







ARTICLE

Comparison of surgical outcomes of posterior surgeries between cervical spondylotic myelopathy and ossification of the posterior longitudinal ligament

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STUDY DESIGN: Retrospective multicenter study.

OBJECTIVE: To compare the surgical outcomes and complications of posterior decompression between individuals with cervical spondylotic myelopathy (CSM) and those with ossification of the posterior longitudinal ligament (OPLL).

SETTING: Seventeen medical institutions in Japan.

METHODS: This study included 814 individuals with CSM ($n = 636$) and OPLL ($n = 178$) who underwent posterior decompression. Propensity score matching of the baseline characteristics was performed to compare surgical outcomes and perioperative complications between the CSM and OPLL groups.

RESULTS: Before propensity score matching, the OPLL group had higher percentage of male individuals, body mass index, and number of stenosis levels and longer duration of symptoms ($P < 0.01$, $P < 0.01$, $P < 0.01$, and $P < 0.01$, respectively). After matching, the baseline characteristics were comparable between the CSM ($n = 98$) and OPLL ($n = 98$) groups. The postoperative Japanese Orthopaedic Association (JOA) scores, preoperative-to-postoperative changes in the JOA scores, and JOA score recovery rates were not significantly different between the groups ($P = 0.42$, $P = 0.47$, and $P = 0.09$, respectively). The postoperative visual analog scale (VAS) score for neck pain and preoperative-to-postoperative changes in the VAS score for neck pain were not significantly different between the groups ($P = 0.25$ and $P = 0.50$, respectively). The incidence of perioperative complications was comparable between groups.

CONCLUSION: Neurological improvement and complication rates after surgery were comparable between individuals with CSM and those with OPLL, suggesting similar effectiveness and safety of posterior decompression for both conditions.

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INTRODUCTION

Degenerative cervical myelopathy (DCM) causes spinal cord dysfunction in the elderly, and it covers a variety of spinal cord compressive diseases such as cervical spondylotic myelopathy (CSM), ossification of the posterior longitudinal ligament (OPLL), and disc herniation [1]. Individuals with minimal symptoms can be treated conservatively; however, those with progressive myelopathy require surgery. Surgical decompression with or without fixation is widely accepted as an effective treatment option for DCM, as it halts the progression of symptoms and improves neurological outcomes and quality of life [2].

CSM is a degenerative disease caused by spondylotic changes of the cervical spine, including disc degeneration, osteophytes,

hyperostosis of the uncovertebral joint, facet hypertrophy, and ligamentum flavum buckling, resulting in circumferential stenosis of the spinal canal [3]. Meanwhile, OPLL is a multifactorial disorder most commonly observed in Asian countries and is caused by ectopic ossification and hyperostosis of the posterior longitudinal ligament [4–6]. Cervical stenosis in individuals with OPLL is mainly induced by anterior factors. Although CSM and OPLL have different underlying mechanisms of disease, posterior cervical decompression is commonly used as a surgical treatment for both diseases. A previous prospective study revealed comparable improvements in neurological function between OPLL and other forms of DCM after miscellaneous surgical techniques, including anterior and/or posterior approach with or without fusion [7].

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In their study, Nakashima et al. reported other forms of DCM, such as heterogenous diseases including myelopathy secondary to spondylosis, disc herniation, subluxation, and hypertrophied ligamentum flavum [7]. However, the authors compared surgical outcomes between OPLL and other forms of DCM after miscellaneous surgical techniques [7], and thus, the neurological outcomes after a single surgical procedure may differ between OPLL and CSM. Although a few studies have compared the surgical outcomes between CSM and OPLL after posterior cervical decompression, their results vary [8–10]. Two studies reported comparable outcomes in both CSM and OPLL individuals [8, 9], but another study showed worse neurological recovery in individuals with OPLL [10]. These inconsistent surgical outcomes may be due to the small sample size of those previous studies. To the best of our knowledge, there are no big-data studies comparing the surgical outcomes between CSM and OPLL after posterior cervical decompression. The present multicenter large cohort study investigated if the difference in the underlying mechanisms of spinal cord compression between CSM and OPLL has an effect on their surgical outcomes. Therefore, propensity score matching was performed to adjust for baseline characteristics. Subsequently, neurological outcomes and perioperative complications after posterior cervical decompression were compared between individuals with CSM and those with OPLL.

MATERIALS AND METHODS

Subjects

A total of 864 consecutive individuals with clinically and radiographically confirmed DCM who underwent posterior decompression in 17 medical institutes between January 2012 and December 2014 were included in our retrospective database. In the present study, 814 individuals with available data on the number of intervertebral levels of stenosis were included. The participant follow-up period was a minimum of 12 months after surgery. The inclusion criteria were DCM with at least 1 clinical sign of myelopathy, radiologic evidence of spinal cord compression on magnetic resonance imaging or cervical myelogram-computed tomography (CT), and no previous history of cervical spine surgery. The exclusion criteria were individuals with radiculopathy but without myelopathy and those who were diagnosed with infection, trauma, rheumatoid arthritis, ankylosing spondylitis, spinal tumors, or concomitant lumbar stenosis. The attending spine surgeon at each institute divided the individuals into two groups: individuals with radiographic evidence of OPLL ($n = 178$) and those with CSM ($n = 636$). We defined OPLL as ossification of the posterior longitudinal ligament with a thickness >2 mm on CT according to previous studies [11, 12]. Osteophytes near the uncovertebral joint or at the corners of the vertebra were not diagnosed as OPLL.

Indication and techniques of surgical decompression

Attending spine surgeons at each institute performed three different surgical techniques of posterior cervical decompression (expansive open-door laminoplasty [ELAP] [13], double-door laminoplasty [DD] [14], and muscle-preserving selective laminectomy [SL] [15, 16]). Surgical indication, technique, and number of intervertebral levels decompressed were decided by spine surgeons at each institute.

Data collection

The attending spine surgeons retrospectively collected the participants' demographic data (age, sex, duration of symptoms, follow-up period, body mass index [BMI], smoking history, medical history, and the Charlson comorbidity index). Surgical details (ELAP, DD, or SL), operation time, blood loss, number of intervertebral levels of stenosis, number of intervertebral levels decompressed, and duration of symptoms were provided by the attending spine surgeon. Subjects were assessed at the preoperative stage and final follow-up using clinical outcome measures, including the Japanese Orthopaedic Association (JOA) score system for cervical myelopathy and the visual analog scale (VAS) score for neck pain. Hirabayashi's method was used to calculate the recovery rate (RR) of the JOA score: $(\text{postoperative JOA scores} - \text{preoperative JOA scores}) / (17 - \text{preoperative JOA scores}) \times 100\%$ [13]. The attending spine surgeon monitored perioperative complications within 30 days after surgery (i.e., C5

palsy, surgical site infection [SSI], hematoma, dural tear, delirium, and neurological deterioration). On a preoperative neutral lateral radiograph, the K-line was defined as the straight line joining the midpoint of the C2 spinal canal to the midpoint of the C7 spinal canal [17]. K-line was defined as (+) when the OPLL mass never crossed the K-line posteriorly, whereas K-line was defined as (–) when the OPLL mass crossed the K-line posteriorly. Using the midsagittal image of the cervical CT, we defined the OPLL occupancy ratio as the anterior-to-posterior ratio of the OPLL diameter on its thickest part to the diameter of the spinal canal [4].

Statistical analysis

Participants' characteristics and perioperative and postoperative data were compared between the CSM and OPLL groups using unpaired *t*-test or Mann–Whitney U test for continuous variables and chi-square test for categorical variables, as appropriate for data distribution. Propensity score matching was performed to compare the surgical outcomes, perioperative complications, and radiological parameters between the groups. A logistic regression model was used to calculate the propensity scores. The moderator variables were age at surgery, sex, BMI, smoking history, diabetes mellitus, the Charlson comorbidity index, duration of symptoms, number of intervertebral levels of stenosis, preoperative JOA scores, and preoperative VAS score for neck pain. To adjust for individual characteristics, we performed 1-to-1 matching with fixed caliper widths (0.03) without replacement. Accordingly, a case in the CSM group was matched to a case in the OPLL group with the same propensity score. Pairs comprising 1 participant with CSM and 1 participant with OPLL were collected to form two groups (the CSM and OPLL groups). The standardized difference was used to measure the covariate balance. A standardized difference of $<10\%$ was regarded as an ignorable difference between the groups. A paired *t*-test was used to compare the JOA scores and VAS scores for neck pain between the preoperative and postoperative stages. All statistical analyses were performed with SPSS version 26.0 (IBM Corp., Armonk, NY). Continuous variables are presented as mean \pm standard deviation or median (interquartile range). Statistical significance was set at $P < 0.05$.

RESULTS

Individuals' characteristics

The present study included 814 DCM individuals consisted of 562 men (69%) and 252 women (31%), with a mean age at surgery (\pm standard deviation) of 66 ± 12 years. Of the study cohort, 636 individuals (78%) were diagnosed with CSM, whereas 178 individuals (22%) displayed OPLL. The percentage of male in participants with OPLL was significantly higher than that of participants with CSM ($P < 0.01$). The BMI in the participants with OPLL was on average 1.7 (95% CI, 1.1 – 2.4) kg/m^2 higher than that in the participants with CSM ($P < 0.01$). The number of intervertebral levels of stenosis in the OPLL group was on average 0.28 (95% CI, 0.10 – 0.46) higher than that in the CSM group ($P < 0.01$). The duration of symptoms was longer in participants with OPLL than in those with CSM ($P < 0.01$). Individuals with OPLL tended to have a high prevalence of diabetes and smoking history ($P = 0.06$ and $P = 0.06$, respectively). There was no significant difference in age at surgery, the Charlson comorbidity index, preoperative JOA scores, and preoperative VAS score for neck pain between the CSM and OPLL groups (Table 1). In the present study, 87% of individuals with OPLL were K-line (+), and the mean OPLL occupancy ratio was $37 \pm 13\%$.

Individuals' perioperative and postoperative data

The number of decompressed intervertebral levels in the OPLL group was on average 0.48 (95% CI, 0.34 – 0.62) higher than that in the CSM group ($P < 0.01$). The operation time in the OPLL group was on average 17 (95% CI, 8.0 – 26) minutes longer than that in the CSM group ($P < 0.01$). The blood loss was greater in the OPLL group than in the CSM group ($P < 0.01$). There was no significant difference between the groups in terms of the surgical technique, complication rates for C5 palsy, SSI, hematoma, dural tear, delirium, or neurological deterioration (Table 2). No significant differences were observed in the postoperative JOA scores,

Table 1. Comparison of patients' characteristics between CSM and OPLL.

	Total	CSM	OPLL	P value
Number of cases	814	636	178	
Age at surgery	66 ± 12	67 ± 12	65 ± 10	0.10
Sex (male, %)	69	66	79	<0.01
Body mass index (kg/m ²)	24 ± 3.8	24 ± 3.6	25 ± 4.3	<0.01
Smoking history (%)	34	29	36	0.06
Diabetes mellitus (%)	22	20	27	0.06
Charlson comorbidity index	0 (0–1.0) 0.53 ± 0.79	0 (0–1.0) 0.53 ± 0.78	0 (0–1.0) 0.52 ± 0.82	0.73
Duration of symptom (month)	9.6 (4.2–33) 28 ± 45	8.6 (3.8–31) 24 ± 38	17 (4.6–40) 40 ± 64	<0.01
Number of the intervertebral levels of stenosis	2.9 ± 1.1	2.9 ± 1.1	3.2 ± 1.1	<0.01
JOA scores (preoperatively)	11 ± 2.7	11 ± 2.7	11 ± 2.8	0.54
VAS scores for neck pain (preoperatively)	3.4 ± 2.8	3.4 ± 2.9	3.3 ± 2.7	0.73

Values are mean ± standard deviation, median (interquartile range) or %.

CSM cervical spondylotic myelopathy, OPLL ossification of the posterior longitudinal ligament, JOA Japanese Orthopaedic Association, VAS visual analogue scale.

Table 2. Comparison of patients' perioperative and postoperative data between CSM and OPLL.

	Total	CSM	OPLL	P value
Number of the intervertebral levels decompressed	4.4 ± 0.87	4.3 ± 0.84	4.8 ± 0.87	<0.01
Surgical techniques (%)				0.727
ELAP	42	41	43	
DD	31	32	29	
SL	27	27	28	
Operation time (min)	104 ± 48	101 ± 44	118 ± 57	<0.01
Blood loss (g)	23 (5.0–60) 54 ± 91	20 (5.0–57) 50 ± 90	40 (5.0–86) 68 ± 91	<0.01
Complications (%)				
C5 palsy	2.1	2.0	2.8	0.66
Surgical site infection	0.61	0.47	1.1	0.45
Hematoma	0.98	1.3	0	0.38
Dural tear	0.12	0.16	0	0.78
Delirium	0.74	0.79	0.56	0.83
Neurological deterioration	2.6	2.8	1.7	0.68
JOA scores				
Postoperatively	14 ± 2.2	14 ± 2.2	14 ± 2.2	0.55
Postoperatively minus preoperatively	3.0 ± 2.2	3.0 ± 2.1	3.0 ± 2.3	0.87
RR (%)	50 ± 31	50 ± 31	49 ± 34	0.92
VAS scores for neck pain				
Postoperatively	2.1 ± 2.4	2.0 ± 2.4	2.3 ± 2.4	0.32
Postoperatively minus preoperatively	−1.3 ± 2.7	−1.4 ± 2.7	−1.0 ± 2.7	0.22

Values are mean ± standard deviation, median (interquartile range) or %.

CSM cervical spondylotic myelopathy, OPLL ossification of the posterior longitudinal ligament, ELAP expansive open-door laminoplasty, DD double-door laminoplasty, SL selective laminectomy, JOA Japanese Orthopaedic Association, RR recovery rate, VAS visual analogue scale.

preoperative-to-postoperative changes in the JOA scores, or JOA score RR between the groups. Both the CSM and OPLL groups showed significant improvement in the JOA scores postoperatively (CSM group: 3.0 ± 2.1, 95% CI, 2.9–3.2, $P < 0.01$; OPLL group: 3.0 ± 2.3, 95% CI, 2.6–3.3, $P < 0.01$). The postoperative VAS scores for neck pain and preoperative-to-postoperative changes in the VAS score for neck pain were not significantly different between the groups. Both the CSM and OPLL groups showed a significant reduction in the VAS score for neck pain postoperatively (CSM group: −1.4 ± 2.7, 95% CI, −1.6 to −1.1, $P < 0.01$; OPLL group: −1.0 ± 2.7, 95% CI, −1.5 to −0.48, $P < 0.01$) (Table 2).

Individuals' characteristics of the matched groups

After propensity score matching of the individuals' characteristics, the CSM and OPLL groups had 98 individuals each. We observed no significant differences between the groups regarding age at surgery, sex ratio, BMI, smoking history, prevalence of diabetes, the Charlson comorbidity index, duration of symptoms, number of intervertebral levels of stenosis, preoperative JOA scores, and preoperative VAS score for neck pain. In the matched cohort, the standardized difference in covariates was <10% (Table 3). After propensity score matching, 87% of individuals with OPLL were K-line (+), and the OPLL occupancy ratio was 38 ± 13%.

Table 3. Propensity score-matched comparison of patients' characteristics between CSM and OPLL.

	CSM	OPLL	P value	Standardized difference (%)
Number of cases	98	98		
Age at surgery	64 ± 12	65 ± 9.6	0.90	1.9
Sex (male, %)	83	81	0.71	5.4
Body mass index (kg/m ²)	25 ± 3.7	25 ± 3.8	0.77	5.4
Smoking history (%)	30	33	0.64	6.7
Diabetes mellitus (%)	21	25	0.61	7.4
Charlson comorbidity index	0 (0–1.0) 0.40 ± 0.61	0 (0–1.0) 0.46 ± 0.75	0.86	8.8
Duration of symptom (month)	9.1 (4.8–26) 26 ± 49	12 (3.6–32) 31 ± 51	0.76	9.5
Number of the intervertebral levels of stenosis	3.2 ± 1.1	3.1 ± 1.0	0.84	2.9
JOA scores (preoperatively)	11 ± 2.7	11 ± 2.8	0.96	0
VAS scores for neck pain (preoperatively)	3.2 ± 2.8	3.3 ± 2.7	0.76	4.4

Values are mean ± standard deviation, median (interquartile range) or %.

CSM cervical spondylotic myelopathy, OPLL ossification of the posterior longitudinal ligament, JOA Japanese Orthopaedic Association, VAS visual analogue scale.

Table 4. Propensity score-matched comparison of patients' perioperative and postoperative data between CSM and OPLL.

	CSM	OPLL	P value
Number of decompressed intervertebral levels	4.6 ± 0.87	4.9 ± 0.80	< 0.01
Surgical techniques (%)			0.55
ELAP	52	47	
DD	42	49	
SL	6.1	4.1	
Operation time (min)	92 ± 44	91 ± 39	0.80
Blood loss (g)	32 (5.0–75) 62 ± 87	40 (5.0–100) 75 ± 100	0.72
Complications (%)			
C5 palsy	3.1	4.1	0.50
Surgical site infection	0	2.0	0.25
Hematoma	2.0	0	0.25
Dural tear	0	0	–
Delirium	0	1.0	0.50
Neurological deterioration	4.1	2.0	0.34
JOA scores			
Postoperatively	14 ± 2.1	14 ± 2.4	0.42
Postoperatively minus preoperatively	3.1 ± 2.2	2.9 ± 2.5	0.47
RR (%)	55 ± 32	47 ± 35	0.09
VAS scores for neck pain			
Postoperatively	1.9 ± 2.2	2.3 ± 2.4	0.25
Postoperatively minus preoperatively	−1.3 ± 2.9	−1.0 ± 2.7	0.50

Values are mean ± standard deviation, median (interquartile range) or %. CSM cervical spondylotic myelopathy, OPLL ossification of the posterior longitudinal ligament, ELAP expansive open-door laminoplasty, DD double-door laminoplasty, SL selective laminectomy, JOA Japanese Orthopaedic Association, RR recovery rate, VAS visual analogue scale.

Individuals' perioperative and postoperative data of the matched groups

The number of decompressed intervertebral levels in the OPLL group was on average 0.35 (95% CI, 0.11–0.58) higher than that in the CSM group ($P < 0.01$) (Table 4). There was no significant difference in surgical technique, operation time, or blood loss between the CSM and OPLL groups. We observed no significant

difference in the complication rates for C5 palsy, SSI, hematoma, delirium, or neurological deterioration between the groups. Dural tear was not observed in either group (Table 4). The postoperative JOA scores, preoperative-to-postoperative changes in the JOA scores, and JOA score RR between the groups were not significantly different. Both the CSM and OPLL groups demonstrated significant improvement in the JOA scores postoperatively (CSM group: 3.1 ± 2.2 , 95% CI, 2.7–3.6, $P < 0.01$; OPLL group: 2.9 ± 2.5 , 95% CI, 2.4–3.4, $P < 0.01$). The postoperative VAS score for neck pain and preoperative-to-postoperative changes in the VAS score for neck pain between the groups were not significantly different. Both the CSM and OPLL groups showed a significant reduction in the VAS score for neck pain postoperatively (CSM group: -1.3 ± 2.9 , 95% CI, -1.9 – 0.7 , $P < 0.01$; OPLL group: -1.0 ± 2.7 , 95% CI, -1.5 to -0.50 , $P < 0.01$) (Table 4).

DISCUSSION

To the best of our knowledge, this is the first multicenter large cohort study to compare the surgical outcomes of posterior cervical decompression between CSM and OPLL using propensity score-matched analysis. The improvements after surgery assessed by the JOA score and VAS score for neck pain seen in participants with OPLL were comparable with those observed in participants with CSM. Moreover, perioperative complication rates were comparable between the groups. This study demonstrated no significant differences in effectiveness and safety of posterior cervical decompression for CSM and OPLL.

In general, cervical OPLL is more commonly observed in the Asian population [5, 18]. The incidence of OPLL in normal populations or asymptomatic individuals based on CT findings was 6.3% in Japan, 5.7% in South Korea, and 1.3–3.2% in the United States [11, 12, 19]. Meanwhile, the prevalence of OPLL in 7210 individuals with DCM was 18% in the Chinese population [20]. Consistently, the prevalence of OPLL was 22% in our Japanese cohort of DCM. We suggest that the incidence of OPLL in individuals with DCM is ~20% in the Asian population. Previous studies reported that the prevalence of DCM in male individuals was statistically higher than that in female individuals [12, 19, 20]. Consistently, 69% of the DCM participants in the present study were male. Moreover, the percentage of male individuals with OPLL (79%) was significantly higher than that in individuals with CSM (66%). Similar results were reported in a previous study in which the percentage of male individuals was higher in those with OPLL (68%) than in those with other forms of DCM (62%) [20]. Similarly, our results suggest that the percentage of male participants is higher in those with OPLL than in those with CSM.

Consistent with the findings of the present study, a previous study observed a higher BMI in individuals with OPLL than in individuals without OPLL [11]. A higher BMI was also reported as a risk factor for OPLL [21]. Another study demonstrated direct correlations of BMI with the extent of OPLL in the multiple regression analysis [22]. Liao et al. reported that the prevalence of OPLL in the overweight population was significantly higher than that in the normal-weight population, and the obesity population showed the highest prevalence [20]. Diabetes was reported as a risk factor of OPLL in previous studies [21–23]. Compared with participants with CSM, a tendency toward a high prevalence of diabetes was observed in participants with OPLL in the present study. Moreover, Akune et al. reported that the extent of OPLL was positively associated with the insulin secretory response [22]. The duration of symptoms was longer in participants with OPLL than in participants with CSM, suggesting that the slow progression of neurological symptoms due to slow growth of ossification influences the longer duration of symptoms before surgery. We observed a higher number of intervertebral levels of stenosis in participants with OPLL than in those with CSM. As the OPLL progresses, ossification extends to the upper and/or lower levels of the cervical spine, resulting in a higher number of stenosis levels in individuals with OPLL.

In the present study, we aimed to reveal the effect of different underlying mechanisms of spinal cord compression between CSM and OPLL on surgical outcomes. As we observed some differences in baseline characteristics, propensity score matching was used to reduce the influence of baseline differences on the comparison of surgical outcomes between individuals with CSM and those with OPLL. As the baseline factors were appropriately adjusted between the CSM and OPLL groups after propensity score matching, the possible confounding factors did not affect the results of this study. Our results showed that the improvements after surgery assessed by the JOA scores and VAS score for neck pain were not significantly different between the participants with CSM and those with OPLL. In general, spine surgeons tend to perform extensive decompression in individuals with OPLL because ossification progression was reported after posterior cervical decompression for OPLL individuals [24, 25]. Indeed, a higher number of intervertebral levels were surgically decompressed in the OPLL group than in the CSM group after matching the number of intervertebral levels of stenosis in the present study. Age and operation time were important predictors of surgical complications [26]. After propensity score matching, no significant difference was observed in age and operation time between participants with CSM and OPLL. We found that the incidence of complications after posterior cervical decompression was comparable between participants with CSM and those with OPLL. Therefore, posterior cervical decompression can be performed equally safely for OPLL and CSM.

The present study has several limitations. First, this was a retrospective study, which inevitably included selection bias. Second, a period of at least 1 year of follow-up after surgery was relatively short for a retrospective study, and long-term surgical outcomes were not assessed. Further studies with a longer follow-up period should be performed to confirm the results of the present study. Third, surgical outcomes, such as health-related quality of life outcomes, were not evaluated with objective individual-based outcome measures. Fourth, the indications for surgery and choice of surgical technique were left to the spine surgeon's discretion at each institute. Fifth, although we accurately analyzed the effect of different underlying mechanisms of spinal cord compression between CSM and OPLL on their surgical outcomes, propensity score matching of disease specific difference in baseline characteristics may affect the results of the study. Finally, propensity score matching may cause some biases because data from unmatched individuals were not analyzed in the comparison of surgical outcomes between individuals with CSM and those with OPLL.

CONCLUSION

Propensity score-matched analysis revealed that neurological improvement and perioperative complication rates after posterior cervical decompression were not significantly different between individuals with CSM and those with OPLL in a short-term follow-up. Posterior cervical decompression is an equally effective and safe treatment option for CSM and OPLL; however, this finding requires further validation.

DATA AVAILABILITY

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

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AUTHOR CONTRIBUTIONS

SN and NN designed the research. SN analyzed the data. SN and NN wrote the paper. SN, KD, TI, HF, KN, YT, and KF performed data collection. NN, SS, YT, OT, MY, MN, MM, KW, KI, and JY supervised the study. All authors reviewed and approved the paper.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL

This study received ethical approval from the institutional review boards of the participating institutions. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this research.

ADDITIONAL INFORMATION

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