	3,067 ± 2,935	2,967 ± 3,701	0.69
Total crystalloid received on the first day	4,969 ± 1,898	4,662 ± 1,867	0.24
Total crystalloid received on the second day	1,783 ± 1,346	2,030 ± 1,764	0.27
Total crystalloid received on the third day	1,243 ± 1,224	1,969 ± 1,349	0.34
Colloid resuscitation (mL)†			
Total colloid received on the first day	1,068 ± 1,002	1,366 ± 1,020	0.04
Total colloid received on the second day	512 ± 667	559 ± 768	0.65
Total colloid received on the third day	305 ± 608	402 ± 794	0.34
Fluid balance at 72 hours (mL)	5,964 ± 4,960	8,133 ± 3,743	0.01
Vasopressors, n (%)			
Norepinephrine	98 (94.2)	97 (95.1)	0.78
Epinephrine	33 (31.7)	39 (38.2)	0.33
Dopamine	2 (1.9)	1 (1)	0.57
Dobutamine	8 (7.7)	3 (2.9)	0.13
Maximum vasopressor dose (mcg/kg/min)	0.38 ± 0.55	0.38 ± 0.45	0.93
Hydrocortisone, n (%)	72 (69.2)	68 (62.3)	0.77
Mechanical ventilator, n (%)	74 (71.2)	67 (65.7)	0.40
Renal replacement therapy, n (%)	32 (30.8)	16 (15.7)	0.01

* Crystalloid solution that was administration for septic shock resuscitation was 0.9% sodium chloride solution

†For patients in albumin group, all of them received 5% albumin solution

TABLE 3. Patient outcomes.

Clinical outcome	Albumin (n = 104)	Gelatin (n = 102)	Unadjusted Odds Ratio (95% confidence interval)	*P*-value	Adjusted Odds Ratio (95% confidence interval)†	*P*-value†
28-day mortality, n (%)	29 (27.9)	39 (38.2)	0.63 (0.35–1.12)	0.11	0.46 (0.24-0.89)	0.02
Hospital mortality, n (%)	41 (39.4)	45 (44.1)	0.82 (0.47–1.44)	0.50	0.60 (0.32-1.12)	0.11
Days alive and free of ventilator support to Day 28	13.5 ± 12.6	13.5 ± 12.8		0.98		
Days alive and free of vasopressor usage to Day 28	15.7 ± 12.0	14.2 ± 12.2		0.35		
Days alive and free of renal replacement therapy to Day 28	13.9 ± 13.3	14.3 ± 13.0		0.83		
Days alive and free of organ support to Day 28	11.2 ± 12.0	11.9 ± 12.0		0.56		
Long term renal replacement therapy requirement after discharge, n renal replacement/n survivors (%)	9/63 (14.3)	4/57 (7.0)	2.21 (0.64-7.61)	0.20	1.80 (0.47-6.95)	0.40

†Performed by Logistic regression analysis adjusted for baseline underlying conditions (hypertension and diabetes mellitus) and site of infection (intra-abdominal infection, urinary tract infection and other sites of infection)

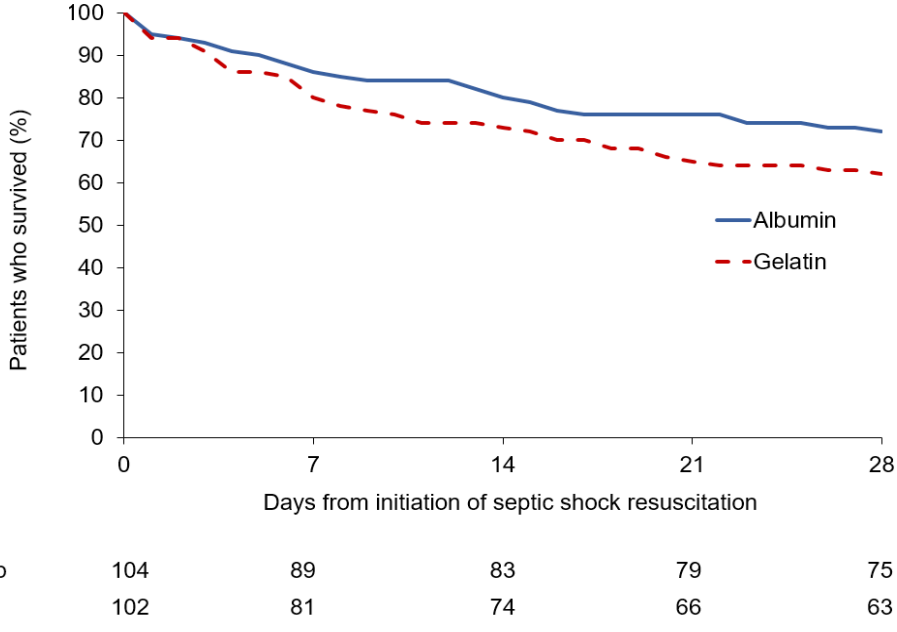


Fig 2. Kaplan–Meier curve of 28-day survival. The hazard ratio for death in patients who received albumin solution (albumin group) compared with patients who received gelatin solution (gelatin group) during septic shock resuscitation was 0.69 (95% confidence interval, 0.42–1.11; $p = 0.12$).

TABLE 4. Subgroup analysis of 28-day mortalities according to clinical parameters.

Clinical parameter	Albumin n deaths/ n per group (%)	Gelatin n deaths/ n per group (%)	Adjusted Odds Ratio (95% confidence interval)†	*P*-value†
APACHE II score*				
< 20	10/46 (21.7)	9/45 (19.6)	1.09 (0.33–3.66)	0.88
≥ 20	19/58 (32.8)	30/57 (53.6)	0.28 (0.12–0.68)	0.005
Baseline serum albumin				
< 3 g/dL	21/81 (25.9)	34/74 (45.9)	0.29 (0.14–0.63)	0.002
≥ 3 g/dL	8/23 (34.8)	5/28 (17.9)	2.87 (0.62–13.31)	0.18
Baseline serum lactate				
< 4 mmol/L	9/55 (16.4)	12/43 (27.9)	0.34 (0.11–1.07)	0.06
≥ 4 mmol/L	20/49 (40.8)	27/59 (45.8)	0.61 (0.27–1.39)	0.24
Epinephrine				
Not receiving	12/71 (16.9)	14/63 (22.2)	0.51 (0.19–4.36)	0.18
Receiving	17/33 (51.5)	25/39 (64.1)	0.56 (0.20–1.57)	0.27
Maximum dose of vasopressors				
< 0.2 mcg/kg/min	12/68 (17.6)	12/56 (21.4)	0.64 (0.23–1.75)	0.39
≥ 0.2 mcg/kg/min	17/36 (47.2)	27/46 (58.7)	0.44(0.17–1.16)	0.10
Ventilator support				
Not receiving	2/30 (6.7)	6/35 (17.1)	0.35 (0.05–2.22)	0.26
Receiving	27/74 (36.5)	33/67 (49.3)	0.46 (0.22–0.96)	0.04
Renal replacement therapy				
Not receiving	13/72 (18.1)	28/86 (32.6)	0.37 (0.16–0.85)	0.02
Receiving	16/32 (50)	11/16 (68.8)	0.49 (0.09–2.53)	0.39

†Performed by Logistic regression analysis adjusted for baseline underlying conditions (hypertension and diabetes mellitus) and site of infection (intra-abdominal infection, urinary tract infection and other sites of infection)

*The APACHE II score, a severity-determining score, ranges from 0 to 71. Higher scores indicate a more severe disease.

Interestingly, the results of our study demonstrated the benefits of using an albumin solution over a gelatin solution for the resuscitation of the more severely ill patients whose baseline APACHE II scores were ≥ 20 and of patients who had a baseline serum albumin level of < 3 g/dL. As to the more severe patients, a subgroup analysis conducted by the ALBIOS study identified that septic shock patients who were resuscitated with albumin were associated with a lower mortality rate than those who were resuscitated with a crystalloid solution.[3] This benefit of albumin was not evident among the severe sepsis patients participating in the ALBIOS trial. In the case of our cohort, all of the enrolled patients had been diagnosed with septic shock, and their hemodynamics had not properly responded to crystalloid resuscitation; it was therefore not possible for our study to compare the efficacies of the use of the albumin and gelatin solutions with the severe sepsis patients. Nonetheless, there were no differences in the efficacies of the two solutions among the patients who received or did not received epinephrine, nor among the patients who required maximum doses of vasopressor of < 0.2 mcg/kg/min or ≥ 0.2 mcg/kg/min (Table 4).

With regard to the influence of the serum albumin level and the benefits of albumin over gelatin solution for septic shock resuscitation, albumin is considered to be the most important plasma protein to maintain oncotic pressure.[19] Septic shock patients with a low baseline serum albumin level and a low oncotic-pressure are at risk of fluid extravasation from the blood vessels into the interstitial tissue (especially the lung parenchyma), which causes hypoxemia due to non-cardiogenic pulmonary edema. The clinical findings from our cohort showed that among the subgroup of septic shock patients who had a baseline serum albumin level of < 3.0 g/dL, the resuscitation with the albumin solution was associated with a lower 28-day mortality than the rate achieved with the gelatin solution. This finding correlates well with information from a prospective randomized controlled study that enrolled critically ill patients with an average baseline serum albumin level of 2.3 g/dL. The results of that research demonstrated that hypoalbuminemia patients who received albumin administration were associated with significantly better Sequential Organ Failure Assessment scores, especially improved PaO_2/FiO_2 ratios, cardiovascular scores, and Glasgow Coma Scale scores.[20] The target serum albumin level of 3 g/dL was also the same as that employed by the ALBIOS trial.

On the other hand, our study demonstrated that there was a trend toward a higher 28-day mortality among septic shock patients with a baseline serum albumin level of ≥ 3 g/dL who received albumin than those who received gelatin, although the difference did not reach statistical significance. This can be partly explained in two possible ways. The first is that cardiac decompensation might occur after rapid volume expansion with exogenous albumin in patients with a high baseline serum albumin, which might lead to an abrupt increase in the venous return and hydrostatic pressure. The second explanation is that in the case of septic shock patients with increased capillary permeability or capillary leak syndrome, albumin administration may become detrimental when albumin and water cross the capillary membrane and aggravate severe hypoxemia due to acute pulmonary edema.[21]

The strength of our study is that it is the first to compare the efficacies of albumin and gelatin solutions for septic shock resuscitation. Given the finding that there was no significant difference in the 28-day mortality rates of the gelatin and albumin groups, gelatin solution could be used as an alternative to colloid fluid for septic shock resuscitation, especially with patients whose baseline serum albumin is ≥ 3.0 g/dL or whose APACHE II score is < 20. As to the cost of each solution, albumin currently costs 7,672 Thai Baht/liter whereas gelatin is priced at 430 Thai Baht/liter. In view of the marked price differential, gelatin solution could be used as an alternative colloid for septic shock patients who cannot afford the cost of albumin resuscitation. On the other hand, albumin should be recommended for patients whose baseline serum albumin is < 3.0 g/dL or APACHE II score is ≥ 20.

There are certain limitations to our study. For one thing, we did not calculate the number of patients who needed to be enrolled to ensure that the study power was adequate. This is because-to our knowledge-no information was available from any previous study directly comparing the efficacies of albumin and gelatin solutions for septic shock resuscitation. Given our research result that the 28-day mortality of the patients in the gelatin group was 38.2%, then 200 patients per group would have needed to be recruited to detect a 10% absolute mortality difference with a power of 20%. Our study may therefore have inadequate power to detect the precise differences between albumin and gelatin resuscitation. Secondly, as this was a retrospective observational study in which the decisions to use particular colloid solutions were made by the individual attending physicians, a selection bias might have occurred. However, after adjusting the patients' baseline data (age, APACHE II scores, MAPs, and baseline serum lactate levels), the baseline characteristics of the two enrolled groups were well-matched. In addition, as this was a single center study, generalization of the results

could be limited. Physicians who would like to apply the results could adjust the context of the study population to better match their own situation. To confirm the benefits of using an albumin solution for septic shock resuscitation and broaden the applicability of the results, a large multicenter randomized study with adequate power to evaluate the mortality differences between albumin and gelatin (and possibly other colloid solutions) could be conducted.

CONCLUSION

For septic shock resuscitation, the albumin and gelatin solutions did not have any differences in their 28-day mortality rates or organ support-free survival days in the overall populations. However, resuscitation with albumin was associated with better outcomes among patients who had hypoalbuminemia or high APACHE II scores. Further study is required to confirm these benefits.

ACKNOWLEDGMENTS

We thank Mr. David Park for language editing and Mr. Suthipol Udompanturak for statistics consultation.

Consent for publication: Not applicable.

Availability of data and materials: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors have no competing interests to declare, or any real or perceived financial interests in any product or commodity mentioned in this paper.

Funding: The trial was funded by Siriraj Critical Care Research Funding.

Abbreviations: MAP: mean arterial blood pressure; RRT: renal replacement therapy; APACHE II score: Acute Physiology and Chronic Health Evaluation II score; SOFA score: Sequential Organ Failure Assessment score

REFERENCES

1. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonellis M, Ferrer R, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Crit Care Med 2017;45:486-552.
2. Finfer S, Bellomo R, Boyce N, French J, Myburgh J, Norton R, et al. A comparison of albumin and saline for fluid resuscitation in the intensive care unit. N Engl J Med 2004;350:2247-56.
3. Caironi P, Tognoni G, Masson S, Fumagalli R, Pesenti A, Romero M, et al. Albumin re-placement in patients with severe sepsis or septic shock. N Engl J Med 2014;370:1412-21.
4. Xu JY, Chen QH, Xie JF, Pan C, Liu SQ, Huang LW, et al. Comparison of the effects of albumin and crystalloid on mortality in adult patients with severe sepsis and septic shock: a meta-analysis of randomized clinical trials. Critical Care 2014;18:702.
5. Jiang L, Jiang S, Zhang M, Zheng Z, Ma Y. Albumin versus Other Fluids for Fluid Resuscitation in Patients with Sepsis: A Meta-Analysis. PLoS ONE 2014;9:1-21.
6. Qureshi SH, Rizvi SI, Patel NN, Murphy GJ. Meta-analysis of colloids versus crystalloids in critically ill, trauma and surgical patients. BJS 2016;103:14-26.
7. Moeller C, Fleischmann C, Thomas-Rueddel D, Vlasakov V, Rochwerg B, Theurer P, et al. How safe is gelatin? A systematic review and meta-analysis of gelatin-containing plasma expanders vs. crystalloids and albumin. J Crit Care 2016;35:75-83.
8. Trof RJ, Sukul SP, Twisk JW, Girbes AR, Groeneveld AB. Greater cardiac response of colloid than saline fluid loading in septic and non-septic critically ill patients with clinical hypovolaemia. Intensive Care Med 2010;36:697-701.
9. Wu JJ, Huang MS, Tang GJ, Kao WF, Shih HC, Su CH, et al. Hemodynamic response of modified fluid gelatin compared with lactated ringer's solution for volume expansion in emergency resuscitation of hypovolemic shock patients: preliminary report of a prospective, randomized trial. World J Surg 2001;25:598-602.
10. Parker MJ, Griffiths R, Boyle A. Preoperative saline versus gelatin for hip fracture patients; a randomized trial of 396 patients. Br J Anaesth 2004;92:67-70.
11. Annane D, Siami S, Jaber S, Martin C, Elatrous S, Declère AD, et al. Effects of fluid resuscitation with colloids vs. crystalloids on mortality in critically ill patients presenting with hypovolemic shock: the CRISTAL randomized trial. JAMA 2013;310: 1809-17.
12. Tongyoo S, Viarasilpa T, Permpikul C. The impact of intensive care unit admissions following early resuscitation on the outcome of patients with severe sepsis and septic shock. J Med Assoc Thai 2014;97 (Suppl 1):S69-76.
13. Permpikul C, Tongyoo S, Ratanarat R, Wilachone W, Poompichet A. Impact of septic shock hemodynamic resuscitation guidelines on rapid early volume replacement and reduced mortality. J Med Assoc Thai 2010;93 (Suppl 1):S102-9.
14. Zarychanski R, Abou-Setta AM, Turgeon AF, Houston BL, McIntyre L, Marshall JC, et al. Association of hydroxyethyl starch administration with mortality and acute kidney injury in critically ill patients requiring volume resuscitation: a systematic review and meta-analysis. JAMA 2013;309:678-88.
15. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, et al. Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock. Intensive Care Med 2013;39:165-228.
16. Permpikul C, Sringam P, Tongyoo S. Therapeutic goal achievements during severe sepsis and septic shock resuscitation and their association with patients' outcomes. J Med Assoc Thai 2014;97 (Suppl 3):S176-83.
17. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the reporting of observational studies in epidemiology. Epidemiology 2007;18:805-35.

18. Russell JA, Lee T, Singer J, De Backer D, Annane D. Days alive and free as an alternative to a mortality outcome in pivotal vasopressor and septic shock trials. Crit Care 2018;47:333-37.
19. Dubois MJ, Orellana-Jimenez C, Melot C, De Backer D, Berre J, Leeman M, et al. Albumin administration improves organ function in critically ill hypoalbuminemic patients: A prospective, randomized, controlled, pilot study. Crit Care Med 2006;34: 2536-40.
20. Weil MH, Henning RJ, Puri VK. Colloid oncotic pressure: clinical significance. Crit Care Med 1979;7:113-6.
21. Milford EM, Reade MC. Resuscitation Fluid Choices to Preserve the Endothelial Glycocalyx. Crit Care 2019;23:77.

Predictor of In-hospital Mortality among Acute Coronary Syndrome Patients after Treatment with an Intra-aortic Balloon Pump in Tertiary Hospital, Thailand

Chorchana Wichian, M.D.*, Thotsaporn Morasert, M.D.*, Surat Tongyoo, M.D., Naruebeth Koson, M.NS.*****

**Department of Internal Medicine, Suratthani Hospital, Suratthani 84000, **Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, ***Department of Cardiovascular and Thoracic Intensive Care Unit, Suratthani Hospital, Surat Thani 84000, Thailand.*

ABSTRACT

Objective: Intra-aortic balloon pump (IABP), a mechanical hemodynamic support device, had widely been used to treat cardiogenic shock patients for several decades. However, the information about the predictive factors associated with mortality was scarce. This study aims to identify the predictive factors associated with in-hospital mortality in acute coronary syndrome (ACS) patients who performed IABP for their hemodynamic support during admission.

Methods: We conduct a retrospective cohort study design. All admission records of ACS patients with IABP at Suratthani Hospital between October 2015 and September 2019 were retrieved.

Results: Overall 75 ACS patients with IABP insertion were enrolled. Thirty-one patients died during admission, in-hospital mortality was 41.3%. From the multivariable analysis, we identified 3 predictors associated with in-hospital mortality included cardiac arrest at presentation (adjusted OR [aOR]=11.18, 95%CI: 2.42-51.57, P=0.002), a higher number of inotropes or vasopressors (aOR 6.10, 95%CI 1.36-27.24, P=0.018) and Killip class IV (aOR 5.64, 95%CI 1.01-31.39, P=0.048).

Conclusion: ACS patients who required IABP support had high mortality. Cardiac arrest, Killip class IV (cardiogenic shock) at presentation and requiring a higher number of inotropes or vasopressors were independent predictive factors of in-hospital mortality.

Keywords: Acute coronary syndrome; in-hospital mortality; intra-aortic balloon pump (Siriraj Med J 2020; 72: 462-469)

INTRODUCTION

Coronary artery disease (CAD), an important non-communicable disease, is a leading cause of death in adults which accounting for one - thirds of all death in the subject over 35 years as reported by the World Health Organization.[1] Data from Thailand reported that CAD caused 31 deaths per 100,000 population.[2] The CAD can be categorized according to the clinical presentation into acute coronary syndrome (ACS) and stable CAD. ACS is divided into ST-segment elevated myocardial infarction (STEMI) and non-ST segmented elevated myocardial infarction (NSTEMI) by initial electrocardiogram. Patients who presented with ACS had a significantly higher mortality rate than those with stable CAD. Data from the Thai acute coronary syndrome (Thai ACS registry), reported high 1-year mortality in both STEMI (14%) and NSTEMI (25%).[3]

Corresponding author: Chorchana Wichian
E-mail: chorchana@gmail.com
Received 30 July 2020 Revised 28 August 2020 Accepted 1 September 2020
ORCID ID: http://orcid.org/0000-0001-8929-644X
http://dx.doi.org/10.33192/Smj.2020.62

Although, the restoration of epicardial coronary artery blood flow by either percutaneous coronary intervention (PCI) or coronary artery bypass graft surgery (CABG) can improve the outcome of many ACS patients but the in-hospital mortality was still high.[3] The previous study identified factors associated with in-hospital mortality among STEMI patients which included age older than 75 years, diabetes mellitus, cardiac arrhythmias and cardiogenic shock.[4] The treatment of cardiogenic shock composed of pharmacological treatment with inotropic drug plus vasopressors and mechanical circulatory support for the critically ill patients.

Mechanical circulatory support (MCS) takes an important role in unstable conditions or high-risk patients, especially patients with left main coronary artery stenosis, severely impaired left venticular systolic function, multivessel coronary artery stenosis disease, elderly patients and anterior wall STEMI.[5] Following the current guidelines[6] recommend using many types of MCS systems include intra-aortic balloon pump (IABP), Tandem heart, Impella, or veno-arterial extracorporeal membrane oxygenation (VA-ECMO). The MCS can increase forward blood flow from the left ventricle, reduce left ventricular end-diastolic pressure result in improving coronary artery blood flow as well as systemic circulation. However, the level of augmented cardiac output difference by each type of MCS. Comparing among the mechanical devices that can be a bed-side, percutaneous insertion; ECMO is a device that can increase systemic blood flow in the highest volume, followed with Impella and IABP. In The recent years, VA-ECMO and Impella had been recommended over IABP for hemodynamic support for cardiogenic shock patients. Although, IABP can increase cardiac output only about 0.5 litres per minute, it still the most common device that is widely used for hemodynamic support among cardiogenic shock patients in Thailand due to its relatively low price and more available of experienced physicians for device insertion and management.

Up to now, in the era that almost every ACS patient received timely reperfusion therapy by either PCI or CABG. To the best of our knowledge there is scarce information about the result of IABP usage for hemodynamic support among cardiogenic shock patients. Therefore, the objective of this study is to evaluate the predictors of in-hospital mortality in ACS patients with cardiogenic shock receiving IABP insertion for hemodynamic support.

MATERIALS AND METHODS

Study design and setting

We conducted a retrospective cohort design in a university-affiliated tertiary care centre, Suratthani Hospital (SH), Thailand. The study was approved by the ethic committee review board of SH. The number of approval was 34/2020.

Study participants

All admission records of ACS patients in the cardiac catheterization unit of SH between October 2015 and September 2019 were screened. The ACS patients, aged 18 years or more and required an IABP insertion were included. The diagnosis of ACS is based on international guidlines.[7-9] All patients were confirmed coronary artery occlusion by the coronary artery angiography. Patients were considered to have cardiogenic shock if their systolic blood pressure (SBP) < 90 mmHg or required any vasopressor to maintain SBP ≥ 90 mmHg. The exclusion criteria were normal coronary angiography (CAG) or diagnosis of non-obstructive coronary disease.

Data collection

The patients' baseline clinical characteristic, initial laboratory data, and complication during admission included sepsis, performed hemodialysis, limb ischemia and stroke were extracted from the medical records. Patients were categorized to Killip classification (I-IV) at the presentation by a cardiologist who was blinded to the patients' survival status. The left ventricular ejection fraction (LVEF) results by transthoracic echocardiography were obtained if they were performed within the first seven days of admission. The timing of IABP insertion and information of revascularization procedures included CABG and PCI were also collected. All patients admission records were separated into two groups: non-survived (all-cause in-hospital death) admission and survived admissions based on the survival status of the patient.

Statistical analysis

We analyzed the characteristics of ACS patients who performed IABP and compared non-survived cases with survived cases. Categorical variables are presented as percentages and were compared using Fisher's exact test. Continuous variables are presented as the mean ± standard deviation (SD) and were compared using the two-sample t-test. Non-parametric continuous variables are presented as interquartile ranges (IQRs) and were compared using the Wilcoxon rank-sum (Mann-Whitney) test. All proportions and P values were calculated based on variables with no missing data. Logistic regression analysis was carried out to determine the factors associated with in-hospital mortality. Odds ratios (ORs) and their 95% confidence intervals (CIs) were estimated.

Variables at P < 0.1 on univariable analysis, as well as those considered a priori as possible associated factors based on previous research, were selected for inclusion in the final multivariable model. Variables with more than 20 per cent missing were excluded from statistical modelling. All analyses were two-sided with a p-value < 0.05 as a critical value for statistical significance.

RESULTS

A total of 76 ACS patients with IABP were enrolled. Of this number, one patient was excluded due to normal CAG. Finally, 75 patients were included for analysis. The patients were predominantly male (70.7%), with the mean age of 63.9 years (±SD 13.0). During the study period, 31 patients died. The in-hospital mortality of the cohort was 41.3% (31/75). Thirty-seven patients (49.3%) were acute STEMI and 38 patients (50.7%) were NSTEMI/UA. The cardiogenic shock, Killip class IV at presentation was found in 44 patients (58.7%) while the 35 patients (46.7%) had a cardiac arrest at presentation. More than half of the patients were revascularized by PCI (64%) and was inserted IABP in peri- or post-revascularization period (65%). The median duration of IABP use was 3 days (IQR 2-4). Almost all patients (94.7%) received at least one vasopressor or inotropic agent.

In comparison with survived cases, non-survived cases were significantly more likely be the elderly (age ≥65 years) (65% vs. 36%), had Killip class IV (87% vs. 39%), had lower SBP (84.6 mmHg vs. 99.6 mmHg), had lower DBP (54.7 mmHg vs. 64.3 mmHg), had lower MAP (64.7 mmHg vs. 76.1 mmHg), presented with cardiac arrest (84% vs. 20%), had higher serum creatinine (1.3 mg/dL vs. 1.0 mg/dL), were revascularized by PCI (84% vs. 52%), were performed IABP in the peri- or post-revascularization period (84% vs. 53%) and had a higher number of inotropic agents or vasopressors use before start IABP (Tables 1 and 2). Among the ACS patients who performed PCI, complete revascularization (PCI all stenotic vessel), the complete revascularized group had lower in-hospital mortality (70.8% vs 28%, p = 0.004).

The complications after IABP insertion was shown in Table 3. Hemodialysis was the leading complication followed by sepsis, stroke and limb ischemia.

On the univariable analysis, the following parameters were considered to be significantly associated with in-hospital mortality: elderly (age ≥65 years), Killip class IV, SBP <90 mmHg, DBP <60 mmHg, MAP <65 mmHg, cardiac arrest at presentation, revascularization by PCI, IABP insertion during the peri-/post-revascularization period and a higher number of inotropic agents or vasopressors use (Table 4). On multivariable analysis, we identified 3 independent predictors associated with in-hospital mortality among ACS patients in IABP included cardiac arrest at presentation (adjusted OR [aOR] 11.18, 95%CI 2.42-51.57, P=0.002), a higher number of inotropes/vasopressors (aOR 6.10, 95%CI 1.36-27.24, P=0.018) and Killip class IV (aOR 5.64, 95%CI 1.01-31.39, P=0.048) (Table 4).

DISCUSSION

In this 4-year retrospective study, patients who presented with acute myocardial infarction and were treated with an IABP had high in-hospital mortality especially in the elderly, cardiogenic shock and cardiac arrest patients. In the multivariable analysis, we identified 3 independent predictive factors of in-hospital mortality which included Killip class IV, cardiac arrest, and number of inotrope or vasopressors use. Furthermore, the adverse events after IABP was reported significantly higher among the patients who died during the admission.

For identify the predictive factors associated with poor outcome among patients who received IABP support, a retrospective study from Japan, enrolled 104 patients who underwent PCI for treatment the coronary artery lesion responsible for acute myocardial infarction and received IABP insertion, showed that shock stage and low glomerular filtration rate were the predictive factors for dead, while inserted IABP before start PCI was the factor associated with survival.[10] However, only 47.1% of patients in the Japanese cohort were in the shock stage. Although, we also found that hemodialysis after IABP was significantly higher among non-survived patients in our study, we did not include this factor into the final model due to we aimed to explore the predictor of in-hospital mortality before IABP insertion. The others independent predictive factors in the current study for in-hospital dead, included Killip class IV, presented with cardiac arrest and high number of inotropic used were all represent the more severe form of myocardial infarction.

Comparing to the participants of IABP Shock II trial, the largest randomized controlled trial which included 600 myocardial infarctions with cardiogenic shock patients who had planned for early reperfusion therapy, our participant were relatively younger, lower proportion of reperfusion therapy with PCI (64.5% vs 96%) but had similar baseline blood pressure and heart rate.[11] The higher proportion of our acute myocardial infarction with cardiogenic patients who received CABG surgery for reperfusion therapy could be mainly explained by the higher incidence of left main stenosis which was 51.3% in our study, compared with only 9.3% in IABP-Shock II

TABLE 1. Clinical characteristics of non-survived and survived ACS patients with IABP (n=75).

Characteristics	Non-survived, (n=31)	Survived, (n=44)	*P*-value
Age			
years, mean (SD)	66.9 (14.7)	61.8 (11.4)	0.091
≥65 years	20 (65)	16 (36)	0.020
Male sex, n (%)	22 (71)	31 (70)	1.000
Diabetes Mellitus, n (%)	14 (45)	21 (48)	1.000
Hypertension, n (%)	21 (68)	33 (75)	0.600
Previous history of CAD, n (%)	7 (23)	11 (25)	1.000
Previous PCI, n (%)	5 (16)	2 (5)	0.120
STEMI, n (%)	17 (55)	20 (45)	0.490
Anterior wall lesion, n (%)	10 (32)	10 (23)	0.430
Non-STEMI or Unstable angina, n (%)	14 (45)	24 (55)	0.490
Left main coronary artery stenosis	16 (52)	23 (52)	1.000
Killip class at presentation, n (%)			<0.001
I	0 (0)	7 (16)	
II	1 (3)	7 (16)	
III	3 (10)	13 (30)	
IV	27 (87)	17 (39)	
Systolic BP			
mmHg, mean (SD)	84.6 (13.4)	99.6 (25.7)	0.004
<90 mmHg, n (%)	22 (71)	17 (39)	0.009
Diastolic BP			
mmHg, mean (SD)	54.7 (11.6)	64.3 (18.8)	0.014
<60 mmHg, n (%)	20 (65)	17 (39)	0.036
Mean arterial BP			
mmHg, mean (SD)	64.7 (11.3)	76.1 (20.4)	0.007
<65 mmHg, n (%)	17 (55)	14 (32)	0.059
Heart rate, per minute, mean (SD)	94.1 (25.3)	88.0 (23.2)	0.280
Cardiac arrest at presentation, n (%)	26 (84)	9 (20)	<0.001
Serum creatinine, mg/dL, median (IQR)	1.3 (1.1, 1.5)	1.0 (0.9, 1.4)	0.017
Initial hemoglobin level, g/dL, mean (SD)	12.7 (2.1)	11.9 (2.1)	0.140
Current statin use	7 (23)	10 (23)	1.000
DAPT in first day	30 (96.8)	44 (97.8)	1.000
High potency statin first day	6 (19.4)	41 (91.1)	0.300
Left ventricular ejection fraction			
%, mean (SD)	42.6 (14.3)	44.2 (15.4)	0.670
missing, n (%)	9 (29)	1 (2)	0.004

All proportions (%) and P values calculated based on variables with no missing data.

Abbreviations: ACS, acute coronary syndrome; BP, blood pressure; CAD, coronary artery disease; IABP, intra-aortic balloon pump; IQR, interquartile range; PCI, percutaneous coronary intervention; SD, standard deviation; STEMI, ST-segment elevation myocardial infarction

TABLE 2. Comparison of treatment between non-survived and survived ACS patients with IABP (n=76).

Type of treatment	Non-survived, (n=31)	Survived, (n=44)	*P*-value
Mode of revascularization			0.015
PCI, n (%)	25 (81)	23 (52)	
CABG, n (%)	6 (19)	21 (48)	
PCI for STEMI, n (%)			
Primary	12 (39)	9 (20)	0.120
Rescue	3 (10)	4 (9)	1.000
PCI for Non-STEMI or Unstable angina, n (%)	10 (32)	10 (23)	0.430
PCI left main, n (%)	9 (29)	9 (20)	0.420
Number of vessel target for CABG			1.000
1	0 (0)	1 (5)	
2	1 (17)	4 (19)	
3	5 (83)	14 (67)	
4	0 (0)	2 (10)	
Valve surgery co-committed	2 (6)	2 (5)	1.000
Timing of IABP insertion, n (%)			0.006
Pre- revascularization	5 (16)	21 (48)	
Peri- and post revascularization	26 (84)	24 (52)	
Duration of IABP, days, median (IQR)	2 (2, 4)	3 (2, 4)	0.140
Inotrope or vasopressor(s) use prior to IABP			
Any	31 (100)	41 (91)	0.140
No. of medication use			<0.001
1	0 (0)	12 (27)	
2	17 (55)	21 (48)	
3	14 (45)	7 (16)	

Abbreviations: ACS, acute coronary syndrome; CABG, coronary artery bypass graft; IABP, intra-aortic balloon pump; IQR, interquartile range; PCI, percutaneous coronary intervention; SD, standard deviation; STEMI, ST-segment elevation myocardial infarction

TABLE 3. In-hospital complication after IABP (n=76).

Complication	Non-survived (n=31)	Survived (n=44)	*P*-value
Hemodialysis, n (%)	10 (32)	2 (5)	0.003
Sepsis, n (%)	5 (16)	1 (2)	0.076
Stroke, n (%)	2 (6)	0 (0)	0.170
Limb ischemia, n (%)	1 (3)	1 (2)	1.000

Abbreviation: IABP, intra-aortic balloon pump

TABLE 4. Predictor of in-hospital mortality among ACS patients with IABP by univariable and multivariable logistic regression analysis.

Predictor	Univariable			Multivariable		
	OR	(95% CI)	*P*-value	aOR	(95% CI)	*P*-value
Age, years						
<65	1	Reference				
≥65	3.18	(1.22-8.30)	0.018	2.45	(0.54-11.03)	0.245
Test for trend (every 1 increased)	1.03	(0.99-1.07)	0.094			
Killip class						
I	1	Reference				
IV	10.72	(3.19-36.05)	<0.001	5.64	(1.01-31.39)	0.048
Systolic BP, mmHg						
≥90	1	Reference				
<90	3.88	(1.45-10.39)	0.007	0.75	(0.09-6.52)	0.799
Test for trend (every 10 decreased)	1.04	(1.01-1.06)	0.008			
Diastolic BP, mmHg						
≥60	1	Reference				
<60	2.89	(1.11-7.49)	0.029	1.50	(0.03-71.13)	0.838
Test for trend (every 10 decreased)	1.04	(1.01-1.07)	0.018			
Mean arterial BP, mmHg						
≥65	1	Reference				
<65	2.60	(1.01-6.73)	0.049	0.92	(0.02-48.21)	0.967
Test for trend (every 10 decreased)	1.04	(1.01-1.07)	0.010			
Cardiac arrest at presentation						
No	1	Reference				
Yes	20.22	(6.06-67.49)	<0.001	11.18	(2.42-51.57)	0.002
Serum Creatinine, mg/dL						
Test for trend (every 0.1 decreased)	1.00	(0.97-1.03)	0.993			
PCI						
No	1	Reference				
Yes	3.80	(1.31-11.09)	0.014	4.41	(0.29-66.96)	0.285
Timing of IABP						
Pre- revascularization	1	Reference				
Peri- and post revascularization	4.75	(1.54-14.63)	0.007	1.90	(0.15-23.38)	0.615
No. of inotrope/vasopressor						
Test for trend (every 1 agent added)	4.80	(2.02-11.43)	<0.001	6.10	(1.36-27.24)	0.018

Abbreviations: ACS, acute coronary syndrome; aOR, adjusted OR; BP, blood pressure; CI, confidence interval; IABP, intra-aortic balloon pump; PCI, percutaneous coronary intervention

trial. Furthermore, the incidence of significant stenosis in 3 vessels was 70% in our patients, compared with 52.3% in IABP-Shock II trail. For the 30-days mortality outcome which was reported about 39.7% among IABP group of IABP-Shock II trail, similar with 42.1% in our population. Recently, there was a study reported 4-years experience at Hua Hin hospital, a large local government hospital in the southern part of Thailand, enrolled 57 acute myocardial infarction with cardiogenic shock and received IABP insertion for hemodynamic support.[12] Most patients (50.9%) presented with acute subendocardial myocardial infarction, following with acute transmural myocardial infarction (45.6%). Only 3.5% of patients had a cardiac arrest at presentation. The overall in-hospital mortality was 23% which was lower than 40.8% in our report. However, considering patients in our study who did not have cardiac arrest, the in-hospital mortality rate was only 25% which was comparable with the mentioned study.

Intra-aortic balloon pump, a percutaneously inserted catheter base mechanical device, had been used for hemodynamic support among cardiogenic shock patients since it first successful clinical application in 1968.[13] Due to IABP ability to improve patients' mean arterial blood pressure, restoration of myocardial perfusion and slight improve cardiac output, this device became the most common mechanical support device usage worldwide for almost 3 decades. Evidence from observational studies and small-sized randomized controlled trial support the benefit of IABP in cardiogenic shock patients caused by acute myocardial infarction for timely reperfusion therapy.[14] However, the recent evidence from a large multicenter randomized controlled trial did not show any benefit of IABP support among acute myocardial infarction with cardiogenic shock who underwent early reperfusion therapy thus the European Society of Cardiology 2018 guideline downgraded the routine using of IABP for hemodynamic support among cardiogenic shock to be class III recommendation.[15]

Our study had some limitations. Firstly, this is a retrospective observational study conducted at a single centre. The number of enrolled patients was limited. So we could unable to identify another potential factor that might be associated with poor outcome among IABP inserted patients. Secondly, the application of information from our study could be considered under the context of the individual institute, and according to the different of patients' baseline characters and their receiving standard of care which may vary from our institute. Thirdly, our mortality rate was reported high, which could be explained by a significantly high proportion of cardiac arrest patients that were enrolled in the study.

CONCLUSION

The acute coronary syndrome patients who required intra-aortic balloon pump support had high mortality. Cardiac arrest, Killip class IV (cardiogenic shock) at presentation and requiring a higher number of inotropes or vasopressors were independent predictive factors of in-hospital mortality.

REFERENCES

1. Sanchis-Gomar F, Perez-Quilis C, Leischik R, Lucia A. Epidemiology of coronary heart disease and acute coronary syndrome. Ann Transl Med 2016;4:256.
2. Disease Don-c. Individual patient Thai database 2018 [Available from: http://www.thaincd.com/2016/mission/documents-detail.php?id=13684&tid=32&gid=1-020.
3. Srimahachota S, Boonyaratavej S, Kanjanavanit R, Sritara P, Krittayaphong R, Kunjara-Na-Ayudhya R, et al. Thai Registry in Acute Coronary Syndrome (TRACS)-an extension of Thai Acute Coronary Syndrome Registry (TACS) group: lower in-hospital but still high mortality at one-year. J Med Assoc Thai 2012;95:508-18.
4. Sanguanwong S, Srimahachota S, Tungsubutra W, Srichaiveth B, Kiatchoosakun S. Predictors of in-hospital mortality in Thai STEMI patients: results from TACSR. J Med Assoc Thai 2007;90(Suppl 1):91-97.
5. Members WC, Levine GN, Bates ER, Blankenship JC, Bailey SR, Bittl JA, et al. 2011 ACCF/AHA/SCAI guideline for percutaneous coronary intervention: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines and the Society for Cardiovascular Angiography and Interventions. Circulation 2011;124:e574-e651.
6. Rihal CS, Naidu SS, Givertz MM, Szeto WY, Burke JA, Kapur NK, et al. 2015 SCAI/ACC/HFSA/STS clinical expert consensus statement on the use of percutaneous mechanical circulatory support devices in cardiovascular care: endorsed by the American Heart Assocation, the Cardiological Society of India, and Sociedad Latino Americana de Cardiologia Intervencion; Affirmation of Value by the Canadian Association of Interventional Cardiology-Association Canadienne de Cardiologie d'intervention. J Am Coll Cardiol 2015;65:e7-e26.
7. Kimura K, Kimura T, Ishihara M, Nakagawa Y, Nakao K, Miyauchi K, et al. JCS 2018 guideline on diagnosis and treatment of acute coronary syndrome. Circ J 2019;83:1085-196.
8. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth universal definition of myocardial infarction (2018). J Am Coll Cardiol 2018;72:2231-64.
9. Neumann FJ, Sousa-Uva M, Ahlsson A, Alfonso F, Banning AP, Benedetto U, et al. 2018 ESC/EACTS guidelines on myocardial revascularization. Eur Heart J 2019;40:87-165.
10. Kasahara T, Sakakura K, Yamamoto K, Taniguchi Y, Tsukui T, Seguchi M, et al. Clinical Factors Associated with In-Hospital Mortality in Patients with Acute Myocardial Infarction Who Required Intra-Aortic Balloon Pumping. Int Heart J 2020;61:209-14.

11. Thiele H, Schuler G, Neumann FJ, Hausleiter J, Olbrich HG, Schwarz B, et al. Intraaortic balloon counterpulsation in acute myocardial infarction complicated by cardiogenic shock: design and rationale of the Intraaortic Balloon Pump in Cardiogenic Shock II (IABP-SHOCK II) trial. Am Heart J 2012;163:938-45.
12. Euswas P. Results of Intra-aortic Balloon Pump (IABP) Therapy in Cardiogenic Shock Patients: 4Years Experience at Hua Hin Hospital. Reg 4-5 Med J 2014;33:107-17.
13. Kantrowitz A, Tjonneland S, Freed P, Philipps S, Butner A. Sherman JL Jr. Initial clinical experience with intraaortic balloon pumping in cardiogenic shock. JAMA 1968;203:113-18.
14. Parissis H, Graham V, Lampridis S, Lau M, Hooks G, Mhandu P. IABP: history-evolution-pathophysiology-indications: what we need to know. J Cardiothorac Surg 2016;11:122.
15. Neumann FJ, Sousa-Uva M, Ahlsson A, Alfonso F, Banning AP, Benedetto U, et al. 2018 ESC/EACTS Guidelines on myocardial revascularization. Eur Heart J 2018;40:87-165.

Short-term Effect of Transcutaneous Electrical Nerve Stimulation (TENS) on Pain in Patients with Bone Metastasis: An Uncontrolled Pretest-Posttest Study

Thipawan Bowornkitiwong, M.D.*, Wanida Srilarnkhom, Pawinee Tawatchot**, Teeraporn Paisan**, Suganyapatch Sirichotpapa***, Sirinya Nanthanangkul**, Teerapol Sommaichaiya, M.D.*****

**Department of Palliative Care Unit, Udonthani Cancer Hospital, **Research and Medical Technology Assessment Department, Udonthani Cancer Hospital, ***Physical Therapy Department, Palliative Care Unit, Udonthani Cancer Hospital, ****Anesthesiology Department, Lerdsin Hospital, Bangkok, Thailand.*

ABSTRACT

Objective: To evaluate the short-term effect of TENS on pain for patients with bone metastasis.

Methods: An experimental descriptive study of 25 eligible advanced cancer patients with bone metastasis. Patients were enrolled in the study from June 1, 2018 to December 31, 2019. Pain intensity measurements were recorded at baseline prior to TENS application, then after 30 minutes and 60 minutes of TENS while the device was switched on. TENS was applied prior to radiotherapy at the same time every day for 5 days. Pain score was evaluated with the Visual Analogue Scale (VAS). Symptom assessment was measured by a Thai version of the Edmonton symptom assessment system (ESAS-Thai) on the first day prior to and five days after TENS application began. The paired t-test and Generalized Estimating Equations (GEE) were used analysis.

Results: Mean VAS scores decreased by 1.08 (-1.08; 95% CI; -1.66 to 0.50, $p < 0.001$) and 1.82 (-1.82; 95 CI; -2.40 to 1.24, $p < 0.001$) after 30 and 60 minutes, respectively, compared to the baseline. Lower VAS scores were also correlated to the number of TENS visits. Mean ESAS scores showed a statistically significant difference before and after TENS application (before: 4.32 (95% CI: 3.60–5.03); after: 3.08 (95% CI: 2.61–3.54), $p = 0.004$). During TENS application there was a reduction in VAS pain scores over time.

Conclusion: TENS is non-invasive, inexpensive and safe. It may be a useful adjunct to the multimodality treatment of pain and may reduce the need for morphine.

Keywords: TENS; cancer with bone metastasis (Siriraj Med J 2020; 72: 470-475)

INTRODUCTION

Bone is the fourth most common site for metastasis.[1] Bone pain is commonly reported among patients with metastatic bone disease.[2,3] Bone pain in most cancer patients initially occurs as intermittent dull aches and then becomes constant and more severe, especially at night.[4] Another characteristic of bone pain is breakthrough (episodic) pain, defined as recurrent episodes of severe pain breaking through the regimen administered to treat background pain.[5] It can be spontaneous or precipitated by some factors especially by movement.

Corresponding author: Thipawan Bowornkitiwong
E-mail: mugajung@hotmail.com
Received 30 July 2020 Revised 17 August 2020 Accepted 1 September 2020
ORCID ID: http://orcid.org/0000-0002-7760-6081
http://dx.doi.org/10.33192/Smj.2020.63

Bone cancer pain, which has both a nociceptive and neuropathic component,[6] is difficult to control with only one modality, typically requiring the use of multiple interventions including radiotherapy, surgery, chemotherapy, bisphosphonate, calcitonin, and analgesics.[7] Patients with pain have decreased quality of life in all domains, including physical functioning, social relationship and mental health.[8]

Standard analgesic approaches and radiotherapy can both be used to treat bone cancer pain.[9] Radiotherapy is estimated to produce complete pain relief at one month in approximately a quarter of patients.[10] Opioid-based therapy is the most common analgesic used to treat cancer bone pain and does not have a ceiling dose until unmanageable adverse effects occur.[11] However, adverse effects of sedation, constipation, nausea, and vomiting are common.[11] Others are confusion, hallucination, nightmares, urinary retention, myoclonus, dizziness, and dysphoria. Other adjunctive medications, such as NSAIDs and bisphosphonates are also associated with undesired side effects.[12,13]

Transcutaneous electrical nerve stimulation (TENS) is a non-invasive intervention used to relieve chronic pain, mostly commonly in non-cancer diseases. Systematic reviews have shown benefits for musculoskeletal and osteoarthritic pain.[14,15] TENS reduces pain through both peripheral and central mechanisms.[16] Its purpose is to selectively activate large diameter non-noxious afferents, which reduces nociceptor cell activity and sensitization in the central nervous system.[17] The afferent input is sent to the central nervous system to activate descending inhibitory systems to reduce hyperalgesia.[18] The peripheral blockade of TENS results in the reduction of substance P in dorsal root ganglia neurons in animals.[18] TENS may also alter the excitability of peripheral nociceptors to reduce afferent input to the central nervous system.[18]

A few studies have examined the effect of TENS on bone pain among cancer patients.[18,19] However, the evidence whether TENS is an effective tool for pain management among cancer patients is inconclusive.[20] Therefore, the objective of this study is to evaluate the short-term effect of TENS on pain for patients with bone metastasis.

MATERIALS AND METHODS

Research design

This study was designed as a pre-experimental pretest-posttest study design without a control group. The study protocol was reviewed and approved by Udonthani Cancer Hospital Ethics Committees for Human Research (reference number UCH–CT 13/2561). Pre-experimental studies are important for informing future decisions about sample size and feasibility when information is limited.[21]

Eligibility

The study population comprised patients aged 15 and older receiving palliative radiotherapy treatment at Udonthani Cancer Hospital for bone metastasis formed the study population. Participating patients were required to have advanced cancer with radiologically confirmed bone metastasis (by plain film, CT, MRI, or bone scan), pain rated at least 3 out of 10 on a numerical pain-intensity scale at rest or on movement, a life expectancy of more than 4 weeks, and a prescription of regular analgesic medication. Patients with a pacemaker, history of seizure, skin infection or abnormal sensation over the area of bone pain were excluded from the study. Each patient's analgesic drug and also non-pharmacological treatment prior to the intervention were maintained over the duration of the study. Patients were enrolled in the study from June 1, 2018 to December 31, 2019.

TENS application protocol

TENS was applied to the site of bone pain by a physical therapist using a dual channel TENS device. TENS pads were 61 x 27 x 96 mm in size and were placed between 5-10 cm apart on the site of skin with a pulse width of 200 microseconds, and pulse frequency of 80 Hz. The intensity was increased over 60 minutes until patients indicated maximum tolerance while still feeling comfortable.

Outcome measures

Pain intensity was assessed by the VAS. Scores were recorded by marking on a horizontal line (100 mm in length) that represented a continuum between "no pain" and "worst pain".

Pain intensity measurements were recorded at baseline prior to TENS application, then after 30 minutes and 60 minutes of TENS while the device was switched on. TENS was switched off and electrode pads were removed immediately after 60 minutes. TENS was applied prior to radiotherapy at the same time every day for 5 days. Participants were observed at the end of each TENS application for adverse reaction.

Symptom assessment was measured by a Thai version of the Edmonton symptom assessment system (ESAS-Thai)[22] on the first day prior to and five days after TENS application began.

Medical records of all patients were reviewed and demographic data were recorded including age, histologic

types of malignancy, sites of metastasis, performance status, prescribed treatments and analgesic drugs.

Statistical analysis

Paired t-test and Generalized Estimating Equations (GEE) were employed to calculate the intensity change which estimated with ESAS and the correlation between active TENS and VAS change respectively. Statistical analysis was carried out in STATA version 15.0 Copyright by Faculty of Public Health, Khon Kaen University. (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

RESULTS

Patient characteristics

In total, 25 patients were recruited in this study. Sixteen of them (64%) were female and 9 (36%) were male. The average age was 50.7 years old (range 22-78) Table 1. The most common site of the primary malignancy was breast (32%), followed by head and neck (12%), colorectal (12%), lung (12%), bile duct (12%), prostate (8%), cervix (8%), and ovaries (4%). ECOG performance status of 1-4 were 13 (52%), 8 (32%), 2 (8%) and 2 (8%), respectively.

Treatment outcome

The mean VAS score at baseline was 5.90 and gradually decreased during the first treatment Table 2. After 30 minutes of TENS treatment the mean VAS was 3.82, and after 60 minutes the mean VAS was 2.68 on the first visit. Similar decreases during treatment were observed in the second through fifth treatments. Additionally, the pre-treatment VAS decreased throughout the treatment series, decreasing from 5.90 at baseline to 2.31 prior to the fifth treatment.

Effect analysis

The duration of TENS treatment was related to VAS pain intensity scores which exhibited statistically significant reduction at 30 and 60 minutes Table 3. Mean VAS pain score decreased by 1.08 at 30 minutes (difference -1.08; 95%CI -1.66, -0.50; $p<0.001$) and decreased by 1.82 at 60 minutes (difference -1.82; 95%CI -2.40, -1.24; $p < 0.001$) compared to baseline at 0 minutes when TENS was initiated. Similarly, the number of applied TENS visit was correlated to VAS. The mean scores reduced by 1.63 (-1.63; 95% CI; -2.07 to -1.19, $p < 0.001$), 2.04 (-2.04; 95% CI; -2.48 to -1.60, $p < 0.001$), 2.39 (-2.39; 95% CI; -2.82 to -1.95, $p < 0.001$), and 2.49 (-2.49; 95% CI; -2.92 to -2.05, $p < 0.001$) at the second, third, fourth and fifth visit respectively compared to the first visit.

The mean ESAS score before TENS application was 4.32, compare to a score of 3.08 post TENS application ($p = 0.004$) Table 4. There were no reports of adverse events related to TENS application.

DISCUSSION

Our study found the benefit of TENS for bone cancer pain and quality of life. VAS scores in patients decreased relative to the duration and number of visit of the TENS application. Symptom assessment scores according to the ESAS decreased after the TENS application. The findings showed a similar efficacy to some previous studies. Loh and colleagues reported reduction in VAS scores by 9.8 on a 0-100 mm scale and NRP scores by 0.8 on a 1-10 scale following TENS application.[23] A smaller study.[24] and case report[18] using TENS for pain reduction among cancer patients have shown that nearly all patients reported reductions in perceived pain, which is consistent with our findings. Furthermore, a multicenter phase III trial of TENS in patients with cancer bone pain showed that TENS relieved pain intensity, particularly during movement as compared to at rest.[19] However, no large randomized controlled trials (RCTs) have been conducted to test improvement in bone pain in cancer patients. A Cochrane systematic review and meta-analysis found only three studies which met the eligible criteria as of 2011. The results were inconclusive due to a lack of suitable RCTs.[20]

The limitations of this study included the small sample size and lack of comparison arm. Therefore, the results may not be generalizable to broader populations, and the independent effect on pain reduction attributable to TENS could not be assessed. Another limitation was that the doctor and patient were not blinded to the treatment. Finally, the follow-up was short-term. Therefore, we could not assess the long-term effect on pain from TENS.

There are two potential confounding factors in this study. First, the intervention of being part of the trial and the attentions of the physiotherapist could have a significant placebo effect each day, and from day-to-day. Second, radiotherapy would be expected to reduce pain (although maybe not immediately) and thus influence the ESAS and final intensity rating.

Bone metastasis is a common cause of pain in cancer patients. The therapeutic goal is not only optimal pain control but also improvement of quality of life. Therefore, multimodality treatment including pharmacological and non-pharmacological treatments is important for patient care.

TABLE 1. Patient demographics and baseline characteristics.

Patient characteristics	No. (N=25)	(%)
Gender		
Female	16	64
Male	9	36
Age (years)		
Mean	50.68 (range 22-78)	
Malignancy		
Breast cancer	8	32
Head and neck cancer	3	12
Colorectal cancer	3	12
Lung cancer	3	12
Cholangiocarcinoma	3	12
Prostate cancer	2	8
Cervix cancer	2	8
Ovarian cancer	1	4
Site of TENS application*		
C spine	1	4
T spine	15	60
L spine	9	36
Pelvis	7	28
Scapula	2	8
Femur	2	8
Performance status**		
ECOG 1	13	52
ECOG 2	8	32
ECOG 3	2	8
ECOG 4	2	8
Metastasis site		
Bone	18	72
Bone, liver	4	16
Bone, lung	2	8
Bone, liver, lung	1	4
Previous treatment		
Chemotherapy and radiotherapy	10	40
Radiotherapy	9	36
Chemotherapy	6	24
Analgesics		
Weak opioid	15	60
Strong opioid	10	40
NSAIDs	5	20
Gabapentin	2	8
TCA	3	12
Acetaminophen	7	28

*One person was able to have TENS applied to more than 1 site.

**Eastern Cooperative Oncology Group performance status.

TABLE 2. Visual analogue scale (VAS); Mean and SD.

Visit	Baseline		30 minutes Active TENS		60 minutes Active TENS	
	Mean	SD	Mean	SD	Mean	SD
1	5.90	2.36	3.82	2.00	2.68	2.07
2	3.36	1.76	2.48	1.78	1.65	1.65
3	2.74	1.45	1.90	1.12	1.63	1.47
4	2.63	1.69	1.72	1.04	0.87	0.78
5	2.31	1.83	1.54	1.35	1.00	0.87

TABLE 3. Correlation between active TENS and VAS change.

Correlation structure	Parameter	Coefficient	Standard error	Z	95% CI Upper	Lower	*P*-value
Exchange correlation	Baseline	Reference					
	30 minutes	-1.08	0.29	-3.65	-1.66	- 0.50	< 0.001
	60 minutes	-1.82	0.29	-6.16	-2.40	- 1.24	< 0.001
	Visit 1	Reference					
	Visit 2	-1.63	0.22	-7.33	-2.07	-1.19	< 0.001
	Visit 3	-2.04	0.22	-9.16	-2.48	-1.60	< 0.001
	Visit 4	-2.39	0.22	-10.74	-2.83	-1.95	< 0.001
	Visit 5	-2.49	0.22	-11.16	-2.92	-2.05	< 0.001

TABLE 4. ESAS difference.

Variable	Mean	95% Confidence interval of the difference		t	*P*-value
		Lower	Upper		
ESAS (baseline)	4.32	3.60	5.03	3.19	0.004
ESAS (after TENS)	3.08	2.61	3.54		

CONCLUSION

This uncontrolled pre-experimental pretest-posttest study found reductions in pain among cancer patients, as reported by visual analog scale. The reductions were reported immediately following TENS treatment, as well as proportional to the number of TENS treatments given. Long-term improvements on pain were not assessed. As a non-invasive, inexpensive, and safe treatment, TENS may be a beneficial component of multimodal pain treatment and may reduce the need for morphine. The results of this study may inform the design of future RCTs, which are needed to measure the efficacy of TENS relative to controls or other treatments.

REFERENCES

1. Budczies J, von Winterfeld M, Klauschen F, Bockmayr M, Lennerz JK, Denkert C, et al. The landscape of metastatic progression patterns across major human cancers. Oncotarget 2015;6:570-83. doi:10.18632/oncotarget.2677
2. Vieira C, Fragoso M, Pereira D, Medeiros R. Pain prevalence and treatment in patients with metastatic bone disease. Oncol Lett 2019;17:3362-70. doi:10.3892/ol.2019.10013
3. Ibrahim T, Farolfi A, Mercatali L, Ricci M, Amadori D. Metastastic bone disease in the era of bone targeted therapy: clinical impact. Tumori 2013;99:1-9.
4. Figura N, Smith J, Yu H. Mechanisms of, and Adjuvants for Bone Pain. Hematol Oncol Clin North Am 2018;32:447-58.
5. Mercadante S. Malignant bone pain: pathophysiology and treatment. Pain 1997;69:1-18.
6. Patrick W. Mantyh. Bone cancer pain: From mechanism to therapy. Curr Opin Support Palliat Care 2014;8:83-90.
7. Mercadante S, Fulfaro F. Management of painful bone metastasis. Curr Opin Oncol 2007;19:308-14.
8. Green CR, Hart-Johnson T, Loeffler DR. Cancer-related chronic pain : examining the quality of life in diverse cancer survivors. Cancer 2011;117:1994-2003.
9. WHO Guidelines for the Pharmacological and Radiotherapeutic Management of Cancer Pain in Adults and Adolescents. World Health Organization; Geneva, Switzerland: 2018.
10. McQuay HJ, Collins SL, Carroll D, Moore RA. Radiotherapy for the palliation of painful bone metastases. Cochrane Database Syst Rev 2000;(2):CD001793.
11. Rita JW. Cancer Pain Management: Opioid Analgesics, Part 2. J Adv Pract Oncol 2017;8:588-607.
12. Sam H , Waheed A, Fakhreddin J. Adverse Effects of Nonsteroidal Antiinflammatory Drugs: An Update of Gastrointestinal, Cardiovascular and Renal Complications. J Pharm Pharm Sci 2013;16:821-47.
13. Shenhong W, Willium LD, James LG. The use of bisphosphonates in cancer patients. Acta Oncologica 2007;46:581-91.
14. Johnson M., Martinson M. Efficacy of electrical nerve stimulation for chronic musculoskeletal pain : a meta-analysis of RCT. Pain 2007;130:157-65.
15. Bjordal JM, Johnson MI, Lopes-Martins RA, Bogen B, Chow R, Ljunggren AE. Short term efficacy of physical interventions in osteoarthritic knee pain. A systematic review and meta-analysis of randomized placebo-controlled trials. BMC Musculoskelet Disord 2007;8:51.
16. Vance CG, Dailey DL, Rakel BA, Sluka KA. Using TENS for pain control: the state of the evidence. Pain Manag 2014;4:197-209.
17. Johnson M. Transcutaneous Electrical Nerve Stimulation: Mechanisms, Clinical Application and Evidence. Rev Pain 2007;1:7-11. doi:10.1177/204946370700100103
18. Searle RD, Bennett MI, Johnson MI, Callin S, Radford H. Transcutaneous electrical nerve stimulation (TENS) for cancer bone pain. J Pain Symptom Manage 2009;37:424-8.
19. Bennett MI, Johnson MI, Brown SR, Radford H, Brown JM, Searle RD. Feasibility Study of transcutaneous electrical nerve stimulation (TENS) for cancer bone pain. J Pain 2010;11:351-9.
20. Hurlow A, Bennett MI, Robb KA, Johnson MI, Simpson KH, Oxberry SG. Transcutaneous electrical nerve stimulation (TENS) for cancer pain in adults. Cochrane Database Syst Rev 2012;(3):CD006276.
21. Anderson GL, Prentice RL. Individually randomized intervention trials for disease prevention and control. Stat Methods Med Res 1999;8:287-309.
22. Montarat C, Darin J, Albert JK, Umaporn U. Reliability and Validity of a Thai Version of the Edmonton Symptom Assessment Scale (ESAS-Thai). J Pain Symptom Manage 2011;42:954-60.
23. Loh J, Gulati A. The use of transcutaneous electrical nerve stimulation (TENS) in a major cancer center for the treatment of severe cancer related pain and associated disability. Pain Med 2015;16:1204-10.
24. Jeffrey L, Amitabh G transcutaneous electrical nerve stimulation for treatment of sarcoma cancer pain. Pain Manag 2013;3:189-99.

Pre-class versus In-class Video Lectures for the Flipped Classroom in Medical Education: A Non-randomized Controlled Trial

Monton Wongwandee, M.D., M.Sc., Panwara Paritakul, M.D., MMedSci MedEd.

Department of Medicine, Faculty of Medicine, Srinakharinwirot University, Nakhon Nayok 26120, Thailand.

ABSTRACT

Objective: The objective was to test the effectiveness of the two different teaching models focusing on pre-class preparation.

Methods: This study was a single-center, post-test only, non-randomized, controlled trial. The fourth-year medical students were assigned to attend either flipped classroom (FC) or in-class video classroom (IVC). The FC students watched a pre-class video lecture individually. In contrast, IVC students viewed the video together during class time. Both groups had the same in-class interactive activities, including case quizzes and discussion. The primary outcomes were a post-test score and student satisfaction.

Results: Of 105 students, 53 were assigned to the FC group and 52 to the IVC group. 77% of the FC students reported video viewing. There was no significant difference in the post-test score between the FC and the IVC groups (p = .107). However, the subgroup analysis showed that the post-test score of the IVC group was significantly higher than the FC subgroup who did not view the video (p = .024). The total satisfaction score was not significantly different between the FC and the IVC groups (p = .945). 83% of the FC who did not view the video claimed they had too many out-of-class workloads.

Conclusion: There were no differences in the effectiveness between the FC and IVC approach. However, the IVC students showed better knowledge acquisition over the FC subgroup, who did not watch the video. Hence our study emphasized an essential role of the knowledge preparation on the successful flipped classroom.

Keywords: Flipped classroom; medical education; nonrandomized controlled trials (Siriraj Med J 2020; 72: 476-482)

INTRODUCTION

Medical knowledge is rapidly expanding, diseases are newly emerged and more complicated, and patient care is increasingly individualized. In medical education, a teaching model like a traditional didactic lecture may not be suitable. In contrast, many believe that an active-learned, critical thinking-based technique such as flipped classrooms (FC) is more efficient. Its characteristics promote higher cognitive learning, not only memorization. The results of a meta-analysis study supported the effectiveness of the FC on learning.[1] It concluded that the FC approach yields a significant improvement in student learning compared with traditional teaching methods. However, its effectiveness in medical education is inconsistent in the previous systematic review.[2] The key to the FC approach's success is that students take responsibility for their learning and prepare before class.[3] The pre-class assignment was aimed to master the factual knowledge

Corresponding author: Monton Wongwandee
E-mail: monton@g.swu.ac.th
Received 9 March 2020 Revised 25 May 2020 Accepted 1 September 2020
ORCID ID: http://orcid.org/0000-0002-0747-0174
http://dx.doi.org/10.33192/Smj.2020.64

in preparation for the in-class interactive experiences. This preparatory work also makes students control their learning process by defining learning goals and monitoring their progress in achieving them.[4] Failure to do so leads to compromise the knowledge acquisition when finishing the FC. The previous studies, including our teaching experience, found that many students did not comply with the pre-class assignment, making the FC fail.[5,6] Our research question was whether the well-prepared teaching material could be effectively used as a pre-class self-study for the flipped classroom. We compared the learning outcome using PowerPoint recording video as the teaching material between pre-class self-study and in-class study (FC vs. in-class video classroom (IVC)).

MATERIALS AND METHODS

Study design and participants

This study was a static-group comparison, post-test only, non-randomized controlled trial. The trial was done at the Faculty of Medicine, Srinakharinwirot University, Thailand. Its six-year Doctor of Medicine (MD) program is split into two parts. The first half is the pre-clinical phase, and the second is the clinical phase. The population of interest was medical students, while the study participants were fourth-year medical students (clerkship). Eligible criteria included students who registered for the Seizures and Epilepsy module during the academic year 2018-19. Students were excluded if they did not attend the class, did not complete a post-test, or did not give the student feedback on teaching. Four student groups (A, B, C, and D) were preexisting formed for medical rotations. Grade point averages (GPAs) in pre-clinical years were evenly distributed across the groups. We assigned these groups to expose two different teaching styles. The study intervention cohort included groups A and C, while the comparison intervention cohort included groups B and D. The study was approved by the Human Ethics Committee of Srinakharinwirot University, Bangkok, Thailand (SWUEC/E-368/2017), and all participants provided written informed consent.

Interventions

The interventions were two different teaching methods regarding student pre-class preparation. The study intervention was an FC approach. Pre-class 30-minute video lecture watching was assigned two weeks in advance. The students could individually view the streaming online video lecture on their own devices via the provided link. We used the PowerPoint slide presentation as a video format. The content encompassed basic knowledge of seizures and epilepsy (definition, etiology, and mechanism), video clips of seizure types, diagnostic approach, and treatment principle. During the 40-minute class time, the students participated in the problem-solving activity and large-group interactive discussion. There were 26 to 28 attendees in each class. This in-class session contained short diagnostic quizzes with answer choices on ten common epileptic case scenarios. The students were able to discuss with nearby classmates and voted via their own devices. The screen in front of the class showed the voting results. The teacher explained the answers while students could freely give the comments or ask the questions.

For the comparison intervention in the IVC, there was no pre-class assignment. Alternatively, students were assigned to view the video lecture together at the beginning of the class. Unlike the FC group, this step ensured that everyone got preparatory knowledge before the interactive session. After that, they did the same as the FC group's in-class activity, including case quizzes with discussion. The critical difference between the study intervention and the comparison intervention was how students prepared for the interactive class, i.e., out-of-class or in-class video lecture viewing. The in-class duration of the FC was 40 minutes, while that of the IVC was 70 minutes (Fig 1). Only one teacher taught all the study groups.

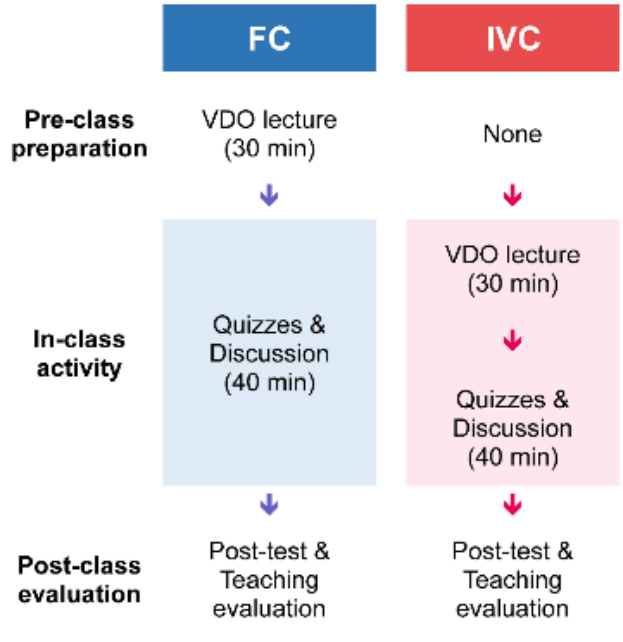


Fig 1. Class protocols
Pre-class video lecture viewing was assigned for the FC group, while IVC students watched the video lecture together at the beginning of the class.
Abbreviations: FC = flipped classroom, IVC = in-class video classroom

Outcomes

There were two primary outcomes, including a post-test score and a total satisfaction score. The students did the 10-item MCQs post-test at the end of the class. All questions were application type. They were told that it was not an examination and done on the purpose of research only. They had to complete the test individually, and the discussion was not allowed at this stage. Student feedback on teaching was also anonymously given at the same time. This feedback included ten aspects of teaching quality using a five-point Likert scale as the point. The total satisfaction score was the sum of these points. The feedback also included the other three open-ended questions about what they were satisfied or not, and the video watching information.

Statistical analysis

There was no sample size calculation. All students who registered for the Seizures and Epilepsy module in the academic year 2018-19 were enrolled. Quantitative variables (post-test score, student satisfaction scores, video watching duration) were reported as mean with SD or median with IQR. Independent-samples t-test or one-way ANOVA was used to compare variables with a normal distribution. Mann-Whitney *U* test or Kruskal-Wallis test was chosen to compared variables with non-normal distribution. A post hoc test was performed using Bonferroni correction. Spearman's rho correlation was selected for the association between the post-test score and the video watching duration. Categorical variables (video watching proportion, reasons for not viewing the video, what students like or dislike about teaching) were reported as a percentage. All statistical analyses were done with IBM SPSS Statistics for Windows, Version 25.0. Statistical significance was set at a two-sided α of 0.05.

RESULTS

Between May 28, 2018, and January 6, 2019, 105 students were assessed for eligibility. All 105 students were enrolled and nonrandomly assigned to attend either an FC (n = 53) or IVC (n = 52). No one was excluded. A total of 105 participants were finally included in the analysis of outcomes (Fig 2). The student characteristics were similar between groups at baseline (Table 1).

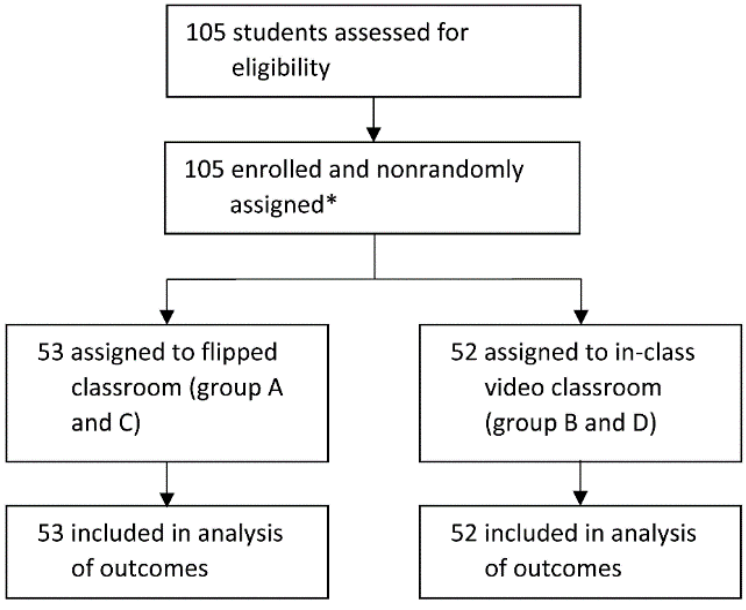


Fig 2. Trial profile
*Groups (A, B, C, and D) were preexisting formed with the purpose of medical rotations.

TABLE 1. Baseline characteristics of the students.

Characteristic	Flipped classroom (n=53)	In-class video classroom (n=52)	*p*-value
Age, years	18 (17-18)	18 (17-18)	0.576
Female sex, n (%)	29 (54.7)	29 (55.8)	0.912
GPAs*	3.09 (0.50)	3.10 (0.46)	0.882

Data are median (IQR) or mean (SD) unless otherwise indicated.
*Grade point averages (GPAs) in the pre-clinical years were presented.

41 (77%) students, who participated in the FC, reported watching the pre-class video lecture. The median viewing duration was 80 (IQR = 40-100) percent of total video length. Of 12 students who did not watch the video, 83% claimed that there was not enough time for viewing, 8% forgot the assignment, and 8% preferred reading a pre-class handout.

The primary outcomes were demonstrated in Table 2. We found no significant difference in the post-test score between the FC group (*Mdn* = 7) and the IVC group (*Mdn* = 8), $U = 1130.00$, $p = .107$, $r = .16$, Mann-Whitney test. The total satisfaction score was not significantly different between the FC group ($M = 43.2$, $SD = 4.4$) and the IVC group ($M = 43.3$, $SD = 4.9$), $t(95) = .07$, $p = .945$, $d = .02$. Moreover, the satisfaction scores for each of the ten aspects of teaching qualities did not show the significant differences between the two groups (Table 2).

The subgroup analysis (FC, who did not view the video; FC, who viewed the video; and IVC) were illustrated in Fig 3. A Kruskal-Wallis H test showed a statistically significant difference in the post-test score between the subgroups, $\chi2(2) = 7.03$, $p = .030$. The post hoc test using Bonferroni correction showed a significant difference in the post-test score only between the FC subgroup, who did not view the video (*Mdn* = 6.5), and the IVC group (*Mdn* = 8), $p = .024$. There was no significant correlation between the duration of pre-class video viewing and the post-test score, as determined by Spearman's rank-order correlation ($r_s = .265$, $p = .103$). There was not a significant difference in total satisfaction score between three subgroups ($F(2,94) = .004$, $p = .996$, one-way ANOVA). The reliability index (KR-20) of the post-test was 0.51.

The students' favorites, including things that need improvement in the FC and IVC groups, were illustrated in Fig 4. Quizzes with discussion were the top favorite in the FC and IVC group (51% vs. 27%). 42% of the FC students listed the pre-class video lecture as the favorite. Of those, the additional positive comments were as follows:

1. The video watching was self-paced.
2. Students could view the video again later.
3. The video helped them understand in-class time easily.

19% of IVC students gave the negative vote on the in-class video lecture. Some said that the video lecture's speed was too fast, and the video could not be rerun.

TABLE 2. Primary outcomes.

Variable	Flipped classroom (n=53)	In-class video classroom (n=52)	r or mean difference (95% CI)*	*p*-value
Post-test score	7 (6-9)	8 (6.25-9)	0.16	0.107
Total satisfaction score	43.2 (4.4)	43.3 (4.9)	0.07 (-1.82 to 1.95)	0.945
Understanding of subject	4 (4-5)	4 (4-5)	0.11	0.271
Applying to the clinical practice	4 (4-5)	4 (4-5)	0.07	0.490
Teacher's knowledge of the subject	5 (4-5)	5 (5-5)	0.08	0.423
Opportunity for asking and discussion	5 (4-5)	5 (4-5)	0.14	0.145
The teacher answers the questions clearly	5 (4-5)	5 (4-5)	0.03	0.724
Teacher demonstrates objectives	4 (4-5)	5 (4-5)	0.14	0.140
Video lecture†	4 (4-5)	4 (4-5)	0.14	0.174
Handout	4 (4-4)	4 (3-4)	0.18	0.072
Classroom environment	4 (4-5)	4 (4-5)	0.08	0.402
Class time	4 (4-5)	4 (4-5)	0.04	0.655

The table shows the post-test scores, total satisfaction scores, and satisfaction scores for each aspect of teaching qualities. Data are median (IQR) or mean (SD) unless otherwise indicated.

*For medians, r is given; for means, the difference is given with 95% CI. The r-value is calculated from $Z/\sqrt{N}$, where the Z value is computed from the Mann–Whitney *U* test, and N is the total number of the samples.

†Pre-class video lecture for the flipped classroom and in-class video lecture for the in-class video classroom

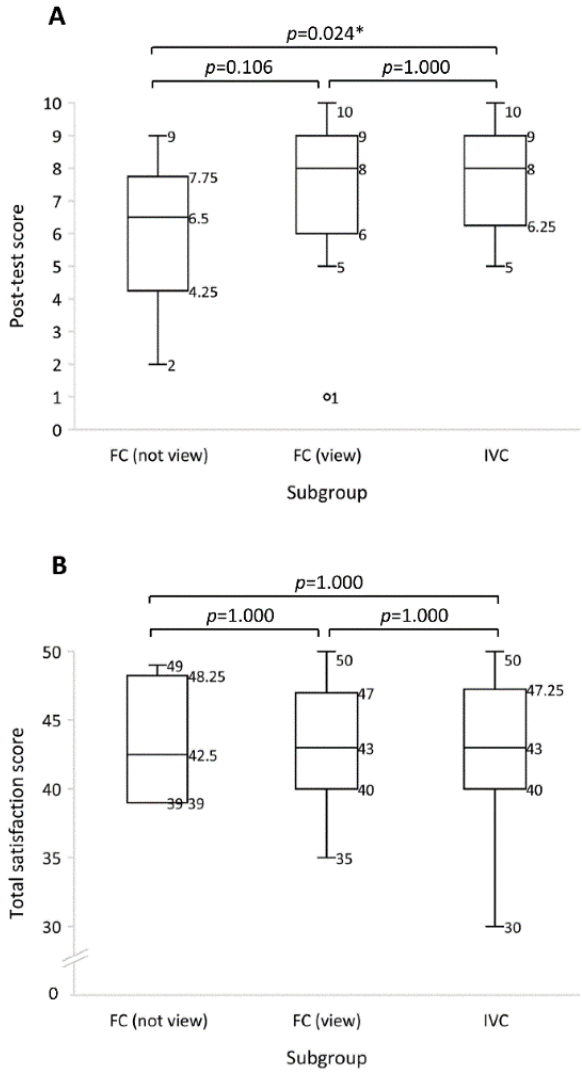


Fig 3. Scores for the subgroups (FC (not view), FC (view), and IVC)
Boxplots show **(A)** post-test scores and **(B)** total satisfaction scores
*Statistically significant difference (Kruskal-Wallis H test, post hoc test using Bonferroni correction)
FC (not view) = flipped classroom subgroup who did not view the video lecture, FC (view) = flipped classroom subgroup who viewed the video lecture, IVC = in-class video classroom

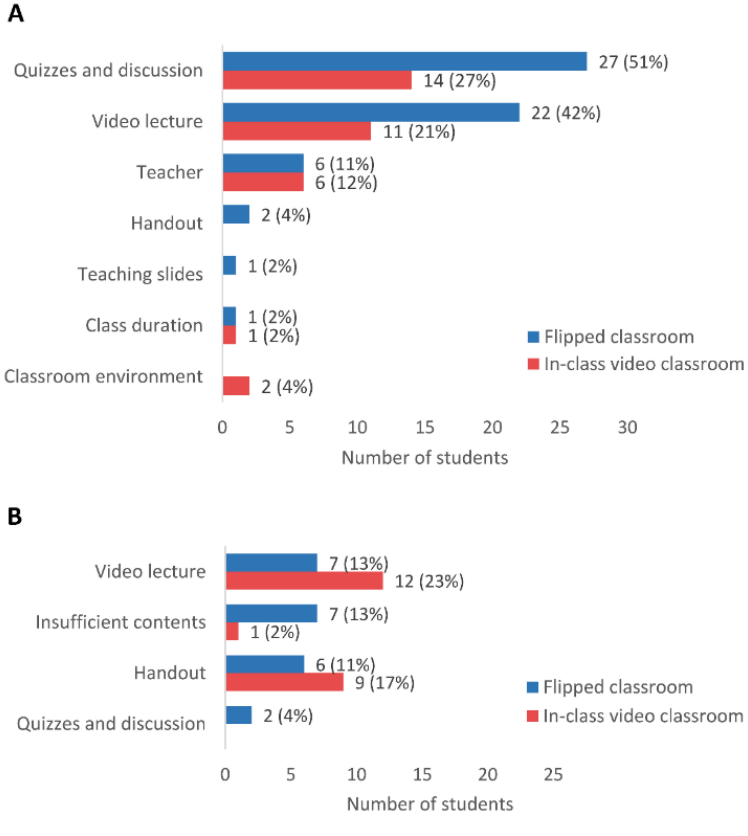


Fig 4. The number of students that give feedback on teaching components in the flipped classroom and in-class video classroom.
Graph shows numbers of students (with percentage) who were satisfied **(A)** and unsatisfied **(B)** with teaching components in two teaching groups.

DISCUSSION

The present study supported the strength of the FC approach. The pre-class online video lecture remained the favorite because students were able to study at their own pace. In other words, they could view the video as often as needed, anytime, and anywhere. Pre-class preparation also helped students understand more easily in class. In-class activity, i.e., case quizzes with interactive discussion, was voted the favorite from the FC students. Its problem-solving style stimulated the learners' critical thinking process. Moreover, some students compared such activity to a game, and they enjoyed it.

Although there were many benefits of the FC, however, the present study showed no differences in post-test scores, including total satisfaction scores between the FC and IVC groups. One explanation is probably related to students' compliance with the FC protocol. Our subgroup analysis found that the post-test score for the IVC group was significantly higher than the FC students who did not watch the pre-class video. These non-compliant students did not master their knowledge before class, so they might not catch up with others during the interactive class. Our result showed a significant non-compliance (23%), which could compromise the FC group's effectiveness.

Several previous studies also found the problems of non-compliance on the FC protocol, where the incidences ranged from 20 to 31.3 percent.[5-7] Many students express concern about time management for the FC. There may be several out-of-class assignments from different modules in the same period. They need to balance the out-of-class assignments and their personal lives, while some saw the pre-class preparation as a burden.[8] One study found that half of the students who would not like to take another FC cited the pre-class work as a reason.[9] As a result, the out-of-class time spent is essential for the success of the FC model. Some non-health professionals FC studies demonstrated that most students spent up to 20 to 25 minutes viewing pre-class video lectures.[10,11] These results supported our data which the estimated time of video watching was 24 minutes, i.e., 80% (median) of 30-minute video length. Future research should focus on the out-of-class workloads and how to improve the compliance of the FC protocol.

Another possible reason why the FC and IVC groups did not show significant differences in knowledge and satisfaction is that the IVC was not the same as a traditional lecture. The in-class activity of the IVC in our study included case quizzes with the interactive discussion, which would enhance student engagement and effectiveness. This explanation is supported by our study's result, which the quizzes were the most favorite for the IVC group.

The present study had several limitations. Firstly, the study design was post-test only, non-randomized controlled study. No randomization means that unknown baseline characteristics between the FC and IVC groups may not be similar; although, GPAs in pre-clinical years were evenly distributed across the groups. No pre-test done indicates that the post-test score only may not reflect the knowledge students gained from the intervention. Secondly, the effectiveness of teaching, i.e., the satisfaction and post-test scores were categorized based on Kirkpatrick's classification as just level 1 (perceptions of intervention) and 2b (changes in knowledge and skills), respectively.[12] The post-test done immediately indicates only knowledge acquisition, which may not correlate with long-term knowledge retention. The higher level of the effects, such as changes in behaviors or professional practice, is more valid and needed in future research. Thirdly, the post-test reliability index was relatively low; however, a small number of the test items (ten) may affect this value.

Moreover, some components of our FC protocol may not meet the quality that delivers the highest effectiveness. The 30-minute duration of the video seemed too long for the students to concentrate, as discussed above. Moreover, the video lecture was the only format we provided for the pre-class assignment. One student who did not view the video said that reading was a preferred assignment. Therefore, giving various forms of study materials would be suitable for different learning styles. The pre-class phase also lacked quizzes that students could ensure their mastery of knowledge before class. Our students' ratio to teachers (26-28:1) was rather large than the ideal suggestion, i.e., 4:1 to 25:1.[13]

Although there were many advantages of the FC model, the present study showed no differences in effectiveness between the FC and IVC approach. Moreover, the IVC group was superior to the FC subgroup, who did not view pre-class video for the knowledge acquisition. Therefore, the present study confirmed the crucial role of compliance with the FC protocol in successful teaching. Most non-compliant students were concerned about the out-of-class assignment. Future research should focus on the FC protocol concerning student compliance and how to improve it.

ACKNOWLEDGMENTS

The authors thank all the students for their participation in this study.

REFERENCES

1. Hew KF, Lo CK. Flipped classroom improves student learning in health professions education: a meta-analysis. BMC Med Educ 2018;18:38.
2. Chen F, Lui AM, Martinelli SM. A systematic review of the effectiveness of flipped classrooms in medical education. Med Educ 2017;51:585-97.
3. Kennedy C. Update on the flipped classroom2013 01/12/19. Available from: https://amee.org/getattachment/Marketing-Toolkit/flipped classroom. 2013 [cited 2019 Dec 1].
4. Rutherfoord RH, Rutherfoord JK, editors. Flipping the classroom: Is it for you? Proceedings of the 14th annual ACM SIGITE conference on Information technology education. 2013. doi: 10.1145/2512276.2512299.
5. Heitz C, Prusakowski M, Willis G, Franck C. Does the concept of the "flipped classroom" extend to the emergency medicine clinical clerkship? West J Emerg Med 2015;16:851-5.
6. Morgan H, McLean K, Chapman C, Fitzgerald J, Yousuf A, Hammoud M. The flipped classroom for medical students. Clin Teach 2015;12:155-60.
7. Maneeratana K, Singhanart T, Singhatanadgid P, editors. A preliminary study on the utilization and effectiveness of a flipped classroom in Thailand. 2016 IEEE International Conference on Teaching, Assessment, and Learning for Engineering (TALE). 2016. doi: 10.1109/TALE.2016.7851819.
8. Cotta KI, Shah S, Almgren MM, Macías-Moriarity LZ, Mody V. Effectiveness of flipped classroom instructional model in teaching pharmaceutical calculations. Curr Pharm Teach Learn 2016;8:646-53.
9. Murphy J, Chang J-M, Suaray K. Student performance and attitudes in a collaborative and flipped linear algebra course. Int J Math Educ Sci Technol 2016;47:653-73.
10. McGivney-Burelle J, Xue F. Flipping calculus. Primus 2013;23:477-86.
11. Vazquez JJ, Chiang EP. Flipping out! A case study on how to flip the principles of economics classroom. IAER. 2015;21:379-90.
12. Boet S, Sharma S, Goldman J, Reeves S. Medical education research: an overview of methods. Can J Anaesth 2012;59:159-70.
13. Prober CG, Khan S. Medical education reimagined: a call to action. Acad Med 2013;88:1407-10.

Correlation of Medical Knowledge and Non-Technical Skills Assessment in Anesthesia Residents

Kasana Raksamani, M.D., Tachawan Jirativanont, M.D., Pavinee Sareenun

Department of Anesthesiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

ABSTRACT

Objective: Non-technical skills training and assessment has been implemented in anesthesia residency training program to improve quality of patient care but have not been properly assessed. We hypothesized that trainees with good knowledge correlated with good cognitive parts of non-technical skills.

Methods: Seventy anesthesia residents (24 PGY-1, 24 PGY-2 and 22 PGY-3) were assessed for their knowledge by 180-item MCQs, 5 key-feature essay questions, and 18-station OSCE's. Subsequently, a perioperative anesthesia crisis situation was set up in the simulation lab for all residents and was video recorded. Non-technical skills were assessed by 2 independent trained raters using Anesthetists' Non-Technical Skills (ANTS) behavioral markers. The residents' scores were calculated to find the correlation within the ANTS rating scale.

Results: The mean scores of knowledge tests were 164.3 ±18.4 out of 300 [165.5 ±18.0, 154.7 ±16.3 and 173.6 ±16.4 for PGY-1, PGY-2 and PGY-3 respectively]. The mean scores of ANTS was divided into 4 categories (rating scale 1 to 4): task management 2.9 (±0.6), teamworking 3.0 (±0.5), situation awareness 2.9 (±0.8) and decision making 2.8 (±0.7). The knowledge test results moderately correlated with ANTS score in task management, situation awareness and decision making [r=0.382 (p<0.01), r=0.433 (p<0.001) and r=0.350 (p<0.01) respectively] and weakly correlated with the teamworking category (r=0.166, p=0.16).

Conclusion: Resident's knowledge scores showed moderate correlation with non-technical skills assessment results except teamwork. Non-technical skills are required to be trained and assessed together with knowledge to enhance the patient's safety and outcome.

Keywords: Non-technical skills; Anesthetists' non-technical skills; Assessment (Siriraj Med J 2020; 72: 483-487)

INTRODUCTION

Non-technical skills have been demonstrated to enhance performance in many medical specialties including anesthesia.[1,2] Assessment tools of non-technical skills have been developed and validated in order to facilitate teaching and learning.[3,4] The Anesthetists' Non-Technical Skills (ANTS) system contained the behavioral markers rating in 4 categories: task management, teamworking, situation awareness and decision making.[4-5]

Non-technical skills training has been implemented in anesthesia residency curriculum as part of competencies in order to improve the quality of patient care and safety.[6-8] However, non-technical skills in anesthesia have integrated with other competencies and has made it difficult to assess these separately. The assessment of non-technical skills is still limited to formative assessment along with knowledge and technical skills.

Corresponding author: Kasana Raksamani
E-mail: kasana.rak@mahidol.edu
Received 9 March 2020 Revised 25 May 2020 Accepted 26 May 2020
ORCID ID: http://orcid.org/0000-0003-4524-4781
http://dx.doi.org/10.33192/Smj.2020.65

When looking closely at non-technical skills, they are divided into cognitive, analytical thinking, and social skills such as communication and teamwork.[9] Evidences suggested non-technical skills enhanced technical performances in surgical and anesthesia training.[10,11] Moreover, the interprofessional team process training improved team outcome in crisis situation without focusing on knowledge.[13,14] However, we hypothesized that trainees with good knowledge are correlated with good cognitive parts of non-technical skills.

The aim of this study was to find the correlations of knowledge assessment results and non-technical skills assessment using ANTS behavioral rating system in anesthesia residents.

MATERIALS AND METHODS

The study protocol was approved by the local ethical committee Siriraj Institutional Review Board, Bangkok, Thailand (Si 170/2015), and voluntary informed consent was obtained. Seventy anesthesia residents were assessed their knowledge at the end of the academic year by 180-item MCQs, 5 key-features essay questions, and 18-station OSCEs using the same assessment tools. Subsequently, a perioperative anesthesia crisis situation was set up in the simulation lab for all residents and video-recorded. Non-technical skills were assessed by 2 independent trained raters using Anesthetists' Non-Technical Skills (ANTS) behavioral assessment tool. This tool was translated to Thai version and found to be valid.[14] Consequently, the residents' knowledge scores were calculated to find the correlation with ANTS rating scale.

A simulation scenario was developed by researchers focusing on the Anesthetists' non-technical skills. The session was established in an operating room setting with the full-body high fidelity patient simulator (SimMan 3G; Laerdal Medical, Stavanger, Norway). The participant was individually and consecutively assigned a role of anesthesiologist in the scene of a 45-year-old female patient, previously healthy, undergoing laparoscopic myomectomy. The scenario started at the end of the procedure, after the reversal agent was given the patient developed pulseless ventricular tachycardia. The sessions were video recorded for assessment purposes.

Statistical analysis was performed using PASW Statistics version 18 (SPSS Inc., Chicago, IL, USA). The demographic data and mean scores for the knowledge test and ANTS behavioral markers were analyzed with descriptive statistics and compared between groups with one-way ANOVA. The correlations among knowledge test results and ANTS categories were calculated using Pearson's correlation. Pearson's correlation was defined as follows: $r < 0.3$, weak; $0.3 < r < 0.7$, moderate; $r > 0.7$, strong. P values of less than 0.05 were deemed to be statistically significant.

RESULTS

Seventy anesthesia residents participated in this study (24 PGY-1, 24 PGY-2 and 22 PGY-3), 12 (17.1%) were male and 58 (82.9%) were female, with mean age 29.3 ±1.6 years. The mean scores of knowledge tests were 161.9 ±20.5 out of 300 [150.7 ±19.1, 159.4 ±17.7 and 177.0 ±15.9 for PGY-1, PGY-2 and PGY-3 respectively] with significant difference between groups. The details of the scores were reported in Table 1.

The mean scores of ANTS was divided into 4 categories (rating scale 1 to 4): task management 2.9 ±0.6, teamworking 3.0 ±0.5, situation awareness 2.9 ±0.8 and decision making 2.8 ±0.7 and significantly different between groups in all categories (Table 2). The knowledge test results moderately correlated with ANTS score in all categories; task management, teamworking, situation awareness and decision making [r=0.48 (p<0.001), r=0.31 (p=0.01), r=0.54 (p<0.001) and r=0.44 (p<0.001) respectively]. The correlation of test score in PGY-1 was moderate in task management, situation awareness and decision making [r=0.31 (p=0.1), r=47, (p=0.03) and r=0.50 (p=0.02) respectively) and weakly correlated in teamwork (r=0.23, p=0.24). There was poor correlation between test scores and ANTS score in all categories for PGY-2 (task management r= -0.03, teamworking r= -0.17, situation awareness r= -0.02, decision making r= -0.09). There was strong correlation for test scores and task management in PGY-3 (r=0.71, p<0.001) with moderate correlation in situation awareness (r=0.58, p<0.01) and decision making (r=0.62, p<0.01), while there was weak correlation in teamworking (r=0.17). The details of the correlation coefficients were demonstrated in the Table 3.

DISCUSSION

Non-technical skills are divided into cognitive and social skill sets.[9] The cognitive skills of Anesthetists' Non-Technical Skills (ANTS) behavioral markers are situation awareness, decision making and task management, while the social skills are teamworking.[4] The cognitive skills of ANTS in anesthesia residents moderately correlated with the knowledge test scores and even had strong correlation with some categories in PGY-3.

Situation awareness is the first and most important step of gathering and interpreting information before making decision in complex and dynamic anesthesia clinical work.[15,16] In this study, the level of situation

TABLE 1. Knowledge test scores of PGY-1, PGY-2 and PGY-3.

Scores	Overall	PGY-1	PGY-2	PGY-3	*P* value
MCQ (100)	56.9 ±8.9	53.1 ±9.5	56.9 ±8.5	60.9 ±7.2	0.01
KFQs (100)	49.4 ±6.7	47.2 ±7.0	47.8 ±5.7	53.6 ±5.7	0.001
OSCE (100)	55.6 ±8.0	50.3 ±5.7	54.7 ±7.0	62.5 ±5.9	<0.001
Total scores (300)	161.9 ±20.5	150.7 ±19.1	159.4 ±17.7	177.0 ±15.9	<0.001

Data presented in mean ±SD

Abbreviations: ANTS, Anesthetists' Non-Technical Skills; PGY, postgraduate year; MCQ, multiple-choice questions; KFQ, Key-features questions; OSCE, objective structured clinical examination

TABLE 2. Mean score of ANTS.

ANTS category	Overall	PGY-1	PGY-2	PGY-3	*P* value
Task management	2.9 ±0.6	2.5 ±0.5	2.7 ±0.7	3.4 ±0.4	<0.001
Teamworking	3.0 ±0.5	2.7 ±0.5	2.9 ±0.5	3.3 ±0.4	<0.001
Situation awareness	2.9 ±0.8	2.5 ±0.8	2.7 ±0.6	3.6 ±0.5	<0.001
Decision making	2.8 ±0.7	2.4 ± 0.6	2.8 ±0.8	3.2 ±0.6	0.001

Data presented in mean ±SD

Abbreviations: ANTS, Anesthetists' Non-Technical Skills; PGY, postgraduate year

awareness was significantly improved in each year of training, and improved most when compared to the other categories. Also, the situation awareness was moderately correlated with all test scores. This could be due to the fact that when the trainees achieved a higher level of training, they collected more knowledge and broadened their situation awareness level. This highlighted the importance of training situation awareness together with knowledge, which occurred to be the predominant cause of anesthesia errors in critical incidence reports.[17] The ANTS behavioral markers had 3 levels of situation awareness of gathering information, recognizing and understanding, and anticipating, which can be used as a framework to facilitate teaching, giving feedback and assessing the learners during both formal training and clinical learning.[5,6]

Decision making consists of 3 elements in the ANTS behavioral rating system: identifying options, balancing risks and selecting options, and re-evaluating.[5] The scores of decision making significantly improved as the residents were in a higher level of training, which is in line with the results of situation awareness because the decision making process usually happened immediately after situation awareness.[18,19] The development of dynamic decision making in anesthesia needed the collection of mental models and knowledge for analytical thinking.[19,20] This could explain the results of moderate correlation of decision making and test scores.

Task management is defined by behaviors such as planning and preparation, prioritization, providing and maintaining standards, identifying and utilizing resources, which are noticeably knowledge based in only one element, providing and maintaining standards.[20] The results were in the same direction as situation awareness and decision making, significantly increased from PGY-1 to PGY-3 and moderately correlated with the test scores.

TABLE 3. Pearson's correlations of the overall scores, MCQ, KFQ, OSCE scores and elements of Anesthetists' non-technical skills in PGY-1, PGY-2 and PGY-3 anesthesia residents.

		All	PGY-1	PGY-2	PGY-3
Task management	Overall score	0.48[c]	0.31	-0.03	0.71[c]
	MCQ	0.37[b]	0.39	-0.05	0.54[b]
	KFQ	0.33[b]	0.07	-0.11	0.59[b]
	OSCE	0.54[c]	0.30	0.07	0.70[c]
Teamworking	Overall score	0.31[b]	0.23	-0.17	0.17
	MCQ	0.016	0.20	-0.23	-0.04
	KFQ	0.26[a]	0.24	-0.23	0.26
	OSCE	0.40[b]	0.15	0.03	0.25
Situation awareness	Overall score	0.54[c]	0.47[a]	-0.02	0.58[b]
	MCQ	0.44[c]	0.54[b]	-0.05	0.36
	KFQ	0.38[b]	0.20	-0.15	0.56[b]
	OSCE	0.58[c]	0.41[a]	0.13	0.57[b]
Decision making	Overall score	0.44[c]	0.50[a]	-0.09	0.62[b]
	MCQ	0.38[b]	0.48[a]	0.04	0.43[a]
	KFQ	0.28[a]	0.25	-0.24	0.60[b]
	OSCE	0.45[c]	0.56[b]	-0.08	0.56[b]

[a] $p<0.05$, [a] p,0.01, [a] $p<0.001$

The widely used methods for teaching and learning task management was in simulation settings due to the controllable tasks to perform.[11,12] The complex tasks can also be practiced in workplace settings in both routine and non-routine events.[21]

Teamworking is the category that the result had lowest correlation coefficient, different from the other categories, while the teamwork scores were significant improved in each level of training, the correlation with test scores was weak in all types of tests except OSCEs. This emphasized the importance of additional training and assessing of teamwork and communication, apart from knowledge and clinical training.[22] These social skills are an important part of anesthesia work in the operating theater to enhance patient safety.[23]

Moreover, the correlation of knowledge scores and level of situation awareness was different between the levels of training, moderate in PGY-1, poor in PGY-2, and moderate in PGY-3. This could be due to the improvement rate of ANTS from increasing clinical training and experiences, higher than the improvement rate of knowledge scores.

The limitation of this study was all the tests were not analyzed according to the learning objectives, which could be the mixed components of knowledge, skills and non-technical skills. Further study would be very valuable to determine the types and components of the tests to assess non-technical skills together with knowledge and technical skills. Another limitation was the lack of evidences of longitudinal development of these knowledge and skills due to the nature of this cross-sectional study that assessed at the end of the year. Time-series study would be answered the development of knowledge and competency of non-technical skills.

CONCLUSION

Residents' knowledge scores have moderate correlation with the all domain of non-technical skills assessment results, task management, teamworking, situation awareness and decision making. The category of non-technical skills that had lowest correlation coefficient was teamwork, which was poorly correlated with MCQs and KFQs, but acceptable correlation with OSCEs. Non-technical skills therefore are required to be trained and assessed together with knowledge to enhance patient safety and outcomes and cannot be assumed that residents with good medical knowledge would have good non-technical skills in all categories.

REFERENCES

1. Hull L, Arora S, Aggarwal R, Darzi A, Vincent C, Sevdalis N. The impact of nontechnical skills on technical performance in surgery: a systematic review. J Am Coll Surg 2012;214:214-30.
2. Glavin RJ. Excellence in Anesthesiology. The Role of Nontechnical Skills. Anesthesiology 2009;110:201-3.
3. Flin R, Patey R, Glavin R, Maran N. Anesthetists' non-technical skills. Br J Anesth 2010;105:38-44.
4. Fletcher G, Flin R, McGeorge P, Glavin R, Maran N, Patey R. Anesthetists' Non-Technical Skills (ANTS): evaluation of a behavioural marker system. Br J Anesth 2003;90:580-8.
5. Fletcher G, Flin R, McGeorge P, Glavin R, Maran N, Patey R. Rating non-technical skills: developing a behavioural marker system for use in Anesthesia. Cognition, Technology & Work 2004;6:165-71.
6. Flin R, Patey R. Non-technical skills for Anesthetists: developing and applying ANTS. Best Pract Res Clin Anaesthesiol 2011;25:215-27.
7. Engel N, Patey RE, Ross S, Wisely L. Non-technical skills. BMJ 2008;337:0812454.
8. Yule S, Maran N, Tsuburaya A. Training methods for non-technical skills. in Flin R, Youngson G, Yule S, Editors, Enhancing surgical performance. A primer on non-technical skills, CRC Press, London; 2015.
9. Gordon M, Baker P, Catchpole K, Darbyshire D, Schocken D. Devising a consensus definition and framework for non-technical skills in healthcare to support educational design: a modified Delphi study. Med Teach 2015;37:572-7.
10. Mishra A, Catchpole K, Dale T, McCulloch P. The influence of non-technical performance on technical outcome in laparoscopic cholecystectomy. Surg Endosc 2008;22:68-73.
11. Riem N, Boet S, Bould MD, Tavares W, Naik VN. Do technical skills correlate with non-technical skills in crisis resource management: a simulation study. Br J Anesth 2012;109:723-8.
12. Doumouras AG, Hamidi M, Lung K, Tarola CL, Tsao MW, Scott JW, et al. Non-technical skills of surgeons and Anesthetists in simulated operating theatre crises. Br J Surg 2017;104:1028-36.
13. Gjeraa K, Jepsen RMHG, Rewers M, Østergaard D, Dieckmann P. Exploring the relationship between anaesthesiologists' non-technical and technical skills. Acta Anaesthesiol Scand 2016;60:36-47.
14. Jirativanont T, Raksamani K, Aroonpruksakul N, Apidechakul P, Suraseranivongse S. Validity evidence of non-technical skills assessment instruments in simulated Anesthesia crisis management. Anesth Intensive Care 2017;45:469-75.
15. Gaba DM, Howard SK, Small SD. Situation awareness in anesthesiology. Hum Factors 1995;37:20-31.
16. Schulz CM, Endsley MR, Kochs EF, Gelb AW, Wagner KJ. Situation awareness in anesthesia: concept and research. Anesthesiology 2013;118:729-42.
17. Schulz CM, Krautheim V, Hackemann A, Kreuzer M, Kochs EF, Wagner KJ. Situation awareness errors in anesthesia and critical care in 200 cases of a critical incident reporting system. BMC Anesthesiol 2015;16:4.
18. Gaba DM. Dynamic decision-making in anesthesiology: cognitive models and training approaches. In: Advanced models of cognition for medical training and practice. Springer, Berlin and Heidelberg, 1992.p.123-47.
19. Stiegler MP, Tung A. Cognitive processes in anesthesiology decision making. Anesthesiology 2014;120:204-17.
20. Weinger MB, Slagle J. Human factors research in anesthesia patient safety: techniques to elucidate factors affecting clinical task performance and decision making. J Am Med Inform Assoc 2002;9(6 Suppl 1): S58-S63.
21. Yee B, Naik VN, Joo HS, Savoldelli GL, Chung DY, Houston PL, et al. Nontechnical skills in anesthesia crisis management with repeated exposure to simulation-based education. Anesthesiology 2005;103:241-8.
22. Wacker J, Kolbe M. Leadership and teamwork in anesthesia–making use of human factors to improve clinical performance. Trends in Anesthesia and Critical Care 2014; 4(6):200-5.
23. Mills P, Neily J, Dunn E. Teamwork and communication in surgical teams: implications for patient safety. J Am Coll Surg 2008;206:107-12.

Pattern Recognition using Morphologies of Anthropophilic and Zoophilic Dermatophytosis Lesions: Comparison between Final-Year Medical Students and Dermatology Residents

Sumanas Bunyaratavej, M.D., Rungsima Kiratiwongwan, M.D., Pichaya Limphoka, M.D., Kamonpan Lertrujiwanit, B.Sc., Charussri Leeyaphan, M.D.

Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

ABSTRACT

Objective: To compared pattern recognition abilities of final-year medical students and dermatology residents to distinguish and classify superficial fungal infections and resembling lesions.

Methods: The study was conducted at the Department of Dermatology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand, in 2019. The participants had to make diagnosis from 78 images including typical and atypical lesions within 50 seconds. No history or any description was given. The answer sheets were reviewed.

Results: Medical students (n = 18) and dermatology residents (n = 19) showed no significant differences in the means of overall accuracy scores. Residents demonstrated a statistically higher mean score than the medical students in diagnoses of anthropophilic infection with mostly presented with typical lesion. However, there were no significant differences in the mean scores for their diagnoses of zoophilic dermatophytosis as atypical lesions and other skin lesions.

Conclusion: Pattern recognition was helpful for the diagnosis of cutaneous dermatophytosis, especially in cases of typical lesions. Nonetheless, pattern recognition alone is insufficient for the diagnosis of atypical dermatophytosis lesions; analytical diagnostic skills should also be enhanced to an increase in the accuracies of atypical-lesion diagnoses.

Keywords: Morphological diagnosis; anthropophilic dermatophytosis; zoophilic dermatophytosis, accuracy; medical students; dermatology residents (Siriraj Med J 2020; 72: 488-491)

INTRODUCTION

Pattern recognition is an important clinical skill in dermatology.[1] Cutaneous dermatophytosis, a common skin infectious disease, is mainly caused by anthropophilic and zoophilic dermatophytes.[2] The recognition and classification of cutaneous dermatophytoses are based on clinical characteristics and lead to appropriate investigations.[3] The typical presentation of cutaneous dermatophytosis is a scaly, red, and slightly elevated lesion with an active border.[3] However, zoophilic infections frequently cause more inflammatory lesions[4] and may resemble other skin diseases, such as eczema and psoriasis, leading to a misdiagnosed pattern.[5] This study therefore compared the abilities of final-year medical students and dermatology residents to distinguish and classify skin lesions for superficial fungal infections and resembling lesions, as well as the participants' confidence levels.

Corresponding author: Charussri Leeyaphan
E-mail: charussrilee@gmail.com
Received 10 June 2020 Revised 20 July 2020 Accepted 19 August 2020
ORCID ID: http://orcid.org/0000-0001-8430-376X
http://dx.doi.org/10.33192/Smj.2020.66

MATERIALS AND METHODS

The investigation was conducted at the Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, in 2019. The study protocol had been approved by the Siriraj Institutional Review Board (Si 197/2020). High-quality, representative, clinical images with proper exposure were selected by two clinical instructors. The 78 clinical images comprised anthropophilic cutaneous lesions (37 cases), zoophilic cutaneous dermatophytosis lesions (33 cases), and other skin lesions resembling cutaneous dermatophytosis (8 cases). Sample pictures that were used as questions were demonstrated in Fig 1. No history or any description was given. The participants had to state whether each image was a dermatophytosis, and they needed to provide a confidence-level score for each decision (1, low confidence; 2, moderate confidence; 3, high confidence). The decision for each image needed to be made within 50 seconds. All cases of anthropophilic and zoophilic dermatophytosis were confirmed with positive branching septate hyphae from potassium hydroxide examination, and with fungal culture results in Sabouraud's dextrose agar with cyclohexamide. The data from the response sheets were consolidated, and a retrospective review was undertaken of the accuracy scores and confidence scores of the final-year medical students and dermatology residents.

Data were analyzed using SPSS Statistics for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA). The unpaired t-test was applied to compare the correct results and confidence scores of the final-year medical students and dermatology residents. A *p*-value of less than 0.05 indicated statistical significance.

RESULTS

A total of 37 participants consisting of 18 final-year medical students (48.6%) and 19 dermatology residents (51.4%) were enrolled. Their accuracy and confidence scores are detailed in Table 1. The final-year medical students and dermatology residents showed no significant differences in the means of their overall accuracy scores (41 ± 8.2 vs. 43.5 ± 10.5, respectively; $p = 0.421$). As to the diagnoses of the anthropophilic lesions, the dermatology residents demonstrated a statistically higher mean score (23 ± 5.9) than the final-year medical students (18.6 ± 5.3; $p = 0.014$). In contrast, there were no significant differences in the mean scores for their diagnoses of zoophilic dermatophytosis and other skin lesions. The dermatology residents had significantly higher confidence scores than the final-year medical students ($p < 0.05$) for the evaluations of the overall, anthropophilic, and zoophilic dermatophytosis images. On the other hand, the confidence scores of the 2 groups for the diagnoses of other dermatological conditions were not statistically different.

DISCUSSION

Pattern recognition is a necessary skill for dermatologic diagnosis.[1] This study revealed that the dermatology residents had a higher accuracy rate and greater confidence than the final-year medical students for the diagnosis of anthropophilic dermatophytosis. "Pattern recognition" refers to the process of matching a present case with examples from previous patients or prototypes of a disease stored in the diagnostician's memory. The process is normally very useful for diagnoses, especially in instances of typical lesions.[6,7] In the current study, the higher correct scores and confidence scores of the dermatology residents for the diagnoses of typical lesions may stem from their having more experience and the use of typical presentations of cutaneous anthropophilic dermatophytosis.

For both groups, the ability to diagnose zoophilic dermatophytosis, which usually presents with atypical

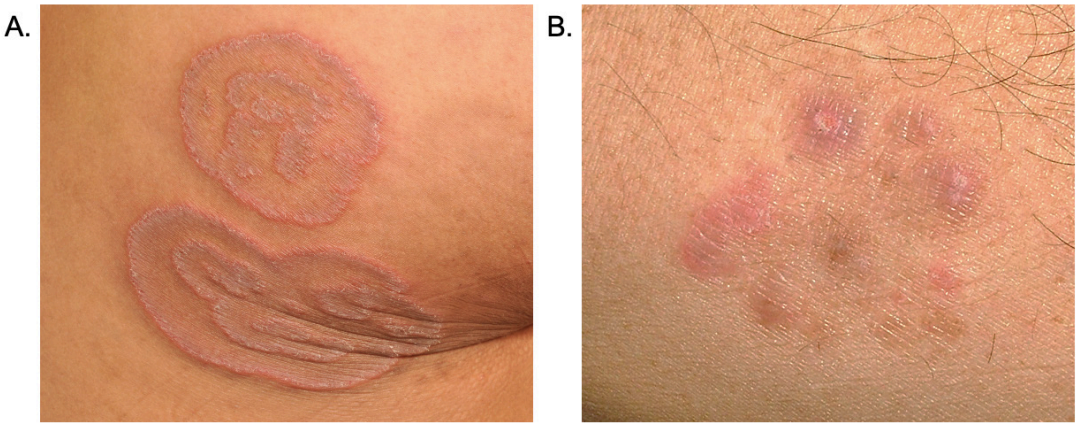


Fig 1. Sample pictures that were used as questions for **(A)** anthropophilic dermatophytosis and **(B)** zoophilic dermatophytosis

TABLE 1. Accuracy and confidence scores of final-year medical students and dermatology residents.

	Total score	Mean score ± SD		*P*-value
		Final-year medical students (n = 18)	Dermatology residents (n = 19)	
Accuracy scores				
Overall	78	41.0 ± 8.2	43.5 ± 10.5	0.421
Anthropophilic dermatophytosis	37	18.2 ± 5.3	23.0 ± 5.9	0.014*
Zoophilic dermatophytosis	33	15.6 ± 5.6	12.8 ± 5.2	0.098
Other dermatological conditions	8	7.2 ± 0.9	7.7 ± 0.7	0.093
Confidence scores				
Overall	234	138.8 ± 27.0	157.4 ± 15.0	0.022*
Anthropophilic dermatophytosis	111	66.8 ± 14.5	75.5 ± 7.3	0.030*
Zoophilic dermatophytosis	99	59.3 ± 11.8	66.5 ± 7.3	0.036*
Other dermatological conditions	24	15.5 ± 6.2	15.4 ± 7.1	0.964

* $p < 0.05$

Abbreviation: SD, standard deviation

lesions, was lower than that for anthropophilic lesions. Although there was no significant different in accuracy scores for the recognition of zoophilic dermatophytosis between final-year medical students and dermatology resident, final-year medical students intended to have a higher mean score. However, the dermatology resident had higher confidence scores than the final-year medical students. This result is similar to that reported by a previous investigation, which revealed that there was no correlation between confidence levels and the accuracy of diagnoses.[8] The misdiagnoses of zoophilic dermatophytosis by both groups may due to atypical presentations of zoophilic dermatophytosis having been used. This condition frequently results in highly inflamed lesions[4] that may resemble other skin diseases, such as eczema and psoriasis.[5] Other research reported that diagnostic errors for atypical dermatology conditions resulted from the ambiguities of atypical clinical lesions; moreover, only a little improvement in accuracy was gained with higher levels of clinician expertise.[7] That study suggested that atypical lesions may contain inadequate information for diagnostic purposes. To increase the diagnostic accuracy of medical students and dermatology residents, we suggest that more instruction with atypical lesions should be given, and with a greater frequency than typical lesions. Nevertheless, pattern recognition alone may not be enough to make diagnoses for atypical lesions. The development of analytical and diagnostic skills within a framework utilizing patients' histories and physical examinations should also be enhanced in order to improve clinicians' abilities to discriminate between atypical skin-lesion types.

The limitation of this study is its small sample size. In addition, cutaneous dermatophytosis was the sole, representative skin disease used in this study. Further study with a larger sample size and a wider variety of skin lesion types is recommended.

In conclusion, pattern recognition was found to be a helpful clinical skill for the diagnosis of cutaneous dermatophytosis, especially in cases of typical lesions. Given that dermatology residents encounter typical skin lesions more frequently than medical students, they can develop a higher degree of pattern recognition skills and, in turn, expertise in the diagnosis of typical skin lesions. As to atypical skin lesions, it is recommended that training in pattern recognition for such lesions should be provided with more frequency than for typical lesions. Nonetheless, pattern recognition alone is insufficient for the diagnosis of atypical lesions: analytical diagnostic skills should also be enhanced. Collectively, such actions should contribute to an increase in the accuracies of atypical-lesion diagnoses.

ACKNOWLEDGMENTS

The authors thank Assist. Prof. Chulaluk Komoltri, DrPH, for her invaluable support with the statistical analyses.

REFERENCES

1. Rimoin L, Altieri L, Craft N, Krasne S, Kellman PJ. Training pattern recognition of skin lesion morphology, configuration, and distribution. J Am Acad Dermatol 2015;72:489-95.
2. Hassanzadeh Rad B, Hashemi SJ, Farasatinasab M, Atighi J. Epidemiological Survey of Human Dermatophytosis due to Zoophilic Species in Tehran, Iran. Iran J Public Health 2018; 47:1930-6.
3. Kaushik N, Pujalte GG, Reese ST. Superficial Fungal Infections. Prim Care 2015;42:501-16.
4. Weitzman I, Summerbell RC. The dermatophytes. Clin Microbiol Rev 1995;8:240-59.
5. Hainer BL. Dermatophyte infections. Am Fam Physician 2003;67:101-8.
6. Costa Filho GB, Moura AS, Brandao PR, Schmidt HG, Mamede S. Effects of deliberate reflection on diagnostic accuracy, confidence and diagnostic calibration in dermatology. Perspect Med Educ 2019;8:230-6.
7. Norman GR, Rosenthal D, Brooks LR, Allen SW, Muzzin LJ. The development of expertise in dermatology. Arch Dermatol 1989;125:1063-8.
8. Landefeld CS, Chren MM, Myers A, Geller R, Robbins S, Goldman L. Diagnostic yield of the autopsy in a university hospital and a community hospital. N Engl J Med 1988;318: 1249-54.

Effects of Four Noble Truths Practice on Hypertension Control

Sadudee Rojanapirom, D.N.S. Candidate*, Siriorn Sindhu, Ph.D., Ketsarin Utriyaprasit, Ph.D. **, Peera Buranakitjaroen, Ph.D. ***, Chukiat Viwatwongkasem, Ph.D. ******

**Doctor of Nursing Science Program, Faculty of Nursing, **Department of Surgical Nursing, Faculty of Nursing, Mahidol University, ***Department of Medicine, Faculty of Medicine, Mahidol University, ****Department of Biostatistics, Faculty of Public Health, Mahidol University, Bangkok 10700, Thailand.*

ABSTRACT

Objective: To examine the effects of Four Noble Truths practice in controlling blood pressure with three-group comparison, involving a Mobile Web group, Guidebook group and usual care group in patients with stage 1 hypertension.

Methods: This randomized controlled trial was conducted in one university hospital setting. 145 participants were recruited into the study by randomized selection and were randomly assigning to the three groups. Of these, 45 participated as Mobile Web users, 50 as Guidebook users and 50 in the control group. The Mobile Web and Guidebook were developed using the guidance of the Four Noble Truths and received a phone call every two weeks for three times while control received a usual care. Then, the outcomes were measured including blood pressure and satisfaction at two-month after recruitment. The average blood pressure and satisfaction were compared by comparative descriptive statistic. Finally, analysis of covariance (ANCOVA) was used to analyze the covariate that may influence the outcomes with the post hoc analysis by Bonferroni.

Results: Participants who received Mobile Web or Guidebook had reduced their blood pressure more than those receiving usual care, with statistical significance ($P < 0.05$). It was found that blood pressures could be lowered to a maximum level of 15.09 ± 9.62 mmHg within 8 weeks of treatment. Participants who attended participated in Mobile Web or Guidebook were satisfied with healthcare services more than those receiving usual care, with statistical significance ($P < 0.05$).

Conclusion: The program could be applied to control high blood pressure in patients with stage 1 hypertension. The outstanding of this program could support overall difference in healthcare of each patient with fast efficacy.

Keywords: Blood pressure control; four noble truths; mobile web (Siriraj Med J 2020; 72: 492-501)

INTRODUCTION

One billion of the current world population suffers from high blood pressure,[1] and half of this population has uncontrolled hypertension.[2] Thailand has also found the same trend among people aged 18 years and older who are likely to have high blood pressure with 53.2% reporting uncontrolled hypertension.[3] Untreated hypertension is notorious for increasing the risk of mortality and is often described as a silent killer. Prehypertension and stage 1 hypertension are also associated with a high risk of atherosclerotic disease by 30% and organ damage by 50% in patients with uncontrolled hypertension within 8-10 years after onset.[4]

Corresponding author: Siriorn Sindhu
E-mail: siriorn.sin@mahidol.ac.th
Received 18 May 2020 Revised 23 June 2020 Accepted 24 June 2020
ORCID ID: http://orcid.org/0000-0001-9326-757X
http://dx.doi.org/10.33192/Smj.2020.67

In its general practice guidelines in 2012, the Thai Hypertension Society focused on behavioral change along with treatment to control blood pressure, but the outcome was not optimal. The numbers of people with uncontrolled hypertension continued to rise nationwide. Over the past decade, previous studies have suggested that uncontrolled hypertension is influenced by three main components including personal factors, health care provider factors, and health service system factors. Interventions for alleviating uncontrolled hypertension by development of these three factors have continued to the present.[5]

In recent studies, efforts have been made to control both blood pressure and behavioral modification in patients with hypertension. In the last 20 years, self-management and self-management support have been addressed in terms of raising awareness about creating self-care, systems and environments. Self-management intervention consisted of health education, self-regulation and support group in order to improve the outcomes of care.[6] Moreover, nowadays, there were an increasing number of self-management intervention using the mobile application.[7] However, those interventions were implemented without considering the social determinants of health in Thai patients with hypertension, so patients remained unable to control blood pressure.

Several interventions used Buddhism as a guidance to develop the intervention in order to control blood pressure level such as meditation. Meditation interventions were effectively reducing blood pressure level when compared with control.[8-9] All uncontrolled hypertension is based on the patient's own behavior[10] and the intervention should focus on the seeking for the truth of problem and develop an intervention related to the cause of problem using of the Four Noble truths guidance. Therefore, interventions aimed at changing the behaviors of patients to reduce blood pressure with the guidance of the Four Noble truths include the following: 1) Dukkha - the truth of suffering; 2) Samudaya - the truth of the cause of suffering; 3) Nirodha - the truth of the end of suffering; and 4) Magga - the truth of the path that frees us from suffering.[11] Effective methods are important and require the inclusion of modern communication technology that is suitable for long-distance patients and large groups of the population.

In this study, an intervention was developed to control blood pressure in people with stage 1 hypertension with a combination of the principles of Buddhism and modern technology such as mobile health technology. Mobile health or mHealth technology enables platform flexibility and improves patients' mHealth application performance, which is suitable for patients in terms of schedule and treatment planning. The popularity of mHealth studies in hypertension and other chronic diseases such as improving self-management,[12] and medication adherence[13] is demonstrated in the improvements in health status made possible by the mHealth application, which is an effective intervention to improve the blood pressure controlling.

The Four Noble Truths are a rational way to understand the realities of suffering and finding a way out of suffering. This will solve the problem of failure to modify behavior that causes uncontrolled hypertension by combining with Mobile Web, which is easy to access, inexpensive, affordable to many people and features a process for effectively reducing the blood pressure of patients. The objective of this study was to determine the effects of practicing the Four Noble Truths in controlling blood pressure. The hypothesis postulated that patients with hypertension in both groups (Mobile Web and Guidebook groups) who attended the Four-Noble Truths Practice on Hypertension Control program would have lower blood pressure and more health service satisfaction than those who received the usual care program only.

MATERIALS AND METHODS

This study was a randomized control trial (RCT) with a three-group pre-test and post-test design. The study was conducted at a university hospital in Thailand. The study protocol was reviewed and approved by the Research Ethical Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University (Si 296/2018). The study participants were male or female patients diagnosed with stage 1 hypertension by a physician and recorded in medical folders by using the international classification of disease codes-9 (ICD-9)* (systolic blood pressure: SBP = 140-159 mmHg or diastolic blood pressure: DBP = 90-99 mmHg.),[14] aged 18 years and over with regular doctor's appointments in the out-patient department of the university hospital where the study was conducted.

The sample size was calculated by performing power analysis with the G*Power program.[15] The differences in the mean scores were compared among the three groups with repeated measure analysis (pre-intervention, first month, and post intervention) with ANCOVA. Thus, the sample size calculation set alpha at 0.05 and assigned power of test at 0.80. According to Cohen's guideline, the medium effect size for ANCOVA was set at 0.25. This study obtained 158 patients (53 subjects per group) and added 10% (16 subjects) to prevent attrition from the sample group, thereby bringing the number of participants in this study to a total of 174 (58 subjects/group).[16]

The inclusion criteria for the participants was as follows: 1) diagnosis with uncontrolled stage 1 hypertension and treatment with antihypertensive drugs for at least six months and 2) SBP ranging between 140-159 mmHg. The exclusion criteria consisted of: 1) 1) diagnosed with diseases in the critical stage in the past 3 months; 2) history of diagnosis with psychiatric problems such as depression and schizophrenia; 3) Pregnancy. The participants who met the criteria were selected for enrollment in the study by the minimizer technique version 1.0[17] for improving external validity as follows: 1) Minimization of covariates related to blood pressure level for controlling confounding factors. The covariates should not exceed three variables. In this study, one covariate, gender, was included; 2) Random assignment into the following three groups: usual care (Usual Care Group), learning by interactive program (Mobile Web Group) and learning by Guidebook (Guidebook Group).

The intervention packages were categorized into the following three types: 1) The guidebook contained parts of self-directed records (i.e. self-evaluation related blood pressure level, self-perception related cause of hypertension, setting up targeted blood pressure level, finding a way to lower blood pressure, and recording activities); 2) The Mobile Web contained mobile phone information including an animation to present how to control blood pressure. The information for the guidebook and Mobile Web were developed based on the Four Noble Truths which aimed to support hypertension control by understanding the truth of suffering (dukkha), the truth of the cause of suffering (samudaya), the truth of the end of suffering (nirhodha) and the truth of the path that frees us from suffering (magga). Therefore, the interventions included self-evaluation related to blood pressure levels, self-perception related to the causes of hypertension, setting up targeted blood pressure levels, finding ways to lower blood pressure and recording activities. The information was implemented into the program following the recommendations of the JNC 8; 3) Usual care received general care from the nurses at the hypertension clinics, including recording blood pressure and weight in addition to following-up with physicians.

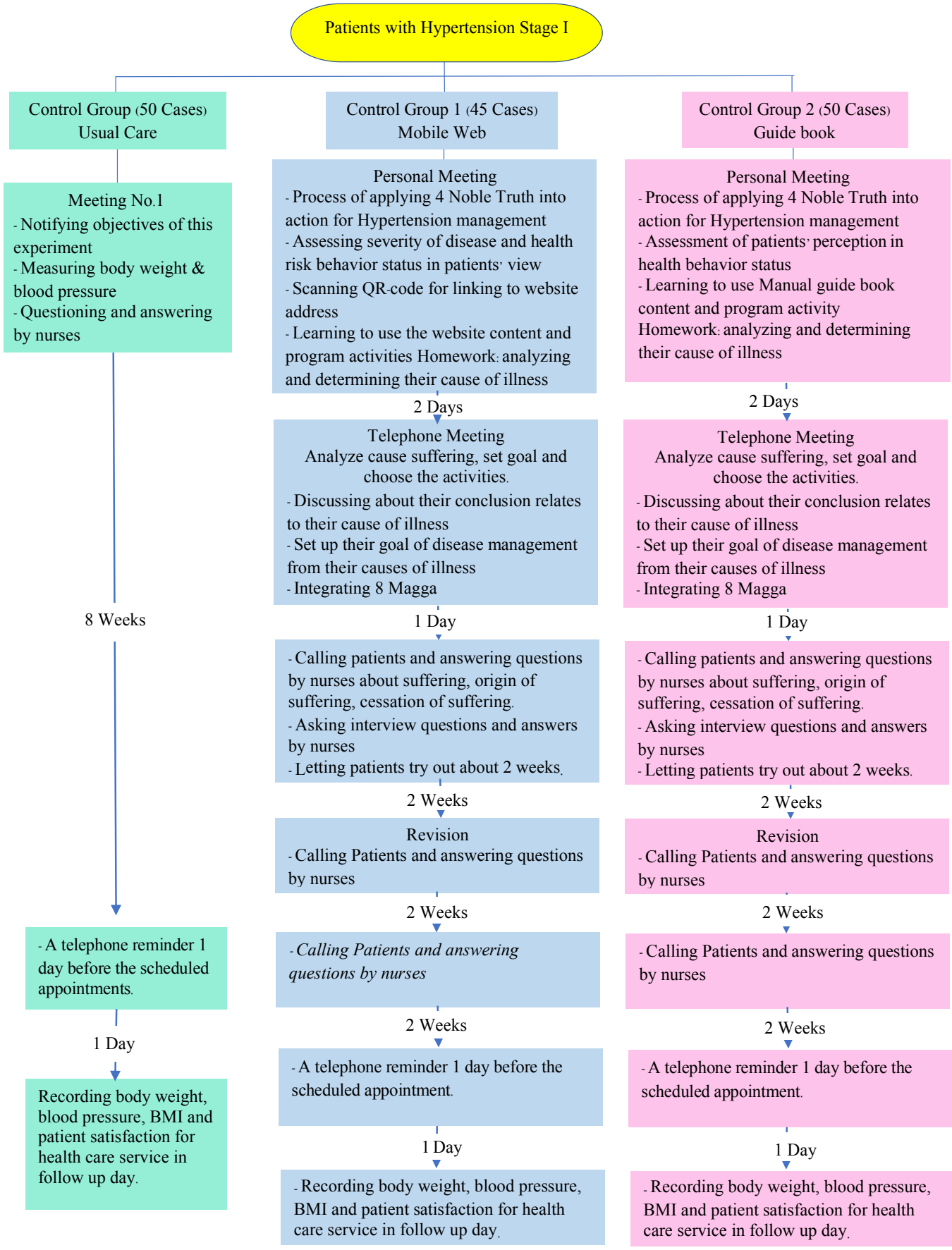
SBP and DBP blood pressure were measured by the average value of two separate measures by using a mercury sphygmomanometer[18] after minutes of relaxation. The measurements were taken on the left arms of the participants. All clothing was removed from the arm and the center of the bladder was placed over the brachial artery. The measurement was taken by a trained nurse. Next, health service evaluation was used to measure patients' satisfaction regarding health service delivery. The instrument was developed by Sindhu, et al. (2004) with a questionnaire containing 20 items on patient perception of health services received from healthcare providers. The overall satisfaction rate was 0-100. In terms of interpretation of the overall items and the overall satisfaction scores, higher scores indicated higher health service satisfaction. The internal consistency (Cronbach's alpha) was 0.89.[19]

The research assistants were trained in following protocol, approaching patients, giving questions and answers every two weeks by phone with the same questions and controlling times for accurate treatment between researcher and research assistants. In the intervention group, the participants met the researcher at the first appointment and received usual care with instructions on how to use the Guidebook or Mobile Web. Every two weeks for three sessions after recruitment, the intervention group received phone calls for empowerment, advice and answers to patients' questions. Finally, in the two-month follow-up phase, the participants had data collected on clinical characteristics, SBP and patients' service satisfaction. On the other hand, the control group received usual care detailing hypertension information and measuring the outcome at the end of the study at two months. The flow chart of the data collection process is presented in Fig 1.

Statistical analysis

The comparison of the demographic data among the three groups used the Kruskal Wallis Test. Then the post hoc analysis employed Dunn's test to perform pairwise comparison of variables. In this case, the expected value was < 5 more than 20%, and chi-square or Fisher's exact testing was used. The average pre- and post- SBP of each intervention were compared by using the Wilcoxon Signed Ranks Test. Then the comparison of each part of satisfaction on healthcare services was compared by Kruskal Wallis Test and followed by pairwise comparison using Dunn's test. Finally, analysis of covariance (ANCOVA) was used to analyze the covariate potentially influencing blood pressure reduction after the program with dependent variables (SBP and patient satisfaction) After adjusting the covariate, if at least one pair of the dependent variables and covariate had statistically significant differences, post hoc analysis by Bonferroni was performed to identify those pairs. The variance among the three groups was analyzed by Levene's Test in which a 2-sided p value of <0.05 was considered statistically significant.

Fig 1. Flow of Data Collection Process.



RESULTS

One hundred and seventy-four patients with stage 1 hypertension who were eligible based on the inclusion and exclusion criteria were recruited into this study. During data collection, 29 patients withdrew from the study due to the difficulty in getting to the hospital and inconvenience in completing the 2-month study program. Thus, a total of 145 participants remained in the study including 45 Mobile Web users, 50 Guidebook users and 50 participants in the control group.

Demographic characteristics

The mean ages in each group were 57.6 ± 11.75 years for the Mobile Web group, 68.04 ± 8.86 years for the guidebook group and 63.70 ± 13.40 for the control group. Most of the participants were females. The percentage of females in each group was 75.6 in the Mobile Web group, 76 in the guidebook group and 66 in the control group. When comparing the personal characteristics of the experimental Mobile Web, Guidebook, and control groups, it was found that gender and type of hypertension medication showed no statistically significant difference. However, there were statistically significant differences in body mass index (BMI), blood pressure before the program, duration after hypertension diagnosis, educational attainment, healthcare coverage, income, employment and comorbidities (Table 1).

Systolic blood pressure

The comparison of mean SBP following the intervention found that the Mobile Web group had an average decrease at the end of the program (2 months), with a mean difference SBP of 15.09 ± 9.62 mmHg, while the Guidebook group had a mean difference in SBP of 13.02 ± 12.19 mmHg and the control group had a mean difference of 9.18 ± 12.95 mmHg. The comparisons of mean SBP at pre- and post-intervention were statistically and significantly different in all three groups as shown in Table 2.

Comparison of SBP among three groups after adjusting for covariates

Analysis of covariance (ANCOVA) was used to analyze the covariates potentially influencing blood pressure reduction after the program, including age, comorbidities, SBP before the program, duration after hypertension diagnosis, educational attainment, healthcare coverage, income, employment and BMI. After adjusting the covariate, it was found that at least one pair of the mean SBP had a statistically significant difference. The post hoc analysis by the Bonferroni method found that the mean SBP of the Mobile Web group was lower than the control group, while the Guidebook group was also lower than the control group with statistical significance as shown in Tables 3 and 4.

Satisfaction scores

The satisfaction score with the selected program found the Mobile Web group to have the highest mean satisfaction score of 93.78 ± 4.97, followed by the control group (93.36 ± 7.26) and the Guidebook group (90.4 ± 5.26).

Comparison of satisfaction scores among the three groups after adjusting for covariates

Analysis of covariance (ANCOVA) was used to analyze the covariate with potential influence over satisfaction scores, including age, comorbidities, SBP before the program, duration after hypertension diagnosis, educational attainment, healthcare coverage, income, employment and BMI. After adjusting the covariate, at least one pair of satisfaction scores was found to have statistically significant differences as shown in Tables 5 and 6.

DISCUSSION

The mobile web and Guidebook groups had lower SBP than the control group after participating in the Practice of Four Noble Truths for Controlling High Blood Pressure program with statistical significance. At the same time, there were differences in diastolic blood pressure in the Mobile Web and Guidebook groups with no statistical significance. Thus, the program was able to have remarkable effects on lowering SBP in both groups. The program introduced the Four Noble Truths of Buddhism combined with the Mobile Web and the Guidebook technology under the management of specialized nurses in high blood pressure.

Both of the experimental groups with Mobile Web and Guidebook users were encouraged to lower their blood pressure accurately, continuously and appropriately according to individual cases. The Four Noble Truths of Buddhism comprise the four truths of suffering and cessation of suffering, which briefly refer to the cessation of suffering from the causes with correct processes. In patients with high blood pressure, the causal effects were from high blood pressure. Hence, the design of this program had to focus on the elimination of those causes in each patient according to the Four Noble Truths, including Dukkha, Samudaya, Nirodha, and Magga[20] as described below:

TABLE 1. Demographic of participants.

Characteristic	Mobile web (n=45)	Guide book (n=50)	Control (n=50)	*P*-value
Age (years)	57.60±11.75	68.04±8.86	63.70±13.40	**<0.001**[1]
Sex				0.455[2]
Male	11 (24.4)	12 (24.0)	17 (34.0)	
Female	34 (75.6)	38 (76.0)	33 (66.0)	
BMI	27.92±6.21	24.58±3.60	26.09±5.02	**0.040**[1]
SBP before participating in the study (mmHg)	144.18±8.26	147.24±7.28	148.40±5.69	**0.050**[1]
Duration after hypertension diagnosis (years)	6.57±5.33	10.12±6.98	13.62±7.81	**<0.001**[1]
Educational attainment				**<0.001**[2]
Uneducated or elementary school	6 (13.3)	28 (56.0)	13 (26.0)	
High school or vocational school	9 (20.0)	7 (14.0)	17 (34.0)	
Bachelor degree or higher	30 (66.7)	15 (30.0)	20 (40.0)	
Healthcare coverage				**0.002**[3]
UHC	1 (2.2)	2 (4.0)	11 (22.0)	
SSS	4 (8.9)	2 (4.0)	8 (16.0)	
CSMBS	28 (62.2)	39 (78.0)	27 (54.0)	
Self-employed	10 (22.2)	6 (12.0)	3 (6.0)	
Other	2 (4.4)	1 (2.0)	1 (2.0)	
Income	19,328.89±11,565.26 20,000 (0-50000)	12,164±11,807.21 8,000 (0-50,000)	13,840±17,289 5,000 (0-70,000)	**0.004**[1]
Employment				**0.004**[3]
Marchant	2 (4.4)	7 (14.0)	3 (6.0)	
Government officer	13 (28.9)	2 (4.0)	4 (8.0)	
Employer	9 (20.0)	4 (8.0)	9 (18.0)	
Unemployed	21 (46.7)	37 (74.0)	34 (68.0)	
Types of hypertension medication				0.595[3]
Never received	0 (0.0)	2 (4.0)	1 (2.0)	
1 type	26 (57.8)	26 (52.0)	23 (46.0)	
2 types	19 (42.2)	22 (44.0)	26 (52.0)	
Comorbidities				**0.023**[2]
None	26 (57.8)	15 (30.0)	23 (46.0)	
Have	19 (42.2)	35 (70.0)	27 (54.0)	
Diabetes	9 (20.0)	22 (44.0)	13 (26.0)	
Hyperlipidemia	10 (22.2)	19 (38.0)	17 (34.0)	
AR	1 (2.2)	1 (2.0)	0 (0.0)	
CKD	1 (2.2)	3 (6.0)	5 (10.0)	
Other	3 (6.7)	4 (8.0)	6 (12.0)	

[1]Kruskal Wallis Test, [2]Chi-square test, [3]Fisher's exact test

Results: mean±SD, median (min-max), n (%)

Abbreviations: BMI = Body Mass Index, SBP = Systolic Blood Pressure, UHC = Universal health coverage, SSS = Social Security Scheme, CSMBS = Civil Servant Medical Benefit Scheme, AR = Allergic Rhinitis, CKD = Chronic Kidney Disease

TABLE 2. The average of SBP before and after intervention with the mean difference of SBP.

Groups	Before SBP	After SBP	*P*-value	Mean SBP difference
Mobile web	144.20±8.27	129.09±7.85	**<0.001**	15.09±9.62
Guide book	147.24±7.29	134.22±10.10	**<0.001**	13.02±12.19
Control	148.40±5.69	139.22±12.85	**<0.001**	9.18±12.95

Wilcoxon signed ranks test
Abbreviation: SBP = Systolic Blood Pressure

TABLE 3. The ANCOVA of mean SBP difference and mean satisfaction difference among groups after adjusting for age, comorbidities, SBP before the program, duration after hypertension diagnosis, educational attainment, healthcare coverage, income, employment and BMI.

Variables	SS	df	MS	F	*P*-value
SBP[a]					
Groups	1464.3549	2	732.1775	6.94	0.001
Age	168.2553	1	168.2553	1.59	0.209
Comorbidities	343.2014	1	343.2014	3.25	0.074
SBP before the program	255.1927	1	255.1927	2.42	0.123
Duration after hypertension diagnosis	228.5107	1	288.5107	2.16	0.144
Educational attainment	30.6850	2	15.3425	0.15	0.865
Healthcare coverage	335.7861	4	83.9465	0.80	0.530
Income	245.8787	1	245.8787	2.33	0.129
Employment	348.2690	3	116.0897	1.10	0.352
BMI	115.8679	1	115.8679	1.10	0.297
Satisfaction[b]					
Groups	287.1816	2	143.5908	3.87	0.023
Age	0.4960	1	0.4960	0.01	0.908
Comorbidities	12.2170	1	12.2170	0.33	0.567
SBP before the program	26.5270	1	26.5270	0.71	0.400
Duration after hypertension diagnosis	137.3572	1	137.3572	3.70	0.057
Educational attainment	36.9258	2	18.4629	0.50	0.609
Healthcare coverage	54.9481	4	13.7370	0.37	0.830
Income	14.7046	1	14.7046	0.40	0.530
Employment	46.2282	3	15.4094	0.41	0.743
BMI	0.4826	1	0.4826	0.01	0.909

[a]Levene's Test of Equality of Error Variances: F=0.647 P-value=0.966
[b]Levene's Test of Equality of Error Variances: F=1.098 P-value=0.346
Abbreviations: SBP = Systolic Blood Pressure, BMI = Body Mass Index

TABLE 4. The mean difference of mean SBP after the program in the mobile-web, the guide-book and the control groups.

Group		Mean Difference	*P*-value
SBP[a]			
Mobile web	Guide book	-3.09	0.711
Mobile web	Control	-9.15	**0.001**
Guide book	Control	-6.06	**0.045**
Satisfaction[b]			
Mobile web	Guide book	2.92	0.183
Mobile web	Control	-1.02	1.000
Guide book	Control	-3.94	**0.023**

[a]Adjustment for multiple comparisons: Bonferroni
[b]Post hoc analysis of satisfaction score

TABLE 5. The ANCOVA of mean satisfaction score among groups after adjusting for age, comorbidities, SBP before the program, duration after hypertension diagnosis, educational attainment, healthcare coverage, income, employment and BMI.

Variables	SS	df	MS	F	*P*-value
Groups	287.1816	2	143.5908	3.87	0.023
Age	0.4960	1	0.4960	0.01	0.908
Comorbidities	12.2170	1	12.2170	0.33	0.567
SBP before the program	26.5270	1	26.5270	0.71	0.400
Duration after hypertension diagnosis	137.3572	1	137.3572	3.70	0.057
Educational attainment	36.9258	2	18.4629	0.50	0.609
Healthcare coverage	54.9481	4	13.7370	0.37	0.830
Income	14.7046	1	14.7046	0.40	0.530
Employment	46.2282	3	15.4094	0.41	0.743
BMI	0.4826	1	0.4826	0.01	0.909

Levene's Test of Equality of Error Variances: F=1.098 P-value=0.346

TABLE 6. Comparison of overall satisfaction on health services.

Characteristics	Mobile-web group (n=38)	Guide-book group (n=38)	Control group (n=42)	*P*-value
Overall satisfaction on health services	95.33±6.94	87±11.11	89.80±12.86	**0.001**[3]

[3]Kruskal Wallis test

Dukkha (knowing symptoms) - Patients understood their own symptoms when they had physical examinations by taking measurements for blood pressure, weight and height in addition to receiving immediate information from the staff. Blood pressure measurement could inform the patients that they had asymptomatic stage 1 hypertension.[21] Thus, they became careless. However, this program would stimulate patients to know and understand, so they had greater awareness and self-care.

Samudaya (knowing causes) - The patients received knowledge, considered, and determined the causes of their own hypertension, particularly the causes by incorrect lifestyles[22] that could be solved and prevented on their own. The causes of each patient were different. Patients reviewed those causes with nurses in this program, thereby allowing patients to understand the causes based on their behaviors and the effects to their blood vessels and systems in the body. The program empowered patients to strengthen their well-being as a source of awareness about the importance of healthcare with true beliefs and knowledge based on health science. The patients could make their decisions to change or maintain the behaviors that support controlling hypertension as advised by nurses and other people involved. Ultimately, there was a development of health with clear outcomes.[19]

This was the reason why the researcher used the Mobile Web or Guidebook, which were designed to allow patients to understand the pathophysiology of fatty tissues that could narrow blood vessels. The patients could then imagine the changes in their bodies as motivation to solve all causes. The pictures in Guidebook or the animation in Mobile Web described the mechanisms of high blood pressure in the body with deep and clear understanding rather than practice by usual care of simply using words to describe and explain. According to the findings, the general introduction of tablet devices could entail a shift in the way students learnt, because the devices provided interactive, media-rich and exciting new environments.[23] When understanding the self-led causes, patients could come up with the idea that they were able to correct themselves and design or choose a lifestyle that would suit them. For instance, they chose to exercise by cycling or walking fast at home, at work, with friends, and at convenient times to do so. Thus, they were able to adopt these activities into real-life practice.[24]

Nirodha - Once the patients understood the root causes of their behaviors, they accepted the idea that they were able to fix those problems themselves. They were the ones who set the right goals and chose the right lifestyles. For example, they chose to exercise by cycling or walking fast at home, at work, or with friends. Setting the right times for themselves could actually be helpful in everyday life. The nurses provided information. There was goal-setting between patients and nurses, which was also a means of empowerment. The study of Anuruang found that participants in the program were healthier and more satisfied with their healthcare services than in the past and better than the control group receiving usual care only with statistical significance ($p < 0.05$).[19] These clear goals led patients to practice continuously until they reached their goals.

Magga (practice) - Implementation could be the key to success. Buddhist concepts tend to focus on practice rather than prayer. The main focuses are on the present time, concentration of mind and conscious actions at all times. It should be important to encourage patients to order and remind themselves constantly. The two experimental groups (100%) were composed of Buddhists who understood these contexts when they were encouraged to do so. In particular, all of the participants were Thai Buddhists and used the principles of Buddhism in their daily lives. Therefore, they could correct their own causes based on the Noble Eightfold Path toward successful achievement of goals.

In terms of overall satisfaction in health care services, it was found that the average satisfaction of the Mobile Web group was statistically and significantly higher than that of the Guidebook group. According to the findings, the patients' satisfaction with Mobile Internet-based Health Services (MIHS) was positively influenced by perceived usefulness and confirmation of MIHS performance expectations.[25] When comparing the satisfaction with the health services of the three groups, the Mobile Web group had the highest satisfaction, followed by the Guidebook group and the control group, respectively.

Limitation

There was some limitation in this study. First, the adherence to antihypertensive drugs may influence the blood pressure level of participants however we did not measure the adherence of participants and control for this extraneous variable. Second, there was the statistically significant differences among groups for baseline characteristics (e.g. age, BMI, education) however we used the random assignment to assign participants into three groups so the difference may happen by change. Third, our study lacked of controlling for changes in anti-hypertensive regimens during the study period so the medication adjustment may influence the blood pressure level.

CONCLUSION

The program, "Practice of Four Noble Truths in Controlling High Blood Pressure" could be applied to controlling high blood pressure in patients with stage 1 hypertension in nursing and health care practice at both public and private public health centers, hospitals and others. The outstanding features of this program were able to support the overall differences in the healthcare of each patient with rapid efficacy with a variety of choices such as interventions (Mobile Web or Guidebook) for patients to choose based on their needs. They could select their own activities that fit their reasons, convenience and preferences. The program could potentially develop nursing roles in providing care for patients with hypertension nationwide. In particular, the project team could collaborate with other healthcare providers to improve the health of the entire population. It could be helpful to use the Four Noble Truths in health service delivery as a reasonable and conceptual practice leading to optimal success.

REFERENCES

1. World Health Organization. Global status report on noncommunicable diseases 2014. World Health Organization; 2014.
2. Kansui Y, Ibaraki A, Goto K, Haga Y, Seki T, Takiguchi T, et al. Trends in blood pressure control and medication use during 20 years in a hypertension clinic in Japan. Clin Exp Hypertens 2016;38:299-304.
3. Rangsin R, Tatsanawiwat P. The evaluation of DM type 2 and hypertension caring in hospital under Ministry of Public Health Control: National Health Security Office; 2012.
4. Whelton PK, Carey RM. The 2017 American College of Cardiology/American Heart Association clinical practice guideline for high blood pressure in adults. JAMA Cardiol 2018;3:352-3.
5. Yoon SS, Gu Q, Nwankwo T, Wright JD, Hong Y, Burt V. Trends in blood pressure among adults with hypertension: United States, 2003 to 2012. Hypertension. 2015;65:54-61.
6. Tapp H, Dulin M, Plescia M. Chronic disease self-management. Chronic Illness Care: Springer; 2018.p.29-40.
7. Brahmagunabhorn P, Evans B. A constitution for living. Bangkok: Dhammasapa Press; 2012.
8. Logan AG. Transforming hypertension management using mobile health technology for telemonitoring and self-care support. Can J Cardiol 2013;29:579-85.
9. Herdaningsih S, Muhtadi A, Lestari K. Consumption time of captopril influenced the outcomes of patients with stage 1 hypertension. Pharmacology and Clinical Pharmacy Research. 2017;2:11:74.
10. Erdfelder E, Faul F, Buchner A. GPOWER: A general power analysis program. Behavior Research Methods, Instruments, & Computers 1996;28:1-11.
11. Cohen J. Statistical power analysis for the behavioral sciences: Academic Press; 2013.
12. Saghaei M. An overview of randomization and minimization programs for randomized clinical trials. J Med Signals Sens 2011;1:55-61.
13. Meidert A, Briegel J, Saugel B. Principles and pitfalls of arterial blood pressure measurement. Der Anaesthesist 2019;68:637-50.
14. Anuruang S, Sindhu S, Wanitkun N, Sriyuktasuth A. The Effects of Empowering Care Program on Health Status and Health Service Satisfaction among Women with Hypertension. Thai Journal of Nursing Council 2009;24:11-23.
15. Carey RM, Whelton PK. Prevention, detection, evaluation, and management of high blood pressure in adults: synopsis of the 2017 American College of Cardiology/American Heart Association Hypertension Guideline. Ann Intern Med 2018;168:351-58.
16. Dreher ML. Dietary Patterns and Hypertension. Dietary Patterns and Whole Plant Foods in Aging and Disease: Springer; 2018.p.371-89.
17. Montrieux H, Vanderlinde R, Schellens T, De Marez L. Teaching and learning with mobile technology: A qualitative explorative study about the introduction of tablet devices in secondary education. PloS One 2015;10:e0144008.
18. Blumenthal JA, Sherwood A, Smith PJ, Mabe S, Watkins L, Lin PH, et al. Lifestyle modification for resistant hypertension: The TRIUMPH randomized clinical trial. Am Heart J 2015;170:986-94.e5.
19. Gu D, Yang X, Li X, Jain HK, Liang C. Understanding the role of mobile internet-based health services on patient satisfaction and word-of-mouth. Int J Environ Res Public Health 2018;15:1972.

Granular Myringitis Treatment at Siriraj Hospital

Kanokrat Suvarnsit, M.D.*, Thansamorn Chantarawiwat, M.D.*, Sarun Prakairungthong, M.D.*, Siriporn Limviriyakul, M.D.*, Suvajana Atipas, M.D.*, Pittayapon Pitathawatchai, M.D.**

**Department of Oto-Rhino-Laryngology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, **Department of Otolaryngology Head and Neck Surgery, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.*

ABSTRACT

Objective: To review the clinical features and management of patients diagnosed with granular myringitis at Siriraj Hospital, during 2014–2016, and their applications in clinical practice.

Methods: The clinical data of 115 patients diagnosed with myringitis at the Department of Otorhinolaryngology, Faculty of Medicine Siriraj Hospital, between September 1, 2014, and September 30, 2016, were retrospectively reviewed. Patients who were lost to follow-up after the first visit or patients who were diagnosed with other diseases, such as bullous myringitis, were excluded, leaving 96 patients included in the study. Patient information and data, including age, gender, underlying disease, history of ear disease and surgery, symptoms, duration, type of treatment, outcome, total follow-up time, complications, and recurrence rate were recorded.

Results: In total, 96 patients (27 men (28.10%) and 69 women (71.90%)) were included in the study. Their ages ranged from 3 to 90 years old (mean, 52.88). Sixty-two patients (64.60%) were diagnosed by otologic staff. The average duration of symptoms from onset was 5.6 months (range, 0.03–60.80 months). The most frequent symptom was otorrhea (55.3%). There were 38 treatment regimens applied. The most common medications used were topical antibiotics with steroids (28.11%), topical antibiotics (24.91%), and diluted vinegar (17.08%). There was no significant difference in the curative rate between these regimens ($p = 0.261$).

Conclusion: Granular myringitis is a poorly understood condition and there is no standard treatment regimen. While there is a great variation in the treatment of granular myringitis at Siriraj Hospital, this retrospective review showed there was no statistical significant difference among the different regimens. Further high-value research is needed to further assess the management strategies.

Keywords: Granular myringitis; chronic myringitis (Siriraj Med J 2020; 72: 502-507)

INTRODUCTION

Granular myringitis is characterized by a chronic inflammation of the tympanic membrane leading to the replacement of its epithelial surface and, occasionally, adjacent deep meatal skin with proliferating granulation tissue[1] (Fig 1). It is possible that this condition may have been underdiagnosed in the past due to the low magnification power available under physical examination by using an otoscope instead of a microscope. Moreover, it may have been misdiagnosed as otitis externa or chronic otitis media, in which case the same treatment would help to improve myringitis as well. Although granular myringitis is a minor illness, it can lead to several complications, such as tympanic membrane perforation, post-inflammatory canal fibrosis, or atresia.[2,3] Patients usually seek treatment because of their annoying symptoms; for example, malodorous otorrhea, intrameatal itching, feeling aural fullness, and earache.[4]

Corresponding author: Suvajana Atipas
E-mail: grsat2@gmail.com
Received 20 October 2017 Revised 14 August 2020 Accepted 15 August 2020
ORCID ID: http://orcid.org/0000-0002-5760-1339
http://dx.doi.org/10.33192/Smj.2020.68

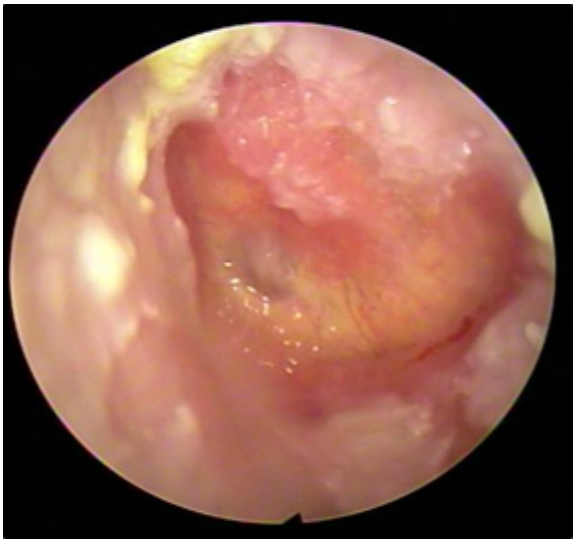


Fig 1. Left granular myringitis - the granulation tissue presents on the surface of the tympanic membrane with associated inflammatory changes to the external auditory canal epithelium.

At present, the etiology of this condition is unclear. It may be an infectious process, inflammation, or local trauma. Clinicians can treat this condition with topical ear drops, which are topical antibiotics with/or without steroid drops, or diluted vinegar solution.[5] Some also use a LASER or other chemical substances, and surgical intervention has even been reported in the previous literature.[6] Recently, the medication of choice has normally depended on the causative organism identified by tissue/fluid cultures, which typically are bacteria or fungi.[4,7] These organisms grow best in a narrow pH range, i.e., from pH 6.5 to 7.5.[8] So, the management of various types of external otitis is to maintain the acidity of the external canal to prevent their growth. In order to acidify the ear canal for treatment involving draining the ear, acetic acid and Burow's solution have long been successfully used.[9,10] As in folk medicine, vinegar can be used to manage external otitis with good effect.[11] It is possible that the acidification of the external ear has a good effect on granular diseases of the tympanic membrane by preventing bacterial growth and the stimulation of squamous re-epithelialization. However, a standard effective regimen for granular myringitis is lacking and remains in need.

In this study, we reviewed the records of patients diagnosed with myringitis at Siriraj Hospital, as a supertertiary care hospital, during 2014-2016. The objective of this review was to determine the optimal management for the treatment and prevention of the recurrence of granular myringitis that could later be applied in clinical practice.

MATERIALS AND METHODS

The research was approved by the Institutional Review Board of the Faculty of Medicine Siriraj Hospital, Mahidol University (Si 728/2559).

The clinical data of 115 patients (34 males and 81 females) diagnosed with myringitis based on ICD-10 code (H73) at the Department of Otorhinolaryngology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand, between September 1, 2014, and September 30, 2016, were retrospectively reviewed.

The exclusion criteria were patients who were lost to follow-up after the first visit and patients who were diagnosed with other diseases, such as bullous myringitis. Patient information and data, including age, gender, underlying diseases, history of ear diseases and surgeries, affected side, symptoms, duration, investigations, type of treatment, outcome, total follow-up time, complications, and recurrence rate, were recorded.

The follow-up outcomes were categorized into 4 grades: "Resolved," when an intact completely epithelialized TM without otorrhea, moistness, or subjective complaints was documented; "Improved," when there was a marked improvement in both the physical signs and subjective symptoms; "Unchanged," when there was little or no improvement of the TM appearance or symptoms; or "Worse," when the subjective symptoms had an exaggerated or increased otorrhea, or the myringitis or granulation tissue occupied a larger area of the TM. We classified resolved and improved as "good outcomes" and unchanged and worse as "poor outcomes."

Statistical analysis

PASW Statistics (SPSS) version 18.0 (SPSS Inc., Chicago, IL., USA) was used for the statistical analysis. Quantitative data are reported herein as the mean +/- SD and qualitative data are reported as the number of patients and as percentages. The Chi-square test was used to compare categorical data. A probability (p) value of less than 0.05 was considered statistically significant.

RESULTS

Demographic data

Between September 1, 2014, and September 30, 2016, 115 patients were identified with a diagnosis of myringitis based on ICD10 (H73) and considered for inclusion in the study; however, 19 patients were excluded as they were also diagnosed with other diseases too, such as bullous myringitis, acute otitis media, or mastoiditis, leaving 96 patients included in the study.

The 96 patients included in the study comprised 27 men (28.10%) and 69 women (71.90%), aged from

3 to 90 years old (mean, 52.88 years old). The right ear was affected in 50 patients (52.10%), the left in 36 (37.50%), and both in 10 (10.40%). Sixty-two patients (64.60%) were diagnosed by otologic staff, while 34 patients (35.40%) were diagnosed by non-otologic staff and residents. The average total follow-up time was 31.2 weeks (range, 0.86–160.86 weeks). The average duration of symptoms was 5.6 months (range, 0.03–60.8 months). The presenting symptoms varied, including otorrhea in 47 patients (55.30%), aural fullness in 40 patients (47.10%), itching in 17 patients (20.00%), earache in 22 patients (25.90%), and tinnitus in 12 patients (14.10%). Recurrence was noted in 12.5% of patients. None of the patients had any complications, such as ear canal stenosis post treatment.

Many patients had associated medical illness; for example, allergic and/or chronic rhinitis (25.00%), hypertension (25.00%), dyslipidemia (18.80%), diabetes mellitus (15.60%), and chronic kidney disease (4.20%).

Also, 43 patients (44.79%) had a history of other ear diseases, such as closed TM perforation (13.54%), TM perforation (12.50%), otitis externa (9.38%), otomycosis (6.25%), otitis media (1.04%), impact cerumen (1.04%), and bilateral superior canal dehiscence (1.04%). The patient who had bilateral superior canal dehiscence developed granular myringitis after myringotomy with a pressure equalization tube (PE tube) placement.

Fifteen patients (15.63%) had a previous TM perforation prior to the diagnosis of granular myringitis. Thirteen patients (13.54%) underwent tympanoplasty and 2 patients (2.08%) underwent a paper patch. Two patients (2.08%) had myringotomy and a PE tube placement and 1 patient (1.04%) had a history of ear wax removal. The duration between otologic procedures and the onset of granular myringitis ranged from 0.53 to 361.33 months. (median, 68.90 months)

Outcome

The total number of visits was 397 visits, involving 96 patients. However, 116 of those visits involved patients ultimately lost to follow-up. Thus, the outcomes of only 281 visits were evaluated. In this review, there were 38 treatment regimens. We categorized these into 4 groups: no treatment (7.47%), single topical ear drop treatment (40.57%) or adjuvant treatment (11.74%), topical ear drop with adjuvant treatment (37.37%), and surgery (2.85%). Adjuvant treatments noted in the literature included antiseptic solutions, chemical cauterization, oral antifungal, oral antibiotics, and intravenous antibiotics. (Tables 2 and 3)

TABLE 1. Demographic data and clinical characteristics of the 96 patients reviewed in detail in the study.

	Number (%) or Mean (Min, Max)
Sex	
Female	69 (71.90)
Male	27 (28.10)
Age (year)	52.88 ± 19.24 (3,90)
Affected side	
Bilateral	10 (10.40)
Right	50 (52.10)
Left	36 (37.50)
Duration of symptoms (month)	5.6 ± 13.69 (0.03 - 60.8)
Total follow-up time (month)	3.18 ± 9.93 (0.22 - 40.22)
Clinician	
Staff	
Otologist	62 (64.60)
Non-otologist	20 (20.80)
Resident	14 (14.60)
Recurrence	
Yes	12 (12.50)
No	55 (57.30)
Could not be evaluated	29 (30.20)

TABLE 2. Topical ear drops.

	Visit (n = 218)	Outcome	
		Poor (%) (n = 67)	Good (%) (n=151)
Antibiotic	70	24 (34.28)	46 (65.71)
Alone	38	15 (39.47)	23 (60.52)
With adjuvant treatment	32	9 (28.13)	23 (71.87)
Antibiotic with steroid	79	25 (31.65)	54 (68.35)
Alone	48	16 (33.33)	32 (66.67)
With adjuvant treatment	31	9 (29.03)	22 (70.97)
Diluted vinegar	48	11 (22.92)	37 (77.08)
Alone	18	5 (27.78)	13 (72.22)
With adjuvant treatment	30	6 (20.00)	24 (80.00)
Antifungal	18	5 (27.78)	13 (72.22)
Alone	7	1 (14.29)	6 (85.71)
With adjuvant treatment	11	4 (36.36)	7 (63.63)
2.5% NaHCO3 in glycerine	3	2 (66.67)	1 (33.33)
Alone	3	2 (66.67)	1 (33.33)
With adjuvant treatment	0	0 (0.00)	0 (0.00)

TABLE 3. Adjuvant treatments.

	Visit (n = 154)	Outcome	
		Poor (%) (n = 43)	Good (%) (n=111)
Topical antiseptic	65	15 (23.08)	50 (76.92)
Alone	23	5 (21.74)	18 (78.26)
With adjuvant treatment	42	10 (23.81)	32 (76.19)
Cauterization	30	7 (23.33)	23 (76.67)
Alone	5	2 (40.00)	3 (60.00)
With adjuvant treatment	25	5 (20.00)	20 (80.00)
Oral antibiotic	51	18 (35.29)	33 (64.71)
Alone	5	2 (40.00)	3 (60.00)
With adjuvant treatment	46	16 (34.78)	30 (65.22)
Oral antifungal	7	3 (42.86)	4 (57.14)
Alone	0	0 (0.00)	0 (0.00)
With adjuvant treatment	7	3 (42.86)	4 (57.14)
IV antibiotic	1	0 (0.00)	1 (100.00)
Alone	0	0 (0.00)	0 (0.00)
With adjuvant treatment	1	0 (0.00)	1 (100.00)

The most common topical ear drops used to treat granular myringitis included topical antibiotics with steroids (28.11%), topical antibiotics (24.91%), and diluted vinegar (17.08%). The adjuvant treatments most frequently used were antiseptics (23.13%), oral antibiotics (18.15%), and cauterization (10.68%). Diluted vinegar was only used by otologists in 48 visits: 37.50% as a single treatment and 62.50% combined with another treatment.

The outcomes of each treatment regimen were compared using Chi-square test. The result showed no statistical significant difference between outcomes among these regimens ($p = 0.261$).

DISCUSSION

Between 2014 and 2016, 115 patients were diagnosed with granular myringitis at the Department of Otorhinolaryngology, Faculty of Medicine, Siriraj Hospital, of whom 96 were included in the present study. The prevalence of granular myringitis was higher in women (71.90%) than in men (28.10%), with a 2.6-fold greater prevalence in women. The most frequent presenting symptom was an otorrhea. Other symptoms recorded include a sensation of fullness, mild pain, or itching in the ear. Recurrence was noted in 12.50% of patients and some were affected bilaterally.

A predisposing health condition may not be associated with granular myringitis according to the small percentage of each underlying condition demonstrated in this study. Generally, injury to the epithelial layer and exposure of the fibrous middle layer causes the formation of granulation tissue in an attempt to heal the defect.[4] Schuknecht described the focal or diffuse replacement of the dermis of the tympanic membrane and adjacent canal wall with granulation tissue as a characteristic of granular myringitis.[12] Blevins and Karmody reported that 60% of patients, both adults and children, had undergone previous otologic procedures.[13] In this study, only 18.75% of patients had undergone an otologic procedure prior to the development of granular myringitis, in which the duration between the otologic procedure and the onset of symptoms ranged from 0.53 to 361.33 months (mean, 68.90 months). This percentage was lower than in previous studies. This was possibly because the average duration was longer than the healing process of the tympanic membrane, so it seems like the healed surgical site was similar to being in a normal condition.

To date, granular myringitis remains a poorly understood condition and the proposed treatments are quite variable. A 2008 systemic review of the management of granular myringitis concluded that there was insufficient high-quality evidence to support any particular management plan or treatment protocol for patients with granular myringitis.[14] In this review, there were many different treatment regimens for granular myringitis at Siriraj Hospital. After meticulous aural toilet, topical antibiotics with steroid drops and topical antibiotic drops were the most frequently used in this study, often combined with another adjuvant treatment, such as a topical antiseptic, oral antibiotic, and chemical cauterization. Diluted vinegar was also used but only by otologists and the results showed a good outcome in 77.10% of cases. Surgical interventions were limited in use, and involved only 2.85% of cases that had failed to respond to medical treatment, but led to a high success rate (87.5%). All the surgical interventions in this study were myringoplasty. However, there was no significant difference in the success rate between each treatment group.

The limitation in this study was due to a small number of patients. A further study of longer data collecting period could yield significant outcome. A study of randomized controlled trial utilizing a larger population is suggested.

CONCLUSION

Granular myringitis is a poorly understood condition and there is no standard effective treatment regimen. Regarding our results, topical ear drops, such as antibiotic ear drops with or without a steroid, or diluted vinegar, showed fair outcomes in the treatment with an about 60–80% success rate, without any statistical significant difference among these regimens. Hence, diluted vinegar solution might be considered as another option for patients with granular myringitis because it is less likely to cause bacterial resistance and also as it is safe, low-cost, and has high availability despite the unfamiliarity with its use among many general practitioners. However, further high-value research (i.e., randomized controlled trials) is needed to further assess and identify the most appropriate management strategies that could both resolve granular myringitis and prevent its recurrence.

ACKNOWLEDGMENTS

We gratefully appreciate the kind contributions of the consulting statistician Miss Julaporn Pooliam as well as the research assistants (Ms. Jeerapa Kerdnoppakhun, Miss Ngamrat Treerassapanich) and all the patients who were involved in this project.

Disclosure of the presence of financial support

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

Conflicts of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Gulya AJ, Minor LB, Glasscock ME, Poe D. Glasscock-Shambaugh Surgery of the Ear: People's Medical Publishing House-USA; 2010.
2. Lavy J, Fagan P. Chronic stenosing external otitis/postinflammatory acquired atresia: a review. Clin Otolaryngol Allied Sci 2000;25: 435-9.
3. Slattery WH 3rd, Saadat P. Postinflammatory medial canal fibrosis. Am J Otol 1997;18:294-7.
4. Stoney P, Kwok P, Hawke M. Granular myringitis: a review. J Otolaryngol 1992;21:129-35.
5. Jung HH, Cho SD, Yoo CK, Lim HH, Chae SW. Vinegar treatment in the management of granular myringitis. J Laryngol Otol 2002;116:176-80.
6. El-Seifi A, Fouad B. Granular myringitis: is it a surgical problem? Am J Otol 2000;21:462-7.
7. Kunachak S. Intractable granular myringitis: possible etiology and management. J Otolaryngol 1992;21:297-8.
8. Aminifarshidmehr N. The management of chronic suppurative otitis media with acid media solution. Am J Otol 1996;17:24-5.
9. Smathers CR. Chemical treatment of external otitis. South Med J 1977;70:543-5.
10. Thorp MA, Kruger J, Oliver S, Nilssen EL, Prescott CA. The antibacterial activity of acetic acid and Burow's solution as topical otological preparations. J Laryngol Otol 1998;112:925-8.
11. Flint PW, Haughey BH, Niparko JK, Richardson MA, Lund VJ, Robbins KT, et al. Cummings Otolaryngology - Head and Neck Surgery: Head and Neck Surgery, 3-Volume Set: Elsevier Health Sciences; 2010.
12. Schuknecht HF. Infections. In: Schuknecht HF, ed. Pathology of the ear, 2nd eds. Cambridge, MA: Harvard University Press; 1974.
13. Blevins NH, Karmody CS. Chronic myringitis: prevalence, presentation, and natural history. Otol Neurotol 2001;22:3-10.
14. Neilson LJ, Hussain SS. Management of granular myringitis: a systemic review. J Laryngol Otol 2008;122:3-10.

Social Transmission of Corona Virus: An Overview

Angurbala Mishra, MBA.*, Subhasmita Biswal, Ph.D.*, Somadatta Das, MA., Rabindra Nath Padhy, Ph.D.**, Bibhuti Bhusan Pradhan, Ph.D.*****

**Department of Humanities and Social Sciences, Institute of Technical Education and Research, Siksha 'O' Anusandhan Deemed to be University, Bhubaneswar-751030, Odisha, India. **Central Research Laboratory, IMS and SUM hospital, Siksha 'O' Anusandhan Deemed to be University, Bhubaneswar-751003, Odisha, India. ***Siksha 'O' Anusandhan Deemed to be University, Bhubaneswar-751003, Odisha, India.*

ABSTRACT

Corona virus (COVID-19) causes an infectious disease of the upper respiratory tract, with terminating illness of failure of lungs. Being a viral disease, no ensconced drug for control is known and it is picked up by a new host the man as droplets in air released by a patient. The virus has been detected in 215 countries and World Health Organization (WHO) declared as an epidemic and pandemic. The common structures of social contact critically determine the spread of the infection from a patient; in the dearth of vaccines, the control of the virus has been its prevention of spread through systematic 'social distancing measures'. Furthermore, the use of cotton facemask in public places and while dealing with one has been the robust method of prevention of spread of the virus from sneezing or talking with anyone. It was seen from January to April 2020 counties followed social distancing procedures without touching; hand-shaking or hugging with any one had least number of COVID-19 affected persons. Hand washing with soap-water frequently to ward off any viral contamination obtained from touches of fomite or furniture etc., is rigorously followed, city disinfection with bleaching water has been additional practice in societies to abate infection from spits from patients in the common spaces. Moreover, the described types of social distancing framework adopted by some countries without additional measures prevented super-infection of the virus in societies. Finally, the findings have important implications for the policy making to be adopted globally as well as, individual-scale preventive methods.

Keywords: COVID-19; social distancing; social transmission; pandemic (Siriraj Med J 2020; 72: 508-511)

INTRODUCTION

The present situation with human corona virus COVID-19 all over, as an emerging infectious virus, causes a major staggering respiratory morbidity for 8-10 days with eventual mortality, worldwide.[1] The virus constantly demonstrates a rising patterns of society-transmission at several Indian cities, despitean effective social isolation (lockdown) through the country. WHO has not recommended any particular drug against COVID-19; in the absence of any emulative drug, social distancing and self-isolation in homes have the most effective steps for the control of the COVID-19 pandemic.[2] Prevention of social contacts in schools/colleges, workplaces and public spheres are the means of control measures. Droplets from nose and mouth serve as the vehicles of transmission of the virus from an infected individual to one and all nearby, within proximity of one to two meters; social interactions within a set of empirical variables including the most basic human behaviour patterns, as, coughing, sneezing, hand-haking, touching and sharing of objects

Corresponding author: Rabindra Nath Padhy
E-mail: rnpadhy54@gmail.com
Received 17 July 2020 Revised 2 September 2020 Accepted 3 September 2020
ORCID ID: http://orcid.org/0000-0002-2522-9843
http://dx.doi.org/10.33192/Smj.2020.69

with some individual any object and fomite.[3-6] Other previous fatal viral infections of upper respiratory tract leading to the fatal lungs infections were due to Severe Acute Respiratory Syndrome (SARS) virus and Middle East Respiratory Syndrome (MERS) virus. These two human viral pathogens had not been seen in India. However, COVID-19 is another fatal virus belonging to "Corona" group of viruses, identified in China in 2019 causing respiratory infections ranging from the common cold to severe disease of lungs, contaminated through air.[7,8] The most common symptoms of COVID-19 are fever, tiredness, shivering, headache, muscle pains, dry cough and gastrointestinal disorders.[8] Sometimes, infection remains cryptic without any kind of visible symptoms, which would be manifested in immune-compromised individuals. Any individual with chronic comorbidities stemming from inherent diabetes or problems of some innards such as, heart, brain liver or kidney, blood pressure, coronary artery disease and a few more other serious illness under some active medication with any mainstream drug(s) develop expression of the fatal lungs infection. Those individuals need hospitalization of uninterrupted supply of oxygen in ICUs even. Thus COVID-19 affects much the elderly senior citizens mush more affect older people, who suffer from medical comorbidities like diabetes, chronic kidney disease or inflammatory lung disease, etc.

Theoretical Framework

The COVID-19 wave influences all sections of the community and is especially harmful to the elderly people who are in the most vulnerable section for virus spread; people of any age group with comorbidities, such as, hearth diseases diabetes, hypercholesterolemia, blood pressure problems, renal of liver problems, and a few more are too vulnerable to infection. Thus, inter-personal or social distance is to be maintained everywhere; consequently. Social gathering for ceremonies, market places, railway stations and bus-stands, etc. to cite for example, are prohibited by government. Maintenance of social distance at workplaces, industries, construction-sites being prevented adversely influence the individual and national economy[7], with concomitant community preservations from viral infections at work place, help men serving as automated moderators. Social distancing is obligatory to minimize the spread of the disease; if not executed accurately, spread would be fast. The expanded social isolation leads to lack of communication with senior citizens who may need support.[8] Government have called on the youth to cuddle the attempt to preserve themselves and overall citizens. By the by, youths help those, who are most vulnerable and enhance social awareness on health in societies. Therefore, youths are critical to limiting the virus spread; consequently, youths influence public health, society and the economy at large.

The first point of precaution is the spreading of virus controlling information in local languages, thus ensuring that services and facilities are appropriate to the specific situation of local people. The large number of local peoples disobeying the specific standard procedure of social precautionary system remains vulnerable, especially if they are dependent on the income from the broader economy-produces, tourism, handicrafts and similar employments in urban areas. Social and physical distancing helps low down of virus spread by causing moratorium in infection chain growths.[7] Physical distance between people, and hand washes with soap minimize contact with contaminated fomite/ furniture/ surfaces, and help escape from infection.[7] Maneuvers for the common people comprise initiating easy going work arrangements like, work at home, e-learning, minimizing and preventing crowdedness, shut down of non-essential facilities and services, which safe guard and prevent virus spread. The adopted government procedure of prevention of going out for some trivial purposes and avoiding crowds for obtaining essential home requirements is restricted. The measures are used in with individual protective methods against COVID-19, such asfrequent hand-washing and the gentle-manly cough-etiquette or using cotton face mask.

All public health maneuvers to cease the spread of disease can be stabilized with easily moulded plan of actions to inspire endurance by society and social connection, governmental protection of incomes and secured the food supply. Countries should stabilize the possible benefits and help eliminate negative outcomes of each obstruction, and dispose plan of actions to stimulate petty community-engagements, acquire mutual trust and limit social harm in the economic sphere. Many action plans can be implemented for community endurance stimulating mental health, by with physical access to essential and non-essential goods and semi-technical services, and limit the economic damage of stay at home maneuvers, where these are considered inevitable. Work at home and distance learning plan of actions in divergent situations exhibit transformation and the role of technology in supporting business continuity and sustaining social connections within 'authority and individuals', 'teachers and students', as well as, 'families and communities'. In a broader sense, enforcement of distancing maneuvers intent to profit from personal and professional community connections by widely accessible means with mobile

phones and computers. E-conferences are and to be conducted across international platforms on medical and technological themes.

In parallel, there were suspected cases of COVID-19 that were to be tested and cases were promptly isolated to newly organised COVID-hospitals; and the particular zones were declared as 'containment zones'with imposed restrictions of movements into or from it. Previous contact tracing is done, and home-quarantification of contact peopleis seriously advised in containment zones for the duration of the incubation period of 14 to 21 days. This is almost universal in counties where prevention is taken as the main task than treatment of COVID-19 patients. Isolation manoeuvre in a society makes the task of contact-tracing of identified easier since the number of infected people rapidly in ceases, by the by, infection case numbers sharply declines. As social measures would be lifted, case-finding, isolation for COVID-19 cases and quarantine of contacts, to respond categorically to resurgent or imported cases shall be emphasised. Reorganization of health and social services should continue to work in coordination to assess and test patient by rapid tests for sue treatments, to minimise least virus spread. WHO describes four levels of COVID-19 transmission (1) no reported case (2) sporadic cases (3) clusters of cases, and (4) community transmission.

COVID-19 spreads through droplets and mucus from a patient, who might not have been hospitalised; thus, droplets and mucus are released. Droplets are very small and remain suspended in air for a while. With a deep breath A typical cough begins during which, lungs eject air in a compressed form with thrust with crackles. A person blows out around one-and-a-half-litre of air in one cough. Moreover, when one coughs or sneezes, saliva contaminated with viruses comes out. Around 3,000 droplets of saliva come out in a single cough; droplets of saliva travel at the speed of up to 80 kmph. At such a speed, one should not be in the range when COVID-19 is pandemic everywhere. Each droplet of saliva contains around 20lakh virus particles. Even if a few of those reach a healthy person's respiratory tract, the particles colonise the person's body and multiply to billions in 6-7 days. The infected individual would start showing symptoms of Covid-19 at end of a week after infection.

Preventive Measures

Some protective advice followed by the local public health agency for protect oneself and others around, knowing the facts and taking proper precautions to avoid the infections of COVID-19:

1. If one is going outside home use of mask is mandatory.
2. Reaching home hands must be cleaned using soap/ hand wash liquid or alcohol-based hand rub.
3. Maintain a safe distance of one to 1.5 m as interpersonal distance mandatory and coughing or sneezing must be done with etiquette.
4. Don't touch your Eyes, nose or mouth need be was he often beforehand.
5. Cover your nose and mouth with your bent elbow or a tissue when you cough or sneeze.
6. Stay home if you feel unwell.
7. Medical attention if showing any kind symptoms such as fever, a cough and difficulty in breathing,
8. Follow the directions of your local health authority.

Avoiding unneeded visits to medical facilities allows healthcare systems to operate more effectively, therefore protecting you and others.

CONCLUSION

In this study, COVID-19 preventive measures were theoretically and empirically taken into account. It is evident that the reproductive patterns of the virus are tightly linked with human social behaviour. Therefore, preventive policies, measures and individual behaviour need be suiting to virus spread. Social isolation and availability of COVID-19 confirmation test facility are mandatory for any country, since those are the most reliable and convergent forms of obtaining solutions to reduce community transmission of virus. Concerning transmission check observed in China and South Korea the other super-spreading patterns observed were the full convergence of nonlinear variables with the adoption of social isolation, COVID-19 confirmation tests availability and the social distancing methods of 1 to 2 meters physical distance with the additional use of masks use and sanitization (city disinfection). Other important points for use of masks were religiously followed; social distancing measures with the use of masks and city disinfection helped preventing dissemination. The isolation of the undetected infected hosts (asymptomatic cases) and prevention of airborne transmission were done. Thus, policy actions are needed to include strategic measures to minimise the natural community transmission to achieve efficiency are, social distancing measures, the use of masks and disinfection measures at public spaces. Dearth of masks and financial costs for disinfection of vulnerable areas, might be limiting factors. The individual dimension of prevention (citizen collaboration and

support) is mandatory for governmental actions to result in community prevention stability. Global efforts from all countries are necessary to help control community infections everywhere by disallowing air travels.

Conflict of interest

Each of the authors has contributed to read and approved this manuscript. None of the authors has any conflict of interest, financial or otherwise.

REFERENCES

1. Sun P, Lu X, Xu C, Sun W, Pan B. Understanding of COVID-19 based on current evidence. J Med Virol 2020;92:548-51.
2. Ferguson NM, Laydon D, Nedjati-Gilani G, Imai N, Ainslie K, Baguelin M, et al. Impact of non-pharmaceutical interventions (NPIs) to reduce COVID-19 mortality and healthcare demand. Imperial College COVID-19 Response Team; 19 March 2020. Available from: https://doi.org/10.25561/77482.
3. Global Surveillance for human infection with novel coronavirus (2019-nCoV): interim guidance, 31 January 2020. World Health Organization; 2020.
4. Rational use of personal protective equipment for coronavirus disease (COVID-19) and considerations during severe shortages. Interim guidance; 6 April 2020. World Health Organization; 2020.
5. Coronavirus disease 2019 (COVID-19) Situation Report – 68. WHO situation report; 28 March 2020. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports
6. Isolation Precautions: Personal Protective Equipment. Elsevier Clinical Skills. GN_08_2. Available from: https://www.elsevier.com/_data/assets/pdf_file/0004/974623/Isolation-Precautions_Personal-Protective-Equipment.pdf
7. Centers for Disease Control and Prevention (CDCP) (2020). www.cdc.gov
8. World Health Organization (2020). www.who.int. 20 April 2020.

miRNAs: Perspective Towards the Use for Body Fluid Identification

Pairoa Praihirunkit, Ph.D.

Department of Forensic Science, Faculty of Allied Health Sciences, Thammasat University, Prathumthani 12121, Thailand.

ABSTRACT

Identification of body fluids provides an important lead for crime investigation by which it can give a clue about the nature of the case and assist crime reconstruction. In the last decade, miRNAs have emerged as promising markers for body fluid identification due to their cell- /tissue-specificities. miRNAs are a class of small noncoding RNAs with ~ 22 nucleotides in length and their small sizes enable them to be resistant to degradation. The possibility to adopt miRNA markers for body fluid identification has been studied in various forensically relevant body fluids. This review aims to give a comprehensive summary of proposed miRNA markers for identifying five body fluids (venous blood, menstrual blood, semen, vaginal secretion and saliva). Based on numerous evaluations of miRNA markers and the development of model analysis using a single panel of miRNAs to identify unknown samples, proposed panels and analysis strategies were gathered and discussed.

Keywords: miRNA; forensically relevant body fluids; body fluid identification; crime; forensic casework (Siriraj Med J 2020; 72: 512-526)

INTRODUCTION

miRNAs are a class of small non-coding RNAs with ~ 22 nucleotides in length that have been found in organisms ranging from viruses, plants, invertebrates, vertebrates to humans as demonstrated in the latest release of miRBase (v22), the online database of miRNA sequences which contains 48,860 miRNA sequences from 271 organisms.[1] It is known that miRNAs regulate gene expression within cells they are generated and they can be secreted into the extracellular space for regulating other cells or for cell-to-cell communication.[2,3] Following physiological activities of the cells and cell death, miRNAs are non-specifically released from cells.[4,5] These extracellular miRNAs have been found in vesicle-like molecules such as exosomes, microvesicles and apoptotic bodies[2,6], whereas some are associated with proteins, particularly AGO2.[4,6,7] This allows miRNAs to be shielded from RNAase degradation and to increase their stability in biological fluids.[4]

Studies on expression profiles of miRNAs revealed that they are differentially expressed in each cell type. In human cells, the Functional Annotation of the Mammalian Genome (FANTOM5) consortium has recently demonstrated that miRNAs are differentially expressed in 121 distinct cell types with the top five expressed miRNAs accounting for ~50% of the overall miRNA in each cell type.[8] These expression patterns of miRNAs suggest that miRNAs could be promising markers for cell type and tissue identification. For body fluid identification, miRNAs have been extensively studied in five forensically relevant body fluids: venous blood,

Corresponding author: Pairoa Praihirunkit
E-mail: pairoa.prai@gmail.com
Received 10 June 2020 Revised 22 July 2020 Accepted 8 August 2020
ORCID ID: http://orcid.org/0000-0002-3202-5240
http://dx.doi.org/10.33192/Smj.2020.70

menstrual blood, semen, vaginal secretion and saliva as previously reviewed in.[9-13] The first review by Courts C. *et al*. mentioned panels of miRNAs specific for the five body fluids from two publications that opened up the view of applying miRNAs for body fluid identification.[12] Subsequent reviews added more recent miRNA markers and covered issues of miRNA profiling methodologies, factors influencing miRNA expression[9], standardisation of analysis procedures (methods to isolate and quantify miRNAs)[10] and the potential use of miRNA in clinical conditions.[11] The present review aims to provide up-to-date work focusing on the five body fluids: venous blood, menstrual blood, semen, vaginal secretion and saliva that are relevant to violent and sexual assaults. The presence of venous blood is generally related to injuries and can be an indicator of offensive scenarios. Significant evidence in sexual assaults includes semen, vaginal secretion, menstrual blood and saliva. This review summarises and discusses model analyses consisting of proposed panels of miRNA to distinguish one kind of body fluids from other forensically relevant body fluids.

miRNA markers in venous and menstrual blood

Discrimination between venous blood and menstrual blood could give a clue about the nature of the case and assist crime reconstruction whether it associates with violent and/or sexual assaults. The presence of venous blood is related to violent assaults causing injury or death. Menstrual blood could be involved in sexual assaults in a certain case. For instance, a woman who is sexually assaulted by a man during her menstruation can transfer the menstrual blood on the suspect's penis. Hanson E.K. *et al*. were the first to propose certain miRNA markers for identifying the forensically relevant body fluids including venous and menstrual blood. Using quantitative real-time polymerase chain reaction (qRT-PCR) as a miRNA profiling and validation method, they demonstrated that miR-451 and miR-16 were differentially expressed in venous blood compared to menstrual blood, semen, vaginal secretion and saliva. In line with this, several studies have shown that miR-451 and miR-16 were specific to venous blood and could be potential markers for venous blood identification[14-21] (Table 1). Based on a number of independent studies using qRT-PCR or microarray for miRNA profiling followed by validation of miRNA expression, an array of potential markers was proposed and repeatedly identified in several studies.[15,17,22-25] With the advance of technologies, more recent studies have conducted massively parallel sequencing (MPS) or next-generation sequencing (NGS) for screening miRNAs at the genome-wide level which allows determination of miRNA quantity and expression patterns. Hence, this enables the discovery of novel miRNAs in biological samples. Wang Z. *et al*. investigated the expression of miRNAs in venous blood using the Ion Personal Genome Machine™ (PGM™) System with the capacity to detect 2,588 annotated mature miRNAs in the human genome (miRBase v21).[18] The authors obtained a miRNA profile and ranked the most abundant miRNAs to search for potential miRNA markers. They showed the top six miRNAs in the blood samples to be miR-486-5p, miR-16-5p, miR-451a, miR-144-3p, miR-126-5p and miR-144-5p. The first four of which had been reported in previous studies as summarised in Table 1. In addition, a study used Illumina HiSeq 4000 to examine the miRNA expression in venous blood.[19] Following analyses of the data, the study identified the top 20 highly expressed miRNAs in which four of them were consistent with previous reports: miR-486-5p, miR-451a, miR-182-5p and miR-16-5p. More recently, a study on miRNA profiling in the venous blood using MPS and validation of miRNA expression via qRT-PCR has shown miR-451a, miR-486-5p, let-7i-5p, let-7b-5p and miR-92a-3p as the five most abundant miRNAs.[21] Strikingly, these miRNAs overlapped with the highly-expressed miRNAs reported by Wang Z. *et al*.[18] and El-Mogy M. *et al*.[19] Altogether, the accumulating data highlight a panel of miRNAs repeatedly found in venous blood and the analysis of such a panel, instead of individual miRNAs, could increase the specificity of venous blood identification. However, it is worth noting that some venous blood-specific miRNAs: miR-451, miR-185-3p and miR-144-3p have also been detected at an equivalent level in menstrual blood. This suggests that the application of miRNA markers for discrimination between venous blood and menstrual blood requires an analysis model in which a threshold or cutoff value is set up to assist the result interpretation.

miRNA profiling in menstrual blood is less extensively studied compared to studies on venous blood. Based on the relevant studies in Table 1, it was suggested that miR-144-3p could be a potential marker for menstrual blood; however, this miRNA was also highly expressed in venous blood.[22,23,25] Particularly, miRNA profiling in venous blood in healthy women during the menstrual cycle revealed that miR-144-3p was the second most abundant miRNA.[15] This underscores that miR-144-3p is commonly found in blood-type samples and its expression is independent of physiological changes during the menstrual cycle. Thus, the appropriate approach to differentiate menstrual blood from venous blood could be the establishment of a decision algorithm comprising miR-144-3p and additional miRNAs, yielding a score to

TABLE 2. Proposed panels of miRNA and model analyses to distinguish body fluids.

Groups	miRNAs in the panel	Types of model analysis	Model analyses	Accuracy/testing of the model	Experimental methods/reference RNA
1. Hanson E.K. *et al.*, 2009 [32]	- Venous blood: miR-451 and miR-16 - Menstrual blood: miR-451 and miR-412 - Semen: miR-135b and miR-10b - Vaginal secretion: miR-124a and miR-372 - Saliva: miR-658 and miR-205	2D scatter plots consisting of dCt of two markers in each type of body fluid	Identify unknown samples by positioning dCt on 2D scatter containing clusters of known samples	100% accuracy for venous blood, semen and saliva; ~90% accuracy for vaginal secretion; ~80% accuracy for menstrual blood	SYBR Green qRT-PCR/U6b
2. Hanson E.K. *et al.*, 2014 [23]	- Menstrual blood: miR-185-5p, miR-144-3p and miR-144-5p	Logistic model	Logit = -(0.005*dCt(miR-185-5p)*dCt (miR-144-3p)* dCt(miR-144-5p)) + (3.718*dCt(miR-185-5p)) – 32.017 Menstrual blood if p>0.5 (Chi-square)	100% accuracy (12 blinded samples tested)	SYBR Green qRT-PCR/miR-940
3. Wang Z. *et al.*, 2015 [34]	- Menstrual blood vaginal secretion and saliva: miR-205-5p - Menstrual blood: miR-214-3p - Saliva and menstrual blood: miR-203a - Semen: miR-891a	Stepwise strategy to identify saliva	Identify saliva using a combination of miRNAs to distinguish saliva from other body fluids	N/A	TaqMan qRT-PCR/ U6
4. Sauer E. *et al.*,2015 [33] 5. Sauer E. *et al.*,2016 [25]	- Semen: miR-891a - Venous and menstrual blood: miR-144-3p and miR-203a-3p - Vaginal secretion and saliva: miR-124-3p and miR-203a-3p	Decision algorithm	Started with quantification of miR-891a to differentiate semen. Non-semen samples were subsequently analysed using D = -1.504-0.127*dCt (miR-144-3p) +0.454*dCt (miR-203a-3p) Venous blood if D>0 and menstrual blood if D<0 D = -3.305-0.743*dCt (miR-203a-3p) +0.582*dCt (miR-124-3p) Saliva if D>0 and vaginal secretion if D<0	90% accuracy (9 out of 10 blinded samples were correctly identified.)	TaqMan qRT-PCR/ SNORD24, SNORD38B and SNORD43

TABLE 1. Summary of reported miRNAs in forensically relevant body fluids, analysed by miRNA profiling and/or validation. (continue)

Groups	Venous blood	Menstrual blood	Semen	Vaginal secretion	Saliva	Screening platforms	Validation methods/reference RNA	Evaluation of miRNA stability in stain samples
6. Rekker K. *et al.*, 2013 [15]	miR-451a* miR-144-3p* miR-16-5p* miR-15a-5p* miR-19b-3p miR-142-3p miR-486-5p miR-92a-3p* miR-20a-5p miR-223-3p miR-103a-3p miR-93-5p miR-106a-5p let-7g-5p* let-7b-5p*	N/A	N/A	N/A	N/A	qRT-PCR (Exiqon miRCURY LNA microRNA) Human panel I)	N/A	N/A
7. Wang Z. *et al.*, 2013 [17]	miR-486* miR-16*	miR-214	miR-888 miR-891a*	N/A	N/A	Microarray	TaqMan qRT-PCR/U6	- Bloodstains stored at RT, 1 month: No significant change in miR-486 and miR-16 expression compared to fresh samples - Menstrual bloodstains stored at RT, 1 month: No significant change in miR-214 expression compared to fresh samples - Semen stains stored at RT, 1 month: No significant change in miR-888 and miR-891 expression compared to fresh samples

TABLE 1. Summary of reported miRNAs in forensically relevant body fluids, analysed by miRNA profiling and/or validation. (continue)

Groups	Venous blood	Menstrual blood	Semen	Vaginal secretion	Saliva	Screening platforms	Validation methods/reference RNA	Evaluation of miRNA stability in stain samples
8. Hanson E.K. *et al.*, 2014 [23]	miR-185-5p* miR-144-3p*	miR-185-5p* miR-144-3p* miR-144-5p*	N/A	N/A	N/A	qRT-PCR	SYBR Green qRT-PCR/miR-940	N/A
9. Park J.L. *et al.*, 2014 [24]	miR-484 miR-182*	N/A	miR-2392 miR-3197	miR-1260b miR-654-5p	miR-223 miR-145	Microarray	SYBR Green qRT-PCR/U6	N/A
10. Sauer E. *et al.*, 2015 [33]	miR-144-3p*	miR-203a-3p*	miR-891a*	miR-124-3p	miR-203a*	N/A	TaqMan qRT-PCR/SNORD24, SNORD38B and SNORD43	N/A
11. Tong D. *et al.*, 2015[38]	N/A	N/A	miR-10b miR-135b	N/A	N/A	N/A	TaqMan qRT-PCR/U6b	- Semen stains stored at 25°C, 1 year: No significant change in miR-10b and miR-135b expression compared to stains at day 1
12. Wang Z. *et al.*, 2015 [34]	N/A	N/A	N/A	N/A	miR-200c-3p* miR-203a* miR-205-5p*	N/A	TaqMan qRT-PCR/U6	N/A
13. Sauer E. *et al.*, 2016 [25]	miR-144-3p*	miR-144-3p*	miR-891a* miR-10a miR-10b miR-135b	N/A	N/A	Microarray	TaqMan qRT-PCR/ SNORD24, SNORD38B and SNORD43	- Bloodstains stored at RT, 1 to 36 years: miR-144-3p was detectable at the level that yielded the correct fluid identification using the decision algorithm. - Semen stains stored at RT, 1 year: miR-891a was detectable at the level that yielded the correct fluid identification using the decision algorithm.

TABLE 1. Summary of reported miRNAs in forensically relevant body fluids, analysed by miRNA profiling and/or validation. (continue)

Groups	Venous blood	Menstrual blood	Semen	Vaginal secretion	Saliva	Screening platforms	Validation methods/reference RNA	Evaluation of miRNA stability in stain samples
14. Seashols-Williams S. *et al.*,2016 [28]	N/A	N/A	miR-891a*	N/A	miR-26b	Massively parallel sequencing (Illumina Hiseq)	SYBR Green qRT-PCR/Let7i and Let-7g	N/A
15. Wang Z.*et al.*, 2016 [18]	miR-486-5p* miR-16-5p* miR-451a* miR-144-3p* miR-126-5p miR-144-5p	N/A	N/A	N/A	miR-203a-3p* miR-205-5p* miR-223-3p miR-200c-3p* miR-141-3p miR-375* miR-34a-5p let-7c-5p miR-27b-3p* miR-125b-5p miR-23b-3p miR-99a-5p* miR-29a-3p miR-23a-3p miR-27a-3p miR-210-3p miR-24-3p* miR-29b-3p miR-22-3p	Massively parallel sequencing (Ion Personal Genome Machine 1 system)	N/A	N/A
16. Li Z. *et al.*, 2017 [39]	N/A	miR-141-3p miR-497-5p miR-143-5p	N/A	N/A	N/A	Microarray	SYBR Green and TaqMan qRT-PCR/U6	N/A

TABLE 1. Summary of reported miRNAs in forensically relevant body fluids, analysed by miRNA profiling and/or validation. (continue)

Groups	Venous blood	Menstrual blood	Semen	Vaginal secretion	Saliva	Screening platforms	Validation methods/reference RNA	Evaluation of miRNA stability in stain samples
17. Wang Z. *et al.*, 2017 [40]	N/A	N/A	miR-891a* miR-888* miR-429 miR-449a miR-34b miR-2392 miR-3197 miR-30a miR-196b	N/A	N/A	Massively parallel sequencing	N/A	N/A
18. El-Mogy M. *et al.*, 2018 [19]	miR-486-5p* let-7f-5p miR-451a* miR-92a-3p* miR-191-5p let-7a-5p let-7i-5p* let-7g-5p miR-182-5p* let-7b-5p* miR-185-5p* miR-16-5p* miR-26a-5p miR-25-3p miR-183-5p miR-181a-5p miR-151a-5p miR-151b miR-101-3p miR-30d-5p miR-30e-5p	N/A	N/A	N/A	miR-143-3p miR-203a-3p* miR191-5p miR-26a-5p let-7f-5p miR-486-5p miR-378a-3p miR-27b-3p* let-7g-5p miR-24-3p* let-7a-5p miR-375* miR-148a-3p miR-21-5p miR-205-5p* miR-320a miR-99a-5p* let-7i-5p miR-92a-3p let-7b-5p	Massively parallel sequencing	N/A	N/A

TABLE 1. Summary of reported miRNAs in forensically relevant body fluids, analysed by miRNA profiling and/or validation. (continue)

Groups	Venous blood	Menstrual blood	Semen	Vaginal secretion	Saliva	Screening platforms	Validation methods/reference RNA	Evaluation of miRNA stability in stain samples
19. O Leary K.R. *et al.*, 2018 [20]	miR-451*	N/A	miR-891a*	N/A	N/A	N/A	TaqMan qRT-PCR/miR-16	N/A
20. Tian H. *et al.*, [0] 2018 [3]	N/A	N/A	miR-891a* miR-888* miR-10a miR-10b miR-135b	N/A	N/A	N/A	TaqMan qRT-PCR/U6	N/A
21. Fang C. *et al.*, 2019 [21]	miR-451a* miR-486-5p* let-7i-5p* let-7b-5p* miR-92a-3p*	N/A	N/A	N/A	N/A	Massively parallel sequencing	SYBR Green qRT-PCR/ Cel-miR-39	- Bloodstains stored at RT, 3 weeks and 37°C, 1 day: No significant change in miR-451a and miR-486-5p expression compared to fresh samples

N/A: Not applicable
RT: Room temperature
Asterisks indicate miRNAs that were repeatedly found in more than one study.
miR-451 is also known as miR-451a.
miR-16 is also known as miR-16-5p.
miR-182 is also known as miR-182-5p.
miR-205 is also known as miR-205-5p.
miR-124a is also known as miR-124-3p.

classify the type of the blood. Indeed, Hanson E.K. *et al.* have generated a probability algorithm using miR-144-3p, miR-144-5p and miR-185-5p and the validation of this model showed 100% accuracy in a set of test samples, which are independent of samples used in the model development.[23] To allow the use of the model in practical work, the authors determined the expression of the miRNAs throughout 28 days of the menstrual cycle. The results from two donors showed that the developed model successfully identified menstrual blood between day 1 through 4 of menstruations. Sauer E. *et al.* also proposed an algorithm using miR-144-3p and miR-203a-3p to distinguish menstrual blood from venous blood with 100% accuracy.[25] Analysis of miRNA stability in aged samples, approximately one to four years old, demonstrated that the menstrual blood samples were successfully classified from venous blood, suggesting the flexible implementation of the algorithm in aged menstrual blood. Because both algorithms were validated in a small sample size: 12 and 2 samples, respectively, further studies may be conducted in a larger sample size prior to application for casework.

To be able to adopt miRNAs as markers for body fluid identification, their stability within the samples is one of the most important concerns. In bloodstains, it was shown that potential markers for venous blood including miR-16, miR-144-3p, miR-486, miR-451a were relatively stable in samples stored at room temperature.[17,21,25] Particularly, miR-144-3p abundance was not significantly altered in bloodstains kept for one year[22] and 36 years.[25] Quantification of miR-451a in bloodstains exposed to natural heat (8.9-61°C), humidity (10-99%) and sunlight for 180 days revealed that miR-451a was detected, though its level was significantly decreased as compared to the control samples kept at room temperature, low humidity and darkness.[26] In line with this, bloodstains treated with 1% sodium hypochlorite solutions showed a significant downregulation of six blood markers (miR-16, miR-486, miR-451a, miR-20a, miR-151a and miR-148a) compared to untreated controls.[21] Based on the studies of miRNA stability in various circumstances, it seems that miRNAs tend to be degraded in the samples under environmental challenges and harsh chemical exposure. Taken together, this suggests that samples for miRNA analysis should be stored in an appropriate condition and be protected from exposure to chemicals. In menstrual bloodstains, expression of miR-214 in samples stored at room temperature for one month was not significantly altered compared to the fresh sample.[17] In contrast, miRNAs in liquid whole blood appeared to be decreased following sample storage at room temperature for 72 hours and at -80°C for nine months.[27] This suggests that sample storage in dehydrated form is preferable to liquid form to protect miRNAs from degradation, and the specimen collection using a dry swab and stains of biological fluids gathered at a crime scene are applicable for miRNA analyses.

miRNA markers in semen

Semen is crucial evidence in sexual assault cases that can be present in the form of stains on objects or fabrics and it can be recovered from a victim's body, e.g. skin and vagina. Semen comprises spermatozoa and fluids from the seminal vesicles, the prostate and the bulbourethral glands. Although spermatozoa are ultimate markers of semen, they do not always exist especially in sterile seminal fluid. To search for alternative markers for identifying semen, miRNA profiling was conducted by the abovementioned studies (Table 1). Among the proposed miRNAs, it was clear that miR-891a was highly and exclusively expressed in semen compared to other forensically relevant body fluids: venous blood, menstrual blood, vaginal secretion and saliva.[17,20,22,25,28] Due to its distinctive expression in semen, miR-891a was suggested to be solely used for semen identification without the necessity of a statistical algorithm.

Evaluation of time-wise miRNA stability of miR-891a using qRT-PCR revealed that semen stains stored at room temperature for one month[17], one year[22] and three years[25] had a similar miRNA expression compared to fresh samples. Furthermore, a study has determined the effects of heat, humidity and sunlight on the stability of miR-891a by which semen stains were kept in an environmental chamber mimicking dry 24 hours in a Virginia summer with a temperature between 45-52°C and humidity of 50% for 14 days.[29] Throughout the experiment, it was observed that expression of miR-891a was not significantly changed in the stain kept in the chamber compared to the control samples kept at room temperature.[29] Whereas, samples treated with dish detergents showed amplification failures of let-7g, a highly expressed reference miRNA that could be because the detergent disrupts the cell membrane lipid composition of miRNA-containing microvesicles.[29] Another study has treated semen stains at a higher temperature and humidity (a temperature between 8.9-61.0°C and humidity ranged from 10-99%) for 180 days. It was revealed that expression of miR-891a was dramatically decreased from day 120, compared to control samples kept at room temperature, low humidity and darkness (a temperature between 19.0-23.7 °C and humidity ranged from 38-52%).[26] This provides examples of environmental

factors and chemical exposure affecting the analysis of miRNA expression in semen. Further studies on other factors are required to understand the limitation of miRNA analysis which would be a great consideration for implementation into casework.

It is likely that miR-891a can be used to identify infertile semen. Although miR-891a was significantly down-regulated in four types of infertile semen (asthenospermia, oligospermia, azoospermia and oligospermia combined with asthenospermia) compared to normal semen, its expression was still higher in the infertile semen when compared to non-semen body fluids (venous blood, menstrual blood, vaginal secretion and saliva).[30] This suggests that miR-891a could be used to discriminate semen from other forensic-related body fluids. Conversely, a subsequent study showed that the expression of miR-891a in azoospermia and asthenospermia was comparable to that in normal semen.[31] The discrepancy between the two studies can be derived from differences in reference RNAs used in the miRNA quantification in which the former study used SNORD24 and SNORD38B, whereas the latter study used 5s-rRNA. Taken together, the findings point out the potential of miR-891a for identifying both normal and infertile semen from other forensically relevant body fluids.

miRNA markers in vaginal secretion and saliva

Vaginal secretion is one of the most important forensically relevant body fluids, particularly in sexual assault cases. It can be present on penile swabs, male underpants and objects with alleged vaginal penetration. Among several studies, it has been shown by two groups that miR-124a (also known as miR-124-3p) was strongly associated with vaginal secretion.[32,33] A miRNA assay combining miR-124a with miR-372 revealed that the assay correctly differentiated ten of a total of eleven vaginal secretion samples from venous blood, menstrual blood, semen and saliva, suggesting that these miRNA markers could be used for an initial screening of unknown samples.[32] Likewise, miR-124a was selected together with miR-203a-3p to develop a decision tree facilitating the determination of vaginal secretion from venous blood, menstrual blood, semen and saliva. Through the validation of the decision tree using blind samples, it was demonstrated that nine out of a total of ten samples were correctly identified.[33] The overlap of miR-124a in these studies suggests that miR-124a could be a promising marker for vaginal secretion.

The identification of saliva can be proof that an assault occurred, especially in sexual assault cases. For example, saliva may be deposited on female underpants or bodies in a disputed licking event. Several independent lines of evidence suggested that the three miRNAs: miR-200c-3p, miR-203a and miR-205-5p were potential markers for saliva.[14,18,19,33,34] Quantification of the three miRNAs in saliva, vaginal secretion, semen, venous blood and menstrual blood revealed that they were also detected in vaginal secretion and menstrual blood at a comparable level to that in saliva, whereas their expression was low in semen and venous blood.[34] This is possible due to the fact that both saliva and vaginal secretion contain mucous membranes and menstrual blood consists of vaginal epithelial cells.[35] Thus, these body fluids share a similar pattern of miRNA expression. Although miR-200c-3p, miR-203a and miR-205-5p were not exclusively expressed in saliva, they tended to be potential markers for saliva identification when they were combined with other body fluid-specific miRNAs to establish a stepwise approach for body fluid identification.[33,34]

Proposed miRNA panels and analysis strategies to identify forensically relevant body fluids

To effectively identify forensically relevant body fluids, an ideal strategy would be the analysis of a panel, which consists of differentially expressed miRNAs. As shown in Table 2, the first panel was proposed by Hanson E.K. *et al.* who quantified two specific markers for each type of body fluid and plotted dCt values in two-dimensional (2D) scatter plot.[32] In this model, the unknown samples could be categorised against the cluster of dCt values on the 2D scatter plot of known samples, allowing identification of the body fluid origin. The authors tested the specificity of the model and showed that the accuracy was in the range of 80-100% for the five body fluids. Although the accuracy was close to 100%, the model may not be able to identify body fluids of which do not clearly belong to a particular cluster. This suggests the requirement of statistical approaches to establish a threshold for the probability of identification. Subsequent studies have addressed this issue and developed statistical models to determine the source of body fluids.[23,25,30,33,41] A significant model was reported by the group of Sauer E. *et al.* who suggested to initially distinguish semen from other body fluids using miR-891a, a truly semen-specific marker.[25,33] If the sample was non-semen, further statistical analysis was applied to differentiate venous blood from menstrual blood and vaginal secretion from saliva. The expression of semen-specific markers (miR-891a, miR-135a and miR-10a) was extended in infertile semen and it was shown that their expression was significantly down-regulated in infertile semen compared to normal semen.[30] However, the reduction in miRNA expression did not affect the

TABLE 2. Proposed panels of miRNA and model analyses to distinguish body fluids.

Groups	miRNAs in the panel	Types of model analysis	Model analyses	Accuracy/testing of the model	Experimental methods/reference RNA
1. Hanson E.K. *et al.*, 2009 [32]	- Venous blood: miR-451 and miR-16 - Menstrual blood: miR-451 and miR-412 - Semen: miR-135b and miR-10b - Vaginal secretion: miR-124a and miR-372 - Saliva: miR-658 and miR-205	2D scatter plots consisting of dCt of two markers in each type of body fluid	Identify unknown samples by positioning dCt on 2D scatter containing clusters of known samples	100% accuracy for venous blood, semen and saliva; ~90% accuracy for vaginal secretion; ~80% accuracy for menstrual blood	SYBR Green qRT-PCR/U6b
2. Hanson E.K. *et al.*, 2014 [23]	- Menstrual blood: miR-185-5p, miR-144-3p and miR-144-5p	Logistic model	Logit = -(0.005*dCt(miR-185-5p)*dCt (miR-144-3p)* dCt(miR-144-5p)) + (3.718*dCt(miR-185-5p)) – 32.017 Menstrual blood if p>0.5 (Chi-square)	100% accuracy (12 blinded samples tested)	SYBR Green qRT-PCR/miR-940
3. Wang Z. *et al.*, 2015 [34]	- Menstrual blood vaginal secretion and saliva: miR-205-5p - Menstrual blood: miR-214-3p - Saliva and menstrual blood: miR-203a - Semen: miR-891a	Stepwise strategy to identify saliva	Identify saliva using a combination of miRNAs to distinguish saliva from other body fluids	N/A	TaqMan qRT-PCR/ U6
4. Sauer E. *et al.*,2015 [33] 5. Sauer E. *et al.*,2016 [25]	- Semen: miR-891a - Venous and menstrual blood: miR-144-3p and miR-203a-3p - Vaginal secretion and saliva: miR-124-3p and miR-203a-3p	Decision algorithm	Started with quantification of miR-891a to differentiate semen. Non-semen samples were subsequently analysed using D = -1.504-0.127*dCt (miR-144-3p) +0.454*dCt (miR-203a-3p) Venous blood if D>0 and menstrual blood if D<0 D = -3.305-0.743*dCt (miR-203a-3p) +0.582*dCt (miR-124-3p) Saliva if D>0 and vaginal secretion if D<0	90% accuracy (9 out of 10 blinded samples were correctly identified.)	TaqMan qRT-PCR/ SNORD24, SNORD38B and SNORD43

TABLE 2. Proposed panels of miRNA and model analyses to distinguish body fluids. (continue)

Groups	miRNAs in the panel	Types of model analysis	Model analyses	Accuracy/testing of the model	Experimental methods/reference RNA
6. Sirker M. *et al.*,2017 [42]	- Venous and menstrual blood: miR-451 - Semen: miR-10b and miR-374 - Vaginal secretion: miR-203 - Saliva: miR-943	Receiver operator characteristic (ROC)	Calculating the area under the ROC curve (AUC) to discriminate between pairs of body fluids	N/A	TaqMan qRT-PCR/ miR-26b, miR-92, miR-484, miR-144
7. Tian H. *et al.,* 2018 [30]	- Semen: miR-891a, miR-135a and miR-10a	Discrimination function analysis	Y2 = -1.152*dCt(miR-10a) + 0.910*dCt(miR-135a) + 1.092*dCt (miR-891a) - 0.639 Semen if Y2 < 0 and non-semen if Y2 > 0.	97.5% accuracy	TaqMan qRT-PCR/ U6
8. Dorum G. *et al.*, 2019 [41]	- Venous blood: miR-126-3p, miR-486-3p and miR-486-5p - Menstrual blood: miR-200a-3p and miR-451a - Semen: miR-375 - Vaginal secretion: miR-203b-5p and miR-205-5p - Saliva and skin: miR-1246	Partial least squares (PLS) and linear discriminant analysis (LDA)	-	65% accuracy (13 out of 20 blinded samples were correctly identified.)	Massively parallel sequencing
9. Fujimoto S. *et al.*,2019 [31]	- Venous blood: miR-144-3p and miR-451a-5p - Semen: miR-888-5p and miR-891a-5p - Vaginal secretion: miR-1260b - Saliva: miR-203a-3p and miR-223-3p	Partial least squares-discriminant analysis (PLS-DA)	-	>90 % posterior probability (13 out of 14 blinded samples were correctly identified.)	SYBR Green qRT-PCR/miR-484, miR-92a-3p and 5S-rRNA

2D: two-dimensional
N/A: Not applicable

discrimination function analysis to differentiate semen from non-semen as follows: Y2 = -1.152*dCt (miR-10a) + 0.910*dCt (miR-135a) +1.092*dCt (miR-891a)-0.639, semen if Y2 < 0 and non-semen if Y2 > 0. Thus, this underscores the potential of miR-891a for identifying semen and suggests possible implementation in casework.

Based on miRNA expression using MPS, a recent study proposed a panel of nine miRNAs for identification of six body fluids/tissues: venous blood, menstrual blood, semen, vaginal secretion, saliva and skin.[41] Notably, three miRNAs (miR-126-3p, miR-486-3p and miR-486-5p) were selected as venous blood-specific markers which is in accordance with previous studies.[14,15,17-19,21] While the panel included miR-205-5p as a vaginal secretion marker, the miRNA has been also shown to be highly expressed in saliva.[14,18,19,32,34] Possibly, both vaginal secretion and saliva are generated from mucosa-like environments, allowing them to share a similar pattern of miRNA expression. The overlap pattern of miRNA expression adds an extra layer of complexity to the development of a miRNA panel. This problem could be overcome by exploring more specific miRNAs and/or generating a statistical algorithm to analyse a differential expression that would permit the prediction of body fluid origins. To date, the existing data provide a good starting point for further work, showing the potential of a miRNA panel to simultaneously analyse several forensically important body fluids. Prior to the implementation of a miRNA panel in forensic casework, it is necessary to validate such a panel in a larger sample size. Besides, the analysis of miRNA via qRT-PCR requires the standardisation of the procedure using robust reference genes for normalisation that would decrease inter-laboratory variations and take miRNAs a step closer from research to casework for body fluid identification.

Conclusions and future directions towards applications of miRNA markers in forensic casework

During the last decade, miRNAs have emerged as promising markers for body fluid identification and several independent lines of evidence have reported a range of potential miRNAs specific to venous blood, menstrual blood, semen, vaginal secretion and saliva, which are commonly significant evidence for crime reconstruction. A great effort has been made to transfer these miRNAs from laboratory benches to applications in forensic casework through an evaluation of the markers in fluid stains and development of model analysis to accurately differentiate a certain body fluid from the others. An ideal strategy involves the establishment of a miRNA panel consisting of differentially expressed miRNAs in forensically relevant body fluids that will allow identification of unknown stains. To achieve this, several studies have developed various model analyses and some of them used overlapped miRNA markers from previous studies. Based on the previous studies, it appears that all panels allow distinct identification of blood in general and semen, whereas the candidate miRNAs showed less association with vaginal secretion and saliva. This suggests that the initial implementation of miRNA markers for routine casework could be applicable for the identification of blood and semen.

To apply a miRNA analysis into routine procedures, it is necessary to establish standardised protocols starting from miRNA extraction, determination of miRNA expression, validation of reference RNAs for qRT-PCR normalisation, result analysis and result interpretation. At the step of miRNA quantification using qRT-PCR, the protocol largely requires a solid normalisation of reference genes, which are non-biological variances to deliver reliable and reproducible results. To address this point, the MIQE-guidelines (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) is recommended to be adopted in forensic genetics for method standardization.[43] The MIQE-guidelines suggest a qRT-PCR checklist covering details of samples and sample processing, assay optimisation/validation, experiment setup and data analysis that should be included into the submitted manuscript along with the full disclosure of oligonucleotide sequences, aiming to increase experimental transparency and reliability of the result.[44,45] This would also help researchers to reproduce the published protocol and unequivocally interpret the result. Besides, the standardised protocols can be conducted in workgroup trials at which a selected miRNA panel is validated in a large sample size. The accumulation of the result from laboratory members would also allow determination of inter-laboratory variances that would be useful for protocol optimisation or development of the assay guideline. At present, identification of forensically relevant body fluids usually relies on mRNA-based analysis. This is likely due to the existence of several collaborative exercises on DNA/mRNA co-analysis which provides guidelines for DNA/mRNA co-extraction, mRNA profiling, data interpretation as well as awareness throughout the technique.[46-49] If inter-laboratory trials of miRNA markers were established, it would be worthwhile to include miRNA/mRNA co-analysis. This would maximise the capacity of the approach in body fluid identification. At the step of the extraction, several studies have demonstrated that miRNAs in forensically relevant body fluids can be co-extracted with DNA using standard DNA extraction

methods.[50-52] More recently, it has been shown that a co-extraction kit, the AllPrep DNA/RNA/miRNA Universal Kit (APU), had comparable efficiency to standard kits for mRNA/DNA or miRNA/DNA co-extraction.[53] The successful co-extraction provides good support for the development of the protocol to analyse miRNAs, mRNAs and DNA, especially when amounts of sample are limited and DNA typing is more preferable. This would allow the method to be flexible and effective in sample consumption.

ACKNOWLEDGMENTS

I would like to thank Dr. Hans Rudi Grams under the program of Research Clinic, the Faculty of Allied Health Sciences, Thammasat University, for language help of the manuscript.

Formatting of funding sources: This review article did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Kozomara A, Birgaoanu M, Griffiths-Jones S. miRBase: from microRNA sequences to function. Nucleic Acids Res 2019; 47:D155-D62.
2. Iftikhar H, Carney GE. Evidence and potential in vivo functions for biofluid miRNAs: From expression profiling to functional testing: Potential roles of extracellular miRNAs as indicators of physiological change and as agents of intercellular information exchange. Bioessays 2016;38:367-78.
3. Turchinovich A, Samatov TR, Tonevitsky AG, Burwinkel B. Circulating miRNAs: cell-cell communication function? Front Genet 2013;4:119.
4. Turchinovich A, Weiz L, Langheinz A, Burwinkel B. Characterization of extracellular circulating microRNA. Nucleic Acids Res 2011; 39:7223-33.
5. Turchinovich A, Weiz L, Burwinkel B. Extracellular miRNAs: the mystery of their origin and function. Trends Biochem Sci 2012;37:460-5.
6. Gallo A, Tandon M, Alevizos I, Illei GG. The Majority of MicroRNAs Detectable in Serum and Saliva Is Concentrated in Exosomes. Plos One 2012;7:e30679.
7. Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. P Natl Acad Sci USA 2011;108:5003-8.
8. de Rie D, Abugessaisa I, Alam T, Arner E, Arner P, Ashoor H, et al. An integrated expression atlas of miRNAs and their promoters in human and mouse. Nat Biotechnol 2017;35: 872-78.
9. Silva SS, Lopes C, Teixeira AL, Carneiro de Sousa MJ, Medeiros R. Forensic miRNA: potential biomarker for body fluids? Forensic Sci Int Genet 2015;14:1-10.
10. Bavykin AS. Circulating microRNAs in the Identification of Biological Fluids: A New Approach to Standardization of Expression-Based Diagnostics. Mol Biol 2017;51:506-13.
11. Dumache R, Rogobete AF, Sandesc D, Bedreag OH, Ciocan V, Muresan C, et al. Use of Circulating and Cellular miRNAs Expression in Forensic Sciences. J Interdiscip Med 2017;2: 235-41.
12. Courts C, Madea B. Micro-RNA - A potential for forensic science? Forensic Sci Int 2010;203:106-11.
13. Sijen T. Molecular approaches for forensic cell type identification: On mRNA, miRNA, DNA methylation and microbial markers. Forensic Sci Int Genet 2015;18:21-32.
14. Courts C, Madea B. Specific Micro-RNA Signatures for the Detection of Saliva and Blood in Forensic Body-fluid Identification. J Forensic Sci 2011;56:1464-70.
15. Rekker K, Saare M, Roost AM, Salumets A, Peters M. Circulating microRNA Profile throughout the menstrual cycle. PLoS One 2013;8:e81166.
16. Wang Z, Luo HB, Pan XF, Liao M, Hou YP. A model for data analysis of microRNA expression in forensic body fluid identification. Forensic Sci Int Genet 2012;6:419-23.
17. Wang Z, Zhang J, Luo HB, Ye Y, Yan J, Hou YP. Screening and confirmation of microRNA markers for forensic body fluid identification. Forensic Sci Int Genet 2013;7:116-23.
18. Wang Z, Zhou D, Cao YD, Hu Z, Zhang SH, Bian YN, et al. Characterization of microRNA expression profiles in blood and saliva using the Ion Personal Genome Machine (R) System (Ion PGM (TM) System). Forensic Sci Int Genet 2016;20:140-6.
19. El-Mogy M, Lam B, Haj-Ahmad TA, McGowan S, Yu D, Nosal L, et al. Diversity and signature of small RNA in different bodily fluids using next generation sequencing. BMC Genomics 2018;19:408.
20. KR OL, Glynn CL. Investigating the Isolation and Amplification of microRNAs for Forensic Body Fluid Identification. Microrna 2018;7:187-94.
21. Fang C, Zhao J, Li JB, Qian JL, Liu X, Sun QF, et al. Massively parallel sequencing of microRNA in bloodstains and evaluation of environmental influences on miRNA candidates using realtime polymerase chain reaction. Forensic Sci Int Genet 2019;38:32-8.
22. Zubakov D, Boersma AW, Choi Y, van Kuijk PF, Wiemer EA, Kayser M. MicroRNA markers for forensic body fluid identification obtained from microarray screening and quantitative RT-PCR confirmation. Int J Legal Med 2010;124:217-26.
23. Hanson EK, Mirza M, Rekab K, Ballantyne J. The identification of menstrual blood in forensic samples by logistic regression modeling of miRNA expression. Electrophoresis 2014;35:3087-95.
24. Park JL, Park SM, Kwon OH, Lee HC, Kim JY, Seok HH, et al. Microarray screening and qRT-PCR evaluation of microRNA markers for forensic body fluid identification. Electrophoresis 2014;35:3062-8.
25. Sauer E, Reinke AK, Courts C. Differentiation of five body fluids from forensic samples by expression analysis of four microRNAs using quantitative PCR. Forensic Sci Int Genet 2016;22:89-99.
26. Mayes C, Houston R, Seashols-Williams S, LaRue B, Hughes-Stamm S. The stability and persistence of blood and semen mRNA and miRNA targets for body fluid identification in environmentally challenged and laundered samples. Legal Med-Tokyo 2019;38: 45-50.
27. Glinge C, Clauss S, Boddum K, Jabbari R, Jabbari J, Risgaard B, et al. Stability of Circulating Blood-Based MicroRNAs - Pre-Analytic Methodological Considerations. Plos One 2017;12:e0167969.

28. Seashols-Williams S, Lewis C, Calloway C, Peace N, Harrison A, Hayes-Nash C, et al. High-throughput miRNA sequencing and identification of biomarkers for forensically relevant biological fluids. Electrophoresis 2016;37:2780-8.
29. Layne TR, Green RA, Lewis CA, Nogales F, Cruz TCD, Zehner ZE, et al. microRNA Detection in Blood, Urine, Semen, and Saliva Stains After Compromising Treatments. J Forensic Sci 2019; 64:1831-7.
30. Tian H, Lv ML, Li ZL, Peng D, Tan Y, Wang H, et al. Semen-specific miRNAs: Suitable for the distinction of infertile semen in the body fluid identification? Forensic Sci Int Genet 2018;33: 161-7.
31. Fujimoto S, Manabe S, Morimoto C, Ozeki M, Hamano Y, Hirai E, et al. Distinct spectrum of microRNA expression in forensically relevant body fluids and probabilistic discriminant approach. Sci Rep 2019;9:14332.
32. Hanson EK, Lubenow H, Ballantyne J. Identification of forensically relevant body fluids using a panel of differentially expressed microRNAs. Anal Biochem 2009;387:303-14.
33. Sauer E, Reinke AK, Courts C. Validation of forensic body fluid identification based on empirically normalized miRNA expression data. Forensic Sci Int Genet 2015;5:E462-E4.
34. Wang Z, Zhang J, Wei W, Zhou D, Luo HB, Chen XG, et al. Identification of Saliva Using MicroRNA Biomarkers for Forensic Purpose. J Forensic Sci 2015;60:702-6.
35. Schenkels LCPM, Veerman ECI, Amerongen AVN. Biochemical-Composition of Human Saliva in Relation to Other Mucosal Fluids. Crit Rev Oral Biol Med 1995;6:161-75.
36. Hanson EK, Ballantyne J. Circulating microRNA for the identification of forensically relevant body fluids. Methods Mol Bio 2013;1024:221-34.
37. Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, et al. The microRNA spectrum in 12 body fluids. Clin Chem 2010;56:1733-41.
38. Tong DY, Jin Y, Xue TY, Ma XY, Zhang JX, Ou XL, et al. Investigation of the Application of miR10b and miR135b in the Identification of Semen Stains. Plos One 2015;10:e0137067.
39. Li ZL, Bai P, Peng D, Wang H, Guo YD, Jiang YJ, et al. Screening and confirmation of microRNA markers for distinguishing between menstrual and peripheral blood. Forensic Sci Int Genet 2017;30:24-33.
40. Wang Z, Zhao XY, Hou YP. Exploring of microRNA markers for semen stains using massively parallel sequencing. Forensic Sci Int Genet 2017;6:E107-E9.
41. Dorum G, Ingold S, Hanson E, Ballantyne J, Russo G, Aluri S, et al. Predicting the origin of stains from whole miRNome massively parallel sequencing data. Forensic Sci Int Genet 2019;40:131-9.
42. Sirker M, Fimmers R, Schneider PM, Gomes I. Evaluating the forensic application of 19 target microRNAs as biomarkers in body fluid and tissue identification. Forensic Sci Int Genet 2017;27:41-9.
43. Courts C, Pfaffl MW, Sauer E, Parson W. Pleading for adherence to the MIQE-Guidelines when reporting quantitative PCR data in forensic genetic research. Forensic Sci Int Genet 2019;42:e21-e4.
44. Bustin SA, Beaulieu JF, Huggett J, Jaggi R, Kibenge FS, Olsvik PA, et al. MIQE precis: Practical implementation of minimum standard guidelines for fluorescence-based quantitative real-time PCR experiments. Bmc Mol Biol 2010;11:74.
45. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 2009;55:611-22.
46. Haas C, Hanson E, Anjos MJ, Bar W, Banemann R, Berti A, et al. RNA/DNA co-analysis from blood stains-Results of a second collaborative EDNAP exercise. Forensic Sci Int Genet 2012;6:70-80.
47. Carnevali E, Lacerenza D, Severini S, Alessandrini F, Bini C, Di Nunzio C, et al. A GEFI collaborative exercise on DNA/RNA co-analysis and mRNA profiling interpretation. Forensic Sci Int Genet 2017;6:E18-E20.
48. van den Berge M, Carracedo A, Gomes I, Graham EAM, Haas C, Hjort B, et al. A collaborative European exercise on mRNA-based body fluid/skin typing and interpretation of DNA and RNA results. Forensic Sci Int Genet 2014;10:40-8.
49. Haas C, Hanson E, Morling N, Ballantyne J. Collaborative EDNAP exercises on messenger RNA/DNA co-analysis for body fluid identification (blood, saliva, semen) and STR profiling. Forensic Sci Int Genet 2011;3:e5-e6.
50. Omelia EJ, Uchimoto ML, Williams G. Quantitative PCR analysis of blood- and saliva-specific microRNA markers following solid-phase DNA extraction. Anal Biochem 2013;435: 120-2.
51. van der Meer D, Uchimoto ML, Williams G. Simultaneous Analysis of Micro-RNA and DNA for Determining the Body Fluid Origin of DNA Profiles. J Forensic Sci 2013;58:967-71.
52. Lewis CA, Layne TR, Seashols-Williams S. Detection of microRNAs in DNA Extractions for Forensic Biological Source Identification. J Forensic Sci 2019;64:1823-30.
53. Watanabe K, Akutsu T. Evaluation of a co-extraction kit for mRNA, miRNA and DNA methylation-based body fluid identification. Leg Med (Tokyo) 2020;42:101630.