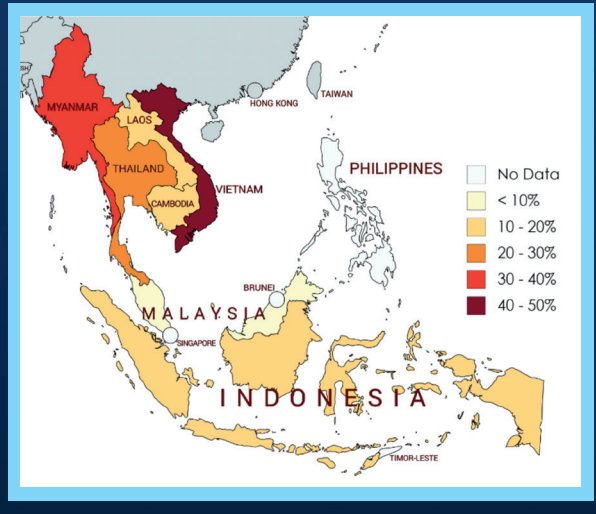
E-ISSN 2228-8082 ISSN 2629-995X
Volume 72, Number 3, May-June 2020

# SMJ
## Siriraj Medical Journal






*By Paul Wasuwanich, et al.*

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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].

# Comparison of the Oncologic Outcomes between Exploratory Laparotomy and Laparoscopic Surgery for Endometrial Cancer: Siriraj Experience

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## ABSTRACT

**Objective:** This study was undertaken to evaluate surgical and oncologic outcomes for patients with endometrial cancer, compared between exploratory laparotomy and laparoscopic surgery.

**Method:** In total, 324 patients who diagnosed with endometrial cancer during January 2007 to December 2016 were enrolled. The comprehensive surgical staging procedures, including total hysterectomy, bilateral salpingo-oophorectomy (BSO), pelvic lymphadenectomy (PL), and/or para-aortic lymphadenectomy (PAL) were undergone. Demographic, clinical, treatment, operative, outcome, and survival outcome were recorded and evaluated.

**Results:** 81 patients performed laparoscopy without conversion. No significant difference in baseline characteristics and pathological characteristics between two groups was observed. When compared with laparotomy group, the laparoscopy group had longer operative time, shorter hospital stays, and lower blood loss. Two-year overall survival (OS) was 97.9% and 95.1% in the laparotomy and laparoscopy groups, respectively (p=0.263). In addition, 2-year disease-free survival (DFS) between both groups was equal (93.7% versus 88.6%, respectively; p=0.309).

**Conclusion:** Laparoscopic surgery is an efficacious, achievable and safe technique for patients with endometrial cancer. Good surgical skills and proper surgical techniques are required to effectuate optimal outcomes.

**Keywords:** Endometrial cancer; oncologic outcomes; laparoscopic surgery (Siriraj Med J 2020; 72: 195-201)

## INTRODUCTION

Currently, endometrial cancer is the cancer that commonly found Thailand. The surgical procedures that can be employed to determine the stage of disease in endometrial cancer includes total hysterectomy, bilateral salpingo-oophorectomy (BSO), pelvic lymphadenectomy (PL) and para-aortic lymphadenectomy (PAL). Traditionally, these procedures are performed by exploratory laparotomy approach. However, during the last decade, laparoscopic surgery has played an important role in comprehensive surgical staging in gynecologic cancer. Laparoscopic surgery not only reduces postoperative pain, wound complication, length of hospital stay, and postoperative adhesion, but it also improves patient quality of life. The result of all these benefits is that patients can receive their adjuvant treatment earlier.

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*http://dx.doi.org/10.33192/Smj.2020.26*

In early stage endometrial cancer, several studies found the same postoperative complications, and survival outcome between exploratory laparotomy and laparoscopic surgery.[1-9] We performed the first total laparoscopic hysterectomy (TLH) at our center in 2004, and we subsequently introduced the Siriraj TLH technique (SiTLH) in 2006.[10] This meticulous technique, has allowed us to safely perform PL and PAL since 2007. Thus, comparison of the outcomes between laparoscopic surgery and exploratory laparotomy in endometrial cancer at Siriraj Hospital was the objective of our present study.

## MATERIALS AND METHODS

The study design was a retrospective cohort study. Medical records were retrieved from the database of the Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand during 5th January 2007 to 27th December 2016 study period. All patients were histologically confirmed as endometrial cancer and primary surgery was scheduled. The histological subtypes included endometrioid adenocarcinoma, papillary serous carcinoma, clear cell carcinoma, and mixed carcinoma. Patients with incomplete medical records were excluded. Informed consent was not required due to the retrospective nature of the study. This study was reviewed and approved by the Ethics Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University before the study initiated (Si 005/2018).

The sample size was calculated using non-inferiority, based on data of previous study[9] that reported 90% survival rate. Calculated sample size using a power of 80% (type II error = 0.20), ratio between two groups was 3.0. Our sample size calculation revealed that a minimum sample size of 300 patients, 225 in exploratory laparotomy group and 75 in laparoscopic surgery group, would be required to achieve a 90% confidence level.

A total of 324 patients who met our criteria were enrolled. Two hundred and forty-three patients underwent exploratory laparotomy, while the others performed surgical staging by laparoscopic surgery. Surgical procedures included total hysterectomy, BSO, PL, and PAL. PL and PAL is indicated in patients with high risk for lymph node metastasis (non-endometrioid histologic subtype, extra-uterine involvement, grade 3 with myometrial invasion of greater than 50%) and can be considered in patients with intermediate risk (invasion of more than half of the myometrium or grade 3 with less than 50% myometrial invasion). However, some patients did not undergo this kind of surgery due to inadequate exposure, morbid obesity, and/or patient comorbidity.

Data collection included preoperative patient characteristics. The duration from skin incision to wound closure labeled as operative time. Major complications were defined as mortality, visceral organ injury, vascular injury, massive blood loss, conversion from laparoscopic surgery to exploratory laparotomy, venous thromboembolic events and wound morbidity. Patients who died within 30 days of surgery classified as mortality. Organ injuries were defined as those requiring surgical correction. Massive blood loss was defined as total blood loss >1,000 ml. Deep vein thrombosis or pulmonary embolism categorized as venous thromboembolic events. Wound morbidity meant wound dehiscence or a deep wound surgical site infection of fascial or muscle layers that required readmission or surgical intervention. Patients with a temperature of greater than 38°C, after the first day of the postoperative period, measured on two separate occasions at least 12 hours apart, labeled as postoperative fever. Minor complications included superficial surgical site infections (skin and subcutaneous infection), urinary tract infections and fever. Loss of blood was calculated from the estimation of blood volume on swabs and the difference between the volume of fluid used during surgery and blood volume in suction containers. After the treatment, all patients received disease surveillance for at least 2 years. The period from the start of treatment to the date of death or the date of the last follow up defined as overall survival (OS). Whereas, the length of time from the start of treatment to the date of recurrence referred as disease-free survival (DFS). Response rate (RR) was defined as the percentage of patients whose cancer shrank or disappeared after treatment.

Comparison of the oncologic outcomes (OS, DFS, RR) between the laparoscopic surgery and exploratory laparotomy was the primary outcome. Surgical outcomes between groups, such as operative time, blood loss, major complications, and length of hospital stay, were also compared.

### *Laparoscopic surgery technique for surgical staging in endometrial cancer*

We developed and introduced the SiTLH technique in 2006.[10] The principles of this technique include early identification of both ureters at the beginning of surgery, dissection at the retroperitoneal space and then restoration of the pelvic anatomy from adhesion-free area to the adhesion area. This technique, allow us to dissect the vital organs safely, and to perform transperitoneal lymphadenectomy.

All patients were placed on the table in the lithotomy position after general anesthesia was performed. A uterine manipulator was placed depending on surgeon discretion. A 10-mm laparoscopic trocar was inserted at the umbilical or supraumbilical area for the optic, and three or four 5-mm trocars were inserted at the iliac, suprapubic, and left paraumbilical regions for ancillary instruments. The 10-step SiTLH BSO was routinely performed. The anatomic boundaries for PL included common iliac bifurcation superiorly, deep circumflex vein inferiorly, iliopsoas muscle laterally, obliterated umbilical artery medially and obturator nerve inferiorly.

Transperitoneal PAL was performed by cutting the peritoneum along the right common iliac artery and aorta. The retroperitoneal space was exposed by hanging the cut peritoneum from the upper abdominal wall. The surgery was performed to at least the level of the inferior mesenteric artery (IMA).

### *Statistical analysis*

SPSS for Windows version 18.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics were analyzed using chi-square test and Fisher's exact test. Data are shown as number and percentage, mean ± standard deviation, or median and range. All calculated *P*-values were two-sided, and a *P*-value < 0.05 was considered statistically significant. DFS and OS were calculated using Kaplan-Meier method. Differences between survival curves were analyzed using log-rank test.

## RESULTS

During the study period, a total of 324 patients underwent surgical staging for endometrial cancer. Of those, 243 patients underwent exploratory laparotomy, and 81 patients underwent laparoscopic surgery. Conversion rate was zero percent. Baseline characteristics and pathological characteristics are shown in Table 1 and Table 2. Those characteristics between groups were the same. Endometrioid adenocarcinoma was the most common histological subtype (91.7%), followed by clear cell carcinoma (4.0%). The most common FIGO stage was stage IA in both groups (57.2% and 60.5% in the laparotomy and laparoscopy group, respectively).

Patients who undertook laparotomy had more estimated blood loss and longer hospital stay, whereas patients who undertook laparoscopy had a longer operative time (Table 3). PAL which is a lengthy operative procedure, was more often performed in the laparoscopy group (77.8% versus 53.9%). However, the numbers of para-aortic lymph node retrieved between both groups were the same (Table 3). The major complication rate seemed to be lower in laparoscopy group (8.2% versus 2.5%, $p$=0.074). The most common complication was superficial wound infection, (a minor complication), as shown in Table 4.

With the clinical complete response rate of 98.8% in the laparoscopy group (Table 5), the 2-year OS was 95.1% which was not significantly different from that of the laparotomy group (Fig 1). DFS in the laparotomy and laparoscopy groups was 93.7% and 88.6%, respectively (Fig 2).

## DISCUSSION

The 2-year OS in the laparoscopy group in the present study was 95.1%, which was not different from the 2-year OS in the laparotomy group. Terao, *et al.*[8] reported a 2-year survival rate of 94.6%, which is comparable to the rate observed in our study. The Gynecologic Oncology Group (GOG) do a study about laparoscopic surgery in endometrial cancer, LAP2 study[5], confirmed laparoscopic surgical staging for uterine cancer to be feasible and safe, with fewer complications. These benefits of laparoscopy also found in our study.

Palomba et al. showed that laparoscopic surgery in early stage endometrial cancer is safe and effective.[6] The long-term data showed no significant difference in OS, DFS or recurrence when compared between exploratory laparotomy and laparoscopic surgery.[7] Furthermore, a prospective analysis in 2012 reported a 5-year survival rate of 89.8%.[11]

No significant difference in DFS between laparoscopic surgery and laparotomy was reported by several studies. In 2012, GOG LAP2 study[11] reported recurrence and survival, the 3-year estimated cumulative incidence of recurrence in the laparoscopy group was 11.39%. Tozzi, *et al.*[4] reported a 2-year DFS of 87.4% in the laparoscopy group versus 91.6% in the laparotomy group - both of which are comparable to our study. However, surgical techniques to prevent tumor spillage during surgery were not used in all cases in our study. Those techniques included no uterine manipulator insertion through the uterine cavity, vaginal vault closure before surgery began, ligation of both fallopian tubes before surgery started, and the use of a specimen retrieval bag during tissue extraction. We expect that DFS will increase even further if these protective techniques can be used in all cases.

Due to the principle of the SiTLH technique, the laparoscopy group had lower blood loss. Early reduction of uterine blood supply at the beginning of the procedure not only reduce blood loss, but also reduces the rate of

**TABLE 1.** Baseline demographic and clinical characteristics (N=324).

| Characteristics | Values[a] | | *P* value |
|---|---|---|---|
| | Laparotomy group (n=243) | Laparoscopy group (n=81) | |
| Age, y | 57.51+10.55 | 56.98+10.10 | 0.692 |
| Body mass index[b] | 26.82+5.49 | 26.74+5.84 | 0.908 |
| Parity | | | 0.702 |
| 0 | 89 (36.6) | 30 (37) | |
| >1 | 154 (63.4) | 51 (63) | |
| Menopause status | | | 0.438 |
| Pre-menopause | 74 (30.5) | 21 (25.9) | |
| Post-menopause | 169 (69.5) | 60 (74.1) | |
| History of hormone replacement therapy | 3 (1.2) | 1 (1.2) | 1.000 |
| ECOG performance status | | | 0.530 |
| 0 | 219 (90.1) | 71 (87.7) | |
| 1 | 24 (9.9) | 10 (12.3) | |

[a] Values are given as mean + standard deviation or number (percentage).
[b] Calculated as weight in kilograms divided by the square of height in meters.

**TABLE 2.** Pathological characteristics (N=324).

| Characteristics | Values[a] | | *P*-value |
|---|---|---|---|
| | Laparotomy group (n=243) | Laparoscopy group (n=81) | |
| Histologic subtype | | | 0.650 |
| Endometrioid adenocarcinoma | 224(92.2) | 73(90.1) | |
| Serous carcinoma | 1(0.4) | 0(0) | |
| Clear cell carcinoma | 10(4.1) | 3(3.7) | |
| Mixed | 8(3.3) | 4(6.2) | |
| Histologic grading | | | 0.752 |
| 1 | 127(52.3) | 39(48.1) | |
| 2 | 73(30.0) | 25(30.9) | |
| 3 | 43(17.7) | 17(21.0) | |
| Presence of LVSI[b] | 46(18.9) | 16(19.8) | 0.870 |
| Positive for peritoneal fluid cytology | 8(3.3) | 4(4.9) | 0.503 |
| Isolated pelvic lymph node metastasis | 16(6.6) | 5(6.2) | 0.869 |
| Isolated para-aortic lymph node metastasis | 1(0.4) | 1(1.2) | 0.438 |
| Pelvic and para-aortic lymph node metastasis | 10(4.1) | 3(3.7) | 1.000 |
| FIGO[c] stage | | | 0.969 |
| IA | 139(57.2) | 49(60.5) | |
| IB | 49(20.2) | 14(17.3) | |
| II | 18(7.4) | 6(7.4) | |
| IIIA | 9(3.7) | 2(2.5) | |
| IIIB | 1(0.4) | 1(1.2) | |
| IIIC1 | 16(6.6) | 5(6.2) | |
| IIIC2 | 11(4.5) | 4(4.9) | |

[a] Values are given as number (percentage)., [b] LVSI, lymphovascular space invasion., [c] FIGO, International Federation of Gynecology and Obstetrics.

**TABLE 3.** Surgical outcomes (N=324).

| Outcomes | Values[a] | | *P*-value |
|---|---|---|---|
| | Laparotomy group (n=243) | Laparoscopy group (n=81) | |
| Estimated blood loss, ml | 306.7 | 128.6 | <0.001 |
| Duration of operation, min | 177.8+52.9 | 259.2+73.8 | <0.001 |
| Length of hospital stay, d | 6(5,8) | 5(4,6) | <0.001 |
| Pelvic lymphadenectomy | 239(98.4) | 80(98.8) | 1.000 |
| Para-aortic lymphadenectomy | 131(53.9) | 63(77.8) | <0.001 |
| Number of lymph node | | | |
| Pelvic lymph node[b] | 14(10,20) | 14(9,20) | 0.593 |
| Para-aortic lymph node[b] | 3(2,5) | 3(2,6) | 0.390 |
| Residual tumour | | | 0.483 |
| no | 237(97.5) | 80(98.8) | |
| <1 cm | 2(0.8) | 1(1.2) | |
| >1 cm | 4(1.6) | 0(0) | |
| Major complication | 20(8.2) | 2(2.5) | 0.074 |
| Adjuvant treatment | 141(58.3) | 40(49.4) | 0.163 |

[a] Values are given as mean + standard deviation, number (percentage), or median (interquartile range).,
[b] calculated only in patients who performed pelvic and para-aortic lymphadenectomy.

**TABLE 4.** Intraoperative complications and postoperative adverse events.

| Complication rate | Values[a] | | *P*-value |
|---|---|---|---|
| | Laparotomy group (n=243) | Laparoscopy group (n=81) | |
| Major complications | | | |
| Bowel injury[b] | 2(0.8) | 0(0) | 1.000 |
| Bladder injury[b] | 1(0.4) | 0(0) | 1.000 |
| Bowel obstruction[b] | 1(0.4) | 0(0) | 1.000 |
| Wound dehiscence[b] | 8(3.3) | 1(1.2) | 0.458 |
| Blood transfusuion[b] | 10(4.1) | 1(1.2) | 0.303 |
| Minor complications | | | |
| Fever[b] | 1(0.4) | 2(2.5) | 0.160 |
| Urinary tract infection[b] | 2(0.8) | 0(0) | 1.000 |
| Bowel ileus[b] | 1(0.4) | 0(0) | 1.000 |
| Superficial wound infection[b] | 18(7.4) | 8(9.9) | 0.498 |

[a] Values are given as number (percentage)., [b] Calculated as number of events divided by total number of patients.

**TABLE 5.** Response of treatment.

| Response rate | Values[a] | |
|---|---|---|
| | Laparotomy group (n=243) | Laparoscopy group (n=81) |
| Complete response | 234(97.9) | 80(98.8) |
| Partial response | 1(0.4) | 1(1.2) |
| Stable disease | 1(0.4) | 0(0) |
| progression | 3(1.3) | 0(0) |

[a] Values are given as number (percentage).

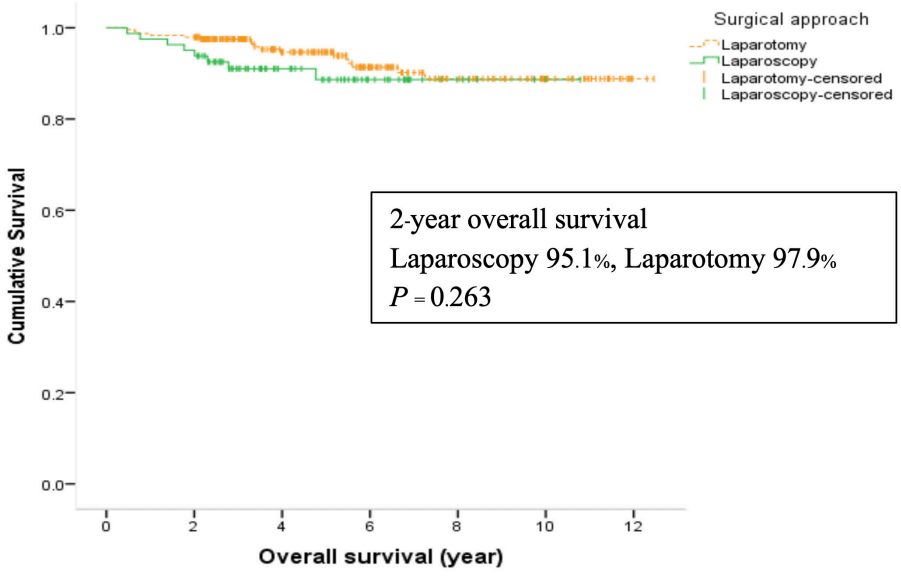


**Fig 1.** Overall survival in 324 endometrial cancer patients stratified by surgical approach (laparoscopy vs. laparotomy)

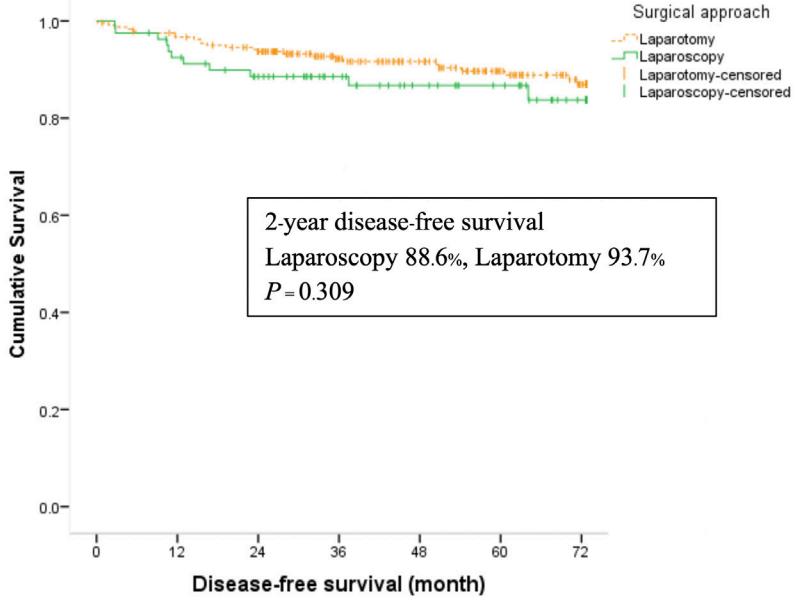


**Fig 2.** Disease-free survival in 324 endometrial cancer patients stratified by surgical approach (laparoscopy vs. laparotomy)

visceral organ injury.[10] This protective feature can be explained by the fact that unnecessary blood loss can obscure surgical field visibility, which leads to increased risk of visceral organ injury and massive blood loss during PL.

The result of our study revealed that PAL was performed more often in the laparoscopy group. This may be due to the fact that laparoscopy provides better visualization and an ability to access the retroperitoneal space, especially in obese patients. Surgeon experience and surgical skill are also the important factors that affect para-aortic lymph node retrieval.

In the present study, there was no statistically significant difference in the number of para-aortic lymph node retrieval between two groups. However, the median number of para-aortic lymph node retrieval was lower than the other studies.[5,9,12-13] We did not perform systematic PAL in all cases. Only para-aortic lymph node sampling was performed in some patients, and limited at the level below IMA. So, this may be causing the lower number of para-aortic lymph node in our study. During the last 2 years, systematic PAL was initiated in our center because surgeons had more experience in this kind of surgery and advanced bipolar electrosurgery was commonly used. The further study which included those patients may provide more information about benefit of systematic PAL in endometrial cancer.

### *Strengths and limitations*

A large sample size and the fact that we include all histologic subtypes were the strengths of this study. The limitation of our study included it retrospective design, and the fact that we included data from a single center. Another limitation is the type of surgical method used was determined at the discretion of each surgeon. Last, the surgical procedure in each surgical technique varies by surgeon, and these variations could have adversely influenced our finding. Importantly, the findings of this study suggest laparoscopic surgery as feasible and safe treatment alternative to laparotomy.

## CONCLUSION

Laparoscopic surgery is an efficacious, achievable and safe technique to treat patients with endometrial cancer. Good surgical skills and proper surgical technique are required to effectuate optimal outcomes.

**Conflict of Interest:** The authors declare no personal or professional conflicts of interest and no financial support from the companies that produce and/or distribute the drugs, devices or materials described in this report.

**Funding disclosure:** This was unfunded study.

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# Characteristics and Clinical Presentations of Patients at the Siriraj Snoring Clinic

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## ABSTRACT

**Objective:** To describe characteristics and clinical presentations of patients in Siriraj snoring clinic and to analyze their relationships with obstructive sleep apnea (OSA) severity.

**Methods:** Three hundred and seventy-three patients'self-administered questionnaires regarding sleep problems recorded between January 2012 and December 2013 and 275 polysomnographic reports were reviewed.

**Results:** Among 373 respondents, there were 247 males (66.2%) and 126 females (33.8%), with an average age of 48 years and body mass index of 28.2 kg/m$^2$. Their most common complaints and comorbidities were snoring ≥3 nights/week (87.9%), worrying about complications from apnea (72.4%), dyslipidemia (36.7%), hypertension (34.3%), and diabetes mellitus (12.1%), respectively. Using apnea-hypopnea index (AHI) of ≥5 and ≥30 events/hour, there were 76.7% and 38.5% of patients diagnosed as OSA and severe OSA, respectively. While using respiratory disturbance index (RDI) with similar cut-off, almost everyone (98.8%) and 60.2% of patents will be diagnosed as OSA and severe OSA, respectively. Characteristics significantly associated with AHI ≥15 events/hour were snoring ≥3 nights/week, witnessed apneas, and nocturia ($p < 0.05$). The comorbidities which significantly associated with OSA group were hypertension, diabetes, and dyslipidemia. There were only weak significant relationships between AHI (and RDI) with ESS and quality of life.

**Conclusion:** The most common complaints in our clinic were loud snoring and worrying about OSA consequences, not excessive daytime sleepiness. Based on RDI criteria, almost everyone were diagnosed as OSA; however, it had poor relationship with patients'symptoms, comorbidities and quality of life. Thus, for better OSA evaluation, we should use data from several aspects, not only AHI nor RDI for proper patient management.

**Keywords:** Obstructive sleep apnea; clinical presentation; characteristic; prevalence (Siriraj Med J 2020; 72: 202-208)

## INTRODUCTION

Obstructive sleep apnea (OSA) is a common disorder that is characterized by narrowing of the upper airway, which leads to abnormal ventilation during sleep.[1] Despite inconsistences in OSA-related epidemiologic data due to differences among the populations being studied and differences in how the disease was identified, substantial evidence has been reported that strongly suggests that untreated OSA may lead to several adverse effects, including cardiovascular diseases, impaired neurocognitive function, decreased quality of life, and increased risk of accidents.[2-8]

Young, *et al.* studied middle-aged adults in the United States and found a prevalence of OSA [defined as apnea-hypopnea index (AHI) ≥5 events/hour (hypopnea

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*http://dx.doi.org/10.33192/Smj.2020.27*

defined as ≥4% oxygen desaturation)] as high as 9% in women and 24% in men.[9] In the same study, the prevalence of OSA syndrome (OSAS) was only 2% in women and 4% in men when OSAS was defined as AHI ≥5 events/hour plus self-reported hypersomnolence. However, a subsequent study by that group found that the prevalence of OSAS plus symptoms of daytime sleepiness had significantly increased over time to 14% in men and 5% of women.[10] In Thailand, the prevalence of OSA and OSAS was reported to be 11.4% (males 15.4%, females 6.3%) and 4.4% (males 4.8%, females 1.9%), respectively.[11] In spite of these reported high rates of prevalence, it is estimated that 93% of women and 82% of men with moderate to severe OSA remain undiagnosed.[12]

The current gold standard method for diagnosing OSA is attended polysomnography (PSG) or type I sleep study that is performed in a sleep lab. However, routine use of PSG in every snoring patient and in every patient with suspected OSA is impractical due to its high cost, long waiting lists, and intensive labor requirements. Furthermore, its reliability and usefulness are increasingly questionable because the results from the sleep study may not associate with the clinical presentations of the patients, i.e. symptoms, signs and quality of life. Waiting for the diagnosis and treatment based on only AHI from the PSG is, thus, possibly inappropriate. To date, the concept of personalized diagnosis and treatment has become more popular. Putting several aspects of information, not only PSG data, may be a better way of patient approach. Understanding comprehensively on the characteristics and clinical presentation of patients may guide us to improve service for the better care.

Although the Snoring Clinic at our center has been established for a decade, data relating to the characteristics and clinical presentations of the patients that attend our clinic are scarce. Accordingly, the aim of this study was to determine the characteristics and clinical presentations of the patients that attend the Siriraj Snoring Clinic, and to investigate association between the identified characteristics and presentations, and severity of obstructive sleep apnea.

## MATERIALS AND METHODS

This retrospective chart review included patients aged ≥18 years who sought treatment for sleep problems at a snoring clinic of the Department of Otorhinolaryngology, Faculty of Medicine Siriraj Hospital, Mahidol University during January 2012 to December 2013. Siriraj Hospital is a 2,300-bed national tertiary referral hospital that is located in Bangkok, Thailand. This study period was selected, because it fell just before PSG scoring definition was changed.[13] The data obtained from patients in the snoring clinic included demographic and clinical data, sleep history, Epworth Sleepiness Scale (ESS) and functional outcomes of sleep questionnaire (FOSQ) data. The PSG data including AHI and respiratory disturbance index (RDI) were obtained from the electronic medical record (hospital intranet). Among patients that had PSG results (AHI and RDI) available for review, those results were investigated for association with ESS and FOSQ scores. Patients with missing or incomplete sleep history and/or physical findings were excluded. The protocol for this study was approved by Siriraj Institutional Review Board (Si 728/2016).

### *Epworth sleepiness scales*

The Epworth Sleepiness Scale (ESS)[14] is an eight-item questionnaire assesses a person's likelihood of falling asleep during eight common situations. Scoring ranges from 0 to 3 for each item for a total possible score of 24 points. A higher score indicates a higher level of sleepiness. In this study, we used the validated Thai version of the ESS.[15]

### *Functional Outcomes of Sleep Questionnaire (FOSQ)*

The Functional Outcomes of Sleep Questionnaire (FOSQ) is a disease-specific health-related quality of life questionnaire. It consists of 30 items that focus on five domains of normal daily life, including general productivity (8 items), vigilance (7 items), social outcome (2 items), activity level (9 items), and sexual relationship (4 items). The mean of each subscale and a global score was reported in a set of scores ranging from 1 to 4 and 5 to 20, respectively. A lower score reflects a greater level of dysfunction or worse quality of life. In this study, we use the validated Thai version of the FOSQ.[16]

### *Polysomnography (PSG)*

Standard technician-attended PSG in this study included the recording of electroencephalography (EEG), electrooculography (EOG), submentalis (chin) electromyography (EMG), electrocardiogram (ECG), thermistors for nasal and oral airflow, thoracic and abdominal impedance belts for respiratory efforts, pulse oximetry, microphone for snoring, and sensors for leg and sleep position. Sleep stages and respiratory parameters were scored according to the recommendations of the American Academy of Sleep Medicine (AASM) manual (2007).[13] Apnea was defined as a 90% drop in oronasal thermal flow lasting at least 10 seconds. Hypopnea was defined as 30% or greater drop in airflow for 10 seconds or longer associated with ≥ 4% oxygen desaturation.

Respiratory event-related arousal (RERA) was defined as is an event during which patients take a series of breaths with increasing respiratory effort that leads to an arousal from sleep that does not satisfy the criteria for apnea or hypopnea. Severity of OSA was classified as mild degree (mild OSA) when the AHI [defined as average of apnea events plus hypopnea events per hour (h) of sleep] was within the range of 5 to 14 events/h. Moderate OSA and severe OSA was defined if the AHI was from 15 to 30 events/h and more than 30 events/h, respectively. Respiratory Disturbance Index (RDI) was defined as the average number of respiratory disturbances (i.e., obstructive apneas, hypopneas, and RERAs) per hour.

### *Statistical analysis*

SPSS Statistics for Windows version 18 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analyses. Continuous data are presented as mean ± standard deviation, and categorical data are shown as frequency and percentage. One-way ANOVA with Bonferroni post hoc test and Chi-square test was used to compare continuous data and categorical data between groups, respectively. Spearman's correlation coefficient analysis was used to compare between PSG findings (AHI or RDI) and both FOSQ scores and ESS scores. Statistical significance was determined at a $p$-value less than 0.05.

## RESULTS

During January 2012 to December 2013, there were 373 patients (247 males and 126 females) with mean age of 48 ± 13.6 years (range: 18-88) who visited our snoring clinic and completed sleep history questionnaire. The mean body mass index (BMI) and ESS score of all participants was 28.2 ± 6.2 kg/m$^2$ and 10.0 ± 4.9, respectively. There were 306 patients who completed the FOSQ, and 350 patients who completed the ESS questionnaire. The most common reasons that patients reported for seeking care at our clinic were snoring at least 3 nights per week (328 patients, 87.9%), worrying about complications from apnea (270 patients, 72.4%), and lacking of energy / tiring during wake time (253 patients, 67.8%),. The three most common comorbidities among this cohort were dyslipidemia (137 patients, 36.7%), hypertension (128 patients, 34.3%), and diabetes (45 patients, 12.1%).

Using one-way ANOVA, there were statistically significant differences of BMI, ESS, AHI, and RDI among various groups of OSA severity. Subsequent analyses with Bonferroni post hoc test demonstrated that ESS scores were significantly different between severe OSA and non-OSA groups and between severe OSA and mild OSA groups. In addition, BMI were significantly different among all groups, except for mild OSA and moderate OSA groups. The clinical characteristics that associated with moderate-to-severe OSA classified by AHI (≥15 events/h) were snoring at least 3 nights per week, snoring bothering other people, witnessed apneas, and nocturia ($p$<0.05). The sole characteristic that associated with moderate-to-severe OSA classified by RDI (≥15 events/h) was worrying about complications from apnea ($p$<0.05).

The comorbidities that had statistical significance in the OSA group according to AHI (AHI ≥5 events/h) were hypertension, diabetes, and dyslipidemia. The sole comorbidity that had statistically significant correlation in the OSA group according to RDI (RDI ≥5 events/h) was hypertension.

The clinical presentations and polysomnographic findings of participants are demonstrated in Tables 1 and 2.

### *Polysomnographic findings*

There were a total 275 patients (177 males and 98 females) who underwent PSG. Of those, 229 underwent full-night PSG, and 46 underwent split-night PSG. PSG findings revealed a diagnosis of OSA according to AHI criteria in 211 (76.7%) patients, a diagnosis of mild OSA in 49 patients (17.8%), moderate OSA in 56 patients (20.4%), and severe OSA in 106 patients (38.5%). Using RDI with a similar cut-off point as AHI to diagnose OSA, 238 patients (98.8%) were diagnosed as OSA, 39 patients (16.2%) as mild OSA, 54 patients (22.4%) as moderate OSA, and 145 patients (60.2%) as severe OSA.

### *Correlation between FOSQ scores, ESS, and polysomnographic findings*

There was no statistically significant difference between FOSQ scores and severity of OSA as shown in Table 3. Spearman's correlation coefficient analysis was used to compare between FOSQ scores, ESS, and polysomnographic findings (both AHI and RDI). Spearman's correlation coefficients between ESS, FOSQ domain scores, and FOSQ global score, and AHI and RDI are demonstrated in Table 4. Scatter diagrams showing correlation between ESS and AHI, and between ESS and RDI are given in Fig 1.

## DISCUSSION

OSA is a highly prevalent disorder among general population. Its common nighttime manifestations include snoring, choking at night, witnessed apneic episodes, nocturia, and frequent arousals; and, its common daytime

**TABLE 1.** Characteristics of 275 patients who underwent polysomnography stratified by OSA severity.

| Characteristics | Non-OSA (N=64) | Mild OSA (N=49) | Moderate OSA (N=56) | Severe OSA (N=106) | *p*-value |
|---|---|---|---|---|---|
| Male gender | 34 (12.4%) | 25 (9.1%) | 36 (13.1%) | 82 (29.8%) | 0.10 |
| Female gender | 30 (10.9%) | 24 (8.7%) | 20 (7.3%) | 24 (8.7%) | 0.10 |
| BMI (kg/m$^2$) | 24.8 ± 3.9 | 28.0 ± 4.4 | 27.8 ± 3.8 | 30.1 ± 7.0 | ***<0.001**** |
| Age (years) | 47.0 ± 14.5 | 50.9 ± 13.9 | 50.5 ± 11.0 | 48.8 ± 12.8 | 0.30 |
| ESS score | 9.1 ± 5.2 | 9.1 ± 4.9 | 10.8 ± 4.7 | 10.9 ± 4.9 | ***0.04**** |
| AHI | 2.4 ± 1.4 | 9.4 ± 2.9 | 21.7 ± 4.3 | 58.2 ± 27.7 | ***<0.001**** |
| RDI | 15.8 ± 10.7 | 26.1 ± 13.5 | 39.7 ± 10.4 | 66.2 ± 20.9 | ***<0.001**** |

Data presented as number and percentage or mean ± standard deviation

**The p-values of <0.05 indicate statistical significance.*

**Abbreviations:** OSA, obstructive sleep apnea; AHI, apnea-hypopnea index; BMI, body mass index; ESS, Epworth Sleepiness Scale; RDI, respiratory disturbance index

**TABLE 2.** Clinical presentation among all study participants (N=373), and among patients who underwent polysomnography (n=275).

| Symptoms | All patients N (%) | AHI <15 N (%) | AHI ≥15 N (%) | *p*-value |
|---|---|---|---|---|
| Worrying about complications from apnea | 270 (72.4) | 76 (27.6) | 121 (44.0) | 0.18 |
| Social consequences due to snoring | 235 (63.0) | 67 (24.4) | 114 (41.5) | 0.06 |
| Excessive daytime sleepiness | 185 (49.6) | 49 (17.8) | 86 (31.3) | 0.11 |
| Snoring ≥3 nights per week | 328 (87.9) | 94 (34.2) | 151 (54.9) | ***0.009**** |
| Snoring bothering other people | 132 (35.4) | 34 (12.4) | 69 (25.1) | ***0.035**** |
| Nocturnal choking/gasping | 204 (54.7) | 57 (20.7) | 98 (35.6) | 0.10 |
| Witnessed apneas | 158 (42.4) | 34 (12.4) | 86 (31.3) | ***<0.001**** |
| Morning headache / Feeling dry | 249 (66.8) | 68 (24.7) | 109 (39.6) | 1.47 |
| Lacking energy / Tiring during wake time | 253 (67.8) | 74 (26.9) | 110 (40.0) | 0.68 |
| Falling asleep while driving | 140 (37.5) | 36 (13.1) | 74 (26.9) | ***0.02**** |
| Deficits in cognition and vigilance | 196 (52.5) | 58 (21.1) | 88 (32.0) | 0.63 |
| Nocturia | 126 (33.8) | 32 (11.6) | 66 (24.0) | ***0.034**** |

**The p-values of <0.05 indicate statistical significance between AHI <15 and AHI ≥15 events/h*

**Abbreviation:** AHI, apnea-hypopnea index

**TABLE 3.** FOSQ domain and global scores stratified by OSA severity (N=275).

| | Non-OSA | Mild OSA | Moderate OSA | Severe OSA | *p*-value |
|---|---|---|---|---|---|
| General productivity | 2.9 ± 1.1 | 3.0 ± 0.8 | 3.3 ± 0.7 | 3.0 ± 0.9 | 0.44 |
| Social outcome | 3.5 ± 0.9 | 3.5 ± 0.9 | 3.6 ± 0.9 | 3.3 ± 1.2 | 0.46 |
| Activity level | 3.1 ± 0.7 | 3.1 ± 0.7 | 3.3 ± 1.1 | 2.9 ± 0.7 | 0.13 |
| Vigilance | 2.7 ± 1.2 | 2.9 ± 1.3 | 2.9 ± 1.1 | 2.8 ± 1.3 | 0.78 |
| Sexual relationship | 1.8 ± 1.5 | 2.2 ± 1.67 | 2.4 ± 1.6 | 2.3 ± 1.4 | 0.16 |
| FOSQ global | 14.0 ± 4.1 | 14.8 ± 3.8 | 15.4 ± 3.5 | 14.3 ± 4.1 | 0.32 |

*The data were presented in mean ± standard deviation (SD) with p-values (p < 0.05, it will indicate statistical significance).*
**Abbreviations:** FOSQ, functional outcomes of sleep questionnaire; OSA, obstructive sleep apnea; AHI, apnea-hypopnea index

**TABLE 4.** Spearman's correlation coefficients between ESS, FOSQ domain scores, and FOSQ global score, and AHI and RDI.

| | Spearman's correlation coefficient | *p*-value (AHI) | Spearman's correlation coefficient | *p*-value (RDI) |
|---|---|---|---|---|
| Epworth Sleepiness Scale (ESS) | 0.2 | ***<0.002**** | 0.12 | ***<0.003*** |
| General productivity | -0.04 | 0.56 | -0.02 | 0.76 |
| Social outcome | -0.08 | 0.25 | -0.02 | 0.76 |
| Activity level | -0.14 | ***0.03**** | -0.15 | ***0.03*** |
| Vigilance | -0.05 | 0.50 | 0.02 | 0.74 |
| Sexual relationship | 0.13 | 0.06 | 0.12 | 0.11 |
| FOSQ global | -0.002 | 0.97 | 0.01 | 0.85 |

**The p-values of <0.05 indicate statistical significance.*
**Abbreviations:** ESS, Epworth Sleepiness Scale; FOSQ, functional outcomes of sleep questionnaire; AHI, apnea-hypopnea index; RDI, respiratory disturbance index

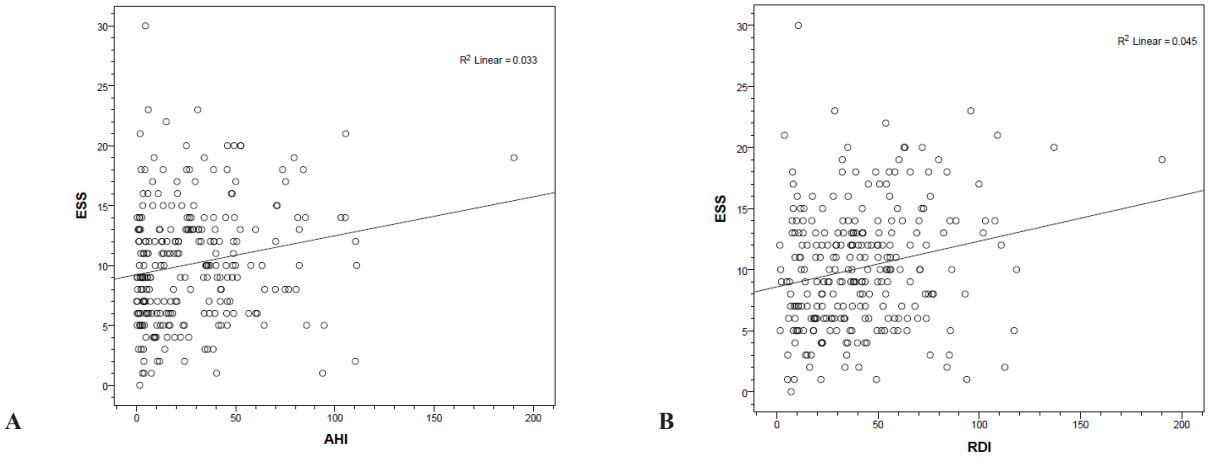


**Fig 1.** **(A)** Scatter diagrams showing correlation between Epworth Sleepiness Scale (ESS) and apnea-hypopnea index (AHI), and **(B)** between ESS and Respiratory Disturbance Index (RDI).

manifestations include excessive daytime somnolence, poor concentration, poor memory, mood changes, and irritability.[17] Not surprisingly, the results of this study showed the most common reason that patients visit our clinic is because of snoring problems. The second most common clinical manifestation was worrying about adverse consequences of untreated OSA, which is different from the second most common cause of seeking treatment reported in previous studies.[18,19] Increasing public awareness about this disease may be one of the reasons why there is such a long waiting list for PSG at our center. Contrary to what we had earlier hypothesized, excessive daytime sleepiness (EDS), although reported as a complaint by 49.6% of patients, was not one of the most commonly reported complaints. Although our results showed ESS scores to be significantly correlated with OSA severity to a modest degree, the level of excessive daytime sleepiness among our cohort was probably not severe enough to motivate them to seek medical attention. Furthermore, it was probably that some patients frequently complained of fatigue, tiredness, and lack of energy rather than sleepiness.[20]

The prevalence of OSA (AHI ≥5 events/h) in our snoring clinic was 76.7%, which is higher than in general population.[9-11,21] This was not unexpected because most patients in our study were symptomatic and/or were at high-risk for being diagnosed as OSA which are different from general population. However, if we used RDI criteria (RDI ≥5 events/h), almost every patient (98.8%) would be diagnosed as OSA, and more patients would be diagnosed as severe OSA. Moreover, our study revealed the characteristics associated with moderate to severe OSA by AHI (AHI ≥15 events/h) to be snoring at least 3 nights per week, snoring that bothers other people, witnessed apneas, and nocturia ($p<0.05$); whereas, the only characteristic associated with moderate to severe OSA by RDI (RDI of ≥15 events/h) was worrying about complication from apnea. This may imply that AHI scored by the recommended hypopnea criteria (30% drop of airflow associated with ≥4% desaturation) from the AASM manual 2007 seems to be more clinically relevant and more specific to OSA symptoms than RDI or possibly AHI from currently recommended hypopnea criteria of AASM manual 2012.

The comorbidities that were different between OSA and non-OSA patients when using AHI were hypertension, diabetes, and dyslipidemia; while no difference in comorbidities was observed between OSA and non-OSA patients when using RDI. Furthermore, no relationship was observed between OSA and cardiovascular diseases, which is different from the findings of other studies.[22-24] Regarding quality of life, we found only a weak relationship between activity level and AHI/RDI, which was slightly different from some studies.[25,26] All of these findings suggest that RDI or AHI alone should not be used to diagnose OSA. Alternatively, whether AHI or RDI are used, they should be used in conjunction with data relating to other aspects of the disease such as patient symptoms, comorbidities, and quality of life.

### *Limitations*

The mentionable limitations of this study include its retrospective design and the fact that we included subjective patient-reported questionnaire data. Further prospective study is needed to confirm and further elucidate the associations between severity of OSA, and patient characteristics and clinical presentations in order to improve diagnosis, treatment, and outcomes. The strength of this study is its relatively large sample size, with a significant proportion of those patients having PSG results available for analysis.

## CONCLUSION

This study showed that most common chief complaints of patients in our clinic were loud snoring and worrying about adverse consequences of untreated OSA, but not EDS. Furthermore, the clinical characteristics that associated with AHI ≥ 15 events/h were snoring at least 3 nights per week, snoring bother other people, witnessed apneas, and nocturia. Using criteria of AHI of ≥ 5 events/h, seventy-six percent of patients were diagnosed as OSA. However, if using criteria of RDI ≥ 5 events/h, almost every patients will be diagnosed as OSA. Given the only weak relationship between AHI (and RDI) with ESS and quality of life. AHI combined with other patient factors was found to be superior to RDI alone for diagnosis of OSA.

## ACKNOWLEDGMENTS

The authors gratefully appreciate the kind contributor of Mr.Suthipol Udompanturak for his assistance with statistical analysis, Ms.Jeerapa Kerdnoppakhun for her data and documentary assistance. The authors also thank all of the patients who were involved in this projects.

**Conflict of interest declaration:** All authors declare no personal or professional conflicts of interest relating to any aspect of this study.

**Funding disclosure:** This was an unfunded study.

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# Incidence of Ocular Toxicity from Iron Chelating Agents at Siriraj Hospital

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## ABSTRACT

**Objective:** To determine the incidences of ocular toxicity and ocular findings, including structural and functional abnormalities, caused by iron chelating agents and detected by an electrophysiologic test at Siriraj Hospital.

**Methods:** A retrospective chart review was conducted of patients receiving multiple blood transfusions and iron chelation therapy who had an eye examination at Siriraj Hospital between January 1995 and December 2017.

**Results:** Ninety-seven charts were reviewed. The 88 patients included comprised 41 males and 47 females. Their ages ranged from 1 year 11 months to 47 years, with children predominant (mean: 8.13 years). Beta thalassemia HbE was the main diagnosis (87.5%). After receiving iron chelating agents, 3 patients had abnormal eye findings with suspected ocular toxicity. Two had retinal pigmentary changes, but only one of those two displayed a mildly decreased response in a scotopic electroretinogram. Although the third patient also showed a decreased electroretinogram response, there were no obvious retinal changes. All three received the iron chelating agents desferrioxamine, deferiprone, and/or deferasirox at different doses and for various durations.

**Conclusion:** Although some pigmentary retinopathy and decreased electroretinogram responses were found, leading to ocular toxicity being suspected, there was a very low incidence of ocular toxicity from the chelating agents. In addition, the dosages of the agents causing ocular toxicity, and the duration of that toxicity, were inconclusive. Moreover, a gold standard for identifying ocular toxicity caused by chelating agents was not able to be established. Consequently, the risks and benefits of employing eye screening coupled with an invasive procedure like an electrophysiologic test will need to be weighed, especially with pediatric patients.

**Keywords:** Ocular toxicity; iron chelating agent; thalassemia; electroretinogram (Siriraj Med J 2020; 72: 209-213)

## INTRODUCTION

Thalassemia, an inherited blood disorder, has a high incidence rate in Thailand. Its treatment depends on the type and severity of the disease involved. Although blood transfusions are a treatment option for patients with anemia, an iron overload can occur following multiple blood transfusions, which can be potentially fatal. Chelation therapy utilizing iron chelating agents helps to remove the excessive iron from the body.

The iron chelating agents used at Siriraj Hospital are desferrioxamine, deferiprone, and deferasirox. Unfortunately, their use may lead to ocular toxicity, presenting in the form of a deterioration in visual acuity and color vision, night blindness, a scotoma or constricted visual field, retinopathy, optic neuropathy, and an abnormal retinal function detectable by electroretinogram (ERG).[1-5] Little information has been reported on the incidences of these ocular toxicities, and there are no standard eye-screening guidelines for patients.

Our study aimed to determine the incidences of ocular toxicity and ocular findings, including both structural and functional abnormalities, detected by ERG and

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*http://dx.doi.org/10.33192/Smj.2020.28*

arising from the use of iron chelating agents at Siriraj Hospital.

## MATERIALS AND METHODS

A retrospective chart review was conducted of patients who received multiple blood transfusions with iron chelation therapy and had an eye examination at Siriraj Hospital between January 1995 and December 2017. Excluded from the study were patients who had abnormal eye conditions before receiving iron chelating agents, such as a previous optic neuropathy or retinopathy due to toxic agents or other causes. This study was conducted after approval by the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University (Si 233/2017).

The baseline visit was defined in two ways. If a patient had not yet been administered any chelating agents, it was deemed to be that visit when the patient had a normal eye examination. However, in cases where a patient had already commenced the use of the agents, the baseline visit was the first visit after the treatment had begun when a normal eye examination was performed. Demographic data (age, sex, body weight, and height) were recorded. The diagnosis, type and dosage of each iron chelating agent received, and serum ferritin level were also recorded. The eye examinations included LogMAR visual acuity measurements, anterior segment and fundus examinations, color vision tests, visual field tests, and electrophysiologic tests. The follow up eye examination is routinely done yearly.

Ocular toxicity from the agents was defined as either a decrease in the best corrected visual acuity (expressed in a LogMAR scale) of more than two steps from a patient's baseline, or any abnormal finding in any part of an eye examination.

### Statistical analysis

A descriptive statistical analysis of the quantitative data was performed by determining the mean, minimum, and maximum values. All analyses were carried out using SPSS Statistics for Windows, version 18 (SPSS Inc., Chicago, IL, USA).

## RESULTS

A 97-chart review was conducted. The 88 included patients comprised 41 males and 47 females. Their ages ranged from 1 year 11 months to 47 years. However, with a mean of 8.13 years, most patients were children. Beta thalassemia HbE was by far the most common diagnosis (87.5%).

After receiving iron chelating agents, 3 patients were found to have abnormal eye findings suggestive of ocular toxicity (Table 1).

***Patient one:***

This was a 9-year-old girl with beta thalassemia HbE. She received deferasirox (21 mg/kg/day) for about 12 months before switching to deferiprone (31.9 mg/kg/day initially, but adjusted to 60 mg/kg/day) for around 17 months. Desferrioxamine (16 mg/kg/day) was subsequently added.

At the toxic visit, she was 17 years old. By that stage, she had received the agents for 7 years. Her serum ferritin level was 4,148 ng/ml. Changes in the pigment of the retina were found in both eyes, but her visual acuity, color vision, and visual field were unremarkable. An ERG was not performed during that visit.

***Patient two:***

This was a 6-year-old boy with beta thalassemia HbE. He received desferrioxamine (20 mg/kg/day) for about 3 months before the dose was increased to 40 mg/kg/day and deferasirox (35 mg/kg/day) was added. He received both agents for approximately 48 months, and then they were stopped. Following a 3-month cessation, the chelation therapy was recommenced using deferasirox (31 mg/kg/day). After 8 months, the agent was switched to deferiprone (71 mg/kg/day) and desferrioxamine (no dosage was recorded).

At the toxic visit, he was 15 years old. His serum ferritin level was 1,912 ng/ml. A pigmentary change was found in the retina of both eyes, and he had a mildly decreased scotopic ERG for both eyes. Other ocular findings were unremarkable.

***Patient three:***

This was a 6-year-old girl with beta thalassemia HbE. She initially received desferrioxamine (no dosage was recorded). Four years later, deferiprone (80 mg/kg/day) was added. Because she developed liver toxicity, the chelating agents were given off and on.

At the toxic visit, she was 20 years old. She received deferiprone (89.5 mg/kg/day). Her serum ferritin level was 894.3 ng/ml. Only an abnormal photopic and scotopic ERG were found. Otherwise, her ocular findings were normal.

## DISCUSSION

Iron chelating agents are used for the treatment of iron overload in patients with hematologic conditions that require frequent blood transfusions to prevent hemosiderosis. If left untreated, hemosiderosis may lead

**TABLE 1.** Three cases were suspected to have ocular toxicity from the iron chelating agents.

| Patient (no.) | Age at 1st visit (year) | Age at toxic visit (year) | Iron chelating agents at toxic visit | Dose of each agent (mg/kg/day) | Serum ferritin at toxic visit (ng/ml) | Abnormal eye findings |
|---|---|---|---|---|---|---|
| 1 | 9 | 17 | Deferiprone<br>Desferrioxamine | 60<br>16 | 4,148 | Pigmentary changes at retina in both eyes |
| 2 | 6 | 15 | Deferiprone<br>Desferrioxamine | 71–85<br>Not recorded | 1,912 | Pigmentary changes at retina in both eyes<br>Mildly decreased scotopic ERG in both eyes |
| 3 | 6 | 20 | Desferrioxamine<br>Deferiprone | Not recorded<br>89.5 | 894.3 | Decreased ERG response in both eyes |

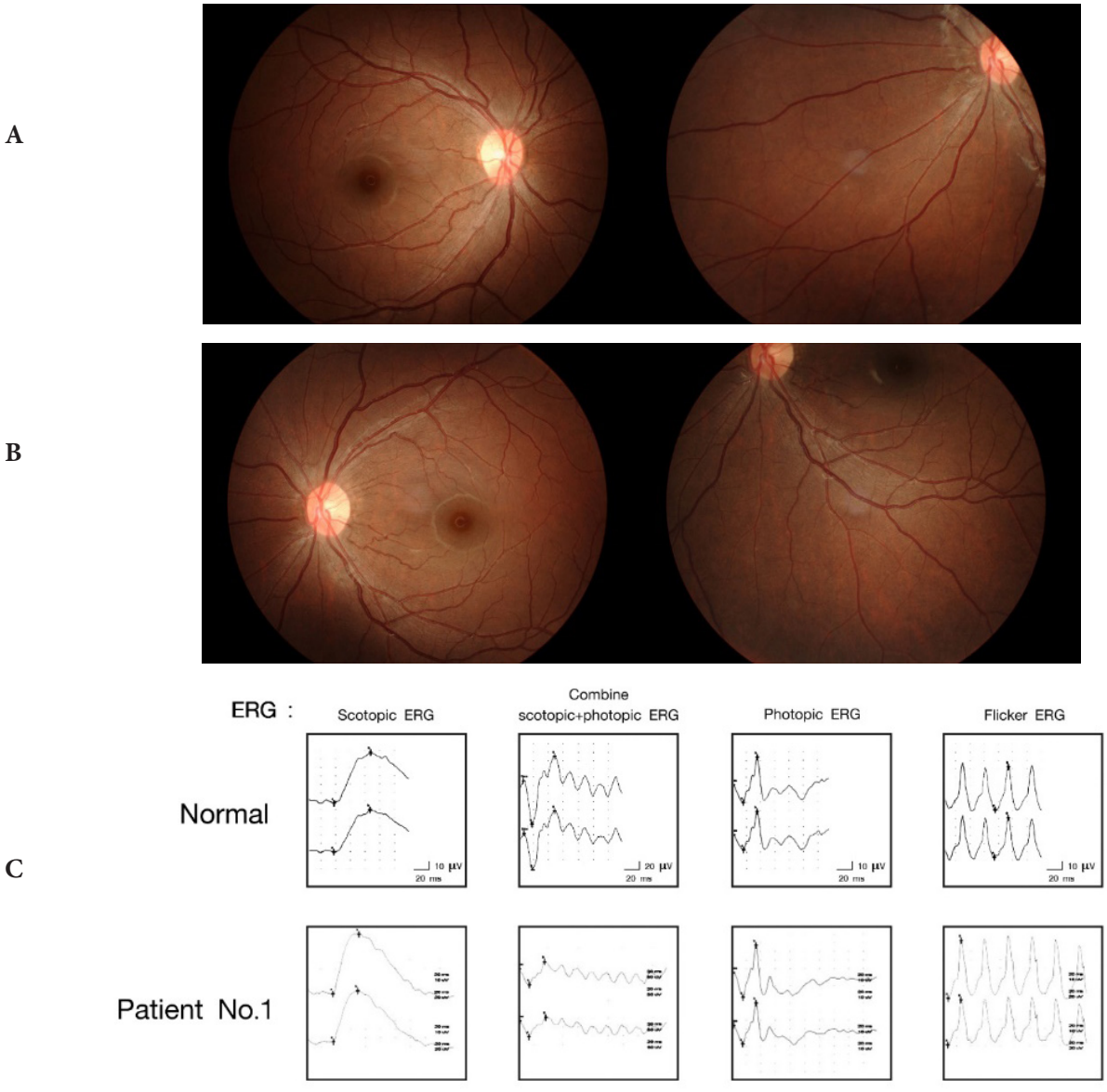


**Fig 1.** Fundus photography of the patient No.1 showed pigmentary retinopathy of the right eye **(A)** and left eye **(B)**. Normal ERG findings in both eyes (**C**: upper=right eye, lower=left eye).

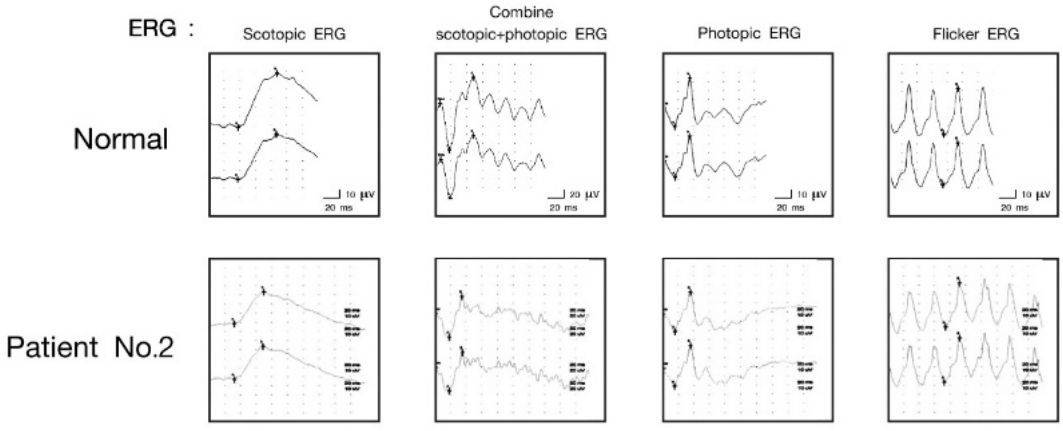


**Fig 2.** ERG findings in patient No.2 showed mildly decreased scotopic ERG in both eyes (upper=right eye, lower=left eye).

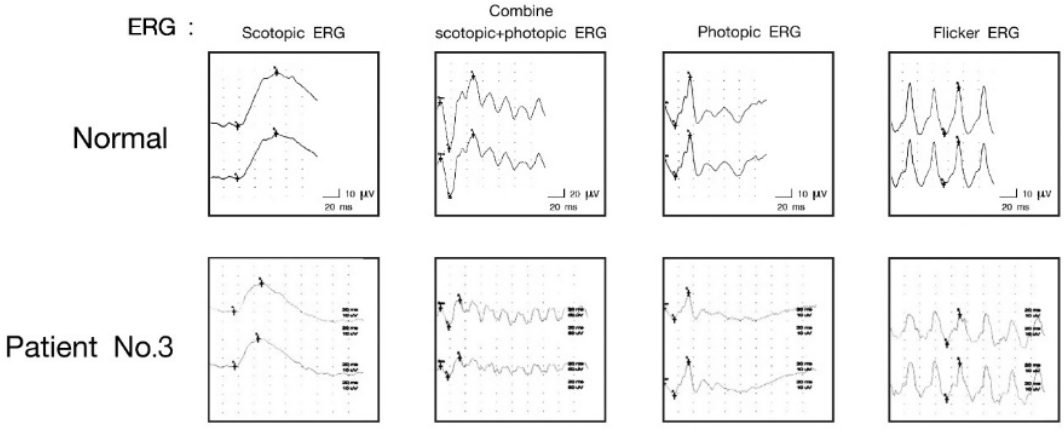


**Fig 3.** ERG findings in patient No.3 showed decreased photopic and scotopic ERG in both eyes (upper=right eye, lower=left eye).

to diabetes, cardiac disease, and hepatic dysfunction. There are many administration routes (for example, oral, subcutaneous and intravenous).

Desferrioxamine is widely used as an iron chelating agent for both intravenous and subcutaneous administration. It has a systemic toxicity effect on the cardiovascular, respiratory, gastrointestinal, cutaneous, and nervous systems. Bone dysplasia and high-frequency sensory neuronal hearing loss have been reported[1]. As to ocular toxicity, desferrioxamine can cause nyctalopia, abnormal color perception, visual field defects, cataract formation, optic neuropathy, and pigmentary retinopathy.[5] Various mechanisms of desferrioxamine toxicity have been hypothesized, such as the induction of oxidation that damages the blood-retinal barrier and reduces the concentration of other metal ions, like $Cu^{2+}$ and $Zn^{2+}$.[6] It is still unclear whether ocular toxicity is dose-dependent or not. However, Simon S et al.,[7] showed that the risk of developing systemic toxicity increased with lower iron loads and with desferrioxamine dosages higher than 50 mg/kg/day.

Deferiprone, an alternative or adjunctive regimen to desferrioxamine, is orally administered. It is able to cross the blood-retinal barrier, and it has been reported to cause damage to the retinal pigment epithelium (RPE).[8]

Deferasirox is a newer, oral-efficient, iron chelator. There has been a case report of deferasirox inducing maculopathy, which was demonstrated by optical coherence tomography.[9]

A study by Baath JS et al.,[10] reported that desferrioxamine-related ocular toxicity was a rare and mild finding. Out of 84 patients who received regular desferrioxamine treatment, only one (1.2%) had desferrioxamine-related ocular toxicity. The researchers found central blurriness and retinal pigmentary changes, shown by examination and decreased central responses in electroretinography. Nevertheless, those changes proved to be completely reversible after a change from intravenous to subcutaneous therapy at a reduced dose. Maura Di Nicola et al.,[1] reported desferrioxamine-related, sight-threatening, ocular toxicity involving the RPE. Damage to the RPE can lead to visual field defects, color vision defects, abnormal electrophysiological

tests, and permanent visual deterioration. Haimovici et al.,[5] described early and unusual features in 16 patients with desferrioxamine-induced retinal toxicity. They found macular and/or peripheral pigmentary changes, reduced electroretinographic amplitudes, and reduced electrooculographic light-peak to dark-tough ratios. Peripapillary, papillomacular, and paramacular patterns of retinal pigment epithelial degeneration were also observed in one patient. Cohen et al.,[11] studied 52 regularly transfused patients who received desferrioxamine by subcutaneous or intravenous infusion. A symptomatic loss of vision and hearing developed in one patient. Both problems improved when chelation therapy was ceased.

In our study, we found 3 patients who were suspected to have ocular toxicity resulting from iron chelating agents. Two had pigment alterations in their retinas, but only one of those two had a mildly decreased response in a scotopic ERG. Although the third patient had a decreased ERG response, there were no obvious retinal changes. All 3 patients had no ocular symptoms or any disturbance in their visual acuity, color vision, or visual field. They all received desferrioxamine, deferiprone, and deferasirox as iron chelating agents at different doses and for a variety of durations.

In conclusion, there is a very low incidence of ocular toxicity arising from the use of these iron chelating agents. Our 22-year chart review revealed only 3 patients who were suspected to have ocular toxicity from objective testing, but without any apparent ocular symptoms. The dosages of these agents that caused the ocular toxicity, and the duration of that toxicity, were still inconclusive, as indicated by our findings and the previous studies mentioned above. No gold standard for identifying the ocular toxicity arising from these agents was able to be established. Consequently, eye screening with an invasive procedure, such as an electrophysiologic test, should be considered after assessing the related risks and benefits, especially with pediatric patients.

We could not calculate the incidence of this toxicity due to a small number of sample size. Our study was a retrospective study and had some limitations. First, there was a lack of detail in the patients' charts about the dosages of the iron chelating agents given. In addition, several agents were frequently given at the same time, which means that the ocular toxicity could have resulted from either any one of them or the particular combination of agents. Second, each patient could not complete all investigations such as lacking of visual field test in young patient. Finally, although the incidence of thalassemia in Thailand is high, eye examinations are not routinely provided; this means that cases of ocular toxicity may go undiagnosed.

## ACKNOWLEDGMENTS

The authors wish to acknowledge the help of Miss Mathuwan Srikong, from the Medical Education Technology Center of the Faculty of Medicine, Mahidol University, for providing us the figures.

**Conflict of interests:** All authors declare no conflict of interests.

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# Activity of Antimicrobial Combinations Against Extensively Drug-Resistant *Acinetobacter baumannii* as Determined by Checkerboard Method and E-test

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## ABSTRACT

**Objective:** Combination therapy is needed to treat extensively drug-resistant (XDR) *Acinetobacter baumannii* infection. Colistin (Col) in combination with another drug is usually used for that purpose. The aim of this study was to determine the activity of antimicrobial combinations against XDR *A. baumannii* using both standard checkerboard (CB) method and E-test. E-test was also evaluated for application in a diagnostic bacteriology laboratory by comparing its efficacy with that of CB method.

**Methods:** Eighty clinical isolates of XDR *A. baumannii* were used to determine the activity of the following antimicrobial combinations by CB method and E-test: Col+cefoperazone/sulbactam (Cps), Cps+moxifloxacin (Mox), and Col+Mox. Comparison of CB and E-test was also evaluated.

**Results:** By CB method, Col+Cps yielded a synergistic effect rate (12.5%) higher than those of the other 2 combinations (CpS+Mox 5% and Col+Mox 0%). The majority of test results revealed additivity. Col+Cps, Cps+Mox, and Col+Mox exhibited additive effect against 78.75%, 85.0%, and 87.5% of isolates, respectively. Overall, E-test and CB yielded only 37.5% concordant rates. However, high concordant rates were specifically observed in additive effect of Col+Cps (73.8%) and Cps+Mox (80.4%).

**Conclusion:** Col+Cps exhibited better activity than the other two combinations against XDR *A. baumannii*. E-test is the method that should be used, but its use is limited to the additive results of Col+Cps and Cps+Mox.

**Keywords:** Synergy test; XDR *Acinetobacter baumannii*; colistin; cefoperazone/sulbactam; moxifloxacin (Siriraj Med J 2020; 72: 214-218)

## INTRODUCTION

*Acinetobacter baumannii*, which is one of the most troublesome pathogens in clinical settings worldwide, has a very high rate of resistance to a wide variety of antimicrobial agents, including aminoglycosides, fluoroquinolones, broad-spectrum beta-lactams, and carbapenems. Extensively drug-resistant (XDR) isolates are common, and pandrug-resistant (PDR) strains have been reported.[1] Therefore, the use of monotherapy is now limited, antimicrobial combinations for the treatment of *A. baumannii* infection are needed. However, appropriate regimens and suitable methods for the determination of *in vitro* synergy in a routine diagnostic laboratory setting are not yet available.

Among the agents that are effective for treating *A. baumannii* infection, colistin (Col) in combination with another drug is usually used for XDR *A. baumannii* therapy. Moxifloxacin (Mox), a fluoroquinolone, has been reported to have better activity than ciprofloxacin against *Acinetobacter* species.[2] For beta-lactam combined with

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*http://dx.doi.org/10.33192/Smj.2020.29*

beta-lactamase inhibitor, combination with sulbactam (*i.e.*, cefoperazone/sulbactam, Cps) seems to have better activity against *A. baumannii* than other combinations since sulbactam has direct antimicrobial activity against the organism.[3,4]

To detect the *in vitro* synergy of antimicrobial combinations, time-kill and checkerboard (CB) tests are standard methods that are commonly used.[5] However, these techniques are time-consuming, labor-intensive, and inappropriate for use in a diagnostic bacteriology laboratory. E-test, which is an easy-to-perform method, has been modified to evaluate antimicrobial combination activity. Therefore, the aims of the present study were to determine the activity of the antimicrobial combinations Col+Cps, Cps+Mox, and Col+Mox against XDR *A. baumannii* by using CB method and E-test, and to evaluate E-test for application in routine clinical service by comparing its efficacy with that of CB method. Since CB method and E-test are based on the same testing principle for determining bacteriostatic activity, CB method was selected as the standard test instead of time-kill method (bactericidal activity determination).

## MATERIALS AND METHODS

Eighty non-repetitive isolates of XDR *A. baumannii* were collected from a culture collection maintained at the Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. They were cultured from various clinical samples (sputum, 56%; blood, 25%; urine, 7.5% and others, 11.5%) from 19 wards during a 6-year period (2006-2011). The isolates were presumptively identified as genus *Acinetobacter* by presence of the following characteristics: gram-negative coccobacilli, catalase-positive, oxidase-negative, glucose-nonfermenter, and ability to grow on MacConkey agar. The ability of isolates to grow at 44°C was further evidence that the isolates were *A. baumannii*. The *A. baumannii* isolates used in this study were resistant to amikacin, gentamicin, cotrimoxazole, ceftazidime, cefotaxime, ceftriazone, cefepime, piperacillin/tazobactam, imipenem, and meropenem, but were susceptible to Col.

Standard powder of antimicrobial agents and E-test strips of Col, Mox, and Cps were kindly provided by Atlantic Pharmaceutical Co., Ltd., Thailand; Bayer Thai Co., Ltd.; and, Pfizer (Thailand) Ltd., respectively. Ethical approval for this study was not required since no human subjects were involved and no patient information was included or reported.

CB method and E-test were performed according to published methods.[5,6] *Pseudomonas aeruginosa* ATCC 27853 was used as the control organism. The interpretive criteria for susceptibility were ≤2 µg/ml, susceptible for Col[7]; 16 µg/ml, susceptible for Cps (16/8, cefoperazone/sulbactam)[3]; and, 2 µg/ml, susceptible for Mox. For CB, two-fold dilutions of Col (2 to 0.031 µg/ml), Mox (32 to 0.125 µg/ml), and Cps (256 to 0.25 µg/ml) were used. These dilutions were prepared fresh immediately prior to use. Three pairs of antimicrobial combinations (i.e., Col+Cps, Col+Mox, and Cps+Mox) were tested by both methods. Minimal inhibitory concentrations (MICs) of each drug alone and in combination with other drugs were read after 20-hours of incubation at 35°C. For the isolates with a MIC exceeding the E-test strip detection limit, the next two-fold dilution was used for fractional inhibitory concentration (FIC) calculation. The FIC index (FICI) was defined as the FIC of drug A plus the FIC of drug B. The FIC of each drug was calculated using the MIC of the drug in combination divided by the MIC of that drug alone. The FICIs were interpreted, as follows: synergy = FICI of ≤0.5; additivity = FICI of >0.5 to ≤1; indifference (no interaction) = FICI of >1 to ≤4; and, antagonism = FICI of >4.

## RESULTS

Susceptibility to Col, Cps, and Mox of all XDR *A. baumannii* isolates tested is demonstrated in Table 1. All XDR isolates in this study were still susceptible to Col, and the majority (56.25%) of them exhibited MIC at 1 µg/ml. All XDR isolates were found to be resistant to Cps and Mox with MIC ranges of 32 to >256 mg/ml and 4 to 32 µg/ml, respectively. The $MIC_{50}$ and $MIC_{90}$ of Cps were 128 µg/ml and 256 µg/ml, respectively; whereas, those MICs of Mox were 16 µg/ml and 32 µg/ml, respectively.

The *in vitro* activities of antimicrobial combinations among Col+Cps, Cps+Mox, and Col+Mox tested by CB and E-test methods against 80 isolates of XDR *A. baumannii* are shown in Table 2. When tested by CB method, synergistic effect was mostly observed from the combinations of Col+Cps (12.5%) and Cps+Mox (5.0%). There was no significant difference ($p$>0.05) between the synergy rates of those two combinations. Synergy was not found in any isolate tested with Col+Mox combination. These results indicate that Cps-based combinations were superior to the others. The most common result interpretation was additivity for all three antimicrobial combinations, including Col+Cps (78.8%), Cps+Mox (85%), and Col+Mox (87.5%).

Based on the MIC values of Cps, all 80 isolates studied could be divided into 3 following groups: ≤64, 128, and ≥256 µg/ml (Tables 3 and 4). For Col+Cps combination, the result showed that better synergistic

**TABLE 1.** Susceptibility results of 80 *Acinetobacter baumannii* isolates to each antimicrobial agent

| Antimicrobial agent | MIC range | $MIC_{50}$ | $MIC_{90}$ | % Susceptibility |
|---|---|---|---|---|
| Col | 0.25 - 2 | 1 | 1 | 100 |
| Cps | 32 - >256 | 128 | 256 | 0 |
| Mox | 4 - 32 | 16 | 32 | 0 |

**Abbreviations:** $MIC_{50}$ = minimum inhibitory concentration for 50% of isolates tested; $MIC_{90}$ = minimum inhibitory concentration for 90% of isolates tested; Col = colistin; Cps = cefoperazone-sulbactam; Mox = moxifloxacin

**TABLE 2.** Results of *in vitro* antimicrobial combination activity determined by checkerboard and E test methods against 80 isolates of *Acinetobacter baumannii*

| Interpretation* | Number of isolates (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Col+Cps | | | Cps+Mox | | | Col+Mox | | |
| | CB | E-test | Con** | CB | E-test | Con** | CB | E-test | Con** |
| S | 10 | 3 | 0 | 4 | 2 | 0 | 0 | 0 | 0 |
| | (12.5) | (3.7) | (0) | (5) | (2.5) | (0) | (0) | (0) | (0) |
| A | 63 | 42 | 31 | 68 | 46 | 37 | 70 | 14 | 7 |
| | (78.8) | (52.5) | (73.8) | (85) | (57.5) | (80.4) | (87.5) | (17.5) | (50) |
| I | 7 | 35 | 2 | 8 | 32 | 2 | 10 | 66 | 11 |
| | (8.7) | (43.8) | (28) | (10) | (40) | (6.3) | (12.5) | (82.5) | (16.7) |
| Total | 80 | 80 | 33 | 80 | 80 | 39 | 80 | 80 | 18 |
| | (100) | (100) | (41.3) | (100) | (100) | (48.8) | (100) | (100) | (22.5) |

**Abbreviations:** CB = checkerboard method; Con = concordance; Col = colistin; Cps = cefoperazone-sulbactam; Mox = moxifloxacin; S = synergy; A = additivity; I = indifference; *No antagonism detected in this study; **Concordant rate was calculated using the E-test result as the total member.

**TABLE 3.** Results of colistin plus cefoperazone-sulbactam activity against each group of *Acinetobacter baumannii* based on cefoperazone-sulbactam minimum inhibitory concentration

| Cps MIC (µg/ml) | Number of isolates | % S | % A | % S+A |
|---|---|---|---|---|
| ≤64 | 22 | 9.09 | 72.73 | 81.82 |
| 128 | 48 | 10.42 | 83.33 | 93.75 |
| ≥256 | 10 | 30 | 70 | 100 |

**Abbreviations:** Cps = cefoperazone-sulbactam; MIC = minimum inhibitory concentration; % S = percent synergy; % A = percent additivity; %S+A = percent synergy plus additivity

**TABLE 4.** Results of cefoperazone-sulbactam plus moxifloxacin activity against each group of *Acinetobacter baumannii* based on cefoperazone-sulbactam minimum inhibitory concentration

| Cps MIC (μg/ml) | Number of isolates | % S | % A | % S+A |
|---|---|---|---|---|
| ≤64 | 22 | 9.09 | 59.09 | 68.18 |
| 128 | 48 | 2.08 | 95.83 | 97.91 |
| ≥256 | 10 | 10 | 90 | 100 |

**Abbreviations:** Cps = cefoperazone-sulbactam; MIC = minimum inhibitory concentration; % S = percent synergy; % A = percent additivity; % S+A = percent synergy plus additivity

effect was obtained from the higher Cps MIC values (Table 3). However, this event was not observed for the Cps+Mox combination (Table 4).

Results obtained from E-test exhibited lower activity than from CB method for all antimicrobial combinations (Table 2). Synergy rates observed from Col+Cps and Cps+Mox by E-test were only 3.7% and 2.5%, respectively. Antagonism was not detected in any antimicrobial combination tested.

Correlation of the results generated by CB method and E-test is also shown in Table 2. Concordant results were commonly observed for the additive effect across all 3 combinations. High additivity concordance was only observed in Col+Cps (73.8%) and Cps+Mox (80.4%). Unfortunately, low concordance of results was observed overall (41.3%, 48.8%, and 22.5% from Col+Cps, Cps+Mox, and Col+Mox, respectively).

## DISCUSSION

Col, which is a drug that was first released for clinical use in 1959 is effective for many kinds of bacteria, including *A. baumannii*. However, clinical use of this agent is limited by its toxicity (nephrotoxicity and neurotoxicity), and this led to the introduction of newer and less toxic agents, such as carbapenems. However, *A. baumannii* continues to develop increasing resistance to these newer effective drugs. Importantly, the majority of XDR strains are still susceptible to Col, but these organisms exhibit rather high MICs that are close to the MIC breakpoint (≤2 μg/ml, susceptible). In the present study, approximately 56% and 6% of isolates had Col MICs of 1 and 2 μg/ml, respectively. High Col MIC values of *A. baumannii* isolates were also reported by another groups of investigators.[8,9] Since Col is considered a last-resort option for treatment of resistant bacterial infection, and Col resistance genes have been reported to spread both vertically and horizontally[10], this drug should be used with both care and caution. It has been recommended that Col should be used in combination with another antimicrobial agent to obtain pharmacological or synergistic effect, lower dose-related toxicity, and lower resistance development rate.

Sulbactam is a member of the serine β-lactamase inhibitor family, and it is unable to inhibit any carbapenemases. The activity of sulbactam against *A. baumannii* clinical isolates was found to be mediated via inhibition of the penicillin-binding proteins (PBPs) PBP1 and PBP3, with very low frequency of resistance.[4] Additionally, the outer membrane of *A. baumannii* appeared to allow good sulbactam uptake, thereby promoting antibacterial activity. However, the pbp3 mutant could result in a high level of resistance to sulbactam. Sulbactam in combination with other antimicrobial agent was shown to exhibit less significant effect than Cps (cefoperazone-sulbactam)-based combination against *A. baumannii*. Cps was included in this study; however, all isolates tested were found to be resistant to the drug with high $MIC_{90}$ (256 μg/ml) (Table 1).

For CB method, Col+Cps was found to exhibit slightly higher *in vitro* activity than the activity observed from the other two combinations against XDR *A. baumannii*. E-test, which is an easy-to-perform method, was found to generate results that largely did not agree with those from CB method. However, E-test usually yielded lower activity of antimicrobial combination than CB, which suggests a low possibility of a very major error result from E-test. Similar results were also reported previously.[11,12] Specifically – due to the generally low concordance rate, E-test may be limited to the additive result of Col+Cps and Cps+Mox.

Many studies[3,13-17] in the *in vitro* activity of Col combined with another agent against *A. baumannii* have

been published. Examples of agents used in combination with Col include carbapenems, sulbactam, fosfomycin, tigecycline, rifampicin, minocycline, lipopeptides, and glycopeptides. Among those combinations, Col+meropenem or Col+imipenem was shown to demonstrate superior activity. To date, up to 90% of *A. baumannii* isolates were found to be resistant to carbapenems. Moreover, the synergistic activity was found to depend on the level of resistance to carbapenems. If the strains were resistant to a high level of those drugs, synergism was not demonstrated.[15] This finding is different from the result observed in the present study. We found the synergy rate from Col+Cps to be increased at a high level of Cps resistance (Table 3). A similar result was obtained from the combination between tigecycline and Cps studied by Li, *et al*.[3] They found the synergistic and additive effects of tigecycline+Cps to be increased with higher tigecycline MIC values. However, the Cps+Mox combination in this study did not demonstrate either of those characteristics. These results indicate different effects of resistance levels on synergy rates depending on each antimicrobial combination. Moreover, it seems that antimicrobial synergy testing may need to be assessed on an isolate-by-isolate basis, and a clinical trial is also needed. In addition and importantly, an effective control measure must be implemented whenever XDR or PDR *A. baumannii* is detected.

## ACKNOWLEDGMENTS

This study was supported by a grant from the Routine to Research Project, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (grant no. 08MI04001/001/08). We thank Atlantic Pharmaceutical Co., Ltd., Thailand; Bayer Thai Co., Ltd.; and, Pfizer (Thailand) Ltd. for providing E-test strips and standard powder of antimicrobial agents. We also thank staff members of the Bacteriology Laboratory, Department of Microbiology, Faculty of Medicine Siriraj Hospital for assistance with the collection of XDR *A. baumannii*.

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# Incidence and Pregnancy Outcomes of Primary Postpartum Hemorrhage Following Implementation of Postpartum Drape with a Calibrated Bag after Normal Vaginal Delivery

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## ABSTRACT

**Objective:** To evaluate the incidence, risk factors, and pregnancy outcomes of primary postpartum hemorrhage (PPH) after the implementation of postpartum drape with a calibrated bag (PDCB) after normal vaginal delivery.

**Methods:** This retrospective chart review compared patients who had normal vaginal delivery in June 2012 prior to PDCB implementation with patients who had normal vaginal delivery in June 2014 after PDCB implementation at Siriraj Hospital.

**Results:** In total, 856 patients were included in this study, with 458 and 398 patients delivered in June 2012 and June 2014, respectively. Baseline characteristics were comparable between the two groups. The incidence of primary PPH increased significantly after the implementation of PDCB (2.8% in 2012 vs. 8.5% in 2014; $p < 0.01$). The incidence of severe PPH was also significantly increased (0.4% in 2012 vs. 2.3% in 2014; $p = 0.02$). Uterine atony was the most common cause and the diagnosis increased after PCDB implementation. The use of additional uterotonic drugs was also significantly increased after PDCB implementation (30.8% in 2012 vs. 85.3% in 2014; $p < 0.01$). The blood transfusion rate was comparable between the two groups. No peripartum hysterectomy or ICU admission was observed in this study. After PDCB implementation, pregnancy-induced hypertension was found to be a significant risk factor for primary PPH ($p < 0.01$).

**Conclusion:** The incidence of primary and severe PPH, and the rate of the use of additional uterotonic drugs were all significantly increased after the implementation of PDCB. Pregnancy-induced hypertension was found to be a significant risk factor for primary PPH.

**Keywords:** Thailand; incidence; pregnancy outcomes; primary postpartum hemorrhage; postpartum drape; calibrated bag; normal vaginal delivery (Siriraj Med J 2020; 72: 219-225)

## INTRODUCTION

Primary postpartum hemorrhage (PPH) is defined as a blood loss of greater than or equal to 500 mL within 24 hours postpartum.[1] PPH is a leading cause of maternal death worldwide, accounting for 27.1% of all maternal mortality.[2] After childbirth, physiologic ligation caused by uterine myometrial contraction is the vital mechanism for the prevention of massive bleeding from

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*http://dx.doi.org/10.33192/Smj.2020.30*

the placental bed, and failure of this mechanism causes uterine atony—the most common cause of primary PPH.[3] To reduce maternal morbidity and mortality, active management of the third stage of labor involving the use of uterotonic agents, controlled cord traction, and uterine massage is widely recommended for the prevention of atonic PPH.[4] However, the main pitfall of the PPH prevention strategy in routine obstetrics practice is an underestimation of postpartum blood loss. Objective measurement of postpartum blood loss is the essential factor that alerts the obstetrician to initiate PPH management. There are many tools for the measurement of postpartum blood loss, including photospectometry, gravimetric method (weighed soaked swabs), collector drape, and visual estimation.[5] Photospectometry is the most accurate method, but its use was found and reported to be impractical in a routine clinical setting.[5,6] Visual estimation was reported to be the least accurate and reliable method of blood loss measurement, as it was found to consistently underestimate blood loss when compared with objective methods.[6-8] Two studies found the visual estimation method to be associated with an error rate of 30% when compared with the gravimetric method or collector drape.[6,8] A study conducted by our team in 2013, which evaluated postpartum blood loss measured in 100 mL discreet categories, confirmed the inaccuracy and underestimation of the visual estimation method when compared with objective measurement using a sterile under-buttock drape (low correspondence and poor agreement, with a Cohen's kappa coefficient of 0.07; $p < 0.05$).[9]

In 2014, our center implemented a new protocol to evaluate postpartum hemorrhage by an objective measurement of postpartum blood loss using a postpartum drape with a calibrated bag (PDCB). In this protocol, postpartum blood loss ≥ 350 mL is considered to be an early warning sign for PPH. The purpose of this study was to evaluate the incidence and risk factors of primary PPH as well as pregnancy outcomes after the implementation of PDCB in 2014 compared with the same following the traditional subjective measurement of blood loss that was performed in 2012 before the implementation of PDCB.

## MATERIALS AND METHODS

### *Study design and population*

This retrospective chart review compared patients who had term normal vaginal delivery in June 2012 prior to PDCB implementation with patients who had term normal vaginal delivery in June 2014 after PDCB implementation at Siriraj Hospital-Thailand's largest national tertiary referral center. Cases with fetal anomalies, stillbirth, multifetal pregnancy, and maternal hematologic diseases that involve clotting mechanisms were excluded. Demographic data, clinical characteristics, pregnancy outcomes, and treatment information were recorded and analyzed. The protocol for this study was approved by the Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (Si 599/2015).

### *Sample size calculation and statistical analysis*

The sample size for this study was calculated using the incidence of PPH from our previous study, which found an increase in postpartum hemorrhage from 3.5% to 9.1% when comparing the subjective visual estimation method with the objective sterile under-buttock drape method, respectively.[9] We used a type 1 error of 0.05 and a type 2 error of 0.2, and the ratio between the groups was 1:1. The calculation plus a 10% increase to compensate for errors of any type yielded a minimum sample size of at least 396 patients per group.

PASW statistics version 18.0 for Windows (SPSS, Inc., Chicago, IL, USA) was used for data analysis. Descriptive data are presented as a number and percentage or the mean ± standard deviation. Comparisons between the groups were performed using the independent t-test for continuous data, and the chi-square test or Fisher's exact test for categorical data. The second and third stages of labor were analyzed using a nonparametric test. Linear regression analysis was used for evaluation of the PPH risk factors. A p-value of less than 0.05 was considered statistically significant.

## RESULTS

In total, 856 patients were enrolled in this study, with 458 patients delivered in the 2012 group and 398 patients delivered in the 2014 group. Demographic data, clinical characteristics, and pregnancy outcomes were comparable between the two groups (Table 1). Table 2 shows the incidence, cause, and treatment relative to primary PPH. It can be seen that postpartum hemorrhage increased significantly after the implementation of PDCB (2.8% in 2012 vs. 8.5% in 2014; $p < 0.01$). The incidence of severe PPH, which is defined as a blood loss greater than 1,000 mL, was also significantly increased after PDCB implementation (0.4% in 2012 vs. 2.3% in 2014; $p = 0.02$). In 2014, the most common cause of PPH was uterine atony, followed with birth passage injury, and retained placenta, respectively (47.1%, 32.4%, and 14.7%). In addition, both atonic and non-atonic PPH had more diagnoses after PDCB implementation and the percentage

**TABLE 1.** Demographic data, clinical characteristics, and pregnancy outcomes in 856 vaginal delivery patients.

| | 2012 deliveries (n=458) Mean±SD | 2014 deliveries (n=398) Mean±SD | *p*-value |
|---|---|---|---|
| Age (years) | 27.0±6.3 | 27.7±6.4 | 0.11 |
| Gestational age (weeks gestation) | 38.1±1.8 | 38.2±1.7 | 0.22 |
| Baseline hematocrit (%) | 34.9±3.3 | 35.2±3.1 | 0.24 |
| Fetal birth weight (grams) | 2,964.5±434.9 | 2,982.5±396.0 | 0.53 |
| | **n (%)** | **n (%)** | ***p*-value** |
| Nulliparous | 212 (46.3%) | 176 (44.2%) | 0.54 |
| Maternal anemia (Hct <30%) | 34 (7.4%) | 20 (5.0%) | 0.15 |
| Gestational diabetes | 22 (4.8%) | 20 (5.0%) | 0.88 |
| Pregnancy-induced hypertension (PIH) | 15 (3.3%) | 24 (6.0%) | 0.05 |
| Infant birth weight | | | 0.24 |
| AGA ($10^{th}$ - $90^{th}$ percentile) | 359 (78.4%) | 329 (82.7%) | |
| SGA (<$10^{th}$ percentile) | 41 (9.0%) | 32 (8.0%) | |
| LGA (>$90^{th}$ percentile) | 58 (12.7%) | 37 (9.3%) | |
| Perinatal asphyxia (Apgar score at 1 min ≤7) | 17 (3.7%) | 13 (3.3%) | 0.72 |
| | **Median (IQR)** | **Median (IQR)** | ***p*-value** |
| Second stage of labor (minutes) | 18 (11, 28) | 17 (11, 29) | 0.66 |
| Third stage of labor (minutes) | 6 (4, 9) | 6 (5, 9) | 0.09 |

A *p*-value <0.05 indicates statistical significance

**Abbreviations:** SD, standard deviation; Hct, hematocrit; AGA, appropriate for gestational age; SGA, small for gestational age; LGA, large for gestational age; IQR, interquartile range

of unidentified causes was reduced (46.1% in 2012 vs. 5.8% in 2014). The use of additional uterotonic drugs was also significantly increased after PDCB implementation (30.8% in 2012 vs. 85.3% in 2014; $p < 0.01$). The blood transfusion rate was comparable between groups (7.7 % in 2012 vs. 11.8% in 2014; $p > 0.05$). There were no cases of massive blood transfusion, peripartum hysterectomy, ICU admission, or maternal death in this study. Tables 3 and 4 show the risk factors associated with primary PPH in the PDCB group, pregnancy-induced hypertension was found to be the only significant risk factor for the development of primary PPH ($p < 0.01$).

## DISCUSSION

In this study, the incidence of primary PPH and severe PPH both increased after the implementation of PDCB. It is known and accepted that the correct measurement of postpartum blood loss can lead to an early diagnosis and management of postpartum hemorrhage. The gravimetric method involving weighing a blood-soaked material has been proved to be more accurate than a visual estimation of postpartum blood loss.[8] However, Ambardekar S, *et al.* performed a randomized trial to compare the efficacy of two different methods for postpartum blood loss measurement (direct method or

**TABLE 2.** Incidence of, cause of, and treatment for PPH between groups (N=856).

| | 2012 deliveries (n=458) n (%) | 2014 deliveries (n=398) n (%) | *p*-value |
|---|---|---|---|
| Incidence | | | |
| Primary PPH (EBL ≥500 mL) | 13 (2.8%) | 34 (8.5%) | *<0.01* |
| Severe PPH (EBL ≥1,000 mL) | 2 (0.4%) | 9 (2.3%) | *0.02* |
| | **2012 (n=13) n (%)** | **2014 (n=34) n (%)** | ***p*-value** |
| Cause | | | 0.19 |
| Uterine atony | 3 (23.1%) | 16 (47.1%) | |
| Non-uterine atony | 4 (30.8%) | 16 (47.1%) | |
| Birth passage injury | 1 (7.7%) | 11 (32.4%) | |
| Retained placenta | 3 (23.1%) | 5 (14.7%) | |
| Unspecified causes | 6 (46.1%) | 2 (5.8%) | |
| Treatment | | | |
| Use of additional uterotonic agents | 4 (30.8%) | 29 (85.3%) | *<0.01* |
| Blood transfusion | 1 (7.7%) | 4 (11.8%) | 1.00 |

A *p*-value<0.05 indicates statistical significance

**Abbreviations:** PPH, postpartum hemorrhage; EBL, estimated blood loss

**TABLE 3.** Risk factors associated with primary PPH after implementation of PDCB (N=398).

| **Risk factors** | **Postpartum hemorrhage n (%) Yes (n=34)** | **No (n=364)** | ***p*-value** |
|---|---|---|---|
| Parity ≥3 1 (2.9%) | 14(3.9%) | 1.00 | |
| Poor ANC (<4 visits) | 1 (2.9%) | 36 (9.9%) | 0.35 |
| Maternal anemia (Hct <30%) | 0 (0.0%) | 16 (4.4%) | 0.38 |
| Gestational diabetes | 3 (8.8 %) | 16 (4.4%) | 0.22 |
| Pregnancy-induced hypertension | 5 (14.7%) | 15 (4.1%) | 0.02 |
| BMI on admission ≥25 kg/m$^2$ | 26 (76.5%) | 235 (64.6%) | 0.19 |
| Prolonged 3$^{rd}$ stage of labor (>30 min) | 4 (11.8%) | 59(16.21%) | 0.63 |
| Operator (medical/nursing students) | 1 (2.9%) | 36 (9.9%) | 0.35 |
| Infant birth weight | | | 0.26 |
| AGA (10$^{th}$ - 90$^{th}$ percentile) | 25 (73.5%) | 304 (83.5%) | |
| SGA (<10$^{th}$ percentile) | 5 (14.7%) | 27 (7.4%) | |
| LGA (>90$^{th}$ percentile) | 4 (11.8%) | 33 (9.1%) | |

A *p*-value <0.05 indicates statistical significance

**Abbreviations:** PPH, postpartum hemorrhage; PDCB, postpartum drape with calibrated bag; ANC, antenatal care; Hct, hematocrit; AGA, appropriate for gestational age; SGA, small for gestational age; LGA, large for gestational age

**TABLE 4.** Linear regression analysis of factors associated with primary PPH after implementation of PDCB.

| | Beta | SE | *p*-value | 95% CI |
|---|---|---|---|---|
| Pregnancy-induced hypertension | 1.306 | 0.555 | 0.02 | 1.24-10.97 |
| Prolonged 3rd stage of labor (>30 min) | -0.359 | 0.556 | 0.518 | 0.24-2.08 |
| BMI on admission ≥25 kg/m$^2$ | 0.530 | 0.423 | 0.210 | 0.74-3.89 |

A *p*-value <0.05 indicates statistical significance

**Abbreviations**: PPH, postpartum hemorrhage; PDCB, postpartum drape with calibrated bag; SE, standard error; CI, confidence interval

blood collecting drape *vs*. indirect method or weighed blood-soaked material) among 1,195 patients. They found that the direct method had a higher efficacy for postpartum blood loss measurement, given that the direct method had a greater mean blood loss and double the incidence of PPH.[10] Previous studies confirmed that objective measurements using a collection bag or drape are appropriate for the measurement of postpartum blood loss.[6,11] In 2016, Bamberg *et al*. conducted a prospective cohort study on the use of a collection bag after vaginal delivery in 809 patients. They found similar results, with an increasing incidence of both PPH and severe PPH. They also recommended this method as a tool for the diagnosis of PPH.[12] In December 2016, the Royal College of Obstetricians and Gynaecologists (RCOG) released a new guideline relative to the prevention and management of PPH. They also confirmed the underestimation of postpartum hemorrhage by the visual estimation method and suggested the use of more reliable methods, including blood collecting drapes or the weighing of soaked swabs after vaginal delivery.[13] Abbaspoor Z, *et al*. reaffirmed the effectiveness of a collection bag in the diagnosis of >500 mL postpartum blood loss (sensitivity of 80%, Specificity = 95. 7%, PPV = 88.9%, and NPV= 91.8%).[14] Accordingly, it is clear that the objective measurement of postpartum blood is superior to the subjective measurement of postpartum blood, especially via the use of PDCB, and consequently, it is presently recommended in routine obstetrics practice, where it is approved as a precise tool for the early diagnosis of primary postpartum hemorrhage.

In this study, the use of 350 mL of postpartum blood loss as an early warning sign for PPH had the effect of increasing the rate of the use of uterotonic agents after the implementation of PDCB. Although uterotonic agents are the main medication for the prevention of postpartum hemorrhage, they all have adverse effects. For example, oxytocin may cause hemodynamic instability or water intoxication, while methylergonovine can cause vasoconstriction leading to hypertension.[15] The risk of these potential adverse effects should be considered and weighed up on a case-by-case basis when using uterotonic agents based on blood loss findings from using the PDCB method. Evaluation of the clinical signs and symptoms, especially the pulse and blood pressure, rather than the blood loss volume alone should be considered as standard practice for the prevention of PPH before prescribing additional uterotonic agents.[13]

Pregnancy-induced hypertension was found to be the only independent risk factor for the development of primary PPH in this study. Many risk factors associated with primary PPH have been reported in the literature, including previous PPH, grand multiparity, macrosomia, prolonged used of oxytocin, and prolonged third stage of labor.[16,17] Shortening the duration of the third stage of labor showed benefit for PPH prevention, which was consistent with the recommended process of active management of the third stage of labor (AMTS).[1,4] Pregnancy-induced hypertension was also included in a review by Sebghati and Wetta *et al*. in 2013, and in a guideline from the Royal College of Obstetricians and Gynaecologists (RCOG), as it causes PPH by disturbing maternal coagulation.[13,17,18] All pregnant women with pregnancy-induced hypertension in our setting, both with or without severe features, were treated with magnesium sulfate for the prevention of seizures that might raise concerns of a tocolytic effect.[17,19] Our previous 2010 study explored the risk factors for PPH by comparing the characteristics of 222 patients between those with and without PPH. We found the duration of the third stage of labor and pregnancy-induced hypertension to be the risk factors for primary PPH, and uterine atony to be the most common cause of PPH-all of which are similar to the findings of this study.[20] However, this study found only pregnancy-induced hypertension to be a significant

risk factor for PPH after the implementation of PDCB. A possible explanation for this may be that PDCB had not yet been introduced in 2010, and consequently, our postpartum blood loss data at that time may have been inaccurate. The incidence of PPH that we reported in 2010 was only 2.4%, which may have been inaccurately low. It is, therefore, possible that the risk factor analysis that we performed in that study may have been based on a rate that was lower than it should have been.

The strength of this study is that our analysis was based on data derived from a real-life clinical setting, using adequate statistical power, and from two different groups: one delivering before and the other after the implementation of PDCB. Therefore, our findings in the present study reflect and support the value and application of PDCB. It is possible that differences in the study population, the healthcare providers, and patient management practices between 2012 and 2014 could have affected the outcomes of this study. However, the demographic and clinical characteristics between the groups were similar, and our study population also covered the period representing the beginning of residency training (June 2012 and June 2014). As such, doctors would have given the same level of care, and patients would have received the same level of care. This, therefore, lowers the opportunity for study bias. Although the size of our study satisfied the sample size calculation-required minimum, our sample size may not have been large enough to identify statistically significant differences between methods for major complications of primary PPH, including massive transfusion, peripartum hysterectomy, ICU admission, and maternal death. A larger sample size or multi-center trial, especially in primary or community settings, should be considered to further elucidate the benefit of PDCB. However, a 2010 cluster randomized controlled trial performed by Zhang *et al.* compared effectiveness between a collection bag and visual estimation for reducing severe PPH in 25,381 patients from 13 European countries after vaginal delivery. They found no significant difference regarding the incidence of severe PPH between groups. They hypothesized that their results suggested a common improper use of the collection bag. Their hypothesis resulted in an increased awareness of the proper use of the collection bag and more vigilant PPH management, with a resulting associated increase in the rate of medical intervention.[21] A systemic review of 36 studies (both quantitative and qualitative) by Hancock *et al.* in 2015 reported similar findings. They found that the use of a collection bag or drape improved the accuracy of blood loss measurement over other methods, but that they did not reduce the rate of severe PPH. They recommended that there are many factors in addition to blood volume that influence outcomes, including and especially the speed of blood flow, nature of blood loss, and patient condition.[22] Taken together, these findings suggest that an early diagnosis of PPH by an objective measurement of postpartum blood loss using PDCB is not the only factor that affects the outcomes of PPH. In fact, multiple factors influence the outcomes of PPH, and all of these factors need to be considered in the decision-making by healthcare providers, including when establishing organizational policy and when designing a local protocol for effective PPH management.

In conclusion, in the present study, it was found that the incidence of primary and severe PPH and the rate of use of additional uterotonic drugs all significantly increased after the implementation of PDCB. Pregnancy-induced hypertension was found to be a significant risk factor for the development of primary PPH. Further study in a larger, multi-center study population is needed to evaluate major complications, especially massive blood transfusion, peripartum hysterectomy, ICU admission, and maternal mortality.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge Dr. Dittakarn Boriboonhiransarn of the Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, for his assistance with suggestion and statistical analysis.

**Conflict of Interest Declaration:** All authors declare they had no personal or professional conflicts of interest, and no financial support from the companies that produce and/or distribute the drugs, devices, or materials described in this report.

**Funding Disclosure:** This study was funded by a grant from the Siriraj Routine to Research (R2R) unit (Grant no. R2R.316/16).

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# Factors Related to the Clinical Outcomes of the Kasai Procedure in Infants with Biliary Atresia

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## ABSTRACT

**Objective:** The Kasai portoenterostomy has been accepted as the primary therapeutic treatment for biliary atresia. However, successful bile drainage does not always promise a long-term native liver survival. Several clinical factors were evaluated to discover associations with the outcomes.

**Methods:** A retrospective chart review was conducted of infants with biliary atresia who underwent the conventional Kasai portoenterostomy at Siriraj Hospital, January 2006-August 2018. The patient demographics, perioperative clinical and laboratory data, adjuvant therapies, complications, and interventions were analyzed in correlation to the short- and long-term outcomes. The short-term outcome evaluated was the resolution of jaundice, while the long-term outcome was remaining jaundice-free with the native liver.

**Results:** The complete medical records of 80 patients were retrospectively reviewed. Their mean age at the time of the Kasai portoenterostomies was 97 days. Overall, 66.3% achieved jaundice clearance. The mean follow-up duration was 50.5 months, with 51.3% of the patients remaining jaundice-free with their native liver. A prolonged duration of a prophylactic antibiotic was significantly related to the jaundice clearance, with a p-value of 0.002. Moreover, a lower number of episodes of cholangitis was significantly related to a good long-term outcome (p-value, 0.024).

**Conclusion:** The prolonged provision of a prophylactic antibiotic as adjuvant therapy after the Kasai portoenterostomy was associated with jaundice clearance. An increased incidence of cholangitis episodes was associated with poor long-term outcomes. Postoperative adjuvant therapy should be standardized and maintained for the care of biliary atresia patients to improve their outcomes.

**Keywords:** Adjuvant therapy; biliary atresia; cholangitis; Kasai portoenterostomy outcome; prophylactic antibiotic
(Siriraj Med J 2020; 72: 226-237)

## INTRODUCTION

Biliary atresia is an idiopathic inflammatory cholangiopathy of infancy. It is characterized by progressive fibrosing obliteration at varying levels of the intrahepatic and extrahepatic bile ducts, and inevitable cirrhosis.[1,2] It is the most common cause of neonatal direct hyperbilirubinemia and cirrhosis in children, and the most common indication for pediatric liver transplantation worldwide.[2,3] The Kasai portoenterostomy[4] has been accepted as the primary therapeutic treatment for the restoration of bile flow and the preservation of the liver. However, the procedure produces variable outcomes. Successful drainage does not always predict a long-term, jaundice-free native liver survival as progressive irreversible liver injury and complications of cirrhosis (mainly portal hypertension) can occur despite adequate bile drainage

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*http://dx.doi.org/10.33192/Smj.2020.31*

being achieved.[3,5-8] Previous studies from large-scale institutions have demonstrated various pathological, clinical, and surgical factors associated with successful bile drainage and long-term, transplant-free survival.[1,3,7-10]

Unfortunately, the majority of infants in developing countries reach tertiary-care centers when they are older than the maximum recommended age of 2 months for the performance of a Kasai portoenterostomy. As well, liver transplantation is not always suitable for every family. Faced with limitations in financial support, transportation, and the provision of continuing care for the immunosuppressed children, a number of biliary atresia children die of end-stage liver disease. We therefore conducted this study to identify any clinical factors that are associated with improved outcomes for Kasai portoenterostomy.

## MATERIALS AND METHODS

A retrospective chart review was conducted of infants with biliary atresia who underwent the conventional Kasai portoenterostomy procedure at Siriraj Hospital, January 2006 - August 2018. Patients who were operated on at another hospital or who developed complications unrelated to biliary atresia or the Kasai operation were excluded. An analysis was made of the patient demographics and laboratory data with respect to the short- and long-term outcomes. The laboratory tests comprised the gamma-glutamyl transpeptidase (GGT) levels prior to, and at specific times after, the operation, as well as the full range of liver function tests (total bilirubin, direct bilirubin, aspartate transaminase, alanine transaminase, and alkaline phosphatase). Also analyzed were the perioperative clinical data (postoperative steroid administration; the duration of the steroid; and intravenous and prophylactic antibiotic administration) to ascertain their impact on the short- and long-term outcomes. The short-term outcome evaluated was the achievement of jaundice clearance, which was defined as a serum total bilirubin level of ≤ 1.2 mg/dL at any time point after the procedure. Patients who did not achieve normalized bilirubin levels were considered to have the poor short-term outcome of no jaundice clearance. The long-term outcomes were categorized as "good" and "poor". A good long-term outcome was defined as a patient being jaundice-free with the native liver for more than 6 months after the Kasai portoenterostomy, with regular monitoring up to the last follow-up appointment. On the other hand, the poor long-term outcomes comprised patients who remained clinically jaundiced or developed cirrhosis with the native liver, patients who underwent a liver transplantation, and disease-related deaths. In cases of patients who achieved jaundice clearance, the durations until the total bilirubin reached normal levels were evaluated. Details of the overall levels of complications that were suspected to affect the long-term outcomes (such as the development of cholangitis, the duration until the first episode of cholangitis, and the number of episodes of cholangitis) were correlated to both the short- and long-term outcomes. The diagnosis of cholangitis after the portoenterostomy procedure was based on clinical symptoms of either progressive jaundice, with or without fever and acholic stool. These were proven by increase bilirubin and GGT levels from baseline in association with leukocytosis and increase C-reactive protein level. The number of salvage interventions (namely, redo-portoenterostomies and percutaneous biliary drainage) were reviewed to establish any associations with the long-term outcomes.

### Statistical analyses

Data were prepared and analyzed using PASW (Predictive Analytics Software) Statistics 18.0 (IBM SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented as mean and standard deviation for normally distributed data, or as median with range in the case of the non-normally distributed data. For both the short- and long-term comparison groups, the independent sample t-test was used to compare the normally distributed data related to the good and poor outcomes, while the Mann–Whitney U test was employed for the non-normally distributed data. Categorical data were analyzed using Pearson's chi-squared test, Yates's continuity correction, or Fisher's exact test to compare the proportional data of the two groups. A receiver operating characteristic curve was employed to identify the optimal cut-off points of age at operation to classify the patients with good outcomes. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy (all with the corresponding 95% confidence interval [95% CI]) were calculated for each cut-off point. Statistical significance was determined to be a p-value of $< 0.05$. The statistically significant factors identified in a univariate analysis were subsequently tested in a multivariate analysis. The latter model used multiple binary logistic regression and adjusted odds ratio (OR) in order to adjust for confounding factors and demonstrate the magnitude of any associations between the independent factors and the outcomes.

### Ethics approval

The protocol for this study was approved by the Institutional Review Board of Siriraj Hospital (Si 641/2018).

## RESULTS

A total of 85 cases with biliary atresia were identified as having been treated at Siriraj Hospital from January 2006 to August 2018. Complete medical records of 80 of those patients were available and formed the basis of the study. There was a slight female predominance (a 3:2 female-to-male ratio), as demonstrated in Table 1. The mean age at Kasai portoenterostomy was 97 days, ranging from 21-204 days. Approximately 94% of the cases received an adjuvant steroid for an average duration of 4 weeks. All cases received intravenous empirical antibiotics, mostly for durations of 2-3 weeks. A prophylactic antibiotic was provided to 97.5% of cases and maintained for durations of 12-24 months after the operation. Altogether, 66.3% of the patients achieved jaundice clearance while the remainder (33.7%) never attained normal bilirubin levels. The mean duration until jaundice clearance was 4 months, ranging from 4-72 weeks after the operation. The incidence of postoperative cholangitis was 87.5%, occurring in 70 out of the total of 80 cases. The average duration to the development of cholangitis was approximately 2 months after the operation, with cases arising as early as within the first week and as late as 12 months. The overall mean number of cholangitis events was 3 episodes throughout the follow-up period. The incidences of the salvage procedures-redo-Kasai operation and percutaneous transhepatic biliary drainage-were 7.5% and 15%, respectively. The mean follow-up duration was 50.5 months, with 51.3% of the patients remaining jaundice-free with their native liver for more than 6 months after the Kasai portoenterostomy.

By using a receiver operating characteristic curve to classify patients with reference to the short-term outcome, the optimal cut-off point for the age at operation was determined to be 90 days. In fact, the accuracy in predicting the long-term outcome was higher for the cut-off point of 80 days. However, as the majority of patients in this study were older, we elected to use the cut-off point of 90 days in the multivariate analysis (Tables 2 and 3). The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy (all with the corresponding 95% CI) of each age group in predicting the outcomes are detailed in Tables 2 and 3.

The univariate analysis of the potential factors related to the good short-term outcome (the achievement of jaundice clearance) is presented in Table 4. The clinical factors significantly associated with jaundice clearance were an age at operation of less than 90 days, a prolonged duration of use of a prophylactic antibiotic, and the absence of postoperative cholangitis. The laboratory factors which were significantly lower among patients with jaundice clearance were the total and direct bilirubin levels, and the hepatic enzymes (aspartate transaminase and alanine transaminase). The only laboratory factor significantly higher among patients with jaundice clearance was the serum GGT level on postoperative day 1. The subsequent multivariate analysis of all of the significant factors associated with jaundice clearance revealed that the only independent factor was the duration of the prophylactic antibiotic, with an adjusted OR of 1.128 (95% CI, 1.045–1.217) and a p-value of 0.002 (Table 5).

The univariate analysis of the potential factors related to the good long-term outcome (remaining jaundice-free with the native liver for more than 6 months after the operation) is demonstrated in Table 6. Approximately half of the study population (41 out of the 80 patients) remained jaundice-free with their native livers, all of which (100%) originally achieved jaundice clearance after the Kasai portoenterostomy. Twelve out of the 53 patients who initially achieved jaundice clearance later became jaundiced or progressed to cirrhosis, accounting for 30.8% of the poor long-term outcome group. The clinical factors found to be significantly associated with the good long-term outcome were the initial achievement of jaundice clearance, an age at operation of less than 80 days, a prolonged duration of use of a prophylactic antibiotic, a shorter duration until the attainment of jaundice clearance, the absence of postoperative cholangitis, and a lower number of episodes of cholangitis.

As previously mentioned, the cut-off point for the age at operation of 80 days more accurately predicted the long-term outcome than the cut-off point for the age of 90 days. Consequently, the age at operation of less than 90 days did not demonstrate any significant correlation to the good long-term outcome in the univariate analysis. As detailed in Table 6, the median age at operation of 77 days for the patients with a good long-term outcome was considerably lower than the median of 99 days for those with a poor long-term outcome (p-value, 0.021). The multivariate analysis of all of the significant factors associated with the good long-term outcome established that the only independent factor was the lower number of episodes of cholangitis, with an adjusted OR of 0.678 (95% CI, 0.484-0.949) and a p-value of 0.024 (Table 7).

As indicated in Tables 5 and 7, the multivariate analyses were not applied to certain factors. This was because those factors were involved in all, or none, of the measuring outcomes. Specifically, jaundice clearance was achieved in 100% of the cases with a good long-term outcome, while postoperative cholangitis occurred in 100% of the cases with poor short- and long-term outcomes. Therefore, the ORs, 95% CIs, and p-values of those factors could not be calculated in the logistic regression analyses.

**TABLE 1.** Patient demographics and clinical data (n = 80).

| Factors | Incidence/mean ± SD | Percentage/range |
|---|---|---|
| Gender | | |
| Female | 49 | 61.3% |
| Male | 31 | 38.7% |
| Age at Kasai operation (days) | 96.7 ± 39.8 | 21–204 |
| Body weight at operation (kilograms) | 5.2 ± 0.9 | 2.8–6.9 |
| Postop steroid administration | | |
| No | 5 | 6.2% |
| Within 7 days | 55 | 68.8% |
| After 7 days | 20 | 25% |
| Duration of steroid administration (days) | 26 ± 9.5 | 0–60 |
| Duration of intravenous antibiotics (days) | 19.8 ± 10.1 | 6–63 |
| Prophylactic antibiotic | | |
| Yes | 78 | 97.5% |
| No | 2 | 2.5% |
| Duration of prophylactic antibiotic (months) | 21.8 ± 22.8 | 0–144 |
| Jaundice clearance | | |
| Yes | 53 | 66.25% |
| No | 27 | 33.75% |
| Duration till jaundice clearance (weeks; n = 53) | 16.4 ± 13.8 | 4–72 |
| Postoperative cholangitis | | |
| Yes | 70 | 87.5% |
| No | 10 | 12.5% |
| Duration till cholangitis (weeks; n = 70) | 9.2 ± 18.5 | 1–144 |
| Cholangitis episodes (n = 70) | 3 ± 2.6 | 0–12 |
| Redo-Kasai operation | 6 | 7.5% |
| PTBD | 12 | 15% |
| Liver transplantation (LT) | 6 | 7.5% |
| Follow up duration (months) | 50.5 ± 45.8 | 1–153 |
| Status (follow-up > 6 months) | | |
| Jaundice-free (NL) | 41 | 51.3% |
| Deceased/cirrhosis/LT | 39 | 48.7% |

**Abbreviations:** PTBD, percutaneous transhepatic biliary drainage; LT, liver transplantation; NL, native liver; SD, standard deviation

**TABLE 2.** Accuracy of ages at different cut-off points in predicting short-term outcome.

| | Sensitivity (95% CI) | Specificity (95% CI) | PPV (95% CI) | NPV (95% CI) | Accuracy (95% CI) |
|---|---|---|---|---|---|
| Age < 80 days | 0.547 | 0.704 | 0.784 | 0.442 | 0.600 |
| n = 37 | (0.415–0.673) | (0.515–0.841) | (0.628–0.886) | (0.304–0.589) | (0.490–0.700) |
| Age < 90 days | 0.642 | 0.667 | 0.791 | 0.486 | 0.650 |
| n = 43 | (0.507–0.757) | (0.478–0.814) | (0.648–0.886) | (0.334–0.641) | (0.541–0.745) |

**Abbreviations:** PPV, positive predictive value; NPV, negative predictive value

**TABLE 3.** Accuracy of ages at different cut-off points in predicting long-term outcome.

| | Sensitivity (95% CI) | Specificity (95% CI) | PPV (95% CI) | NPV (95% CI) | Accuracy (95% CI) |
|---|---|---|---|---|---|
| Age < 80 days | 0.585 | 0.667 | 0.649 | 0.605 | 0.625 |
| n = 37 | (0.434–0.722) | (0.510–0.794) | (0.488–0.782) | (0.456–0.736) | (0.515–0.723) |
| Age < 90 days | 0.634 | 0.564 | 0.605 | 0.595 | 0.600 |
| n = 43 | (0.481–0.764) | (0.410–0.707) | (0.456–0.736) | (0.435–0.737) | (0.490–0.700) |

**Abbreviations:** PPV, positive predictive value; NPV, negative predictive value

## DISCUSSION

The current research is a continuation of an earlier study at the same single center[11], but it included more participants and monitored them for a longer period. Previous studies have reported the impact of patient age at the time of surgery on the outcomes of the Kasai portoenterostomy.[7,12,13] Our review obtained a finding consistent with that of many other studies, which is that a younger age at surgery improves the rates of jaundice clearance and transplant-free survival with the native liver.[3,7,9,10,12,13] In more detail, this study found that a patient age of less than 90 days was significantly associated with the good short-term outcome of the achievement of jaundice clearance. Moreover, those aged less than 80 days were significantly associated with the good long-term outcome (maintaining the jaundice-free status with the native liver for more than 6 months after the operation). In Thailand, the majority of patients receive a Kasai portoenterostomy later than the maximum recommended age of 2 months. Concerned about the burdensome need to provide constant care to those patients, the authors were motivated to find any significant controllable factors impacting on the improvement of their care and the achievement of better outcomes. Despite the present study's findings, it is not our hospital's policy to withdraw salvage surgery when infants with biliary atresia present at an age over 90 days. On the contrary, the hospital encourages the Kasai portoenterostomy in children presenting as late as 4 or 5 months of age with less than a hard liver consistency. The aim is to provide the opportunity-no matter how limited-for the children to grow, and for their families to appreciate the children's requirements and understand the long-term care needed prior to the imminent liver transplantation. The Kasai portoenterostomy procedure may provide more time for those patients and their families for whom a liver transplantation may not be feasible in the future for a range of reasons.

**TABLE 4.** Univariate analysis of laboratory and clinical factors related to good short-term outcome (jaundice clearance).

| Factor | Jaundice clearance | | *P*-value |
|---|---|---|---|
| | Yes (n = 53) | No (n = 27) | |
| Gender, n (%) | | | 0.985 |
| Female | 33 (62.3%) | 16 (59.3%) | |
| Male | 20 (37.7%) | 11 (40.7%) | |
| Age at Kasai operation (days) | | | |
| Mean ± SD | 87.2 ± 35.8 | 115.3 ± 41.3 | |
| Age < 90, n (%) | 34 (64.2%) | 9 (33.3%) | **0.002** |
| Age ≥ 90, n (%) | 19 (35.8%) | 18 (66.7%) | **0.017** |
| Body weight at operation (kg) | | | 0.503 |
| Mean ± SD | 5.3 ± 0.94 | 5.2 ± 0.98 | |
| Liver function test, median (min-max) | | | |
| Total bilirubin (TB) | 9.1 (4.3–17.5) | 10.6 (6.85–31.3) | **0.003** |
| Direct bilirubin (DB) | 8.0 (3.2–14.4) | 9.1 (5–26.1) | **0.015** |
| Aspartate transaminase (AST) | 205 (3–1101) | 286 (114–843) | **0.001** |
| Alanine transaminase (ALT) | 129 (10–505) | 193 (42–554) | **0.023** |
| Alkaline phosphatase (ALP) | 492 (270–871) | 487 (383–846) | 0.171 |
| Gamma-glutamyl transpeptidase (GGT) | 705 (121–2491) | 450 (84–2184) | 0.132 |
| GGT on postoperative day 1 | n = 49 | n = 23 | **0.014** |
| Median (min-max) | 913 (114–3031) | 478 (79–3062) | |
| GGT at 3 months after operation | n = 34 | n = 18 | 0.397 |
| Median (min-max) | 650.5 (15–1796) | 524 (34–1990) | |
| GGT at 4 months after operation | n = 32 | n = 13 | 0.764 |
| Median (min-max) | 435.5 (20–2752) | 316 (27–2302) | |
| GGT at 5 months after operation | n = 27 | n = 9 | 0.144 |
| Median (min-max) | 315 (20–2480) | 784 (136–1311) | |
| GGT at 6 months after operation | n = 26 | n = 8 | 0.239 |
| Median (min-max) | 212 (18–1990) | 369 (70–1280) | |
| Postoperative steroid administration | | | 0.671 |
| None, n (%) | 4 (7.5%) | 1 (3.7%) | |
| Within 7 days, n (%) | 37 (69.8%) | 18 (66.7%) | |
| After 7 days, n (%) | 12 (22.7%) | 8 (29.6%) | |
| Duration of steroid (days), median (min-max) | 28 (0–60) | 28 (0–38) | 0.724 |
| Duration of intravenous antibiotic (days) | | | |
| Median (min-max) | 18 (7–42) | 14 (6–63) | 0.477 |
| Prophylactic ATB (n = 78), n (%) | 52 (98.1%) | 26 (96.3%) | 1.000 |
| Duration of prophylactic antibiotic (months) | | | **< 0.001** |
| Median (min-max) | 24 (0–144) | 5 (0–60) | |
| Postoperative cholangitis, n (%) | 43 (81.1%) | 27 (100%) | **0.014** |
| Duration till cholangitis (weeks) | n = 43 | n = 27 | |
| Median (min, max) | 5 (1–144) | 4 (1–28) | 0.666 |
| Episodes of cholangitis, median (min, max) | 2 (0–12) | 2 (1–8) | 0.124 |

**Abbreviations:** ATB, antibiotic; SD, standard deviation

**TABLE 5.** Univariate and multivariate analyses adjusted for significant factors associated with jaundice clearance, plus postoperative cholangitis.

| Factor | Unadjusted OR (95% CI) | *P*-value | Adjusted OR (95% CI) | *P*-value |
|---|---|---|---|---|
| Age < 90 days | 3.579 (1.347–9.512) | 0.011 | 2.817 (0.630–12.592) | 0.175 |
| TB | 0.803 (0.680–0.949) | 0.010 | 0.581 (0.245–1.380) | 0.219 |
| DB | 0.795 (0.653–0.968) | 0.022 | 1.893 (0.680–5.264) | 0.222 |
| AST | 0.997 (0.994–0.999) | 0.013 | 1.001 (0.995–1.007) | 0.822 |
| ALT | 0.994 (0.989–1.000) | 0.034 | 0.991 (0.976–1.005) | 0.217 |
| Postop GGT | 1.001 (1.000–1.002) | 0.048 | 1.000 (0.999–1.002) | 0.528 |
| Duration of prophylactic ATB | 1.118 (1.051–1.189) | < 0.001 | 1.128 (1.045–1.217) | 0.002 |
| Postoperative cholangitis | Not applicable | | Not applicable | |

**Abbreviations:** TB, total bilirubin; DB, direct bilirubin; AST, aspartate transaminase; ALT, alanine transaminase; Postop GGT, postoperative gamma-glutamyl transpeptidase; ATB, antibiotic

The study identified several probable factors associated with jaundice resolution after a Kasai portoenterostomy. Significant laboratory results were found with the good-short-term-outcome group, with lower levels of bilirubin and liver enzymes being found with earlier, rather than later, ages at diagnosis and at operation. For that reason, the multivariate analysis did not find those factors to be significant independent correlates of the short-term outcome. The only independent factor associated with the achievement of jaundice clearance was the prolonged provision of a prophylactic antibiotic. Nevertheless, a conclusion could not be made that the administration of a prophylactic antibiotic secured a better short-term outcome. This is because a number of individuals with poor outcomes expired prior to the scheduled discontinuation of the prophylactic antibiotic at 18-24 months after the operation, resulting in an overall shorter duration of prophylactic antibiotic usage for the poor-outcome group.

Postoperative cholangitis, a common and serious complication following the Kasai portoenterostomy, has been varyingly reported to occur in between 40% and 93% of cases.[5,10,14-17] Moreover, its occurrence has been found to significantly reduce the survival rate of patients with either good or inadequate bile flow[7,16,17], and repeated episodes-especially within the first 2 years of surgery-have been associated with a decrease in native liver survival and, ultimately, with the need for liver transplantation.[18-21] In the current study, the overall incidence of cholangitis was 87.5%, and perioperative intravenous antibiotics were universally provided (usually for 1-2 weeks, but even longer if the cholangitis worsened). The majority of the antibiotics were combinations of third generation cephalosporins and metronidazole. A postoperative, adjuvant, prophylactic antibiotic was subsequently provided to prevent recurrence of the cholangitis, most episodes of which typically occur within 12 months of the Kasai procedure.[16,21,22] Nevertheless, recent trials and systematic reviews have yielded inconclusive results for determining the effectiveness of prophylactic antibiotics for the prevention of cholangitis after the Kasai portoenterostomy.[19,22–24] In addition, there is no strong evidence that the prolonged use of oral prophylactic antibiotics beyond the early postoperative period offers any greater protection against cholangitis.[5,19,23] Still, most experienced institutions provide oral prophylactic antibiotics for periods of 6 months or longer, similar to the requirements of our hospital's protocol. A randomized control trial by Bu et al.[22] found that there was a significantly lower rate of recurrent cholangitis among patients provided with prophylactic antibiotics than that for a historical control group who did not receive antibiotics. Although a Dutch national cohort by deVries et al.[24] did not identify any reduction in the cholangitis rate of the prophylactic antibiotic group, it did demonstrate that the prophylactic usage was associated with a higher, 4-year, transplant-free

**TABLE 6.** Univariate analyses of laboratory and clinical factors related to good long-term outcome (remaining jaundice-free with native liver).

| Factor | Jaundice-free with native liver | | *P*-value |
|---|---|---|---|
| | Yes (n = 41) | No (n = 39) | |
| Jaundice clearance (n = 53), n (%) | 41 (100%) | 12 (30.8%) | **< 0.001** |
| Age at operation (days), median (min-max) | 77 (31–162) | 99 (21–204) | **0.021** |
| < 90 days, n (%) | 26 (63.4%) | 17 (43.6%) | 0.116 |
| ≥ 90 days, n (%) | 15 (36.6%) | 22 (56.4%) | |
| < 80 days, n (%) | 24 (58.5%) | 13 (33.3%) | **0.027** |
| ≥ 80 days, n (%) | 17 (41.5%) | 26 (66.7%) | |
| Preoperative GGT | n = 35 | n = 36 | 0.765 |
| Median (min-max) | 705 (121–2335) | 596 (84–2491) | |
| Postoperative GGT | n = 38 | n = 34 | 0.250 |
| Median (min-max) | 874.5 (114–2345) | 732 (79–3062) | |
| GGT at 3 months after operation | n = 26 | n = 26 | 0.985 |
| Median (min-max) | 581 (15–1796) | 578 (34–1990) | |
| GGT at 4 months after operation | n = 27 | n = 18 | 0.926 |
| Median (min-max) | 439 (20–2752) | 305.5 (27–2302) | |
| GGT at 5 months after operation | n = 21 | n = 15 | 0.067 |
| Median (min-max) | 282 (20–2480) | 624 (100–1311) | |
| GGT at 6 months after operation | n = 20 | n = 14 | 0.069 |
| Median (min-max) | 198.5 (18–1990) | 383.5 (70–1280) | |
| Postoperative steroid administration | | | 0.495 |
| No, n (%) | 3 (7.3%) | 2 (5.1%) | |
| Within 7 days, n (%) | 30 (73.2%) | 25 (64.1%) | |
| After 7 days, n (%) | 8 (19.5%) | 12 (30.8%) | |
| Duration of steroid (days), median (min-max) | 28 (0–60) | 28 (0–38) | 0.074 |
| Duration of intravenous antibiotics (days) | | | 0.674 |
| Median (min-max) | 17 (7–42) | 18 (6–63) | |
| Prophylactic antibiotic (n = 78), n (%) | 40 (97.6%) | 38 (97.4%) | 1.000 |
| Duration of prophylactic antibiotic (months) | | | **0.006** |
| Median (min-max) | 21 (0–88) | 11 (0–144) | |
| Duration till jaundice clearance (weeks) | n = 41 | n = 12 | **0.006** |
| Median (min-max) | 9 (4–72) | 20.5 (9–60) | |
| Postoperative cholangitis, n (%) | 31 (75.6%) | 39 (100%) | 0.001 |
| Duration till cholangitis (weeks) | | | 0.368 |
| Median (min-max) | 3 (1–29) | 4 (1–144) | |
| Episodes of cholangitis, median (min-max) | 1 (0–12) | 3 (1–11) | **< 0.001** |
| Redo-Kasai operation, n (%) | 2 (4.9%) | 4 (10.3%) | 0.426 |
| PTBD, n (%) | 4 (9.8%) | 8 (20.5%) | 0.301 |

**Abbreviations:** GGT, gamma-glutamyl transpeptidase; PTBD, percutaneous transhepatic biliary drainage

**TABLE 7.** Univariate and multivariate analyses of all significant factors associated with good long-term outcome, plus jaundice clearance and postoperative cholangitis.

| Factor | Unadjusted OR (95% CI) | P-value | Adjusted OR (95% CI) | P-value |
|---|---|---|---|---|
| Jaundice clearance | Not applicable | | Not applicable | |
| Age < 90 days | 2.243 (0.915–5.500) | 0.077 | 0.700 (0.139–3.536) | 0.666 |
| Duration of prophylaxis antibiotic | 1.009 (0.988–1.030) | 0.391 | 0.982 (0.950–1.016) | 0.304 |
| Duration till jaundice clearance | 0.962 (0.920–1.005) | 0.085 | 1.000 (0.941–0.062) | 0.998 |
| Postoperative cholangitis | Not applicable | | Not applicable | |
| Episodes of cholangitis | 0.703 (0.562–0.880) | 0.002 | 0.678 (0.484–0.949) | 0.024 |

survival rate of 54% compared to 34% for the control group. Our protocol of providing a prophylactic antibiotic for 18-24 months after the Kasai operation is based on several lines of evidence. Firstly, recurrent cholangitis frequently occurs during the first 2 years of the Kasai procedure. In addition, the auto-anastomosis of the internal fistula between the bile ductules in the portal plate and the intestinal mucosa matures within 6 weeks of the operation.[15] Finally, wound maturation takes 18-24 months in general. Another reasonable approach would be to provide protection during the immunosuppression phase of concomitant steroids and discontinue their use after 1 year, i.e., once the cholangitis risk is significantly decreased.[19] In the present study, the multivariate analysis of the factors associated with jaundice clearance was not able to be applied to postoperative cholangitis due to the 100% involvement of the postoperative cholangitis in the group of patients without jaundice clearance (Table 4).

In the analysis of the factors associated with a good long-term outcome (remaining jaundice-free with the native liver for more than 6 months after the operation), the only independent factor that was found was the lower number of episodes of cholangitis. Interestingly, the episodes were not a significant factor related to the short-term outcome of jaundice clearance. This is because the incidences of cholangitis occurred for a median of 2 episodes in the group with jaundice resolution as well as in the group without jaundice resolution. On the other hand, the patients who remained jaundice-free with their native liver developed cholangitis for a median of 1 episode, while those who had a poor long-term outcome had a median of 3 episodes of cholangitis throughout their follow-up period (adjusted OR, 0.678; 95% CI, 0.484–0.949; p-value, 0.024). These findings are consistent with previous studies which reported that, firstly, an increased number of cholangitis episodes negatively affected the native liver survival, and secondly, having episodes more than 2 years after the Kasai portoenterostomy appeared to be a prognostic marker for a future liver transplantation.[18,20,21]

In addition to cholangitis, the univariate analysis demonstrated other significant factors affecting the long-term outcome, namely, an age at operation of less than 80 days, the achievement of jaundice clearance, a prolonged provision of a prophylactic antibiotic, and a shorter duration to attain jaundice clearance. In a long-term study of native liver patients who had survived for more than 20 years after the Kasai operation, Nio et al.[25] concluded that the age at operation, the early development of cholangitis, and the time to jaundice resolution were prognostic factors influencing long-term native liver survival. In contrast, the multivariate analysis performed for the current study found that the number of cholangitis episodes was the only independent, significant factor affecting the long-term outcomes. Jaundice clearance was present in 100% of the patients who achieved a good long-term outcome, while the development of cholangitis was present in 100% of the patients who had poor long-term outcomes. Therefore, a logistic regression analysis could not be performed for the 2 variables, jaundice clearance and the development of cholangitis.

Consistent with other studies[26,27], it is evident from the present study that jaundice clearance following the Kasai portoenterostomy was essential for long-term native liver survival. If the age at the operation is the

first controllable factor in the management of biliary atresia, surgical technical standardization would be the second factor impacting on the resolution of jaundice. To maintain a jaundice-free, native liver survival, postoperative adjuvant therapy would be the third key factor. Our surgical technique was based on the original Kasai operation.[4] It was modified in accordance with the Tohoku University standardization protocol, which entails a moderate dissection when transecting the fibrous remnants of the porta hepatis in the same plane as the liver capsule.[26]

Another important adjuvant therapy is the provision of corticosteroids during the postoperative care period following a Kasai portoenterostomy. The results of randomized controlled trials[3,28,29] and meta-analyses[30,31] have indicated that the administration of moderate- to high-dose steroids is beneficial by increasing the rate of jaundice clearance during the first 6 months after the operation. The majority of those trials found that the elevated rate of jaundice clearance occurred only in patients operated on at a younger age. Even so, those patients did not demonstrate any improvement in their long-term, native liver survival rates. The meta-analysis by Chen et al.[31] also revealed that a longer duration of steroid therapy following the Kasai portoenterostomy failed to elicit any further beneficial outcomes. Our postoperative steroid regimen typically consisted of a low dose of oral prednisolone (2 mg/kg/day), which was tapered by half each week for 4 consecutive weeks; that dosage was provided to 94% of the patients in the current study. No differences were evident in either the short- or long-term outcomes of the steroid and non-steroid groups; however, this was most likely the result of the very low number of participants in the non-steroid group. Furthermore, variations in the timing of the initiation of the steroids did not produce any significant differences in the outcomes of the no-steroid, early-steroid, and late-steroid groups. Nevertheless, at our institution, the use of steroids is deemed to be an important adjuvant therapy during the postoperative care period. They are also used for the treatment of cholangitis after a portoenterostomy, given the evident inflammatory process presented and the very rare incidence of side effects associated with the steroids.

The incidences of the salvage procedures (redo-portoenterostomy and percutaneous biliary drainage) were quite low. The redo-portoenterostomy would be provided for patients with a history of biliary drainage or jaundice clearance after the first portoenterostomy, which later developed recurrent jaundice. The percutaneous biliary drainage was the intervention for those with recurrent jaundice or cholangitis, which imaging proved biliary collection or biloma. The analysis of their correlation to the long-term outcomes did not demonstrate any statistical significance.

Finally, the analysis of GGT activity was included in the present study to discover any correlation the activity might have with bile drainage or any prognostic value that would facilitate the prediction of portoenterostomy outcomes. Serum GGT has been utilized to indicate cholestasis, and an elevated level has been reported to be highly accurate in differentiating biliary atresia from other causes of neonatal cholestasis.[32,33] Interestingly, the present study found that the median serum GGT level on postoperative day 1 was significantly higher for the patients with jaundice resolution than for those without (913 and 478 IU/L, respectively; p-value, 0.014). Furthermore, in our comparison of the good and poor long-term outcomes, the median serum GGT levels on postoperative day 1 were 874.5 vs. 732 IU/L, respectively, although with no statistical significance. Despite several drop-out variables at each time point, the serum GGT activity of the patients with jaundice clearance tended to peak on day 1 before gradually decreasing below the serum GGT levels for the patients who had persistent jaundice during months 5 and 6 post-surgery, albeit without statistical significance. By contrast, the GGT activity of the patients with persistent jaundice tended to start in the high 300-500s IU/L and either persist at that level or climb to a peak in month 5 after surgery. Having examined the correlation between GGT activity and the outcomes after the Kasai portoenterostomy, Ihn et al.[34] reported that a concentration exceeding 550 IU/L at month 5 was one of the independent risk factors for decreased native liver survival. Our review of the serum GGT level did not elicit any statistical significance in both the short- and long-term outcome analysis. Nevertheless, given appropriate long-term care, a decreasing serum GGT level is expected in month 5 for the patients, especially in the case of those who achieved a normalized bilirubin level within 4 months of the operation. Thus, it is highly desirable to collect a larger and more complete set of data to analyze the GGT activity in patients with long-term native liver survival, even though the results would be mainly of prognostic value.

With the inherent limitations of a retrospective review, this is another descriptive study on the outcomes of the treatment of biliary atresia. It is difficult for a single center with a limited number of patients to reach statistical significance in the analysis of the listed factors. Still, the study demonstrated comparable outcomes to research by other large-scale centers, with its 66.3%

jaundice clearance rate; a 51.3% jaundice-free, native liver survival within a mean follow-up duration of 50 months; and an essentially acceptable treatment regimen. Further clarification and collaboration with other centers may help standardize the treatment protocol for this rare disease.

## CONCLUSION

The prolonged provision of a prophylactic antibiotic as adjuvant therapy during the postoperative care of biliary atresia patients after the Kasai portoenterostomy was associated with a good short-term outcome (the achievement of jaundice clearance). The increased number of cholangitis episodes was associated with poor long-term outcomes, including poor native liver survival. Postoperative adjuvant therapy should be standardized and maintained for the care of biliary atresia patients to improve their outcomes.

## ACKNOWLEDGMENTS

The authors thank Dr. Sasima Tongsai from the Clinical Epidemiology Unit Research Center, Faculty of Medicine Siriraj Hospital, for her contribution in the form of an extraordinary statistical analysis for the study.

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# Stress and Coping Strategies among Thai Medical Students in a Southern Medical School

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## ABSTRACT

**Objective:** To assess stress, and coping strategies and related factors among medical students.

**Methods:** This cross-sectional study surveyed all 1st to 6th year medical students at the Faculty of Medicine, Prince of Songkla University, from March to May, 2019. Three questionnaires were employed: 1) Demographic data 2) The Suanprung stress test 3) The Brief COPE inventory Thai version. Data were analyzed using descriptive statistics, and the results were presented as percentage, frequency, average and standard deviation. Factors associated with coping strategies were analyzed by means of chi-square or kruskal-wallis test.

**Results:** There were 827 respondents from 1,109 medical students, and 74.6% response rate. The majority of medical students were female (60.7%) with moderate and high stress level scores (44.9% and 38.6%, respectively). The medical students commonly used adaptive coping strategies (self-distraction, acceptance, active coping, and positive reframing) rather than maladaptive coping strategies (denial and substance use). According to the association between general demographic characteristics and coping strategies, we found that; gender, GPA, religion and medical illness had significant correlation with adaptive coping strategies. Whereas, high stress levels were significantly associated with maladaptive coping strategies.

**Conclusion:** Most medical students use adaptive coping strategies. Gender, GPA, religion and medical illness had significant correlation with adaptive coping strategies.

**Keywords:** Medical students; stress; coping strategies (Siriraj Med J 2020; 72: 238-244)

## INTRODUCTION

Stress is the result of an individual's perception that they lack resources to cope with a perceived situation occurring in the past, present or foreseeable future. Stress occurs when the individual is confronted with a situation that is perceived as overwhelming and with which they cannot cope.[1]

A previous study found medical students had higher levels of stress than other groups of the general population.[2] The prevalence of stress suffering among medical students was 54.0% for 3rdacademic year and 55.0% for 4thacademic year students.[3] In Thailand, the prevalence of those suffering from stress among medical students in Ramathibodi Hospital was 61.4%[4], and in Khon Kaen University, 55.8%.[5]

Response to stress can be categorized into: 1) Emotional aspects: fear, anxiety, worry, guilt, depression and irritability; 2) Cognitive reactions: their appraisal of stressful situations and strategies; 3) Behavioral responses: crying, abuse of self or others, smoking and drinking; 4) Physiological reactions: sweating, trembling, stuttering, headaches, weight loss or gain, and body aches.[6] Coping

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*http://dx.doi.org/10.33192/Smj.2020.32*

strategies that involve engagement can reduce both anxiety and depression as well as their effects on mental and physical health.[7] However, coping strategies which center on disengagement; such as: problem avoidance, wishful thinking, social withdrawal and self-criticism have negative consequences, and correlate with depression, anxiety, poor mental health[7] and physical problems.[8]

In 2007, a cross-sectional survey, which explored 4,287 students at 7 medical schools in the United States, found burnout was reported by 49.0% of medical students, and 11.2% reported suicidal ideation within the past year.[9] A study conducted in Thailand in 2007, found coping strategies among clinical medical students at Khon Kaen University included: tension reduction, seeking social support, positive thinking and planful problem solving.[5] Whilst in 2010, among 2nd academic year medical students at Thammasat University, common coping strategies were: investing in close friends, relaxing, being humorous, and developing self-reliance and optimism.[10]

In 2008, a cross-sectional study at the Faculty of Medicine, Prince of Songkla University, discovered 29.1% of medical students had mental health problems. Factors related to mental health problems were: female, 2nd academic year and rural home province.[11] Another study in 2015, among clinical medical students, found that no history of drinking and having a history of exercise had a higher happiness status than that of other groups.[12] Of medical students, 53.3% were drinkers, with the gender proportion of alcohol usages at 60.0% in males, and 48.1% in females. Gender as well as substance usage were significant correlated factors with high-risk drinking.[13] According to this information, the questions are: "What are the stress coping strategies among medical students?"; and: "Are substance use or alcohol consumption other stress coping strategies of medical students?". In the past, there were no in-depth studies that explored these questions among medical students at Prince of Songkla University. Therefore, the purpose of the study was to determine stress, coping strategies and related factors among medical students, and enhance the focusing of these problems.

## MATERIALS AND METHODS

The Ethics Committee of the Faculty of Medicine, Prince of Songkla University approved this cross-sectional study (REC: 60-472-03-4). The study explored all 1st to 6th academic year medical students that studying at the Faculty of Medicine, Prince of Songkla University, Hat Yai Hospital Medical Education Center, and Yala Hospital Medical Education Center, from March to May, 2019. There were 1,109 medical students, categorized by 1st to 6th academic year as follows: 189, 182, 185, 184, 189 and 180, respectively. The inclusion criterion was being a medical student who could complete all questionnaire. The exclusion criterion was medical students who being foreign students or on a leave of absence.

### Methodology

The medical students were communicated by a research assistant in their classes beforehand. After the end of class, the medical students had free time of 1 hour, so a research assistant invited them to participate, by introducing the rationale and overview information of this research. In cases where cooperation was successful, the research assistant distributed self-reporting questionnaires consisting of 3 parts, and thoroughly explained them in detail to participants. The medical students took 5 minutes to consider whether or not to join in the study. After this, the research assistant distributed the documentation, whilst assuring the volunteers that their identities would be protected. Then, the signatures of participants were not required, and all participants retained the right to withdraw from the study at any time.

All participants were allowed to finish and return the questionnaires immediately or later time. They were permitted to submit the questionnaire two options: drop it in the box at the front of the classroom, or bring it back and place it in the box located at the Psychiatry Department. Thus, participant confidentiality was protected.

### Instruments

The questionnaire comprised of 3 parts:

1) General information consisting of: gender, age, academic year, religion, accumulative GPA, income, parental marriage status, hometown, and underlying diseases.

2) The Suanprung stress test, which uses questions to determine stress levels for the last six months contains 20 items, rated on a 5-point Likert scale; with item responses ranging from: "1" (no stress) to "5" (extremely high stress). Total scores were classified into four levels: 0 to 23 as mild, 24 to 41 as moderate, 42 to 61 as high and more than 61 as severe stress. The Suanprung stress test was shown to have an overall Cronbach's alpha greater than 0.7.[14]

3) The Brief COPE inventory Thai version, consisted of 28 questions, and 14 subscales of coping strategies. The Brief COPE scale was designed to assess a broad range of coping responses among adults for all diseases. It contains 28 items, and is rated by a four-point Likert scale, ranging from: "I haven't been doing this at all" (score one) to "I have been doing this a lot" (score four).[15]

Cronbach's alpha of the Brief COPE inventory Thai version was 0.7.[16]

In the stress-coping model, specific coping behaviors are understood as predominantly adaptive or maladaptive. Adaptive coping strategies are: active coping, planning, positive reframing, acceptance, humor, religion, using emotional support and using instrumental support. Whereas, maladaptive coping strategies include: self-distraction, denial, venting negative emotions, substance use, behavioral disengagement and self-blame.[15,17]

### Statistical analysis

All data were analyzed, in order to present the sample's behavior using descriptive statistics. The results are described as: percentage, frequency, average and standard deviation. The correlated factors with coping strategies were analyzed using Chi-square or Kruskal-wallis test.

## RESULTS

### Demographic data

Medical students who completed the questionnaires were 827; the response rate was 74.6%. Of the participants, 502 were female (60.7%) (Table 1). Mean age was 21.4±2.0 years, with mean cumulative grade point average (GPA) being 3.5±0.4. Median income (IQR) was 8,000 (6,000-10,000) baht, per month.

### Stress level and coping strategies

The majority of medical students had moderate and high stress level scores (44.9% and 38.6%, respectively) (Fig 1). Coping strategies frequently performed by medical students were adaptive types; acceptance, active coping, positive reframing and maladaptive types; self-distraction. Whereas maladaptive types; denial and substance use were coping strategies that medical students did not perform at all (Fig 2).

### The association between demographic data, stress level and coping strategies

The association between general demographic characteristics and coping strategies are described (Table 2). Using adaptive coping strategies for controlling stress levels was significantly associated with: gender, religion, GPA, and medical illness. According to gender and GPA factors, the study found that females and those with a high GPA (≥3.5) used adaptive coping strategies more than males and those with a low GPA (<3.5), respectively. There was no significant difference in the use of both adaptive and maladaptive coping strategies between pre-clinic and clinic medical students. In addition, medical students who had a medical illness (such as allergy, dyspepsia, migraine) often used adaptive coping strategies.

Analysis of the association between demographic characteristics and stress levels, showed that only the variable concerning academic year of medical study (pre-clinical and clinical year) was significantly related to stress levels ($p<0.001$). Pre-clinical medical students, had high to severe stress levels, more so than clinical medical students (52.9% and 39.5%, respectively) (Fig 3).

## DISCUSSION

This study found the prevalence of moderate and high stress level scores among medical students were 44.9% and 38.6%, respectively. In Thailand, a previous study at the Faculty of Medicine, Ramathibodi Hospital reported 61.4% of medical students had experienced some degree of stress.[4] At the Faculty of Medicine, Khon Kaen University, it was found that 55.8% of medical students had morbid stress.[5] However, a study at Siriraj Hospital reported only 17.9% of medical students had stress.[18] The cause of these different results may be that the subjects of this study only included 3rd year medical students.[19] In other countries, 56.0% of Malaysian[19], and 63.0% of Saudi Arabian medical students also had a high level of stress. Academic problems were the most common stressors of studying in medical training. Reasons for this could be that academic achievement has always been the top priority for medical students. Medical students with stress also reported significantly more academic problems than students without stress.[4]

In our study, medical students used adaptive coping (self-distraction, acceptance, active coping and positive reframing) rather than maladaptive coping strategies (denial, behavioral disengagement and substance use). These were the same coping strategies commonly used among medical students at Thammasat University,[10] in India[20] and other countries.[21,22] However, females utilized adaptive coping strategies more than males, which may be caused by female appraisal of threatening events as more stressful; hence, they were also more affected by the stress of those around them. This in turn led them to be more emotionally involved than their male counterparts. As they used more emotional coping strategies to deal with stress than males, this leads to more adaptive coping strategies being used in females.[23]

Among medical students who have high grades (GPA≥3.5), the use of adaptive coping strategies is similar to a study from Argentina.[24] The reason may be the adaptive ability to study, and more coping strategies and problem solving skills than others. Additionally, this may lead

**TABLE 1.** Demographic characteristics (n=827).

| Demographic characteristics | Number (%) |
|---|---|
| **Gender** | |
| Female | 502 (60.7) |
| Male | 323 (39.1) |
| Unreported | 2 (0.2) |
| **Academic year of medical student** | |
| 1 | 168 (20.3) |
| 2 | 121 (14.6) |
| 3 | 161 (19.5) |
| 4 | 98 (11.9) |
| 5 | 161 (19.5) |
| 6 | 118 (14.3) |
| **Religion** | |
| Buddhism | 700 (84.6) |
| Islam | 31 (3.7) |
| Islam 3 southern border provinces | 30 (3.6) |
| Christianity/other | 32 (3.9) |
| Unreported | 34 (4.1) |
| **Home province** | |
| Songkhla | 336 (40.6) |
| 3 southern border provinces | 126 (15.2) |
| Other | 352 (42.6) |
| Unreported | 13 (1.6) |
| **Parental marriage status** | |
| Couple | 707 (85.5) |
| Divorce/pass away | 112 (13.5) |
| Unreported | 8 (1.0) |
| **Underlying disease** | |
| **Medical illness** | |
| No | 694 (83.9) |
| Yes | 130 (15.7) |
| Unreported | 3 (0.4) |
| **Psychiatric illness** | |
| No | 784 (94.8) |
| Yes | 39 (4.7) |
| Unreported | 4 (0.5) |

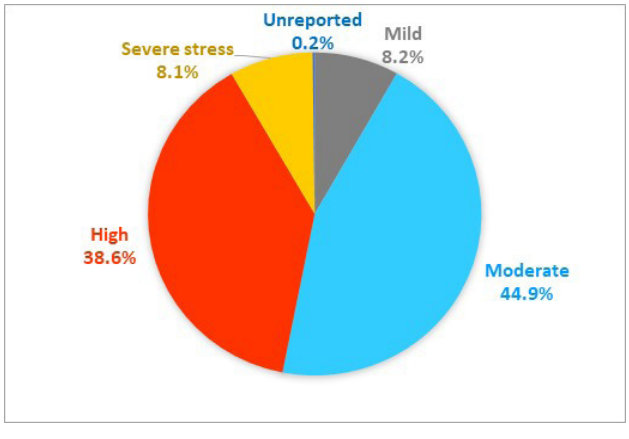


**Fig 1.** Percentage of stress level among medical students (n= 827).

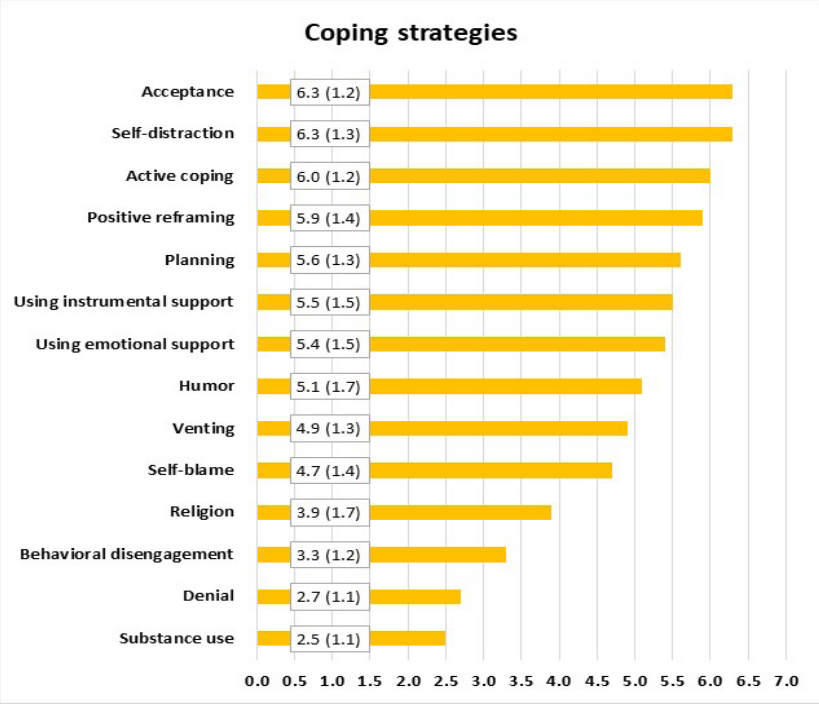


**Fig 2.** Coping strategies score (n=827).

*Mean score interpretations were as below: 2.00 = have not been doing this at all, 2.01 to 4.00 = have been doing this a little bit, 4.01 to 6.00 = have been doing this a medium amount, 6.01 to 8.00 = have been doing this a lot

**TABLE 2.** Multiple linear regression of the association between demographic data and coping strategies.

| Demographic | Adaptive | | | Maladaptive | | |
|---|---|---|---|---|---|---|
| | Median (IQR) | Beta (coefficient) | *P*-value | Median (IQR) | Beta (coefficient) | *P*-value |
| **Gender** | | | | | | |
| Male | 2.7 (2.4, 3.0) | reference | | 2.0 (1.8, 2.2) | | |
| Female | 2.8 (2.5, 3.1) | 0.105 | 0.003 | 2.1 (1.8, 2.2) | | |
| **Year of medical student** | | | | | | |
| Pre-clinic | 2.8 (2.4, 3.1) | | | 2.1 (1.8, 2.2) | | |
| Clinic | 2.8 (2.4, 3.0) | | | 2.0 (1.8, 2.2) | | |
| **Religion** | | | | | | |
| Buddhism/christianity/ others | 2.8 (2.4, 3.0) | reference | | 2.0 (1.8, 2.2) | | |
| Islam 3 provinces | 3.0 (2.8, 3.3) | 0.288 | 0.002 | 2.2 (1.9, 2.3) | | |
| Islam | 2.9 (2.7, 3.0) | 0.096 | 0.265 | 2.1 (1.8, 2.2) | | |
| **Grade point average** | | | | | | |
| <3.5 | 2.7 (2.4, 3.0) | reference | | 2.0 (1.8, 2.3) | | |
| >3.5 | 2.8 (2.5, 3.1) | 0.099 | 0.005 | 2.1 (1.8, 2.3) | | |
| **Medical illness** | | | | | | |
| No | 2.8 (2.4, 3.0) | reference | | 2.0 (1.8, 2.2) | reference | |
| Yes | 2.9 (2.5, 3.1) | 0.116 | 0.018 | 2.1 (1.8, 2.3) | 0.027 | 0.367 |
| **Stress level** | | | | | | |
| Mild-moderate | 2.8 (2.4, 3.1) | reference | | 1.9 (1.8, 2.1) | reference | |
| High-severe | 2.8 (2.5, 3.0) | 0.021 | 0.553 | 2.2 (1.9, 2.4) | 0.258 | <0.001 |

*Significant (p-value <0.05)

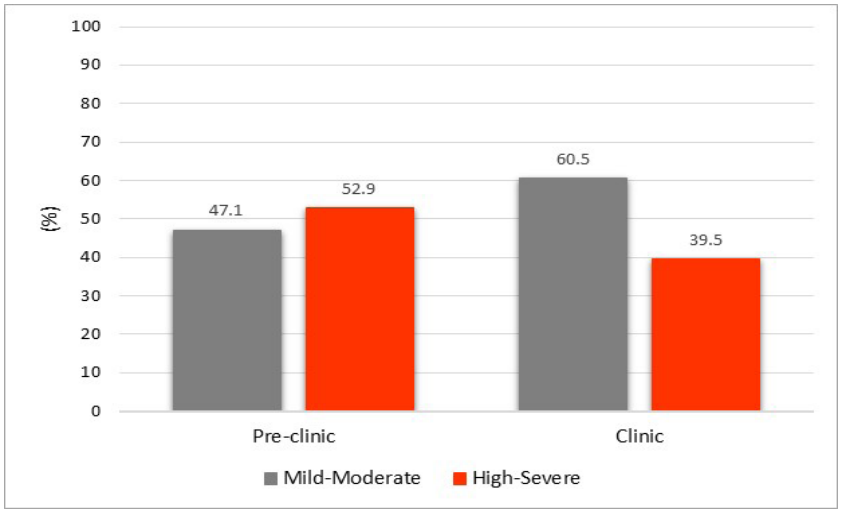


**Fig 3.** Stress level and year of medical student.

them to acquire better academic performance. Besides, this study found Muslim students in 3 southern border provinces used religious coping strategies more than others. This result is similar to studies in Malaysia.[19,22] This may be related to medical students' strong religious beliefs, providing guidance on how to live and giving individuals meaning and identity.[25] In addition, medical students who had high-severe levels of stress tended to use maladaptive coping strategies, rather than those of the mild-moderate group, since performance and coping skills could be markedly impaired in persons who perceived greater stresses in certain situations.[26]

Among the academic year, the clinical medical students stress score was less than pre-clinical medical students. These results were similar to studies in India[27] and Morocco.[28] Pre-clinical medical students may be stressed by the overwhelming amount of information they have to learn, whilst handling the lifestyle of a medical college.[27] However, there was no significant difference in coping strategies between pre-clinical and clinical medical students. This finding might be because of an adaptive or mature defense mechanism being an innate or part of developing changes of both pre-clinical and clinical medical students. In addition, a mature defense mechanism is a part of medical professionalism development in medical education curricula. Thus, a longitudinal and prospective study about individual changes, from pre-clinical to clinical phase, in coping strategies and other areas would be interesting for further study.

Furthermore, there is correlation between high level stress and maladaptive coping strategies. The results of maladaptive coping strategies may eventually have a negative impact on physical and mental health. Therefore, faculty policy should be reviewed to focus on stress in medical students, indicating a need for stress management programs within their medical education.

### Limitations

This study was of a cross-sectional survey, and employed self-reporting for individual perception assessment. Besides its high response rate (74.6%), the information might not have led to finding bias. However, the population was limited to only medical students in the Faculty of Medicine, Prince of Songkla University. Then, it is too soon to generalize these data to a nationwide setting.

### Implications and future recommendations

Further studies should cover more medical schools within Thailand and employ a more quantitative method. In other words, a multi-center survey is recommended.

## CONCLUSION

Half of the medical students perceived stress, and they use mainly adaptive coping strategies; rather than maladaptive coping strategies. Gender, GPA, academic year, medical illness and religion had significant correlations with coping strategies. High level stress was correlated with maladaptive coping strategies. In the future, focusing on medical students' stress coupled with coping strategies could prevent the harmful effects of stress on health and academic performance.

## ACKNOWLEDGMENTS

This study was fully funded by the Faculty of Medicine, Prince of Songkla University, Thailand. The authors are very thankful for the data analysis operated by Mrs.Nisan Werachattawan and Ms.Kruewan Jongborwanwiwat. Moreover, we would like to show our gratitude to all medical student who cooperated in this survey.

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# The Relationship between Resilience Quotient, Social Support and Spiritual Well-Being of Caregivers of Patients with Hemiplegia

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## ABSTRACT

**Objective:** There are three main objectives of this study. The first is to study the levels of resilience quotient, social support and spiritual well-being of caregivers of patients with hemiplegia. The second is to study the relationship between resilience quotient and spiritual well-being of caregivers of patients with hemiplegia. The third objective is to study the relationship between social support and spiritual well-being of caregivers of patients with hemiplegia.

**Methods:** The sample of this study is composed of 170 caregivers of patients with hemiplegia who received treatment at the Department of Rehabilitation Medicine, Faculty of Medicine Siriraj Hospital. Data for this study were collected using a psychological questionnaire, and then by adopting analytical statistics, which are percentage, mean, standard deviation and Pearson's Product Moment Correlation Coefficient.

**Results:** After data collection and analysis, caregivers of patients with hemiplegia were found to have relatively high levels of resilience quotient and social support. Moreover, they have a high level of spiritual well-being. Taken together, these results suggest that there is a positive correlation between resilience quotient and the spiritual well-being of caregivers of patients with hemiplegia, with statistical significance at the .01 level. There is also a positive correlation between social support and the spiritual well-being of caregivers of patients with hemiplegia, with a statistical significance at the .01 level.

**Conclusion:** This research results can be used as a guideline for efforts to enhance the resilient quotient, social support and spiritual well-being of caregivers by providing knowledge, information and caregiving equipment to them. Moreover, caregivers should be encouraged to enjoy their free time by engaging in creative activities and religious activities, which are believed to help increase mental peace.

**Keywords:** Resilience quotient; social support; spiritual well-being; caregiver of patients with hemiplegia (Siriraj Med J 2020; 72: 245-252)

## INTRODUCTION

The development of the quality of life of people with disabilities in Thailand has changed over time. In order to enhance the effectiveness of development of people with disabilities, a strategy was established in the 5th National Development Plan for Life Quality of the Disabled to promote and strengthen the effectiveness of efforts to assist people with disabilities and related organizations. Specifically, guidelines and processes are to be provided to enhance the effectiveness and offer support

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for caregivers of people with disabilities.[1] According to the national statistics on people with disabilities, the number of people with physical disabilities rose from 880,662 people in September 2017[2] to 1,004,733 people in September 2018, an increase of 14.09%.[3] The number of people with physical disabilities significantly increased within a year because hemiplegia is a disorder caused by stroke, which damages the brain function suddenly. It is the number one cause of disability and death in Thailand, where it is estimated that 50,000 people die from hemiplegia and it disables 250,000 people each year.[4] The result is an increasing number of relatives of people with disabilities who need to adjust to new and unexpected roles as caregivers. The phenomenon of adjusting to these new roles can cause stress and anxiety to new caregivers in the long run. Far too often, the new caregivers are likely to confront physical and mental problems which they find it difficult to handle, and which may cause them to suffer a loss of confidence and self-esteem in their lives.

**Resilience quotient** is the capability of a person to adjust or adapt oneself and recover when facing crises.[5] A crisis can happen when a family member becomes disabled suddenly and requires a caregiver who has ability to cope with the situation. This caregiver has to be resilient to deal with suffering and disappointment that the unexpected situation might have on the health expectations of person with disabilities. Therefore, a caregiver needs a high resilience quotient in order to understand both what the disabled person wants, and how to handle the situation so that the caregiver can eventually recover from the suffering brought on by the crisis. Additionally, there is a chance that a person with disabilities will face a health complication, thus, a caregiver needs to be capable of emotional-self regulation and the ability to handle things morally and conscientiously. Yet, a caregiver should also have the ability to bring one's own potential and skills, and use them productively in giving care to a person with disabilities.

**Social support** is a support given by means of delivering information, material or mental support from a person, a group of people, or an institution. This social support should have a positive result in any actions taken by the support receiver.[6] Caregivers, acting alone, often have difficulties in taking care of people with disabilities, and social support is considered to be an important factor in successfully supporting caregivers of people with disabilities. This social support typically originates with family members, who provide attention and caring for caregivers of people with disabilities. Furthermore, social support can be expanded to include other close relatives who are able to offer material or financial support, or engage in positive relationships with the caregiver. In addition, social support can come from a group of caregivers, themselves, who may share information between them, and contribute to better understandings and knowledge that helps each individual cope with his or her situation. Social support can have an effect on the thoughts, attitudes and behavior[7] of caregivers towards the act of giving care to people with disabilities. More than that, hospitals or other institutions can provide information or material supports that helps in facilitating caregiver in giving care to people with disabilities.

**Spiritual well-being** is a state in which ones has both physical and psychological integrity, positive perspectives in living a meaningful life, and satisfaction in personal beliefs, including religion.[8] Additionally, people with spiritual well-being are those who have clear life goals, hope, creativity, and feel the need to give and receive love.[9] Thus, a caregiver of people with disabilities must have spiritual well-being in order to be a successful caregiver, able to enhance the lives of people with disabilities. The caregiver is responsible for creating and organizing activities for people with disabilities so that they can appreciate and live a meaningful life. Religion is one of the spiritual anchors that can hold together one's mind, and offers important principles that can guide one through the difficulties that arise in life. Also, religion teaches people to forgive, give love, sacrifice oneself for others, follow important principles in life, and be delighted and pleased in what they are doing as caregivers.

For this reason, the researcher is interested in studying the resilience quotient, social support and spiritual well-being of caregivers of patients with hemiplegia who are receiving treatment at the Department of Rehabilitation Medicine, Faculty of Medicine Siriraj Hospital. Siriraj Hospital was selected because of its holistic care system that provides physical, mental and social treatment, all of which are provided by personnel through a multidisciplinary approach which involves a number of professionals working together: rehabilitation physician, rehabilitation nurse, physical therapist, occupational therapist, speech therapist, orthotics, psychologist and social worker. The researcher hopes that this research will be useful for clinicians, physicians, scholars, hospital staff and administrators, and the personnel of any related institutions in enhancing the spiritual well-being of people with disabilities and those who care for them. In this study, it is hypothesized that there is a correlation between resilience quotient and spiritual well-being, as well as a correlation between social support and the spiritual well-being of caregivers of patients with hemiplegia.

**Research Framework**

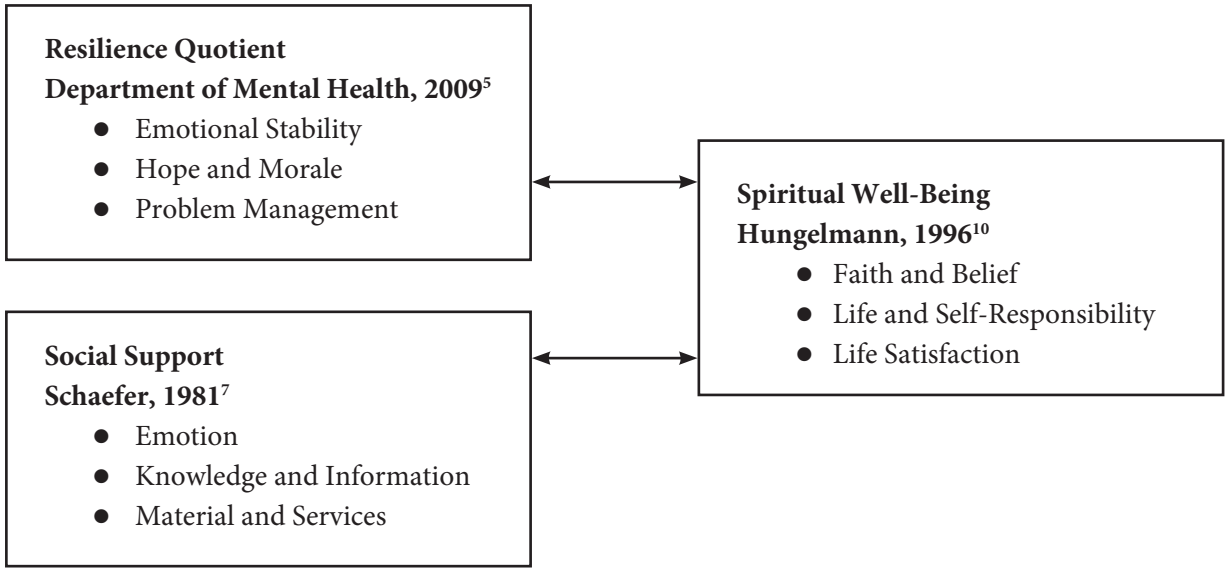


**Fig 1.** From the research framework, the researcher studied the correlation between resilient quotient and spiritual well-being and social support and spiritual well-being.

## MATERIALS AND METHODS

### Study design and population

This research study is a descriptive study and is certified for human research ethics by the Siriraj Institution Review Board (SIRB) of the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (Si 279/2019). The study involved the collection of data from caregivers aged of 18 years old and older who are the main caregivers of patients with hemiplegia that receive treatment at the Department of Rehabilitation Medicine, Faculty of Medicine, Siriraj Hospital. Each month in 2018, there was an average of around 229 patients, counted by the number of unique patients who come to receive treatment individually, without repeated counting.

### Data collection

The sample of this study comprises 168 caregivers of patients with hemiplegia, which is calculated by Yamane's sample size formula (1973)[11] from the accidental sampling. The data were collected through the use of a psychological questionnaire designed by the researcher; and the validity and reliability assessment of the questionnaire was also performed by the researcher. There were 170 returned questionnaires, which exceeds the number of the sample because two more caregivers of patients with hemiplegia were found on the last day of data collection. Since they met the standards for qualification, their data wasn't eliminated.

### Research instrument

The research instrument contains five sections. The first section collects personal information of caregivers such as sex, age, education, relationship with patients, duration of caregiving, occupation, monthly family income, balance of income and expense, health condition, family members, number of family members who need support, and caregiver counselling. The second section addresses characteristics of patients with hemiplegia, and includes the demographic characteristics of patients with hemiplegia who are taken care of by the respondents. The characteristics include the length of time that the patient has undergone treatment, sex, age, health condition, medical care and education. The third section is a set of questions used to develop the resilience quotient, adopted from the concept of resilience quotient from Department of Mental Health[5] which covers three elements: emotional stability, hope and morale, and problem management. There are 18 questions in this section; for each question, the respondent needs to choose the answer that best describes him or herself. The validity index of this set of questions is .93 and the reliability index is .98. In the fourth section, the questionnaire covers all three components of Schaefer's[7] concept of social support, which are emotion, knowledge and information, and material and services. There are 18 questions in this section; for each one, the respondent needs to choose the answer best describes him or herself. The validity

index of this set of questionnaires is .96 and the reliability index is .94. Lastly, the questionnaire addresses the level of spiritual well-being developed from the concept of Hungelmann[10] that described three elements of spiritual well-being as faith and belief, life and self-responsibility, and life satisfaction. There are 18 questions on this section; for each question the respondent needs to choose the answer best describes him or herself. The validity index of this set of questionnaires is .96 and the reliability index is .97.

**Statistical analysis**

The researcher used descriptive analysis to analyze general information and study the level of resilience quotient, social support and spiritual well-being of caregivers of patients with disabilities. In this study, descriptive analysis is combined with frequency, percentage, mean and standard deviation. In addition, the researcher adopts Pearson's Product Moment Correlation Coefficient in order to analyze the relationships between resilience quotient, social support, and spiritual well-being.

## RESULTS

The researcher found that 69.42% of the caregivers are women and 30.69% of caregivers are men. The average age of the caregivers was 52.10, the youngest is 19 years of age; the oldest is 83. Additionally, the highest proportion of respondents, 27.64%, were over the age of 60, while 27.06% were aged between 51 and 60, 25.89% aged between 41-50, and 19.41% aged between 19-40. With regard to education, 52.94% of caregivers have completed a bachelor's degree or higher, 17.06% completed upper secondary education, 12.94% completed lower secondary education, and 17.06% completed primary education. Forty percent of caregivers are children of the patients, 23.53% are spouses of the patients, 15.88% are relatives of the patients, 11.18% are parents of the patients, and 9.41% are paid caregivers.

The average duration of caregiving is 4 years; the shortest duration is 1 month, and the longest duration is 30 years. It was found that almost half of the caregivers had been giving care to patients for more than 24 months, which accounted for 48.80% of samples, followed by those with a caregiving duration between 13 and 24 months, at 24.70%. Approximately 18.80% had been giving care for less than six months, while only 7.70% had been caregivers for between 6 and 12 months. With respect to occupation, 25.88% of caregivers are housewives and househusbands, 21.18% are merchants, 20.59% are paid employees in the business sector, 17.06% are unemployed, and 15.29% are civil servants. The caregivers have an average monthly family income of 33,308.82 baht. The lowest average monthly family income is 5,000 baht, and the highest is 100,000 baht. Furthermore, 44.12% of caregivers have an average family income between 5,000 and 20,000 bath per month, 29.41% have an average family income of more than 40,000 baht, and 26.47% have an average family income between 20,001 and 40,000 baht. Finally, it was reported that 42.35% of caregivers have a surplus of income compared to expenses, while 30.59% reported a deficit between income and expenses, and 27.06% said that income and expenses are equal.

Most of the caregivers (61.76%) do not have congenital diseases, while the remaining 38.42% of caregivers reported that they do. The average number of members in the families of caregivers is 4.76, with the lowest number being 2 and the highest number of family member being 10. Proportionally, 27.65% of caregivers have 4 family members, 27.65% have a 6-10 family members, 24.70% have 5 family members, and 20% have 2-3 family members. The average number of family members who needs financial support is 1.65, with the lowest being 1 and the highest being 6. Proportionally, 60.59% of caregivers' family have 1 family member who needs financial support, while 39.41% have 2 family member who need financial support. 72.94% of caregivers have received advice about caregiving and only 27.06% have not.

The information about patient's characteristics, shows that the average length of time that patients have been receiving treatment at the Department of Rehabilitation Medicine is 3.34 years; the shortest duration is 1 month, and the longest is 25 years. Proportionally, 32.35% of patients have been receiving treatment between 1 and 2 years, 25.88% have been receiving treatment for more than 5 years, and 24.12% have been receiving treatment for less than a year. Most of the patients are male, accounting for 66.47%, while only 33.53% of patients are female. The average patient age is 64.15; the youngest patient is 18 and the oldest is 92. Proportionally, 31.76% of patients aged between 18-59, 28.24% are aged between 60-69, 22.35% are aged between 70-79, and 17.65% are at least 80 years old. The health condition of patients can be divided into 2 groups. The first is the group of patients who are unable to walk, accounting for 54.12%. The second group comprises patients who are able to walk, accounting for 45.88%. When it comes to paying for treatment, 40% of all patients are using health insurance for the treatment, 38.24% are using civil servants' medical insurance, 11.18% are using a benefits card for persons with disabilities, 5.88% are using a social security card, and only 4.70% are paying on their own. For the education

of patients, 39.41% of patients completed secondary education, 33.53% completed primary education, and 27.06% completed at least a bachelor's degree.

The research results show that the caregivers of patients with hemiplegia have a relatively high resilience quotient, which means that they have flexibility in their lives and have the ability to adapt themselves and handle problems during crises. In the same way, it is shown that the social support for caregivers of patients with hemiplegia is at a relatively high level, which indicates that the caregivers receive good information, material and encouragement supports. Moreover, the spiritual well-being of the caregivers is at a high level, which can be interpreted to mean that they have great physical and mental well-being, they are satisfied with their lives, and value themselves. These results can be seen in Table 1 below.

The research shows that resilience quotient has a positive correlation with the spiritual well-being of caregivers of patients with hemiplegia, with statistical significance at the .01 level (r = .71) as shown in Table 2.

The overall dimension of the resilience quotient and its determinants, which are emotional stability, hope and moral, and problem management, is shown to have positive correlation with spiritual well-being at the overall dimension as well as each determinant, including faith and belief, life and self-responsibility, and life satisfaction at the .01 level of statistical significance. The results indicate that there is a positive correlation between social support and spiritual well-being of caregivers of patients with hemiplegia with a statistical significance at the .01 level (r = .71) as can be seen from the data in Table 3.

The overall dimension and each determinant of social support, which are emotion, knowledge and information, and material and services are revealed to have positive correlation with spiritual well-being at the overall dimension along with each determinant of spiritual well-being, which are faith and belief, life and self-responsibility, and life satisfaction at the .01 level of statistical significance.

**TABLE 1.** Mean, standard deviation and level of resilience quotient, social support and spiritual well-being of caregivers of patients with hemiplegia.

| Variable | $\bar{x}$ | S.D. | Level |
|---|---|---|---|
| Resilience quotient | 4.06 | .41 | Relatively high |
| Social support | 4.13 | .45 | Relatively high |
| Spiritual well-being | 4.25 | .41 | High |

**TABLE 2.** Correlation Coefficient (r) and p-value of resilience quotient and spiritual well-being of caregivers of patients with hemiplegia.

| Resilience quotient | Spiritual well-being | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Faith and belief | | Life and self-responsibility | | Life satisfaction | | Overall | |
| | r | p | r | p | r | p | r | p |
| Emotional stability | .46** | .00 | .52** | .00 | .57** | .00 | .58** | .00 |
| Hope and morale | .58** | .00 | .56** | .00 | .57** | .00 | .64** | .00 |
| Problem management | .48** | .00 | .57** | .00 | .51** | .00 | .58** | .00 |
| Overall | .59** | .00 | .65** | .00 | .65** | .00 | .71** | .00 |

**means having a statistical significance at the 0.1 level

**TABLE 3.** Correlation Coefficient (r) and p-value of social support and spiritual well-being of caregivers of patients with hemiplegia.

| Social support | Spiritual well-being | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Faith and belief | | Life and self-responsibility | | Life satisfaction | | Overall | |
| | r | p | r | p | r | p | r | p |
| Emotion | .58** | .00 | .62** | .00 | .65** | .00 | .69** | .00 |
| Knowledge and information | .52** | .00 | .47** | .00 | .54** | .00 | .57** | .00 |
| Material and services | .46** | .00 | .40** | .00 | .52** | .00 | .52** | .00 |
| Overall | .62** | .00 | .59** | .00 | .68** | .00 | .71** | .00 |

**means having a statistical significance at the 0.1 level

## DISCUSSION

In this study, it is found that the resilience quotient of caregivers of patients with hemiplegia is at a relatively high level ( $\bar{x}$ = 4.06). This might be because the average age of caregivers of patients with hemiplegia is 52.10 years old, meaning that they are typically adults who have experienced several problems previously, so they tend to be mature and patient. This corresponds with data from the Department of Mental Health,[5] which indicate that those who have experienced life problems and crises are people who have higher resilience quotient than those who have not. Moreover, patience in adults can help them adjust and adapt themselves promptly when facing serious situations or crises, and they are likely to have fewer physical impacts from mental issues than people with lower patience. The social support of caregivers of patients with hemiplegia is at a relatively high level ( $\bar{x}$ = 4.13). Based on this result, it appears that the caregivers have received sufficient social support, assistance, and information, leading to good results in giving care of patients with hemiplegia. These supports include material and financial supports that enhance the ability of caregivers, and which, in the long run, may benefit the lives of patients with hemiplegia in positive way, in accordance with National Development Plans for Life Quality of the Disabled. The spiritual well-being of caregivers of patients with hemiplegia is also at a high level ( $\bar{x}$ = 4.25), which may be because the caregivers understand the life of people with disabilities and how these people struggle daily, so the caregivers desire to give the best care to these people. The caregivers see how meaningful life is, so they are willing to devote their lives to be caregivers for people with hemiplegia. Furthermore, religion can be a spiritual anchor to caregivers.

The correlation analysis shows that there is a positive correlation between the resilience quotient and spiritual well-being of caregivers of patients with hemiplegia, with statistical significance at the .01 level, which is in line with the hypothesis. It can be interpreted that the higher the resilience quotient caregivers of patients with hemiplegia have, the more spiritual well-being they will have. This is likely because caregivers have the ability to manage and cope with problems, crises, and pressure, so they are able to deal with changes and become productive caregivers. Moreover, religion is another factor that affects the way caregivers perform as the religion teaches people to be good and do good things. For caregivers, helping others by devoting themselves in giving care is in line with this religious teaching. These results are consistent with data in the previous study, "The effect of the E&R Program on mental health of caregivers of children with developmental and intellectual disabilities Rajanukun Development Center (Muangkae) Rajanukun Institute" by Anchalee Watthong and Sala Techameena[12] (E&R Program is a training program that enhances the resilient quotient to people for them to overcome life crises as well as to strengthen people's spiritual encouragement). This research revealed that supporting the resilience of caregivers of children with developmental and intellectual disabilities leads to better performance of caregivers in giving care to children, because the caregivers gain better understanding of problems and know how to manage those problems with creative solutions. Yet, it is a characteristic of caregivers who have spiritual well-

being to participate in creative activities to make their lives meaningful, which is also in accordance with the notion of Pravet Wasee: "If what we are doing is correct and good, we will achieve spiritual well-being."[13]

Furthermore, the correlation analysis shows that there is a positive correlation between social support and spiritual well-being of caregivers of patients with hemiplegia, with a statistical significance at the .01 level. This indicates that the more social support caregivers receive, the more spiritual well-being they will enjoy. In other words, social support given to caregivers will help them accomplish what they are doing; thus, the caregiving is likely to be successful and effective. When people accomplish what they are doing, they tend to be proud and satisfied; this is what is called spiritual well-being. Giving social support to caregivers by providing information about caregiving for patients is essential and has a high positive impact on caregivers' performance. This finding was also reported by Sukheeluk, Nuanchana and Rabudda[14] in their research, "The result of developing caregiver potential for stroke patients at home in Maharaj district, Phranakhon Si Ayuthhaya province." In this research, it was identified that caregivers of patients of stroke gained confidence and courage in giving care wholeheartedly and that they were able to manage problems when they received social support, including joining activities, training, and exchanging knowledge and information on caregiving of patients. Therefore, caregivers who receive social support are likely to have higher motivation, possess greater courage, and be better caregivers. More than that, a family is one of the main supports for caregivers, providing material support, financial support and courage that can mitigate the difficulties from caregiving that may result from patients' unstable emotional states or from the expectations from the patient's family. This also accords with the previous study by Panphadung, Nilmanut and Kitrungrote,[15] "Spiritual Well-Being of Family Caregivers of Hospitalized Patients with Advanced Gynecological Cancer." In this study, it was reported that caregivers need to cope with the misunderstandings and ignorance that could arise from the patient's family or relatives about certain aspects of caregiving. Hence a lot of effort is required for caregivers to provide favorable caregiving, which can have an effect on the spiritual well-being of caregivers.

In this study, there was a time constraint on collecting data from the caregivers of patients with hemiplegia who are receiving treatment at the Department of Rehabilitation Medicine, Faculty of Medicine Siriraj Hospital. Due to the small number of caregivers at the hospital each day, it took two months to get all the designated samples. Notwithstanding this limitation, the study suggests that resilience quotient and social support have correlations with spiritual well-being. Siriraj Hospital has a holistic care system that offers physical, mental and social treatment which are provided by personnel involved in a multidisciplinary approach which includes a rehabilitation physician, rehabilitation nurse, physical therapist, occupational therapist, speech therapist, orthotist, psychologist and social worker. Therefore, with the holistic care, the patients receive comprehensive treatment, which may have positive effects on the caregivers of patients with hemiplegia. These positive effects include a high level of resilience quotient acquired after the caregivers have been able to get through crises and have also been able to bring the patients to receive effective and timely treatment. In addition, the caregivers receive social supports that help them greatly in enhancing their skills and knowledge about giving care to patients with hemiplegia. When the caregivers have a sufficiently high resilience quotient and receive social supports, they are likely to have higher spiritual well-being because resilience quotient and social support both have positive correlations with spiritual well-being. Thus, the caregivers are very likely to have good mental health, be selfless, satisfied in their lives, and feel that what they are doing is valuable to themselves and others. However, the findings in this study are based solely on information collected from the caregivers of patients with hemiplegia at Department of Rehabilitation Medicine, Faculty of Medicine Siriraj Hospital. It is impossible to know if the results of this study are applicable to caregivers of patients with other health conditions, or those from different areas or cultural backgrounds, or whether similar studies of different populations will be relevant or get the same results. Therefore, further studies in different conditions need to be conducted in the future.

The research results can be applied to benefit caregivers in a number of ways. Resilience should be promoted for caregivers of patients with hemiplegia so they can get through problems and difficulties that happen during caregiving which could undermine their courage. Additionally, creative activities and relaxing activities such as drawing, reading, doing sports, crafting or religious activities should be promoted for caregivers to enjoy. Also, social support should be made easily available to caregivers of patients with hemiplegia by providing necessary materials such as wheelchairs and walking sticks, providing guiding information and advice about caregiving for patients with hemiplegia, and arranging experience exchanges among caregivers of patients with

hemiplegia. Exchanging and sharing experiences among caregivers of patients with hemiplegia is considered to be very important because it has a positive impact on the spiritual well-being of caregivers through the acts of giving and receiving support to each other, which helps caregivers to appreciate themselves and gain motivation and confidence to give the best care to patients

## ACKNOWLEDGMENTS

The researcher would like to thank the nurses and staff at the Department of Rehabilitation Medicine, Faculty of Medicine Siriraj Hospital for their facilitation in data collection at the department. Finally, great appreciation is expressed to the sample group of caregivers of patients with hemiplegia for the excellent cooperation offered in answering the questionnaire.

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# Evaluation of Combined Rapid Immunoglobulin M and Immunoglobulin G Lateral Flow Assays for the Diagnosis of Leptospirosis, Scrub Typhus, and Hantavirus Infection

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## ABSTRACT

**Objective:** Leptospirosis, scrub typhus, and hantavirus infection are commonly identified as causes of acute undifferentiated fever in rural parts of Asia. Although the characteristic presentations of these infections are well described, many of them present with nonspecific manifestations. Diagnosis is usually made by combined history of exposure, clinical features and positive antibody detection. The development of rapid antibody detection assay, using an immunochromatographic test (ICT) for the diagnosis of multi-diseases, has provided tools for more accurate diagnosis and appropriate antibiotic treatment of the acute undifferentiated fever syndrome.

**Methods:** We evaluated the diagnostic performance of a commercially available combined rapid ICT for the diagnosis of leptospirosis, scrub typhus, and hantavirus infection, using archived blood samples from 434 patients with laboratory-confirmed leptospirosis (131) or scrub typhus (128), and from patients with other causes of fever as the negative control (175). Polysaccharide of nonpathogenic *Leptospira patoc*, a chimeric recombinant protein cr56 and two other recombinant proteins, r21 and kr56, from different serotypes of *Orientia tsutsugamushi*, and 21kDa species-specific antigen and recombinant CNP antigen derived from the Soochong virus were used as antigens for the diagnosis of leptospirosis, scrub typhus, and hantavirus infection in the combined ICT used in this study.

**Results:** For the diagnosis of leptospirosis; in acute phase, the sensitivity and specificity of the ICT detection of IgM/IgG were 38.2% (95% CI, 29.9- 46.5%), and 99.0% (95% CI 97.9-100%); while in convalescent phase, the same were 84.6% (95%CI, 77.1- 92.0%), and 96.2% (95%CI, 92.5- 99.8%), respectively. For scrub typhus, in acute phase, the sensitivity and specificity of the ICT detection of IgM/IgG were 71.9% (95% CI, 64.1- 79.7%), and 97.4% (95% CI 95.6 - 99.2%); while in convalescent phase, the same were 84.6% (95%CI, 74.8- 94.4%), and 90.2% (95%CI, 85.3- 95.1%) respectively. For hantavirus infection, nine patients had detectable IgM for hantavirus infection. All these cases were diagnosed as scrub typhus by indirect immunofluorescent assay.

**Conclusion:** The performance of this combined ICT for leptospirosis and scrub typhus were comparable to those published data of other ICTs. However, the rapid test for the diagnosis of leptospirosis, using antigen detection, is needed. Hantavirus infection was not detected in this study population.

**Keywords:** Immunochromatographic assay; leptospirosis; scrub typhus; hantavirus (Siriraj Med J 2020; 72: 253-258)

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*http://dx.doi.org/10.33192/Smj.2020.34*

## INTRODUCTION

Acute undifferentiated febrile illness is a leading cause of hospital visit among adults in rural areas of South–East Asia. The common causes of such fever include scrub typhus, murine typhus, leptospirosis, and hantavirus infection.[1,2] Leptospirosis is a worldwide zoonotic disease, caused by pathogenic members of the genus *Leptospira*.[3] Human infection occurs through the direct or indirect exposure to the organism excreted in the urine of both wild and domestic mammals. Scrub typhus is the most common rickettsial infection in the Asia- Pacific region. It is caused by *Orientia tsutsugamushi*, an obligate intracellular Gram-negative bacterium.[4] Human accidentally infected through the bite of the infected chiggers. Over a billion people are at risk of scrub typhus and approximately one million cases occur annually.[4,5] In Thailand, scrub and leptospirosis are the main causes of acute undifferentiated fever, after dengue infection is excluded.[1,6]

Hemorrhagic fever with renal syndrome (HFRS) is caused by various hantaviruses in the genus Hantavirus of the family Bunyaviridae. Seoul virus (Seoul orthohantavirus, SEOV), the only species of the genus Hantavirus that is found to be globally spread as hantavirus, is a common cause of HFRS. HFRS is a viral zoonosis transmitted by rodents.[7] To date, only 2 patients with hantavirus infection have been reported in Thailand.[8,9] However, the incidence of HFRS could be underestimated in Thailand due to the unavailability of a diagnostic test.

Although the characteristic clinical presentations of leptospirosis, scrub typhus, and HFRS are well described, many patients present with protean and nonspecific symptoms and signs.[2] Consequently, the diagnoses of either of these infections are usually made by combination of a history of exposure, well recognized symptoms and signs, and positive antibody detection.[5,6] In addition, hantavirus infection and leptospirosis can share similar clinical and exposure risks.[10] The availability of rapid antibody tests using an immunochromatographic test (ICT) has provided tools for point-of-care serologic testing. ImmuneMed AFI Rapid® is one such commercially available rapid ICT for the qualitative detection of both IgM and IgG antibodies to hantavirus, *O. tsutsugamushi*, and *Leptospira spp*. in a patient's serum, plasma, or whole blood. In this study, we conducted the study to determine the diagnostic performance of this assay, using the stored serum/ plasma samples of patients who presented with an acute febrile illness caused by leptospirosis, scrub typhus, or other diagnoses.

## MATERIALS AND METHODS

### Patients with leptospirosis or scrub typhus

Blood samples (n=259) were collected from patients (male : female = 2 :1), aged 15- 84 years old (median age 45 years old) who presented with acute febrile illness at four hospitals in Thailand between January 2000 and December 2018. Three hospitals are located in the northeastern region of the country (Maharaj Nakhon Ratchasima Hospital, Loei Provincial Hospital and Banmai Chaiyapod Hospital, Burirum Province). In these hospitals, blood samples were collected as part of the epidemiological and clinical studies of patients with acute undifferentiated fever.[6,11,12] Included in these studies were adult patients (>18 years) who presented with acute fever (oral temperature, >38.0°C for less than 15 days) in the absence of an obvious focus of infection. Blood samples were also collected from patients, using the same inclusion criteria, at Siriraj Hospital, Bangkok, Thailand. All of these clinical studies were conducted after the approval of the Ethical Review Subcommittee, Public Health Ministry of Thailand and the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University (Si 014/2019). All patients provided the informed written consent before enrollment to the study. Blood samples were collected on the day of admission, and/or during convalescence or after discharge from the hospital. Plasma was divided into that needed for immediate use and a leftover sample was stored at –70°C.

### Non-scrub typhus and non-leptospirosis samples

Blood samples (n= 175) from patients with other tropical febrile illnesses (laboratory-confirmed) were collected from the same hospitals as the patients with leptospirosis and scrub typhus. All of these samples were tested by indirect immunofluorescent assay (IFA) and were shown to be negative for *O. tsutsugamushi* and *Leptospira*. The diagnoses of patients in this control group were dengue infection in 59 patients; zika virus infection in 16 patients; influenza A or influenza B in 26 patients; murine typhus in 60 patients; other bacterial infections, such as Escherichia coli septicemia, melioidosis, and salmonellosis in 8 patients; and *Plasmodium falciparum* malaria in 6 patients.

The IFA assay for the laboratory confirmation of leptospirosis, scrub typhus, and murine typhus was performed as described previously.[6,11,12] The *Leptospira interrogans*, serovar autumnalis; pooled *O. tsutsugamushi* from Karp, Kato and Gilliam strains; and Rickettsia typhi (Wilmington strain) were used as the antigens for the detection of IgM and IgG antibodies for the

diagnosis of leptospirosis, scrub typhus, and murine typhus respectively. Samples with inconclusive IFA results such as suspected co-infection or low antibody titers were not included in this study.

### Combined ICT for leptospirosis, scrub typhus, and hantavirus infection

Polysaccharide of nonpathogenic *Leptospira patoc*, a chimeric recombinant antigen cr56 and two other recombinant antigens, r21 and kr56, from various serotypes of *O. tsutsugamushi*, and 21kDa genus-specific protein and recombinant CNP protein derived from the Soochong virus of the genus Hantavirus were used as the antigens for the detection of IgM/ IgG for the diagnosis of leptospirosis, scrub typhus, and hantavirus infection respectively.[13-15] The ImmuneMed AFI Rapid® (ImmuneMed, Inc., South Korea) test was performed according to the manufacturer's instructions. In brief, approximately 3 μL of serum was applied to the ICT sample well, and then approximately 7 drops (300 μL) of the sample diluent was added into the sample well immediately. Results of the assay were interpreted visually at 15 minutes, as either negative if only the control band was stained or as positive when both the test and control bands were clearly stained (Fig 1).

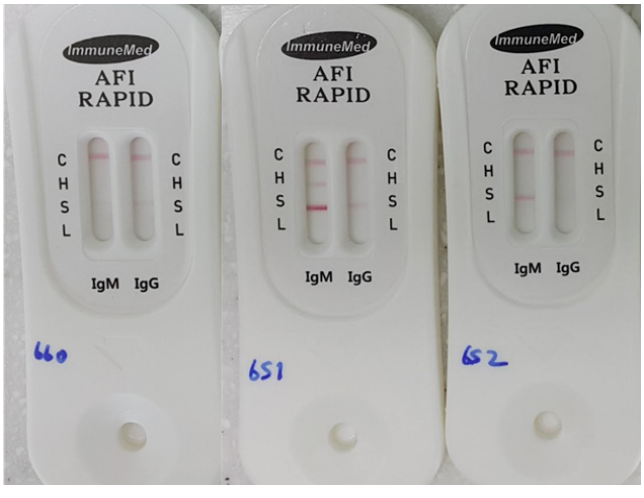


**Fig 1.** Examples of the visually interpretation of results from three patients with negative result (no 660, left panel), positive band for both scrub typhus IgM, IgG and hantavirus IgM (no 651, middle panel), and positive scrub typhus IgM (no 652, right panel). C, H, S, L represented control, hantavirus, scrub typhus, and leptospirosis respectively.

### Data analysis

The diagnostic performance was determined by comparing the IgM and IgG ICT results with the result from the IFA for each patient. The diagnostic criterion for the reference IFA assay for scrub typhus and leptospirosis was either an IgM or IgG IFA assay titer ≥ 1:400 at an acute phase sample or a four-fold increase between paired acute and convalescent phase samples. Inconclusive results were considered negative in the statistical analysis. A two-by-two table was constructed, in which the IFA results, as a gold standard test were cross-tabulated with the ICT assay result to calculate the percentage of true-positive, false-positive, false-negative, and true-negative results. The standard diagnostic accuracy indices of the sensitivity and specificity with 95% confidence intervals (CIs) were calculated, using the SPSS 21.0 software (SPSS Inc., Chicago, IL, USA).

## RESULTS

Overall there were 434 patients included in this study, with the median duration of fever was 4 days (ranged from 3 to 14 days). Of these 193 patients' convalescent samples were available. The median duration between acute and convalescent periods of 10 days (IQR, 6 to 14 days). Among 131 patients with laboratory-confirmed leptospirosis, convalescent samples were available in 91 patients. In acute- phase samples, the sensitivities of the ICT tests for the detection of IgM and IgG antibodies for the diagnosis of leptospirosis were 37.4% (95% CI, 29.1- 45.7%), and 9.2% (95% CI 4.3-14.1%) respectively (Table 1). The specificities of IgM and IgG were 99.0% (95%CI, 97.9- 100%), and 100% (95%CI, 88.2-100%) respectively. False positive IgM/IgG antibody detection was found in only 3 patients (in 2 patients with scrub typhus, and 1 patient with murine typhus) among 303 acute patients. The sensitivities of the test were improved to 84.6% in the convalescent phase, with false positive IgM/IgG antibody detection in 4 patients (3 patients with influenza infection) among 104 convalescent patients.

Among 128 patients with IFA-confirmed scrub typhus, convalescent samples were available in 52 patients. The sensitivities of the ICT tests for the detection of IgM and IgG antibodies against *O. tsutsugamushi* in the acute- phase samples were 68.0% (95% CI, 55.9- 76.1%), and 41.4% (95% CI 32.9- 49.9%) respectively. In acute-phase, the specificities of IgM and IgG antibodies against *O. tsutsugamushi* were 97.4% (95%CI, 95.6-99.2%), and 99.7% (95%CI, 99.1-100%) respectively. False positive IgM/IgG antibody detection was found in 8 patients (in 7 patients with leptospirosis and in 1 patient with *S. aureus* bacteremia) among 306 acute patients. The sensitivities of this ICT test was improved as 84.6% when the test was performed using convalescent samples. In the convalescent samples, false positive IgM/ IgG antibody detection was found in 14 patients, comprising; 12 patients with leptospirosis (3 of them also had a positive IFA for scrub

**TABLE 1.** Sensitivities and specificities of the ICT for the diagnosis of leptospirosis and scrub typhus in acute and convalescent samples.

| | Acute phase samples | | | Convalescent phase samples | | |
|---|---|---|---|---|---|---|
| | IgM | IgG | IgM/IgG | IgM | IgG | IgM/IgG |
| **Leptospirosis** | 49/131 | 12/131 | 50/131 | 77/91 | 43/91 | 77/91 |
| Others | 3/303 | 0/303 | 3/303 | 4/104 | 0/104 | 4/104 |
| Sensitivity, % | 37.4 | 9.2 | 38.2 | 84.6 | 47.3 | 84.6 |
| 95%CI | 29.1-45.7 | 4.3-14.1 | 29.9- 46.5 | 77.1-92 | 37-57.5 | 77.1-92.0 |
| Specificity, % | 99.0 | 100 | 99.0 | 96.2 | 100 | 96.2 |
| 95%CI | 97.9-100 | 88.2-100 | 97.9-100 | 92.5- 99.8 | NA | 92.5- 99.8 |
| **Scrub typhus** | 87/128 | 53/128 | 92/128 | 44/52 | 32/52 | 44/52 |
| Others | 8/306 | 1/306 | 8/306 | 14/143 | 1/143 | 14/143 |
| Sensitivity, % | 68.0 | 41.4 | 71.9 | 84.6 | 61.5 | 84.6 |
| 95%CI | 55.9-76.1 | 32.9-49.9 | 64.1-79.7 | 74.8-94.4 | 48.3-74.7 | 74.8-94.4 |
| Specificity, % | 97.4 | 99.7 | 97.4 | 90.2 | 99.3 | 90.2 |
| 95%CI | 95.6-99.2 | 99.1-100 | 95.6-99.2 | 85.3-95.1 | 97.9-100 | 85.3-95.1 |

typhus), and 2 patients with influenza infection, among 143 convalescent patients. Details of the sensitivities and specificities of IgM/IgG for the diagnosis of leptospirosis and scrub typhus, in the acute and convalescent samples are shown in Table 1.

For hantavirus infection, overall there were 9 patients with a positive IgM test, comprising 2 patients who were positive in both acute and convalescent samples, 4 patients who were negative in the acute phase but positive in the convalescent sample, and 3 patients who were positive in only acute samples. None of the acute and convalescent tested positive for IgG. All patients who had positive IgM for hantavirus infection also had positive IgM for scrub typhus. All the patients were empirically treated with oral doxycycline and became afebrile during follow-up. As an example, one woman among the positive patients to hantavirus but who was negative to dengue IgM/IgG had fever for 5 days with a normal white blood cell count, but increased in atypical lymphocytes, and thrombocytopenia. This patient fully recovered after the treatment with doxycycline. Only an acute- phase sample was available in this patient. Her IFA IgM/ IgG antibody titers against *O. tsutsugamushi* were 1:800 and 1:200, respectively. These positive hantavirus IgM samples were retested by another IFA for the detection of hantavirus infection at the ImmuneMed laboratory, Korea. None of them was confirmed hantavirus infection with the second IFA.

## DISCUSSION

Among patients with non-malaria fever, leptospirosis and scrub typhus were diagnosed in approximately 10% to 30% of them.[1,6,7] The awareness and an early diagnosis of both leptospirosis and scrub typhus has impact on choice of antibiotic treatment during the acute phase of illness. Empirical treatment with oral doxycycline is considered to be the most cost-effective strategy for the initial treatment of patients with clinically suspected leptospirosis or scrub typhus.[16] However, in the absence of rapid and reliable laboratory tests for both diseases, misdiagnosis and delayed appropriate patient management occur frequently. Consequently, there is an urgent need for a more accurate and easy to perform point-of-care leptospirosis and scrub typhus diagnostics.

For the syndromic approach of acute fever, this rapid ICT for the simultaneous detection of IgM/IgG for the diagnosis of leptospirosis, scrub typhus, and hantavirus infection demonstrated comparable sensitivity and specificity to the previously reported individual ICTs for either leptospirosis[17] or scrub typhus.[18]

The results of this study confirmed that in the acute phase of leptospirosis (less than 7 days of illness), serological diagnosis using either IgM/IgG detection is not sensitive. The antibody against *Leptospira spp*. only become detectable in the late acute phase of the disease.[17] Molecular diagnosis by either conventional or real- time polymerase chain reaction is the most common laboratory test for the confirmation of leptospirosis in this early phase. The main use of serological testing is for confirmation of a diagnosis of leptospirosis when the convalescent sample is available. Therefore, an ICT for the detection of IgG/IgM against *Leptospira spp*. alone might not be cost- effective for routine implementation. Thus rapid point- of- care for antigen detection is urgently needed for the early diagnosis of leptospirosis.

The assay demonstrated better sensitivity for the diagnosis of scrub typhus than leptospirosis in the acute phase of infection. *O. tsutsugamushi* re-infection is not uncommon in endemic areas of scrub typhus.[5] The pattern of antibody response to Orientia re-infection mimics that found in those who have had re-infection by dengue virus. Therefore, the detection of both IgM and IgG antibodies at the same time provided higher sensitivity than the assays for IgM or IgG alone.

For the diagnosis of either leptospirosis or scrub typhus, both IgM and IgG could be detected in more than 80% of samples collected after day 6 to day 14 of fever. False- positives may be caused by many factors, including cross-reactivity between antibodies among *O. tsutsugamushi, Leptospira spp.*, or other pathogens, such as influenza, or by the persistence of an antibody following recovery from previous scrub typhus or leptospirosis. For hantavirus infection, only IgM was detectable in 9 patients. However, all of them were diagnosed with scrub typhus by IFA and as all patients recovered after doxycycline treatment, further investigation at the ImmuneMed laboratory, confirmed that all of these samples were not hantavirus infection. Thus cross reaction of hantavirus infection and scrub typhus was the most likely explanation of results found in this study.

We did not calculate the positive and negative predictive values for this ICT because we used the stored samples collected from various hospitals, at different periods of time. As a result, the proportion of samples from patients with leptospirosis, scrub typhus, and other diagnoses did not represent the true prevalence of these diseases among patients with acute fever in Thailand. Overall, it would be more cost effective to implement this ICT for the simultaneous diagnosis of either leptospirosis or scrub typhus (or hantavirus infection) than the application of individual ICTs for these diseases sequentially. Although the determination of the diagnostic performance of a newly developed assay is commonly performed by using well stored samples, prospective clinical studies are needed to determine the more accurate diagnostic performance of the assay as discrepancies between stored samples and prospective studies with the same ICT assays may exist. More studies are also needed to confirm that hantavirus infection is rare in Thailand.

## CONCLUSION

For the syndromic approach to acute fever, this rapid ICT for the simultaneous detection of IgM/IgG for the diagnosis of leptospirosis, scrub typhus, and hantavirus infection, demonstrated comparable sensitivity and specificity to the previously reported individual ICTs for either leptospirosis or scrub typhus in endemic areas. However, we did not detect hantavirus infection in this study, and a rapid test for the diagnosis of leptospirosis, using antigen detection, is still needed.

## ACKNOWLEDGMENTS

The authors thank ImmuneMed Inc, Korea for their kind donation of ICT (ImmuneMed AFI Rapid®) used in this study. The company played no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Funding:** YS was supported by a Siriraj Chalermphrakiat Grant, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript

**Conflict of Interest:** Professor Yoon-Won Kim advised the scientific contribution when ImmuneMed AFI Rapid® has been developed.

**Abbreviations**

HFRS: Hemorrhagic fever with renal syndrome, ICT: Immunochromatographic test, IFA: Indirect immunofluorescent assay

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# Hepatitis E in Southeast Asia

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## ABSTRACT

Hepatitis E is a major cause of acute viral hepatitis in the world. The causative agent of hepatitis E is hepatitis E virus (HEV). In Southeast Asia, the seroprevalence of HEV and the most prevalent genotype of HEV are largely unclear and the available data is either limited or outdated. After a systematic review of literature, we found the seroprevalence of HEV and the most prevalent genotype of HEV appear to vary greatly by countries. The seroprevalence is likely between 17% to 42% and the prevalent genotypes across Southeast Asia are likely 1, 3, and 4, but not 2 as no cases of genotype 2 have been reported in this region. As HEV remains widespread in Southeast Asia and the clinical implications of HEV can be severe, surveillance programs for HEV should be implemented.

**Keywords:** Epidemiology; genotype; seroepidemiologic studies; vaccination; swine (Siriraj Med J 2020; 72: 259-264)

## INTRODUCTION

### Hepatitis E virus

Globally, hepatitis E is a major cause of acute viral hepatitis.[1,2] The causative agent of this liver disease is the hepatitis E virus (HEV).[3] HEV is a non-enveloped, single-stranded, positive-sense RNA virus with similar physical characteristics to the hepatitis A virus.[4,5] Similar to the hepatitis A virus, HEV is transmitted via the oral-fecal route through contaminated water and it can also be transmitted via zoonosis.[6,7] There are five genotypes of HEV known to infect humans, genotypes 1-4 and recently genotype 7 however, for those five genotypes, there is only one serotype.[8,9] HEV appears to have genotype-specific complications. Infection by genotype 1 and 2 can result in severe complications such as death and stillbirth while infection by genotype 3 typically results in mild to non-existent complications in immunocompetent individuals.[10-14]

Genotypes 1 and 2 have only been reported in humans while genotypes 3 and 4 are zoonotic and are known to be carried by swine and other animals depending on the regions. Transmission of HEV from infected swine can be due to direct contact with the swine, consumption of undercooked swine meat, or exposure to swine feces. Exposure to the swine feces can be either directly through contact or indirectly through contaminated water or contaminated shellfish.[6,7,15] Although swine are the most commonly reported source of zoonotic transmission, deer and other animal species have been reported to transmit genotype 3 to humans as well.[16]

### Review methodology

We conducted a systematic search on PubMed and Google Scholars for research articles with the main keywords "hepatitis E," "genotype," and "seroprevalence." These keywords were supplemented with the "Southeast Asia,"

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"Brunei," "Burma," "Myanmar," "Cambodia," "Timor-Leste," "Indonesia," "Laos," "Malaysia," "Philippines," "Singapore," "Thailand," and "Vietnam." Non-English research articles without English translations were not reviewed. All relevant research articles found in the search were reviewed. Recent publications were favored; however, publication dates were not used as an exclusion factor.

**Epidemiology**

In Southeast Asia, previous studies have reported genotype 1 and 4 to be prevalent in humans.[17,18] However, recent evidence found conflicting results suggesting that those previous studies may be outdated.[19-24] The prevalent genotype of HEV is largely unclear and varies greatly by countries.

In Thailand, multiple studies have found that the local swine carry HEV genotype 3, exclusively.[25-28] And in humans, genotype 3 have also been found.[19-24] The seroprevalence of HEV, defined in this paper as the presence of anti-HEV immunoglobin G, was found to be 14% nationally in 2007-2008, but the seroprevalence was not homogenous, varying greatly within the country.[29] The study found lower HEV seroprevalence in Muslim dominated regions where pork consumption is relatively scarce.[29,30] A decade later, a recently published study found that the seroprevalence of HEV to be much higher, 29.7% in the general Thai population.[31] This study also found lower HEV seroprevalence in Muslim dominated region.[31] Recent reports, including a recently published national study, have only found genotype 3 in Thailand.[20,21] Because of these consistent reports, the prevalent HEV genotype that is circulating in Thailand is almost certainly genotype.[3]

In Cambodia, studies have reported various genotypes circulating in the country. In humans, genotypes 3 and 4 have been found.[22,32] And swine have been found to carry HEV genotype 1, 3, and 4.[32,33] Genotype 3 has also been found in the river water.[34] In a study in 2015, the seroprevalence of HEV was found to be 18.4% in the general population.[22]

In Laos, the swine population has been found to carry genotype 4.[35-37] However, there are currently no genotyping studies of HEV in humans in Laos. The seroprevalence in humans may be 17% based on controls used in a study by Bounlu et al.[38]

In Malaysia, a 1999 study reported the seroprevalence of HEV to be only 2% in the general population, strangely low.[39] Another study around the same time found the seroprevalence to be 10% in a population of patients infected by the human immunodeficiency virus.[40] This suggests that seroprevalence of HEV used to be very low, however, these studies are outdated. Genotype information for both humans and swine have not been reported in the literature.

In Myanmar (previous known as Burma), the predominant genotype appears to be genotype 1. A few studies have reported finding genotype 1 in humans.[4,41] The statuses of other genotypes are unknown and swine genotypes have not been studied. A study in 2001 found the seroprevalence of HEV in Myanmar to be 31.5% in humans and 24% in swine.[42]

In Vietnam, studies have reported genotype 4 in humans.[43,44] Genotype 1 has been suspected of being prevalent in Vietnam due to waterborne outbreaks of HEV, however, genotypic analysis to explicitly confirm the presence of genotype 1 HEV does not exist.[45] Animal reservoirs for HEV in Vietnam have not been reported in the literature. This seroprevalence is unknown in the whole country, but in the capital city of Ho Chi Minh City, it is very high at 42%.[46]

In Indonesia, genotype 4 has been found in humans.[47] Genotype 4 has also been reported in swine.[47,48] A study in 2005 of 2,450 pregnant women found that the seroprevalence of HEV was 18% in this population nationally, and that in Muslim dominated areas, where pork consumption is relatively scarce, the seroprevalence was much lower at 2%.[49] There have been no reports of genotype 1, 2, or 3 in Indonesia suggesting that the prevalent genotype may be genotype 4, likely due to zoonotic transmission from swine.

In Singapore, studies on the seroprevalence or genotype of HEV do not exist based on our search criteria. However, the incidence rate of cases is very low at 0.92 per 100,000 people between 2009-2011.[50] Such a low incidence rate is likely related to the fact that Singapore is the most developed country in Southeast Asia, which typically comes with good hygiene and well-funded public health programs.

In the Philippines, a recent study found that the swine carry genotype 3 exclusively.[51] In the river water, HEV RNA was detected and samples were all found to be genotype 3.[23] However, we could not find any seroprevalence or genotype reports on HEV in humans.

There were no or very limited HEV epidemiology data from Brunei and Timor-Leste. However, in Timor-Leste, HEV is likely still endemic.[52]

The seroprevalence of HEV in humans is still unknown in most areas of Southeast Asian countries. The seroprevalence in the region may be between 17% in Laos to 42% in Ho Chi Minh City, Vietnam (Figs 1, 2, and 3).


**Fig 1.** Map of hepatitis E virus genotype distribution found in humans in Southeast Asia.


**Fig 2.** Map of hepatitis E virus genotype distribution found in swine in Southeast Asia.

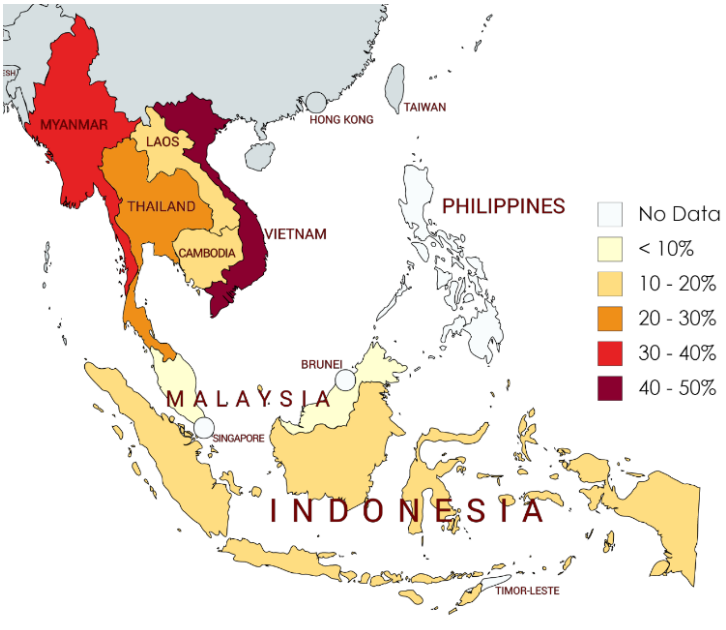


**Fig 3.** Seroprevalence of hepatitis E virus in humans in Southeast Asia.

### Complications and consequences

In the general population, the complications of HEV are typically moderate such as fatigue, vomiting, and jaundice.[53] However, in certain groups, the complications can be severe. In people with chronic liver diseases, acute-on-chronic liver failure, a serious condition with high short-term mortality, can occur as a result of genotype 1 HEV infection.[11] In pregnant women, high morbidity and mortality for both the mother and the fetus have been reported. A study in 2007 on pregnant women in India reported a mortality rate of 41% of those infected with HEV compared to 7% of those non-infected.[10] That study also reported 54% of births from women infected with HEV were stillbirths compared to 1% in non-infected women.[10] Hepatitis E is generally an acute disease, however, in populations with a compromised immune system such as organ transplant recipients or HIV populations, the disease can become chronic.[54-56] In Southeast Asia, studies on the effects of HEV on these vulnerable groups are lacking. A study by Rein et al. estimated the burden of HEV in one year, 2005, in Southeast Asia to be 1,984,235 incident infections, 357,086 symptomatic cases, 7,347 deaths, and 148 stillbirths.[57] Rein et al., however, only explored genotypes 1 and 2 of HEV, thus, their report of burden are likely underestimates.[57]

### Diagnosis

Clinically, hepatitis E is practically indistinguishable from infection from other hepatitis virus such as hepatitis B and C. Thus, confident diagnosis of hepatitis E requires serological tests. Antibody assays for anti-HEV immunoglobin M and anti-HEV immunoglobin G and nucleic acid assays for HEV RNA in blood are commonly used in hepatitis E diagnosis.[58] Ideally, all three tests are done for a comprehensive evaluation, however, resources in many Southeast Asian countries may be too scarce to permit this. The reverse transcriptase polymerase chain reaction for HEV RNA is the most resource intensive, but it is a direct method for detecting HEV and can provide information on whether a patient has a current infection. HEV RNA can be detected in feces as well, but this method is not commonly used. Antibody assays for anti-HEV immunoglobin M and anti-HEV immunoglobin G, such as the Wantai assays by Beijing Wantai Pharmacy Enterprise Co., Ltd. (Beijing, China), are relatively inexpensive, simple to use, and both high in sensitivity and specificity.[59,60] Anti-HEV immunoglobin M and anti-HEV immunoglobin G are indirect methods for detecting HEV. The presence of anti-HEV immunoglobin M indicates either current or recent infection of up to approximately 5 months prior.[61,62] The presence of anti-HEV immunoglobin G indicates a distant past infection of up to 14 years prior in some reports.[63] One of the facilities able to test for HEV, both the antibody and the RNA, is the Armed Forces Research Institute of Medical Sciences (AFRIMS) which have centers in many Southeast Asian countries, especially Thailand.

Recently, new assays were developed by Pisanic et al. for detecting anti-HEV immunoglobin A (for current or recent infection) and anti-HEV immunoglobin G in saliva. The study showed high sensitivity and specificity for anti-HEV immunoglobin G and low sensitivity but high specificity for anti-HEV immunoglobin A.[64] Because these assays are non-invasive and also inexpensive and rapid like the Wantai assays, they have the potential to be useful for HEV screening.

### Vaccination

Since 2012, there exists an approved recombinant HEV vaccine called HEV 239 or Hecolin developed by Xiamen Innovax Biotech in China.[65] However, it is currently only approved for use in China. The vaccine was designed to protect against HEV genotype 1; however, a phase 3 clinical trial demonstrated its effectiveness in protecting against genotype 4 as well.[65] The study reported an efficacy of 100% against HEV genotypes 1 and 4 over a 12-month period.[65] However, there is no data on Hecolin's effectiveness in protecting against genotypes 2 and 3. The vaccine has been shown to be effective for at least 4.5 years for 87% of healthy adults.[66] Because the burden of HEV across Southeast Asia is unclear, the need to evaluate Hecolin for domestic use is also unclear.

## CONCLUSION

HEV genotype 1, which has been typically associated with South Asian countries, does not appear to be prevalent in Southeast Asia based on genotyping studies. All genotypes except for genotype 2 have been reported in Southeast Asia. However, this data and the data on seroprevalences were not ideal as the studies were often outdated or had limited populations of study. Large, national studies or surveillance programs are few. As the seroprevalence of HEV remains high throughout Southeast Asia and the impact of HEV could be significant, surveillance programs for HEV should be implemented.

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# Immersive Technology for Medical Education: Technology Enhance Immersive Learning Experiences

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## ABSTRACT

Immersive Technology was a trend of the interesting technology in presentation, demonstration, imitation. These techniques could be used in medical education. This article would present immersive technology knowledge which consisted of Augmented Reality (AR), Virtual Reality (VR) and Mixed Reality (MR). Immersive technology could also be applied to medical education in 5 parts such as Treatment, Education, Rehabilitation, Training, Surgery, and equipment for medical education media development.

**Keywords:** Immersive technology; virtual reality; augmented reality; mixed reality; immersive learning experience (Siriraj Med J 2020; 72: 265-271)

## INTRODUCTION

The rapid development of technology takes part in the daily life and technology for changes, opportunities, a new relationship (This article will tell about immersive technology.), the development of assessment, connection, imitation of 3-dimensional graphics to classroom to promote the virtual learning by using many instruments. Applying the virtual technology to education urges learners to analyze, solve problems and enhance skills especially for; Immersive Technology for Medical Education. This technology can increase efficiency in learning such as anatomy which is difficult and complicated. Learners can understand and decide effectively which can decrease the mistakes and boost proficiency in learning. Immersive technology for medical education is an application of wireless network in classroom or laboratory. Learners can gain experience, urge their attention in virtual condition. This technology is also the creative design for the development of medical education.

### Meaning of immersive technology

Immersive Technology means experiences and reactions of Virtual Reality (VR). This technology is an important approach that takes parts in the publication of knowledge in science for nonscientists and motivates students to the laboratory. It can change the processes of working in the laboratory and urge development in science.[1] Impression in technology (immersive technology), this technology can apply technology to 3-dimensional different data such as Virtual Reality (VR) and Augmented Reality (AR) for providing 3-dimensional conditions on the computer. Learners can understand the immersive

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*http://dx.doi.org/10.33192/Smj.2020.36*

objects in the real world.[2] Impression in the attention of technology in real-world and virtual conditions.[3] Impression of technology consists of Virtual Reality (VR), Augmented Reality (AR) and Mixed Reality (MR). Learners can apply this technology to react and control. The continuity of immersive and physical condition depends on the levels of immersive condition. Learners can learn through virtual digital content to practice risky situations in the classroom and laboratory under safe conditions.[4] Instruments for immersive technology can train basic knowledge automatically without pressures from patients.[5]

In conclusion, immersive technology means imitation the virtual instruments in a real situation that can show on the computer. This technology applies Virtual Reality (VR), Augmented Reality (AR) to Mixed Reality (MR). It can create an impressive experience to urge attention which can react and control this immersive situation.

**Immersive technology**

Immersive technology is a technology in the virtual world that is quite similar to the real world. Immersive Technology cover to AR, VR and Mixed Reality (MR).

**Kinds of immersive technology**

**Augmented Reality (AR)**

Augmented Reality is a form of Virtual Environment (VE) in the synthesized condition. Augmented Reality (AR) is the increase of realistic things more than virtual reality. AR will imitate the virtual objects then overlap them to the real objects which are in the same area.[6] The activation of technology in real-world and immersive world purposes to create the perfect new condition from computer.[7] The view of physical condition and the real world can be edited by adding more information about the reality of each person from the computer. This information may be applied to create experience for each person in real condition.[8] It is the modern technology that has various displays such as message, number, alphabets, symbol or graphic as well as the view of users in the real world. The augmented reality has the real-time relationship to the immersive condition.[9]

As a result, In AR objects are enhanced in the interactive experience compared to in standard virtual reality.

**Virtual Reality (VR)**

Virtual Reality is a condition that is created by software then present to users. This kind of reality will imitate the steps of operation in real-time processes. Virtual Reality (VR) can be used to demonstrate the steps of operation by using the computer to train, show the models of anatomy and many processes of operation from stereo laparoscopy. The forms of Virtual Reality – Internet of Thing (VR-IoT) will increase in the future because they can apply the capacities in the real world.[10] Virtual reality can be created by 3-dimensional computers. It consists of reflection of real-world to make objects to be similar to real objects and it also improves processes then applies to the teaching materials, learning as well as medical training.[11] Virtual Reality is a model in the synthesized condition to create a real-time virtual world by using computer graphics. This system can react in many ports in censor perception then react to users in gestures and commands. The important factor of virtual reality is to record the data of users then create the virtual world immediately. It is the reacted graphic connection.[12]

**Mixed Reality (MR)**

Mix reality is the combination of elements and perception between the physical and digital worlds.[13] This reality will be impressive when it combines Augmented Reality (AR) and Virtual Reality (VR). AR is the combination of synthesis and realism then blends the physical world to data in the computer. Users can know, react to the condition. Virtual Reality (VR) is the immersive reality from the computer which may not related to the real world.[14] The combination of reality will combine the Augmented Reality (AR) and Virtual Reality (VR) in application which can be edited in the real world. There are many ways of combination between Augmented Reality (AR) and Virtual Reality (VR) in an application.[15] Mix Reality (MR) is the combination of 2 kinds of technology: Augmented Reality (AR) can add more information to the experience in real-world and Virtual Reality (VR) will imitate the situation from computer to urge users to experience.[16]

In conclusion, Mixed Reality (MR) combines 2 realities such as Augmented Reality (AR) and Virtual Reality (VR). It imitates the condition which is overlaps by synthesized objects in the same area.

**Elements of Immersive Technology**

Immersive technology consists of these elements. Immersion is an impressive experience. Users will wear a headphone kit which is designed to protect the light outside the room. Stereoscopic vision will imitate 3D vision. Motion capture, this feature will capture the users' motion accurately.[1] The virtual world will imitate the area by using the computer to be similar to the situation in the real-world then imitate the physical and

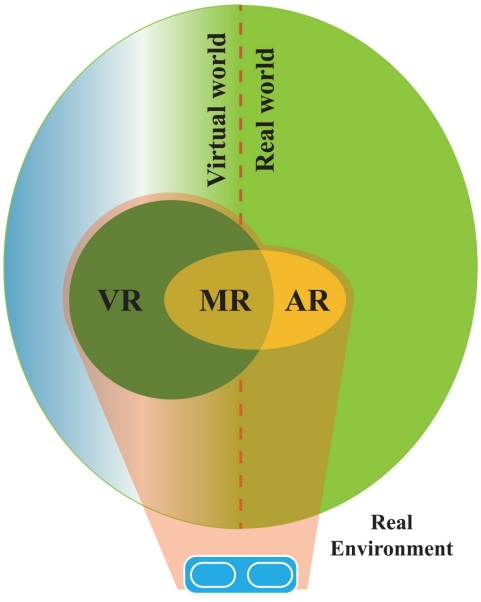


**Fig 1.** Simplified representation of the Augmented Reality (AR), Virtual Reality (VR) and Mixed Reality (MR)
**By:** Mathuwan Srikong

mental condition of being in the virtual world. Sensory feedback, this feature will give feedback according to physical positions. Interactivity will work when the system reacts to users.[17] This part will work when the system connects Head Mounted Display (HMD), Headphones for sound to the system for headphones and decreasing noise. Applying immersive technology in 3D condition will experience users to impress in the virtual world.[18] Elements of software will be different in the virtual environment and the real environment.[19]

## Immersive Learning Experience

Technology in education began to focus on the Augmented reality (AR) and Virtual Reality (VR) because of the ability to support immersive learning teamwork and teaching through treatment to interact with the computer. It is used in various types of training programs and is especially useful for the participation and motivation of learners. It is an effective technology to support learning. To enhance the learning experience can be applied to medicine from a study of Immersive Learning Experiences: Technology-Enhanced Instruction. Users report increased engagement with informal learning environments.[20] In the study of immersive learning for enhanced organic chemistry instruction, the system design and development with immersive and immersive virtual reality technology for teaching organic chemistry in the environment encourage participation, motivation and high interest within an immersive environment, users are disengaged from physical disturbances and systems and can, therefore, be useful for resolving important problems of student participation.[21] In the study of caregiving between careers and the development of communication abilities in the healthcare professional curriculum through realistic, realistic experiences participants have learned positively, feel their status and show empathy through immersive experiences. Understand technology and want to learn in an environment that is updated with technology. There is an increased awareness of learning through virtual reality.[22] Therefore, Immersive technology learning is the application of technology to create immersive learning experiences for future studies.

## Applying Immersive Technology to Medical Science

This part will tell about the latest development and challenges in the future of Mix Reality (MR) which is interesting to medical science development. There are 5 forms of applying immersive technology to medical science[23] as following;

## Treatment

The advantage in medical science: Educational virtual reality videos in improving bowel preparation quality and satisfaction of outpatients undergoing colonoscopy: protocol of a randomized controlled trial. The protocol of research was randomized experiment. The result found that virtual reality video could imitate the experience of intestine preparation improvement to patients. Virtual Reality (VR) could enhance their concentration and result. Moreover, it reduced the patients' anxiety before operation.[24] but its efficacy is closely related to the quality of bowel preparation. Poor patient compliance is a major risk factor for inadequate bowel preparation likely due to poor patient education. Such an education is usually provided via either oral or written instructions by clinicians. However, multiple education methods, such as smartphone applications, have been proved useful in aiding patients through bowel preparation. Also, it was reported that a large proportion of patients feel anxious before colonoscopy. Virtual reality (VR Mix reality (MR) usually applied to the operating system. However, there were some complicated medical instruments. This limitation could decrease the proficiency and it is not suitable for the operation in real situations. Doctors identified the position of wounds from virtual reality which was the combination of the operation by virtual images from recording data and operation in real situations. This approach analyzed the position of wounds before operation. It reduced the risk and improved the security in operation.[25]

**Education**

The advantage in medical education: Applying the virtual reality in medical education. This project was successful in applying the invention for physical therapists and nursing curriculum. The result found that Virtual Reality (VR) was a part that could help students to understand some symptoms related to age and it could increase the attention to elderly persons in vision and Alzheimer's disease.[26]

**Rehabilitation**

The advantage in rehabilitation for medical science: Connection in medical science to Virtual Reality (VR) and Augmented Reality (AR). This work was a combination of Augmented Reality (AR) and Virtual Reality (VR). Specialists used 2D and 3D data for analyzing the operation and communicating between specialists and patients. Case study: Planning for dental implantation by using Augmented Reality (AR) in mobile phones and tablets for dental students. The processes was shown in a display above the patient's head. Endoscopy operation could combine the surgeries' hands to show the positions in anatomy and Augmented Reality (AR) could restore patients in therapy by physical therapists. Patients could give comments to this system.[27] Designing the virtual system to enhance the emotional skill for autism spectrum disorder children purposed to check whether their faces were appropriate to each situation. These children would be evaluated skills which they practiced in Virtual Reality (VR) to the real world. The result found that skills which they practiced in Virtual Reality (VR) could transfer to the real world and their progression was developed when they did not use Virtual Reality (VR).[19]

**Training**

Study of effect of cataract operation to the errors after cataract operation by the first- and second-year trainees. This research studied the error in the capsule after lens during operation which might cause complications; for example, Retinal detachment during operation which caused the rate of vision for patients decreased. The result found that virtual reality in ophthalmology was efficient to the patients who were done cataract operation. The rate of complication after cataract operation decreased by 38%.[28] Application of virtual reality in clinical medical science in training some approaches to manage the patients' painfulness and mind symptoms. Virtual Reality (VR) could increase the patients' obsession which was similar to the virtual hypnosis to make patients endure the painfulness.[18] The efficiency of virtual reality training in orthopedic surgery approved that Virtual Reality (VR) was an instrument for the development of orthopedic surgery techniques.[29]

**Surgery**

Doctors could use Augmented Reality (AR) as the instruments for training the surgery which showed the patients' view. It was beneficial to medical science in the operation room. They could check some qualifications by their bared eyes which could not look in MRI or CT scanning. On the other hand, doctors could use Augmented Reality (AR) to access data.[6] The trend of applying virtual reality and imitating situation was to use in the operation in the virtual situation from the computer then transferred it to patients by using Virtual Reality (VR). Every situation was created by calculating and Augmented Reality (AR) to overlap the virtual objects from the computer to give alternative views of operation. It enhanced the efficiency of the operation.[30]

In conclusion, applying immersive technology to medical science had 5 categories: treatment, education, rehabilitation, training, and surgery. This technology could create the virtual situation in different contexts.

**Instruments for developing immersive in medical science**

At this time, the development of software in medical science recognized to the combination of medical photographing to a more realistic image. 3D structured objects are important to solving clinical problems. Complex imitations can be create to show how 3D objects are in a given situation. In processing, doctors need to have knowledge of anatomy and physiology as well as relational and physical qualifications. The 3D graphic is the main element in the accuracy of imitating a 3D image. The important things for medical science focus on reality and real-time reaction. Software package for these calculations is ANSYS software kit (Ansys Inc., Canonsburg, PA, United States). Creating 3D models are done by SOLIDWORKS software (Dassault Systems, Concord, MA., United States) and creating mechanical objects and imitation (Materialize NV, Leuven, Belgium) for anatomy virtual model.[31]

The most famous instruments for developers for creating applications are Unity 3D and Unreal engine. They can create the immersive model like Virtual Reality (VR) and Augmented Reality (AR) as following; Unity 3D (Unity Technologies, San Francisco, CA, United States) The platform which is flexible for calculating 3D graphic is Unity. This application has various instruments for editing and showing examples in real-time. It is available ON Windows Mac and Linux for a virtual experience. This application can use script to develop

content which can reach each other.[32] Unreal Engine (Epic Games, Cary, NC, United States) is a well-known graphic application. It is not complicated. Moreover, this application is available for free which is a good thing for 3D graphic development. Basis of this application can apply to solve the complicated reasoning, presenting anatomy data in 2D and 3D to help the users to understand the overall condition while the real-time 3D graphic is appropriate to the anatomy.

Planning, pre-analysis, advising on the operation and after the operation, caring can be seen by 3D microscope in Augmented Reality (AR) and Virtual Reality (VR) to encourage the surgeons who purpose to the operation and anatomy by applying optical navigating system or electrical magnet to follow the real-time operation as following; Dextoscope (Braco): processing about planning the operation and applying AR in 3D objects and it can react to users. The database will be registered automatically for creating images. The surface will be divided to categorize the structure to determine the relationship in anatomy. Immersive control is easy to use. It can adjust to display each object to get the best images. The system uses controller and stylus which overlap keyboard and mouse which cause the reaction and management of images to work more accurate. Interface of image shows the relationship of complicated anatomy. Dextoscope can create AR stereoscopic in the 3D world by overlapping the 3D analysis image to the correct position of skin and bones and it can also find the position of diseases. HoloSurgical (Chicago, Illinois, USA): The navigation system for AR and AI operation. This application can correct the limitations about the navigation system for operation and AI system. ARAI uses AR-state-of-the-art to overlap the 3D image in hologram type for the patients' anatomy. Doctors can understand the 3D anatomy structures and complete patients' anatomy structures. This system decreases the complexity of operation. The navigation system HoloSurgical ARAI will scan before operating with AI and its algorithm of this application to know the anatomy structure. VPI Reveal is the 3D creating kit for medical science data. VPI presents the real-time volume analysis for CT and MR data in a 3D monitor. This application is also available on desktop platforms and mobile phone which is easy to use. VPI Reveal can work on the different processes for 3D scanning by using any input which doctors use such as the keyboard and touchscreen mouse. The navigation system and processes in the 3D monitors are supported by various display systems then present the patients' hologram to scan the data and model. Synaptive Medical (Synaptive Medical Inc., Toronto, Ontario, Canada) is a platform for applying to neurosurgery. Neurosurgeons can understand clearly when they look at the images and find the possible ways for neurosurgery. This platform has many ways to work according to the hand-free combination of accurate MR images and it shows the real-time structures. It also has a function that solves the complex reasoning for surgeons by using 3D images with automatic evaluation and 3D graphic rendering. As a result, they can plan before the operation.[33]

**Instruments that apply immersive technology in medical science for medical practitioners**

Augmented Reality is the technology which users can interact between virtual objects and real life. The applications can provide real experience and virtual objects to users such as Google Glass, Microsoft HoloLens because these objects can overlap to the immersive world. Augmented Reality (AR) is different from Virtual Reality (VR) which facilitates users to experience a virtual experience such as Oculus Rift, HTC Vive. The instruments for Augmented Reality (AR) and Virtual Reality (VR) have the monitors which have headphones. Headphones can interact between users and the computer. Microsoft HoloLens is the headphone kit which will overlap virtual objects to the real condition of users to have various experiences. According to the research "Study of Effect for immersive technology by using Microsoft HoloLens Anatomical Pathology", specialists in this area investigated by wearing HoloLens kit and command them in the far distance. This program also provided a real-time diagram, description and voice-command. 3D primary sample in pathology could be viewed in hologram. Users could access to the pathologist for asking advice in their interesting area such as advice during operation. This advice could apply to improve the searching in pathology and the slides navigation would evaluate in pathology.[34] Another research was "Study of Result in anatomy in learning to the achievement of the medical practitioners in the topic: neuroanatomy". This research collected virtual objects and approved users to interact by using mobile phones. Learning via mobile phones could facilitate students to learn and they could also access media in every time they wanted to access.[35]

**Instruments that apply immersive technology in medical science for patients**

The instrument will present the virtual model of the laboratory which is developed for patients. Patients will be operated in angiogram which will use the photographing system to investigate patients' blood heart vessels. They can know about the processes of operation in virtual

condition, so their fear is decreased. The patients can interact with the virtual object via the wireless controller. Doctors introduce patients by using the recorded script. When patients follow the processes, they will describe some side effects to patients. After that, doctors will ask patients to put their hand up. Interaction part: This part will facilitate patients to respond easier by using the button "trigger" to interact. It is their natural movement to grab and move objects according to the processes by doctors. Patients will focus on processes which happen in the virtual world instead of focusing on the real world. Design part: Patients will in the virtual condition to see what they want to interact with. It needs a limited area. Technique part: The Imitated situation in the laboratory is developed by Unity game engine, C# programming, and Autodesk Maya. They can produce a 3D model in medical science and there is some equipment to create a soundtrack.[36]

**Example of applying immersive technology to medical science in ophthalmology**

Applying immersive technology to medical science in ophthalmology, virtual reality (VR) instruments in retinal surgery and cataract surgery proceeds by using the 3D microscope in operation. During the imitation process of the microscope, instruments will be controlled by a pedal with a virtual interface platform that imitates retinal surgery and cataract surgery. It starts from basic surgery to complicated surgery until solving side effects. Doctors can evaluate, analyze the results of students' skills according to many indicators then summarize to scores and announce to them to show their achievement. These results can improve the skills according to students' difference.

## CONCLUSION

Virtual technology is a new strategy in medical science. This technology will imitate situations that rarely happen in a clinical experiment. It is a complicated skill which needs to practice a lot of time until users will be expert and solve problems according to medical ethics. Doctors will be more confident in operation. By the way, rapid development of hardware, software and programming skill for immersive media can facilitate doctors to work. Patients can respond in real-time method and doctors can apply these responses to the studying in each branch in medical science.

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