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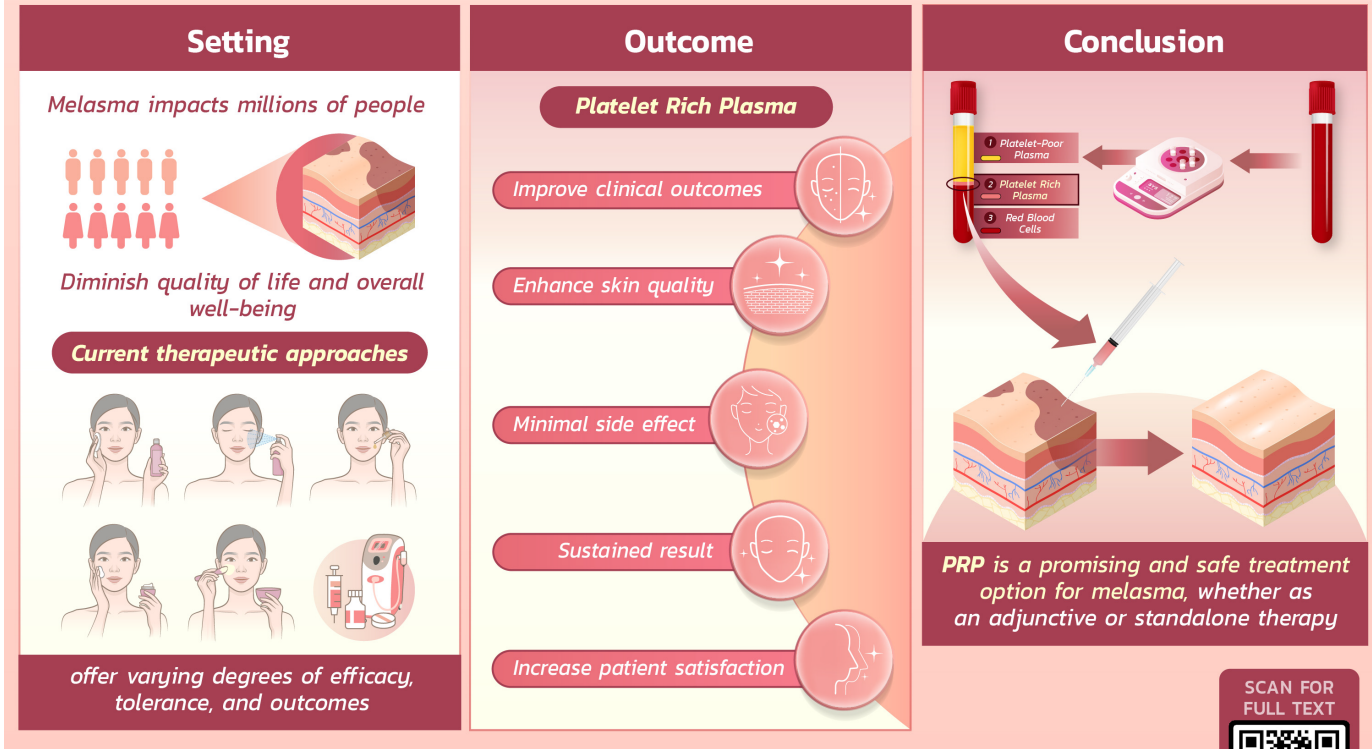

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Prevalence of Soft Tissue and Bone Tumor Diagnostic Discrepancies from Initial to Referral Sarcoma Center

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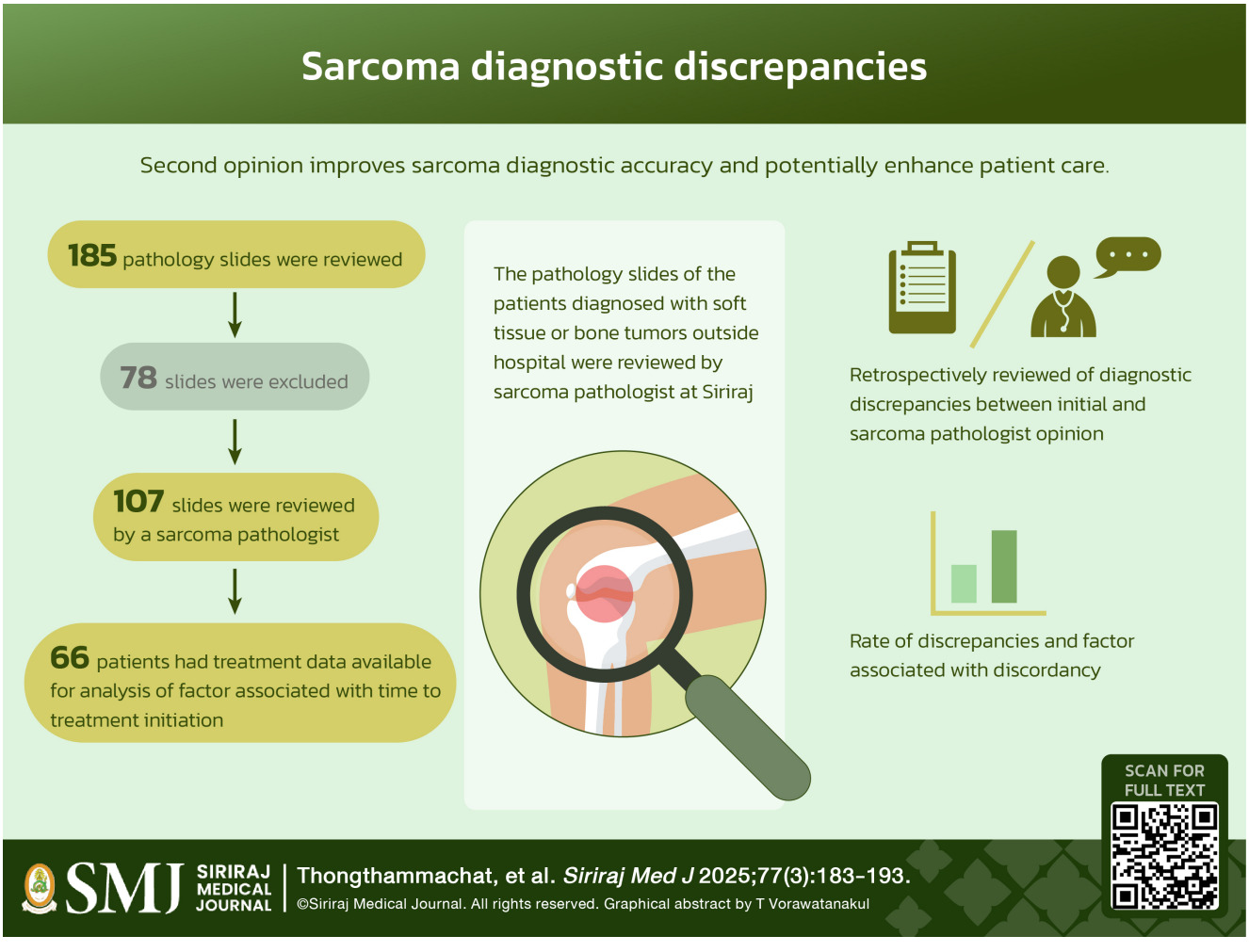

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ABSTRACT

Objective: Diagnosing sarcoma can be challenging. This study evaluates pathological reviews of all sarcoma cases diagnosed at Siriraj Hospital, comparing initial diagnoses with those confirmed by a dedicated sarcoma pathologist

Materials and Methods: Histopathological data from sarcoma patients at Siriraj Hospital were collected over five years. Initial diagnoses were compared to those determined by specialized sarcoma pathologists.

Results: Among the 185 patients, 107 (57%) met the inclusion criteria and were then analyzed. Full concordance (perfect agreement between initial and sarcoma specialized pathologist) was observed in 28 (26.1%) cases, partial concordance (identical diagnosis with differences in classification and / or histopathological subtype differences) in 18 (16.8%) cases, and zero concordance (benign to malignant or vice versa, different histopathological type or invalidation of sarcoma diagnosis) in 61 (57%) cases. The rate of complete concordance was significantly higher in cases with initial complete immunohistochemical (IHC) studies (HR 4.17 and 95% CI 1.43-12.12; p = 0.009), tumors size 100 mm or more (HR 0.32 and 95% CI 0.10-0.99; p = 0.04) and younger than 18 years (HR 5.48, 95% CI 1.49-20; p = 0.01). The main discrepancies were histopathological type (n = 53, 49.5%), subtype (n = 8, 7.5%) and grade plus subtype (n = 4, 3.7%). The mean duration from diagnosis to treatment was 68 days (range: 0-272).

Conclusion: The second opinion modified 73.8% of the initial diagnoses. However, no significant association was found between concordance of diagnosis and time to treatment initiation. Second opinion improves diagnostic accuracy and potentially enhance patient care.

Keywords: Concordance evaluation; histological review; medical decision; sarcoma (Siriraj Med J 2025; 77: 183-193)

INTRODUCTION

Soft tissue and bone sarcomas are rare types of cancer of mesenchymal origin. The incidence in Asian populations is approximately 2.8 per 100,000 people per year.[1] Given the existence of more than 170 soft tissue sarcoma subtypes (STS) according to the WHO 2020 classification[2], it is difficult to make an accurate diagnosis. The delay in correct diagnosis causes delay in treatment and may result in the loss of a curative opportunity. Studies that examine the rate of diagnostic discordances among different pathologists have reported variations ranging from 25% to 40%.[3,4] Due to the potential for incorrect diagnoses and the rarity of the disease, improper treatment is a significant concern.[1-3] Therefore, we systematically compared an initial histopathological diagnosis provided by a pathologist (first opinion, FO) with a diagnosis by a second specialized sarcoma pathologist (second opinion, SO). We analyzed patients suspected of having soft tissue or bone tumors during the years 2014 to 2019.

MATERIALS AND METHODS

Objectives

The main objective of this study was to assess the rate of pathological diagnostic discordance in soft tissue and bone tumors. The secondary objective was to investigate the duration between the date of the pathological diagnosis and the start of specific treatments, such as surgery, chemotherapy, or radiation (individually or in combination). Furthermore, our objective was to explore the factors associated with diagnostic discordance.

Study design

We conducted a retrospective study involving patients diagnosed with soft tissue or bone tumors between 2014 and 2019 at Siriraj Hospital. The initial pathological diagnosis, provided by an external pathologist (referred to as the "first opinion's diagnosis), was subsequently reviewed by a specialized sarcoma pathologist at Siriraj Hospital (referred to as the "second opinion diagnosis"). The revised pathological diagnosis was based on the WHO 2013 classification of Tumours of Soft Tissue and Bone (4th edition). We collected data on patient demographics, the date of the initial pathological diagnosis by the first opinion, the date of pathological diagnostic confirmation by the second opinion at Siriraj Hospital, and the date of the initiation of specific treatments, such as surgery, radiotherapy, chemotherapy, or other systemic therapies prescribed at Siriraj Hospital. Furthermore, we retrieved the results of histopathological diagnosis and pathological information, including tumor type (histological category), subgroup (family, subtype, variant), mitotic rate, size, and margin status, from the Department of Pathology database of the Faculty of Medicine Siriraj Hospital, Mahidol University.

Definition of outcomes

We categorized and evaluated the discordances between the initial pathological diagnosis provided by the 'first opinion (FO)' and the revised diagnosis given by the sarcoma specialized pathologist, called the 'second opinion (SO)', using a three-point scale.

'Zero concordance' was defined when the initial pathological diagnosis (by the FO) indicated a benign condition, but the revised diagnosis indicated malignancy (sarcoma), or vice versa. It was also applied when the tumor was classified into different histological types, such as a change from synovial sarcoma to liposarcoma. 'Partial concordance' was defined when both pathologists (FO and SO) agreed on the same histological type. However, there was a discordant result regarding either grading (including grades not initially reported) or a different subtype, for instance, a change from dedifferentiated liposarcoma to myxoid round cell liposarcoma).

'Full concordance' was when there was a perfect agreement between the FO and SO, encompassing histological type, subtype and grading.

The 'Time from initial report to initiation of treatment' was defined as the interval between the date of the first report (FO), including the preliminary diagnosis, and the date of the beginning of specific cancer treatment, such as surgery, systemic therapy, radiation or ablation.

Statistical analysis

The sample size was calculated using the nQuery Advisor program with the formula for estimating a population proportion, considering a tolerance of 40% for the prevalence of diagnostic differences[11], and a two-sided 95% confidence interval of 5%. We collected data for 369 cases, accounting for approximately 10% of patients with incomplete information, resulting in a total of 400 patient data points.

To evaluate the characteristics and diagnostic concordance, we analyzed categorical data using Pearson's χ^2 test or Fisher's exact test, as appropriate for parametric data. The Kolmogorov-Smirnov test was used to assess normal and non-normal data distributions. The Mann-Whitney U test (also known as the Wilcoxon rank-sum test) was used to compare a non-parametric data. Continuous data were analyzed using the Student's t test. The level of statistical significance was established at P = 0.05 for a two-sided test. The $\chi 2$ test was used to determine the level of concordance and the type of discordance. All analyzes were performed using SPSS® (version 23.0). A multivariate Cox proportional hazards regression analysis was performed using factors associated with concordance.

RESULTS

Baseline characteristics

All values reported subsequently for grade (graded using the Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) Sarcoma Group), histopathology, and the type or site of sarcoma are based on findings obtained from the second review.

Of all cases, a total of 185 patients were initially collected by pathology laboratories. Among these, 69 were reviewed by other pathologists, and 9 were not initially diagnosed with sarcoma. After applying the inclusion criteria, the final analysis included 107 patients. The initial diagnosis of soft tissue or bone tumor was made by private laboratories in 24 (24.3%) cases and by public pathological laboratories in 81 (75.5%). The characteristics of the patients are summarized in Table 1. Among the 107 patients analyzed, 54 were male (50.5%) and 53 were female (49.5%). The median age was 49 years (range, 1-97), with 24 cases (22.4%) involving visceral sarcomas. Initial immunohistochemical (IHC) results were available for 49 cases (45.7%), while 25 cases received a complete diagnosis without planned further IHC. Only one case in our study was confirmed by fluorescent in situ hybridization (FISH) testing for the rearrangement of *EWSR1*.

Concordance analysis

Concordance analysis was performed on 63 (58.9%) biopsied and 44 (41.1%) surgical samples. Table 2 shows the histopathological grade, type of tumor sample (biopsied sample vs. surgical specimen), and type of pathological laboratory (private vs. public). The ***full concordance*** between the diagnoses of the first and second opinion was observed in 28 (26.1%) cases, ***partial concordance*** (identical diagnosis of soft tissue or bone tumor but different in either grade or subtype) in 18 (16.8%) cases, and grade was not initially reported in 61 cases. ***Zero concordance*** (from benign to malignant tumor or vice versa or different histopathological type) was found in 61 cases (57%). Of the 64 specimens for which the classification was not initially reported, 44 were given an FNCLCC classification by the second pathologist, while 20 specimens could not be classified. The complete concordance rate was higher in patients 18 years or younger (p 0.002), in those with initial available IHC (yes vs. no = 36.7% vs. 17.2%) (p 0.027), and those with tumor size of 100 mm or more (yes vs. no = 33.3 vs. 15.3) (p = 0.04). The main discrepancies were related to histopathological type (n = 53, 49.5%) and subtype (n = 8, 7.5%), as shown in Table 1. The most common zero-concordance histopathology diagnosed by a second

TABLE 1. Patient characteristics.

		Frequency	Percentage
Age	Median (range) years	49 (1-97)	-
	>18 years old	90	84.1
Gender	Male	54	50.5
	Female	53	49.5
Diagnosis	First diagnosis	82	76.6
	Recurrent disease	25	23.4
Available initial IHC	Yes	49	45.7
	No	58	54.2
Type of sample	Core needle biopsy	5	4.7
	Surgical biopsy	58	54.2
	Tumor resection	44	41.1
Type of laboratory	Public	81	75.7
	Private	26	24.3
Type of sarcoma	Soft tissue	68	63.5
	Visceral tissue	24	22.4
	Bone tissue	15	14.0
Tumor site	Lower limb	41	38.3
	Upper limb	17	15.9
	Pelvis	12	11.2
	Thorax	10	9.3
	Head and neck	8	7.5
	Body wall	5	4.7
	Abdomen	5	4.7
	Retroperitoneum	3	2.8
	Axial skeleton	3	2.8
	Multiple locations	1	1.9
	CNS	1	1.9
Histological type reviewed by SO	Undifferentiated pleomorphic sarcoma/ undifferentiated sarcoma	25	23.3
	Synovial sarcoma	11	10.3
	Ewing sarcoma	7	6.5
	Leiomyosarcoma	7	6.5
	Osteosarcoma	6	5.6
	Myxofibrosarcoma	5	4.6
	Epithelioid sarcoma	5	4.6
	Alveolar soft part sarcoma	4	3.7
	Liposarcoma	4	3.7
	Spindle cell sarcoma	4	3.7
	MPNST**	3	2.8
	Solitary fibrous tumor	3	2.8
	Carcinoma	3	2.8

TABLE 1. Patient characteristics. (Continue)

		Frequency	Percentage
	Neoplasm	3	2.8
	Dermal sarcoma	2	1.9
	Fibrosarcoma	2	1.9
	Myofibroblastic sarcoma	2	1.9
	Rhabdomyosarcoma	2	1.9
	Chondrosarcoma	1	0.9
	Desmoid fibromatosis	1	0.9
	Endometrial stromal sarcoma	1	0.9
	Kaposi sarcoma	1	0.9
	Clear cell neoplasm	1	0.9
	Clear cell sarcoma	1	0.9
	No residual sarcoma	1	0.9
	Sarcoma with osteoblastic differentiation	1	0.9
	Undifferentiated uterine sarcoma	1	.9
Tumor size	Median (range), (IQR) mm	70 (5-250), (43-108.5)	-
	Less than 100 mm	66	61.7
	100 mm or larger	39	36.4
	Not report	2	1.8
Grade reviewed by SO	1	6	5.6
	2	24	22.4
	3	53	49.5
	Not report	24	22.4
Type of concordance	Zero	61	57
	Partial	18	16.8
	Full	28	26.1
Factor of discordance	Histological type only	20	18.6
	Grade only	11	10.3
	Subtype only	6	5.6
	Grade and histological type	33	30.8
	Grade and subtype	2	1.9
	Different lineage	4	3.7
	Benign vs. Malignant	3	2.8
	No discordance	28	26.1
First modality of treatment	Surgery	55	51.4
	Chemotherapy	19	17.7
	Radiotherapy	4	3.7
	Palliative	2	1.9
	No follow-up data	25	23.4
	Surveillance[#]	2	1.9

**MPNST = malignant peripheral nerve sheath tumor

[#] one patient with Kaposi's sarcoma on HAART, and one with closed observation

TABLE 2. Concordance analysis of clinical variables and degree of concordance.

Concordance analysis		Zero and partial		Full		P value
		Frequency	Percent	Frequency	Percent	
Age (years)	More than 18	66	73.3	24	26.7	0.002
	18 or less	6	35.3	11	64.7	
Available initial IHC	No	48	82.8	10	17.2	0.02
	Yes	31	63.2	18	36.7	
Type of laboratory	Public	53	65.4	28	34.6	0.92
	Private	19	73.1	7	26.9	
Type of sample	Core needle biopsy	3	60	2	40	0.83
	Surgical biopsy	40	69	18	31	
	Tumor resection	29	65.9	15	34.1	
Grading by SO	1,2	22	73.3	8	26.6	0.28
	3	43	81.1	10	18.8	
Type of sarcoma	Soft tissue	49	72	19	28	0.22
	Visceral tissue	15	62.5	9	37.5	
	Bone tissue	8	53.3	7	46.7	
Size (mm.)	100 or more	44	66.6	22	33.3	0.04
	Less than 100	33	84.6	6	15.3	

pathologist was an undifferentiated sarcoma (n=17). Details of the confirmed diagnoses made by the second opinion among the zero concordance group are reported in Table 3. Among 25 patients with complete initial IHC, 12 cases (48%) had complete concordance, while 6 cases had major discrepancies including 1 from sarcoma was revised to be carcinoma, 1 medullary carcinoma to rhabdomyosarcoma, 1 lymphoma to Ewing sarcoma, 1 neuroendocrine tumor to Ewing sarcoma, 1 lipoma to myxofibrosarcoma, 1 liposarcoma to no residual tumor. (Table 3)

Analysis of factors associated with a shorter time from pathological diagnosis to initiation of specific treatment (DDT).

Of 82 de novo patients, 66 had treatment records available. The mean duration from diagnosis to treatment was 68 (range: 0-272). In particular, most cases of visceral sarcoma (15 of 16, 93.7%) had a time from initial pathological diagnosis to initiation of specific treatment of less than 68 days. No significant differences were found according to the availability of initial IHC, the type of laboratory and the complete concordance to achieve a treatment time shorter than 68 days.

Details on the time from the initial pathological diagnosis to the initiation of specific treatment are presented in Table 4.

Univariate analysis of concordance was conducted using previously established prognostic factors. Factors that demonstrated statistically significant associations with better concordance in this analysis included age less than 18 years ($p = 0.004$), complete initial IHC ($p = 0.005$) and tumor size 100 mm or more ($p = 0.012$). Subsequently, a multivariate Cox proportional hazards regression analysis of concordance was performed using the factors mentioned above. This analysis revealed that age less than 18 years, complete initial IHC and tumor size 100 mm or more were associated with better concordance (HR 5.48, 95% CI 1.49-20; $p = 0.01$, HR 4.17 and 95% CI 1.43-12.12; $p = 0.009$, HR 0.32 and 95%CI 0.10-0.99; $p = 0.04$, respectively).

TABLE 3. Diagnostic discrepancies in cases with zero concordance.

Initial diagnosis	Second diagnosis	Frequency
Malignant round cell neoplasm	Alveolar rhabdomyosarcoma	1
Alveolar rhabdomyosarcoma	Alveolar soft part sarcoma	1
Sarcoma	Carcinoma*	1
Malignant peripheral nerve sheath tumor	Clear cell neoplasm	1
Spindle cell neoplasm	Clear cell sarcoma	1
Undifferentiated sarcoma	Dedifferentiated leiomyosarcoma	1
Spindle cell neoplasm	Dedifferentiated Liposarcoma	1
Spindle cell sarcoma	Dermal sarcoma	1
Undifferentiated sarcoma	Dermal sarcoma	1
Fibroblastic neoplasm	Desmoid-type fibromatosis	1
Malignant myxoid tumor	Embryonal rhabdomyosarcoma	1
Undifferentiated pleomorphic sarcoma	Epithelioid sarcoma	1
Spindle cell neoplasm	Epithelioid sarcoma	1
Sarcoma	Epithelioid sarcoma	1
Neuroendocrine tumor	Ewing sarcoma*	1
Malignant round cell neoplasm	Ewing sarcoma	2
Lymphoma	Ewing sarcoma*	1
Spindle cell neoplasm	Leiomyosarcoma	1
Spindle cell sarcoma	Malignant peripheral nerve sheath tumor	2
Malignant peripheral nerve sheath tumor	Malignant spindle cell neoplasm	1
Rhabdomyosarcoma	Medullary carcinoma*	1
Leiomyoma	Myofibroblastic sarcoma	1
Spindle cell neoplasm	Myofibroblastic sarcoma	1
Spindle cell neoplasm	Myxofibrosarcoma	1
Lipoma	Myxofibrosarcoma*	1
Spindle cell sarcoma	Myxofibrosarcoma	1
Myxoid liposarcoma	Myxoid neoplasm	1
Myxoid spindle cell tumor	Myxoid synovial sarcoma	1
Liposarcoma	No residual tumor*	1
Epithelioid sarcoma	Osteosarcoma	1
Pleomorphic and spindle cell neoplasm	Pleomorphic and spindle cell sarcoma	1
Malignant fibrous histiocytoma	Pleomorphic liposarcoma	1
Spindle cell neoplasm	Pleomorphic sarcoma	1
Sarcoma	Pleomorphic sarcoma	1
Spindle cell neoplasm suspicious sarcoma	Sarcomatoid renal cell carcinoma	1
GIST	Solitary fibrous tumor	1
Osteosarcoma	Sarcoma with osteoblastic differentiate	1

TABLE 3. Diagnostic discrepancies in cases with zero concordance. (Continue)

Initial diagnosis	Second diagnosis	Frequency
Spindle cell neoplasm	Spindle cell sarcoma	1
Malignant fibrous histiocytoma	Spindle cell sarcoma	1
Neurofibrosarcoma	Spindle cell sarcoma	1
Spindle cell neoplasm	Superficial pleomorphic sarcoma	1
Spindle cell neoplasm	Synovial sarcoma	2
Sarcoma	Synovial sarcoma	1
Spindle cell carcinoma	Synovial sarcoma	1
Malignant epithelioid neoplasm	Undifferentiated pleomorphic sarcoma	1
Malignant solitary fibrous tumor	Undifferentiated pleomorphic sarcoma	1
Spindle cell sarcoma	Undifferentiated pleomorphic sarcoma	4
Unclassified high grade sarcoma	Undifferentiated pleomorphic sarcoma	1
Pleomorphic malignant neoplasm	Undifferentiated pleomorphic sarcoma	1
Pleomorphic malignant neoplasm	Undifferentiated sarcoma	1
Leiomyosarcoma	Undifferentiated sarcoma	1
Spindle cell neoplasm	Undifferentiated sarcoma	1
Undifferentiated malignant neoplasm	Undifferentiated sarcoma	1
Endometrial stromal sarcoma	Undifferentiated uterine sarcoma	1
Leiomyosarcoma	Undifferentiated uterine sarcoma	1

* zero concordance with major discrepancies

TABLE 4. Time from initial pathological diagnosis to the initiation of specific treatment in 66 de novo cases.

DDT* (N= 66)**	Mean	Less than 68 days		68 or more days		
		Frequency	Percentage	Frequency	Percentage	P
Available initial IHC	No	23	62.5	13	37.5	0.58
	Yes	19	61.7	11	38.2	
Type of laboratory	Public	32	66.7	16	33.3	0.4
	Private	10	55.5	8	44.5	
Type of sarcoma	Soft tissue/bone	28	54.9	23	45.0	0.006
	Visceral	14	93.3	1	6.6	
Full concordance	No	31	65.9	16	34.1	0.58
	Yes	11	57.8	8	42.1	

*DDT = time from initial pathological diagnosis to the initiation of specific treatment
** 16 patients had only review of slides without treatment record.

TABLE 5. Univariate and multivariate analysis of concordance.

Concordance analysis		N	Univariate analysis		Multivariate analysis	
			HR (95%CI)	P	HR (95%CI)	P
Age (years)	More than 18	90	Ref		Ref	
	18 or less	17	5.04 (1.68-15.13)	0.004	5.48 (1.49-20.0)	0.01
Complete initial IHC	No	82	Ref		Ref	
	Yes	25	3.75 (1.50-9.34)	0.005	4.17 (1.43-12.12)	0.009
Type of sarcoma	Soft tissue	68	Ref		Ref	
	Visceral tissue	24	1.57 (0.59-4.21)	0.36	1.43 (0.38-5.35)	0.58
	Bone tissue	15	2.63 (0.81-8.51)	0.1	1.27 (0.28-5.84)	0.75
Tumor size (mm.)	100 or more	66	Ref		Ref	
	Less than 100	39	0.29 (0.11-0.76)	0.012	0.32 (0.10-0.99)	0.049

DISCUSSION

The primary objective of this study was to assess the prevalence of diagnostic discordance and the role of centralized histological review. An accurate diagnosis is crucial for prognosis and appropriate treatment. Recent literature reports diagnostic discrepancies in the histopathological diagnosis of soft tissue sarcomas ranging from 14% to 47%.[4-8] We analyzed the concordance between the initial evaluation and the second opinion, along with various factors associated with clinical and pathological conditions. Using the criteria established by the WHO 2013 Classification of Tumors of Soft Tissue and Bone, our findings indicate a full concordance rate of 33%. Additionally, the observed discordance rate of 67%, with a major discrepancy of 56% and a minor discrepancy of 11%, is higher than that reported in other sarcoma studies.[5,9-11]

This study confirms that establishing a sarcoma diagnosis is highly challenging, as more than 74% of initial diagnoses changed upon the second pathological review. The main result suggests that concordance appears to depend on age, initial IHC, and tumor size. These results emphasize that if the first-opinion pathologist performs immunohistochemical (IHC) studies completely prior to referral, the rate of concordance can be higher and prevent delay in diagnosis. The rationale for younger age being associated with higher concordance is unclear, possibly because most pediatric sarcoma subtypes have distinct histomorphology that most pathologists are familiar with. The most frequent discrepancies were related to histological type. Major discrepancies were based on hematoxylin and eosin- (H&E-) stained slides, with diagnostic discrepancies associated with the difficulty of rare and unusual neoplasms. Such discrepancies were often related to undifferentiated sarcomas diagnosed as spindle cell sarcomas, malignant solitary fibrous tumors, unclassified malignant tumors, pleomorphic spindle cell sarcoma, leiomyosarcomas, endometrial stromal sarcomas, and unclassified malignant tumor. Among the cases with 'full concordance', osteosarcoma was the most common histopathology. However, only 25 patients completed the initial IHC before referral, possibly because most patients were referred soon after the preliminary diagnosis without waiting for a final diagnosis. Of these 25 cases, 6 had zero concordance, including a case where leoimyosarcoma changed to undifferentiated sarcoma, a case where a solitary fibrous tumor became undifferentiated pleomorphic sarcoma, 1 case where neurofibrosarcoma became spindle cell sarcoma, NOS, 1 case of rhabdomyosarcoma to medullary carcinoma, 1 case of sarcoma to carcinoma and 1 case of undifferentiated sarcoma to dermal sarcoma. These discrepancies occurred due to disagreements in IHC interpretation. Among the 61 specimens, 4 received the FNCLCC grade by the second pathologist, while 20 remained ungraded due limited amount of tissue or slide quality. Our study revealed higher histological and classification discrepancies compared to those reported in the SSG sarcoma group, where 25% of cases had their sarcoma histologic type reclassified, and 40% saw a change

in malignancy grade.[12] However, it is important to note that most of the specimens referred had incomplete IHC.

We also identified 24 cases of soft tissue sarcoma arising from visceral sites. The most common histological type was leiomyosarcoma (n=5), consistent with previous studies.[14-16] However, one case changed its diagnosis from leiomyosarcoma to undifferentiated stromal sarcoma. Among the histopathologies with full concordance, leiomyosarcoma was the most common (4 of 5), followed by hemangiopericytoma (2 of 2).

Our study also examined the time from diagnosis to the initiation of specific treatment. The mean duration from the initial diagnosis date to the start of definitive treatment, including surgery, chemotherapy or radiation, for newly diagnosed patients was 68 days. Patients with visceral primary tumors had a shorter time from diagnosis to initial specific treatment compared to those with extremity sites, possibly due to a higher rate of upfront surgery without prior biopsy, driven by abdominal symptoms. The maximum duration from diagnosis to treatment in our study was 272 days, primarily due to a lengthy referral process. However, we did not find any significant associations between factors such as complete initial IHC, histological type, or full concordance and the time to definitive treatment. We were unable to establish a clear association between concordance (full versus zero/partial, or full/partial versus zero) and the time to treatment. Several confounding factors influence the time to treatment, including the time required for imaging studies, the duration of retrieving pathology slides from the original hospital, and the time from the initial visit to the specialist.

The introduction of molecular genetic testing can significantly impact diagnostic accuracy, even in centers with established expertise in sarcoma.[13,14] However, unlike previous studies reporting confirmation rates of 10-14% of samples diagnosed by a second pathologist through additional tests or molecular tests[2,10,11,15], our study only performed such testing in one case initially diagnosed as Ewing's sarcoma. This limitation was largely due to reimbursement problems during the time of diagnosis, as many patients were unable to afford the costs associated with these tests. However, in cases with pathognomonic histomorphology and IHC results suggestive of small round cell sarcoma, a preliminary diagnosis may be sufficient to conclude the diagnosis without the need for additional ancillary tests.

This study has several limitations. First, a significant number of specimens had incomplete IHC prior to review, which could introduce bias in the diagnostic capability of the initial center and impact the rate of diagnostic discrepancy of sarcomas. Second, patients with ultrarare sarcomas or certain histopathological types are usually referred to specialized sarcoma centers, whereas common sarcomas with pathognomonic or specific morphology features can be diagnosed by the first pathologist without the need for referral. Third, pathologists who work in referral centers may already receive some guidance from external reports, such as indications of "suspicion of sarcoma" or "unlikely subtype". Last, there are uncontrolled factors in the treatment process, including the time of referral, the time of specialist consultations, and the time of complete staging scans before surgery, all of which can potentially lead to delays in initiating specific treatment.

CONCLUSION

The diagnostic discrepancy rate of 74% observed in second opinion cases highlights the importance of obtaining opinions from soft tissue pathologists. However, a lingering question remains: is a centralized pathological diagnosis necessary before referral? Although a quick and accurate diagnosis can expedite the initiation of treatment in centers with access to sarcoma pathologists, we were unable to establish a clear association between diagnosis concordance and the timing of the initiation of treatment for patients initially diagnosed with sarcoma from outside hospitals.

Data Availability Statement

The data supporting this study are available upon request from the corresponding author

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DECLARATION

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could appear to influence the work reported in this paper.

Registration Number of Clinical Trial

None.

Author Contributions

Conceptualization and methodology, J.C, K.T, and A.N. ; Pathology consultation, S.M; Formal analysis, J.C. and K.T. ; Visualization and writing – original draft, K.T. ; Writing – review and editing, J.C., and K.T ; Supervision, C.A., C.C, R.P. All authors have read and agreed to the final version of the manuscript.

Ethics Approval

This study was carried out according to the guidelines established in the Declaration of Helsinki and approved by the Institutional Review Board of Siriraj Hospital, Mahidol University. (approval number; COA no. Si 465/2021)

Use of Artificial Intelligence

None

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Differential Fusion Techniques in Uninstrumented Posterolateral Lumbar Fusion: A Retrospective Self-Control Study, Technical Notes and Radiological Outcomes

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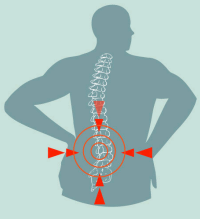
Reverse sandwich technique for uninstrumented posterolateral lumbar fusion

Dual-sided approach in the "reverse sandwich" technique, may improve vascularization and foster a more favorable biological environment for fusion without apparent biomechanical differences observed in this study.



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Lumbar spinal stenosis or spondylolisthesis patients

Undergoing uninstrumented posterolateral lumbar fusion by a senior spine surgeon using posterior midline approaches with iliac bone grafting customized to each patient in single tertiary care center.

The fusion technique was tailored for each patient, with a slight variation between sides. On the left side, the "bread and jam technique" was employed, where the cortical side of the iliac bone graft faced laterally, accompanied by scattered autograft on the medial side.

Conversely, on the right side, the "reverse sandwich technique" was used, wherein the cortical side of the iliac bone graft was positioned medially, with scattered autograft on the medial side.

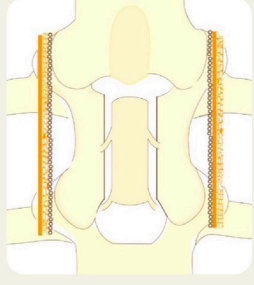

Fig 1. Schemas of fusion techniques

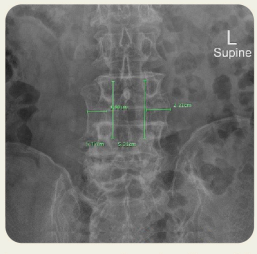

Fig 2. Demonstration of radiographic measurement

Radiographic assessments at 3, 6, 9, 12, and 24 months postoperatively evaluated fusion mass sizes.

The "reverse sandwich" technique, incorporating cancellous bone on both sides of the graft, potentially enhances fusion outcomes compared to the "bread and jam" technique, which uses cancellous bone on one side.

SCAN FOR FULL TEXT

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ABSTRACT

Objective: To compare two surgical techniques, the "bread and jam" and "reverse sandwich," for uninstrumented posterolateral lumbar fusion in treating degenerative lumbar spine disease, focusing on their impact on fusion outcomes.

Materials and Methods: This retrospective study at Siriraj Hospital analyzed data from patients undergoing uninstrumented posterolateral lumbar fusion between January 2002 and December 2016. Procedures were performed by a senior spine surgeon using posterior midline approaches with iliac bone grafting customized to each patient. Radiographic assessments at 3, 6, 9, 12, and 24 months postoperatively evaluated fusion mass sizes, assessed independently for reliability.

Results: Sixty patients (50 women, mean age 63.17 years) were included, predominantly diagnosed with spinal stenosis with spondylolisthesis (79.66%). The main symptoms were radiculopathy (47.46%) and neurogenic claudication (40.68%). Most surgeries targeted one spinal level (71.19%). Fusion mass size differed significantly between the right and left sides at 3, 9, and 24 months, with the right side consistently larger. However, no significant differences were noted at 6 and 12 months.

Conclusion: The "reverse sandwich" technique, incorporating cancellous bone on both sides of the graft, potentially enhances fusion outcomes compared to the "bread and jam" technique, which uses cancellous bone on one side. This dual-sided approach in the "reverse sandwich" technique, may improve vascularization and foster a more favorable biological environment for fusion without apparent biomechanical differences observed in this study. Further research is needed to corroborate these findings and elucidate their biological mechanisms.

Keywords: Lumbar fusion; posterolateral fusion; bone graft; surgical technique; degenerative lumbar spine disease
(Siriraj Med J 2025; 77: 194-199)

INTRODUCTION

Lumbar fusion is commonly executed in patients experiencing lumbar instability due to structural anomalies (such as lumbar spondylolysis and spondylolisthesis) or degenerative conditions (like degenerative lumbar spondylolisthesis and intervertebral disc issues). Various surgical techniques for lumbar fusion encompass posterior fusion, posterolateral fusion, interbody fusion via various approaches (posterior lumbar interbody fusion (PLIF), transforaminal lumbar interbody fusion (TLIF), oblique lumbar interbody fusion/anterior to psoas (OLIF/ATP), lateral lumbar interbody fusion (LLIF) and anterior lumbar interbody fusion (ALIF).[1,2]

In 2023, Andresen reported the efficacy of uninstrumented posterolateral fusion for lumbar spondylolisthesis, no difference in patient-reported outcomes was found between 2 groups (Oswestry Disability Index, visual analogue scale, EuroQol-5 Dimension-3 Level, Short Form-36).[3] Posterolateral fusion has shown a successful fusion rate up to 84%.[4,5] The posterolateral fusion is usually performed with an iliac bone graft and laminectomy bone. The surgical technique has been reported with various techniques since 1953.[6]

In addition to uninstrumented techniques, multi-level instrumented fusion has also been shown to provide good clinical outcomes and is considered safe for patients with extensive degenerative lumbar disease, as demonstrated in studies focusing on the use of posterior long-segment fusion.[7]

Our study aims to evaluate the 2 surgical techniques of posterolateral fusion without instrumentation in 1-3 levels of degenerative lumbar spine surgery.

MATERIALS AND METHODS

We performed the retrospective study using data from the medical database of the Department of Orthopaedic Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University. The study was approved by the Siriraj Institutional Review Board (COA no. Si 368/2019). Due to the retrospective nature of this study, written informed consent was not obtained.

All patients with degenerative spinal stenosis or spondylolisthesis who underwent primary 1-3 level of uninstrumented posterolateral lumbar fusion at Siriraj hospital during January 2002 to December 2016 were enrolled. The exclusion criteria consisted of patients with previous lumbar spine surgery, previously diagnosed postoperative deep surgical site infection, unable to collect data up to 1-year follow-up, and patients who received revision surgery within 1 year.

The primary outcome was the fusion mass size which was measured by plain radiograph of the lumbar spine

in anteroposterior view at 3 months, 6 months, and 1 year post-operative period. Baseline characteristics of patients were collected, which are age, gender, level of surgery, comorbidities, and history of smoking.

Surgical technique

The surgery was performed by a senior spine surgeon, fellowship-trained with 30 years of experience. All patients underwent a standard posterior midline approach, which included laminectomy and posterolateral fusion with an iliac bone graft, but without spinal instrumentation.

The iliac bone graft was harvested from the left iliac wing via a separate incision. A bone segment, measuring 1 cm by 1 cm and length matching the length from the upper to the lower facet, was extracted using a chisel. To preserve iliac crest integrity, the harvest site was maintained at least 1-2 cm from its edge. Cancellous bone was also collected using a bone chisel.

To prepare the grafting site, soft tissue was meticulously detached from the vertebral arch beneath the periosteum, exposing transverse process from the uppermost to the lowest level. Cortical bone on the exterior of the transverse and articular processes was scraped away to expose spongy bone and create a pocket for graft placement. The lateral one-third of the facet joint was hinged open to form an additional pocket. The harvested iliac bone graft and the bone chips from laminectomy, were placed into these pockets.

The fusion technique was tailored for each patient, with a slight variation between sides. On the left side, the "bread and jam technique" was employed, where the cortical side of the iliac bone graft faced laterally, accompanied by scattered autograft on the medial side. Conversely, on the right side, the "reverse sandwich technique" was used, wherein the cortical side of the iliac bone graft was positioned medially, with scattered autograft on the medial side. Illustrations of the surgical techniques are presented in Fig 1.

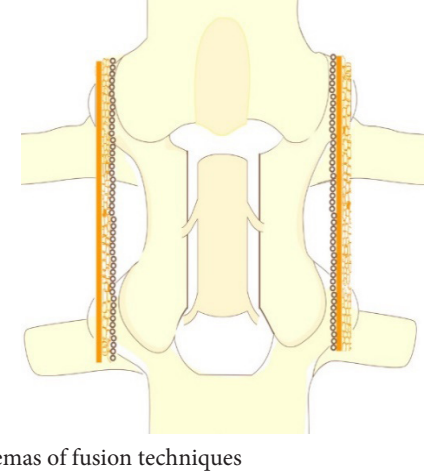

Fig 1. Schemas of fusion techniques

Radiographic assessment

Plain radiographs were obtained for all patients at a minimum follow-up of two years. The presence and extent of the fusion mass were evaluated on anteroposterior radiographs by one orthopedic surgical resident and one spine surgery fellow. These evaluations were conducted twice, with a two-week interval between assessments. Both assessors reviewed the postoperative radiographs blindly, without prior knowledge of the fusion techniques employed on either side. The width of the fusion mass was measured from the midpoint between the two pedicles at the uppermost and lowermost operated levels, as demonstrated in Fig 2.

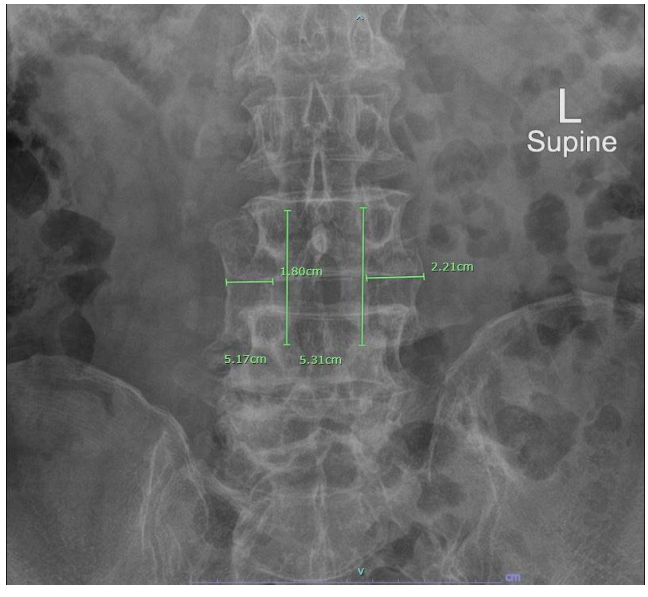

Fig 2. Demonstration of radiographic measurement

The intra-observer reliability for the assessment of fusion mass size was high, with ICCs ranging from 0.98 to 1.00, indicating excellent agreement between repeated measurements by the same rater. The inter-observer reliability was moderate, with ICCs ranging from 0.40 to 0.59, suggesting a fair degree of agreement between different raters.

Statistical analysis

To determine differences between the left and right sides, paired t-tests or Wilcoxon signed-rank tests were utilized, depending on the distribution of the data as determined by the Shapiro-Wilk test. The reliability of the radiographic measurements was assessed using the Intraclass Correlation Coefficient (ICC) to gauge both inter-rater and intra-rater consistency. Validity was established by correlating measurements with recognized benchmarks, and agreement on fusion classification between raters was measured using Cohen's Kappa. All statistical analyses were

performed with SPSS Statistics for Windows, Version 17.0 (SPSS Inc., Chicago, IL, 2008), and a p-value of less than 0.05 was considered statistically significant.

RESULTS

We included 60 patients in our study. There were 50 women (84.57%) and the average age of all participants was 63.17 ± 9.51 years. 3 patients (5.08%), had a history of smoking. The majority of patients, 47 out of 60 (79.66%), were diagnosed with spinal stenosis with spondylolisthesis, while the remaining 12 (20.34%) had spinal stenosis. The main symptoms were radiculopathy (28 patients, 47.46%) and neurogenic claudication (24 patients, 40.68%). Surgery was mostly done at one level of the spine (42 patients, 71.19%), with fewer having two (12 patients, 20.34%) or three levels operated on (5 patients, 8.47%).

Radiographic outcome

Fusion mass was measured from plain radiograph in anteroposterior view at 3,6,9,12,24 months postoperatively. At 3 months, the right side fusion masses were significantly larger (2.65 ± 0.57 cm) than the left (2.46 ± 0.40 cm) with a p-value of 0.01. However, at 6 and 12 months, the fusion mass sizes were not significantly different, with p-values of 0.39 and 0.49, respectively. At 9 months, a significant difference reappeared with the right side being larger (2.95 ± 0.56 cm) compared to the left (2.68 ± 0.47 cm), p-value 0.01. This trend of significant difference persisted at the 24-month assessment, where the right side again showed a larger size (2.86 ± 0.54 cm) than the left (2.71 ± 0.55 cm), with a p-value of 0.01, indicating a statistically significant difference at longer-term follow-ups.

DISCUSSION

The study results indicate that the "reverse sandwich" technique, used on the right side, resulted in significantly larger fusion masses at 3, 9, and 24 months postoperatively compared to the "bread and jam" technique used on the left side. This suggests that the "reverse sandwich" technique may be more effective in promoting bone fusion in the both early and long-term postoperative periods.

In a retrospective study conducted by Brodsky et al., a correlation of 64% was shown between pre-operative plain radiography and surgical exploration. The study focused on 214 lumbar fusion exploration procedures performed on patients who had previously had posterior lumbar fusion (PLF).[7] In their study, Kant et al. (1995) found that plain radiography demonstrated a sensitivity of 89% and specificity of 60% in its ability to predict solid fusion. The radiographic images, when analyzed for fusion, had a positive predictive value (PPV) of 76%.[8]

TABLE 1. Baseline characteristics.

N = 59	N (%) | Mean ± SD
Female	50 (84.75%)
Age (years)	63.17±9.51
History of smoking	3 (5.08%)
Diagnosis	
Spinal stenosis with spondylolisthesis	47 (79.66%)
Spinal stenosis	12 (20.34%)
Main Clinical presentation	
Radiculopathy	28 (47.46%)
Neurogenic claudication	24 (40.68%)
Mechanical low-back	6 (10.17%)
Neurological deficit	1 (1.69%)
Number of level of surgery	
1 level	42 (71.19%)
2 levels	12 (20.34%)
3 levels	5 (8.47%)

TABLE 2. Radiographic outcome.

	Right fusion mass size (cm)	Left fusion mass size (cm)	P-value
3 Month	2.65 ±0.57	2.46 ±0.40	0.01*
6 Month	2.57 ±0.61	2.56 ±0.56	0.39[a]
9 Month	2.95 ±0.56	2.68 ±0.47	0.01*
12 Month	2.75 ±0.56	2.67 ±0.50	0.49[a]
24 Month	2.86 ±0.54	2.71 ±0.55	0.01*[a]

The high intra-observer and inter-observer reliability for fusion assessment demonstrates the consistency and reproducibility of the radiographic evaluation method used in this study. This strengthens the validity of the findings and suggests that the assessment method can be reliably used in future studies.

Differences in fusion mass size between the 'reverse sandwich' and 'bread and jam' techniques may result from bone graft placement and its impact on the biological environment, rather than biomechanical factors. During the bone graft healing process, the establishment of an adequate blood supply is important for the formation of new bone.[9] In the "reverse sandwich" technique, the cancellous bone is situated on both sides of the graft, potentially providing a more favorable environment for fusion due to increased blood supply and osteogenic potential. This bilateral cancellous placement may enhance the vascularization of the graft site, increase surface area, promoting more robust bone growth and fusion. The use of autologous bone graft, particularly iliac crest bone, is effective in promoting fusion due to its osteoconductive, osteoinductive, and osteogenic properties. The "reverse sandwich" technique may further leverage these properties by creating a more surface area conducive environment for bone healing and remodeling. The structural strength and rigidity of cortical bone contribute to graft stabilization and minimize the risk of migration, while vascularized cancellous bone enhances new bone formation through improved vascular and cellular integration, thereby accelerating the fusion process.

This study has several limitations that should be acknowledged. First, the sample size was relatively small, with only 60 patients included. While the self-controlled design minimized inter-patient variability, the limited number of cases reduces the generalizability of the findings and may have impacted the statistical power to detect differences in fusion outcomes across the techniques. Future studies with larger cohorts and multicenter trials are needed to confirm the results and strengthen the evidence. Additionally, while the current study suggests that the "reverse sandwich" technique might be a preferred method for uninstrumented fusion to different bone graft placement technique, further research is necessary to confirm these findings in a larger patient population and to explore the biological mechanisms including cellular and molecular processes, involved in bone growth and fusion that could responsible for the observed differences between the two different bone graft placement techniques. Moreover, long-term clinical outcomes of patients undergoing these different techniques should be evaluated to determine if the larger fusion mass associated with the "reverse sandwich" technique translates into improved clinical outcomes, such as pain relief and functional improvement. Additionally, comparing these techniques of uninstrumented posterolateral fusion with instrumented posterolateral fusion could provide a broader context of difference between instrumented and uninstrumented fusion.

Furthermore, it is important to note that while plain radiographs were used in this study, their accuracy in assessing fusion can vary. Our hospital did not routinely perform CT scans to detect fusion mass during the study period, relying instead on plain radiographs, which has limitations in accurately assessing fusion. Future research may benefit from utilizing computed tomography (CT) scans for more precise evaluation. Including patient-reported outcome measures (PROMs) in future studies would also provide valuable insights into the impact of different fusion techniques on patients' quality of life and functional status.

CONCLUSION

In conclusion, the "reverse sandwich" technique, with cancellous bone on both sides of the graft, may

provide a superior environment for fusion compared to the "bread and jam" technique, in which cancellous bone is limited to one side. Enhanced vascularization on both sides in the "reverse sandwich" technique may improve the biological environment for fusion without significant differences in biomechanics. Further research is warranted to validate these findings and elucidate the underlying biological processes.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available due to privacy or ethical restrictions.

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DECLARATION

Grants and Funding Information

None

Conflict of Interest

The authors hereby declare no personal or professional conflicts of interest relating to any aspect of this study.

Registration Number of Clinical Trial

This study is retrospective, clinical registration is not required.

Author Contributions

C.C. Project administration, conceptualization, and illustrated figure. H.K. Collecting data and editing manuscripts. K.M. Collecting data, analyzing data, and editing manuscripts. B.S. Project administration, conceptualization, collecting and analyzing data, and writing and editing manuscripts.

Use of Artificial Intelligence

None

Ethics approval

The protocol for this study was approved by the Siriraj Institutional Review Board (SIRB) (COA no. Si 368/2019), and written informed consent was not obtained due to the retrospective nature of this study.

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Immunohistochemical Markers Associated with Meningioma Recurrence: A Systematic Review

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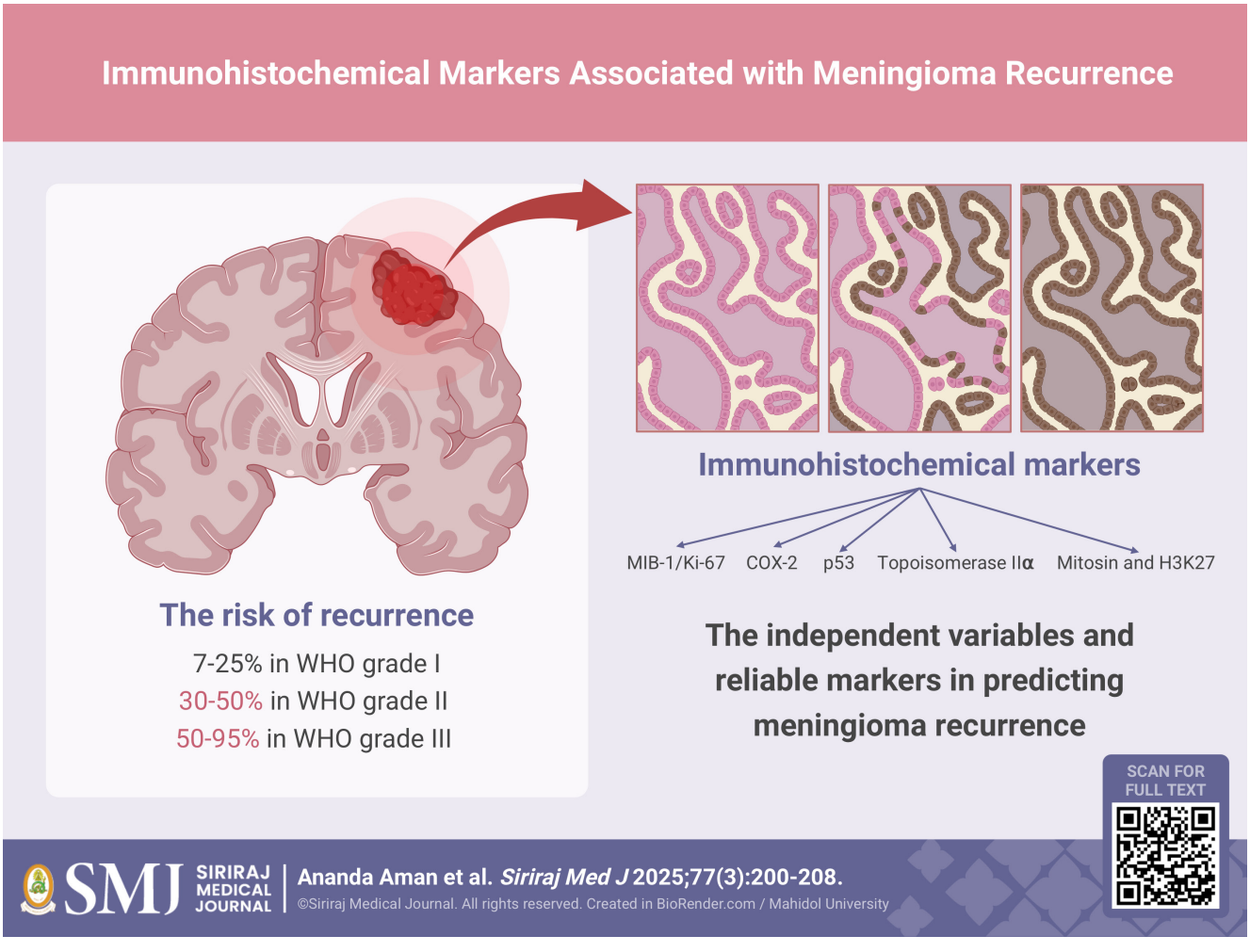

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ABSTRACT

Objective: This study aims to know the role of immunohistochemical markers in the recurrence of surgically treated meningiomas.

Material and Methods: We conducted a comprehensive search of the PubMed, ScienceDirect, Cochrane Library, and Google Scholar databases to locate studies published within the past decade. The inclusion criteria for this study were patients aged 18 years or older who had undergone surgical treatment for meningioma. Studies that were not written in English, case report studies, case series studies, literature review studies, and studies involving patients who received treatment other than surgery or multimodal therapy were excluded. All studies that met the inclusion criteria were subjected to critical appraisal.

Results: Four studies comprising 3176 cases of meningioma cases were included in the analysis. Multivariate analysis showed that two immunohistochemical markers (COX-2 and MIB-1/Ki-67) were independent variables for meningioma recurrence. This study also found no statistical differences between grade I and grade II meningiomas with respect to the overexpression of COX-2 and MIB-1/Ki-67. The second study compared the nonrecurrence/relapse (non-R/R) and recurrence/relapse (R/R) groups and found a significant correlation with MIB-1 percentage, intensity, histoscore, and p53 percentage, regardless of tumor grade. The third study found that mitosin and topoisomerase IIα were significant predictors of recurrence but not MIB-1. The fourth study demonstrated that H3K27me3 loss is significantly associated with more aggressive meningiomas.

Conclusion: Our study concluded that MIB-1/Ki-67, COX-2, p53, topoisomerase IIa, mitosin and H3K27 were independent variables and reliable markers in predicting meningioma recurrence.

Keywords: Immunohistochemical marker; meningioma; recurrence (Siriraj Med J 2025; 77: 200-208)

INTRODUCTION

Meningioma accounts for 20-30% of all primary brain tumors; it is considered the most common central nervous system tumor with benign characteristics and an excellent prognosis if resected utterly.[1] Meningiomas are stratified into three groups, according to the 2016 World Health Organization (WHO) classification scheme: grade I (benign), grade II (atypical), and grade III (anaplastic).[2] The risk of recurrence is 7-25% in WHO grade I, 30-50% in WHO grade II and 50-95% in WHO grade III.[3] Gross total microsurgical resection is the initial choice of treatment, and mostly the outcomes improved post-surgery.[4] The completeness of removal is classified according to the Simpson Grading System on a 5-tier scale. Grade I is the complete removal of the tumor with resection of the dura and bone that are involved; Grade II is the resection of the tumor entirely with coagulation of the dural attachment; Grade III is total removal of the intradural tumor without resection or coagulation of the dura or extradural component; Grade IV is subtotally tumor removal; and Grade V is simple decompression. The recurrence rates increased by Simpson Grades; they were 9, 19, 29, and 40% at ten years in Grade I to IV.[5] Recently, one study showed Simpson Grade IV was significantly correlated with a high risk of recurrence compared to Simpson Grade I, II, and III in meningioma WHO Grade I.[6] Another paper concluded that patients who underwent Simpson Grade I and II surgery gained improvement in recurrence-free survival (RFS) significantly compared to patients with Simpson Grade III and IV.[7]

Despite the removal of the entire tumor (Simpson Grade I-III), the recurrence of benign meningioma remains a concern. A study revealed that recurrence in completely resected Grade I meningiomas accounted for 15% of the 10-year follow-up. In addition to histopathological findings and Simpson grade, immunohistochemical markers are increasingly being recognized as contributing factors to meningioma recurrence. We conducted a systematic review to assess the role of immunohistochemical markers in the recurrence of surgically treated meningiomas.

MATERIALS AND METHODS

Search strategy

We thoroughly searched for available literature from the last ten years in the electronic databases PubMed, ScienceDirect, Cochrane Library, and Google Scholar through December 31, 2023. The inclusion criteria were surgically treated meningioma patients and age > 18 years. Non-English articles, case report studies, case series studies, literature review studies, and studies on patients who received non-surgical treatment or multimodality therapy were excluded. Keywords used to find relevant literature: "meningioma" AND "recurrence OR free-recurrence

survival" AND "biomarkers OR immunohistochemical markers OR markers". An initial search identified 105 articles from PubMed, ScienceDirect, the Cochrane Library, and Google Scholar. Two reviewers (DRS and SAHP) independently screened the titles and abstracts of all the initially identified studies and read the full text to determine whether the studies were relevant to the research question. Any inconsistencies were resolved through a discussion with the first author (RAA). We did not review ongoing relevant studies.

Quality assessment

The reviewers critically appraised the selected articles using a critical appraisal tool for prognostic studies. Cohort and case control studies assessed using Newcastle and Ottawa Quality Assessment Scale. Systematic review and meta-analysis appraised using Oxford Critical Appraisal Worksheet. Any disagreements were resolved through discussion between the two reviewers or by consulting with other authors.

RESULTS

Searching result

The detailed literature selection process is presented in Fig 1. Six journals relevant to our research question were retrieved after the titles and abstracts were screened, full-text read, and double-excluded. Three articles were excluded after being critically appraised, resulting in four studies being included in this systematic review. Quality assessment reported in Table 3 and Table 4.

Study characteristics

Four studies, comprising 3176 cases of meningioma cases were selected. Three studies were conducted in Europe and one in the USA, and all were single-center studies. All four studies were retrospective observational cohort studies conducted between 2012-2022. The sample size ranged from 70 to 292 patients, all surgically treated between 1980 and 2022. Two papers contained meningioma WHO Grades I and II without WHO Grade III, and the other two studies constituted samples of meningioma WHO Grades I, II, and III. Tumors of 1448 patients from two studies underwent total removal (Simpson Grade I-III), whereas the tumors of 660 patients from two other studies underwent total or subtotal resection. All patients in each study were followed up; the duration of follow-up in one study was 0-96 months with a median of 96 months, the second study did not mention the duration of the follow-up but evaluated the recurrence of meningioma in 5 years, the third study followed up patients within a range of 1.5 – 25 years with a mean of eight years and two months, and the fourth study followed up patients within the range of 3 – 17.5 years. Radiological workup in all studies evaluated tumor recurrence, and one study used post-mortem studies to assess recurrence.

TABLE 1. Research question.

Study Component	
Patient	Meningioma
Intervention/Exposure	Immunohistochemical marker
Comparison	-
Outcome	Recurrence/Free-Recurrence Survival

TABLE 2. Inclusion and exclusion criteria.

Inclusion	Exclusion
Surgically treated meningioma patients	Case report study
Age ≥ 18 years old	Case series study
Last ten years of study	Literature review study
English language articles	Received treatment other than surgery of multimodality therapy

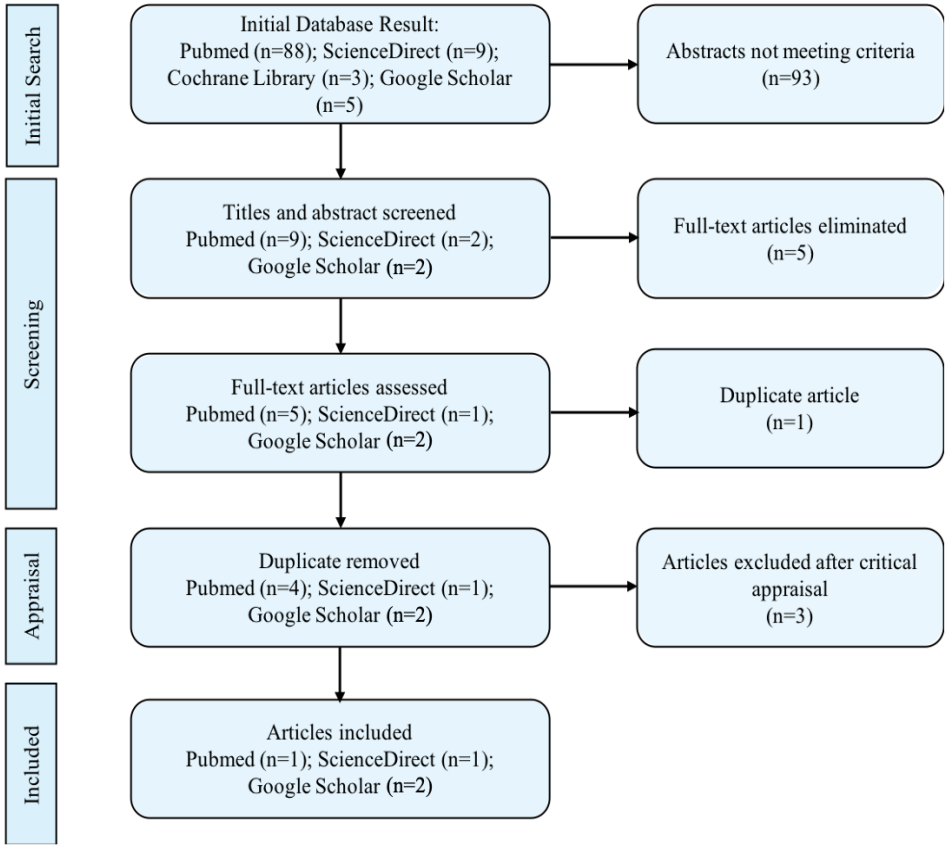

Fig 1. Flowchart of a screening strategy for included studies.

TABLE 3. Newcastle and Ottawa Quality Assessment for Cohort and Case Control Study.

Reference	Year	Design Study	Appraisal Selection	Comparability	Outcome	Score	Quality
Sanz et al[8]	2013	Cohort	****	*	***	8	Good
Csonka et al[9]	2016	Case Control	****	*	***	8	Good
Winther et al[10]	2017	Case Control	***	*	***	7	Good

TABLE 4. Oxford Quality Assessment for Meta-Analysis Study.

Appraisal Cello et al (2023)[11]	Answer
What question (PICO) did the systematic review address?	Yes
Is it unlikely that important, relevant studies were missed?	Yes
Were the criteria used to select articles for inclusion appropriate?	Yes
Were the included studies sufficiently valid for the type of question asked?	Yes
Were the results similar from study to study?	Yes

TABLE 5. Characteristics of Eligible Studies.

Reference	Year Published	Country	Sample Size	Histopathological Grade	Extent of Resection	Follow-Up
Sanz et al.[8]	2013	Spain	135	I, II	Gross Total Resection	1.5 - 25 years (mean: 8 years two months)
Csonka et al.[9]	2016	Hungary	70	I, II, III	Gross Total Resection	Five years
Winther et al.[10]	2017	Norway	160	I, II	Gross Total Resection, Subtotal Resection	0 - 96 months (median: 96 months)
Cello et al.[11]	2023	USA	2811	I, II, III	Gross Total Resection, Subtotal Resection	3 – 17.5 years

Immunohistochemical Markers Relevant to Recurrence

Csonka et al. established two study groups: patients with one or more recurrence/relapses (R/R group), and patients with meningioma without any radiological or postmortem evidence of recurrence/relapse (non-R/R group). The research did not mention the follow-up duration but evaluated the recurrence outcome within five years after resection. Of seventy meningioma patients with meningioma underwent complete surgical removal. The value of markers was measured as the percentage of cell immunopositivity, average labeling intensity score (0-3+), and histoscore (multiple of the percentage of positive cells and average intensity). WHO tumor grade was correlated significantly with MIB-1 Labelling Index (LI) (%) ($p < 0.001$), MIB-1 staining intensity ($p = 0.001$), MIB-1 histoscore ($p < 0.001$), p53 staining intensity ($p < 0.001$), p53 histoscore ($p = 0.031$), Progesterone Receptor (PR) LI (%) ($p < 0.001$), PR intensity ($p < 0.001$), PR histoscores ($p < 0.001$). Comparing the non-R/R and R/R groups, regardless of the grades of the R/R group, there was a significant correlation with the MIB-1 LI (%) ($p < 0.001$), MIB-1 intensity ($p = 0.004$), MIB-1 histoscore ($p < 0.001$), and p53 LI (%) ($p = 0.027$). The study also found a significant correlation between the non-R/R and R/R groups, with only WHO Grade I tumors in the R/R group, with MIB-1 LI (%) ($p = 0.009$), MIB-1 histoscore ($p = 0.029$), p53 LI (%) ($p = 0.032$), and p53 histoscore ($p = 0.038$). The authors concluded that p53 and MIB-1 were sufficient to characterize meningioma immunohistochemically in terms of recurrence risk.[9]

TABLE 6. Summary Table of Tumor Recurrence in Association with Immunohistochemical Markers.

Author, Year	Markers	Tumor Grade	Time of Follow-Up	Effect Size
Sanz et al., 2012[8]	COX-2	I, II	3, 5, and 10-year	95% CI 1.1-9.68; p=0.01
	MIB1/ki67	I, II	3, 5, and 10-year	98% CI 1.12-10.55; p=0.031
Csonka et al., 2016*[9]	p53	I, II, III	5-year	p=0.027
	MIB1/ki67	I, II, III	5-year	p<0.001
	p53	I	5-year	p=0.009
	MIB1/ki67	I	5-year	p=0.032
Winther et al., 2017[10]	MIB1/ki67	I, II	8-year**	95% CI 0.84–4.22; p=0.127
	Topoisomerase IIα	I, II	8-year	95% CI 1.04–4.47; p=0.04
	Mitosin	I, II	8-year	95% CI 1.87–852; p<0.01
Cello et al., 2023[11]	HK327	I, II, III	3, 6, 15 and 17.5 year	(95% CI 1.35–2.15); p<0.01

*markers measured with percentage of cells immunopositivity
**median 96 months

A study by Sanz et al. evaluated 135 patients who underwent complete surgical resection of meningioma (Simpson Grade I, II, III) with a mean follow-up of 8 and two months (range one year and six months to 25 years). Markers were measured as the percentage of cells. COX-2 was considered positive if >10%, Cyclin A considered positive if >3%, MIB-1/Ki-67 was considered positive if >4%, Topoisomerase IIα was considered positive if >4, and TIMP2 was considered positive if >0. Some factors were relevant to RFS, including the expression of immunohistochemical markers COX-2 (9 positive cases and 126 negative cases, p = 0.001), Cyclin A (9 out of 130 valid cases, p = 0.002), MIB-1/Ki-67 (6 out of 133, p = 0.01), Topoisomerase IIα (8 out of 133, p = 0.04), and TIMP2 (16 out of 131, p = 0.04). Multivariate analysis was performed; the study did not include Topoisomerase IIα because of significant co-variation with MIB-1/Ki-67 (p < 0.001) and similar prognostic information. It revealed COX-2 (HR 3.28; 95% CI 1.10-9.68; p = 0.032) and MIB-1/Ki-67 (HR 3.44; 98% CI 1.12-10.55; p = 0.031) were independent variables. A table in the study showed several patients with positive COX-2 related to recurrence over time: 20% of patients had recurrence in 3 years, 30% in 5 years, and 58% in 10 years. The same table also shows the correlation between the positivity of MIB-1/Ki-67 and recurrence: 16.7% of patients had a recurrence at 3 years, 37.5% at 5 years, and 58.3% at 10 years. Another table shows that the expression of COX-2 and MIB-1 was not significantly correlated with the histopathological grade of meningioma (WHO Grade I and II) and grade of resection (Simpson Grade I, II, and III). A Kaplan-Meier curve showed the antagonistic effect of COX-2: patients with negative COX-2 expression had significantly longer RFS than those with positive COX-2 expression. Other markers in this study, such as pAKT, Bcl-2, Cadherin E, Caspase 3a, β-catenin, cathepsin D, CD44, EGFR, HER2, MDM2, MMP9, p21, PDGF, PTEN, progesterone receptor, Survivin, TGF, and VEGF, were not independent variables for recurrence. This study concluded that COX-2 and MIB-1 were independent prognostic factors for recurrence-free survival, and the expression of these markers could predict meningioma recurrence.[8]

Winther et al. included 160 patients, of whom 75.6% underwent gross total resection surgery (Simpson Grade I or II) and 24.4% underwent subtotal resection. The duration of follow-up to evaluate RFS was 0-96 months (median: 96 months). Markers were scored using proliferation indices (PI) based on the percentage of positive immunoreactive nuclei among 1000 tumor cell nuclei with the most significant proliferative activity (hot spots). All analyzed markers (MiB-1, topoisomerase IIα, and mitosin) were expressed at higher levels in atypical meningiomas than in benign meningiomas (p < 0.028). This study showed that topoisomerase IIα and mitosin PI were significant predictors of recurrent tumors (p < 0.039); however, MIB-1 PI was not a significant predictor. (p < 0.497). Among the two markers, mitosin expression was the most accurate discriminator between recurrent and nonrecurrent tumors. Topoisomerase IIα and mitosin expression were significant variables associated with RFS (p < 0.001). In another study, MIB-1 expression and histopathological grade were not associated with RFS (p < 0.127). The table shows that Topoisomerase IIα and mitosin expression were significantly higher in meningioma WHO Grade II than Grade I. Mitosin was the only factor that could significantly predict RFS in multivariate analysis (hazard ratio = 4.80, p < 0.001). In contrast, the association between topoisomerase IIα expression and RFS was not statistically significant (p = 0.052). The MIB-1 PI remained a non-significant factor (p < 0.158).[10]

DISCUSSION

Meningiomas are the most common intracranial primary tumors found in the central nervous system; around 36.4% of all CNS tumors generally appear as benign, slow-growing, and non-infiltrating lesions.[12] However, approximately 10% of the cases appear as histologically malignant lesions and/or are overgrown. Meningiomas originate from arachnoidal cap cells of the leptomeninges.[13] As classified by the World Health Organization (WHO), meningiomas are classified into three grades: benign (grade I), atypical (grade II), and anaplastic/malignant (grade III). Meningiomas that express a malignant phenotype are most susceptible to recurrence. Atypical meningiomas occur in about 4.7-7.2% of all cases and generally have recurrence rates ranging from 29-52% after resection. Anaplastic meningioma only occurs around 1.0-2.8% of all meningioma cases but has a recurrence rate of around 50-94% of cases.[14] The knowledge about immunohistochemistry is needed for patients' education about prognosis and recurrence of meningioma, especially higher grade meningioma. Immunohistochemistry results are also required for effective communication between pathologists and physicians to achieve a diagnosis of malignancy.[15]

Histological level and extent of resection are the two most important predictive factors for recurrence. In higher-grade tumors (i.e., grades II and III), there is an increase in cellularity and a higher level of mitotic and necrotic lesions, which can predict an increased

probability of recurrence, thereby generally resulting in unfavorable outcomes. Based on this, atypical meningiomas have recurrence-free survival, and the median time for recurrence is significantly longer than that for anaplastic meningiomas. Despite recurrence is not only the appearance that occurs in high-grade meningiomas because this can also occur in low-grade meningiomas, even with a lower frequency of occurrence.[16]

The recurrence rate of meningiomas depends on the extent of the resection. The recurrence rate after resection in various studies has shown varying results, ranging from approximately 15% to 25%. Simpson classified the extent of tumor resection into five grades. This resection-level classification system has shown that maximum meningioma treatment can be achieved by gross-total resection, including extensive resection of the attached dura and underlying bone.[17]

Previous research has shown a strong correlation between the risk of recurrence in meningiomas and their molecular profile. In a 2019 study by Ros-Sanjuan et al., atypical meningiomas were observed, and it was found that a high Ki-67 index or histological appearance was commonly present in cases of meningioma recurrence.[18] A study by Sumkovski et al. concluded that the mitotic index is an independent predictor of meningioma recurrence, and the anti-Ki-67 antibody was used to determine the mitotic index.[19] Ning Liu et al. conducted a systematic review, which provided evidence of Ki-67's role in meningioma recurrence.[20] This review found that the marker had a significant association with worse recurrence-free survival and overall survival. A systematic review also showed that Ki-67 was significantly associated with recurrence, except for one study in Norway, which concluded that Ki-67 was not significantly associated with recurrence. Despite this, Ki-67 has been proven in various extensive studies as an immunohistochemical marker that plays a role in recurrence. Ki-67 protein is expressed in cells undergoing mitosis and is detected in routine pathological diagnostics using an MIB-1 antibody clone. Therefore, Ki-67 protein is an excellent marker for detecting cell proliferation in a specific cell population.[16]

The research conducted by Winther et al. demonstrated that topoisomerase IIa is a more effective predictor of recurrence than Ki-67.[10] Their study is supported by numerous previous studies, including one conducted by Korshunov et al., which showed that both Ki-67 and topoisomerase IIa were significantly associated with recurrence. However, the multivariate analysis demonstrated that only topoisomerase IIa had a significant association with recurrence.[21] Other studies by Kunishio et al. showed that topoisomerase IIα was a better predictor of recurrence than Ki-67, but these studies concluded that the two markers had a significant association with recurrence-free survival.[22] Research conducted by Konstantinidou et al. showed that mitosin, a cell proliferation marker, was a significant predictor of early recurrence.[17] Nonetheless, only a few studies have examined the role of mitosin in predicting the prognosis of patients with meningiomas.

Another frequently examined immunohistochemical marker is p53. More than 50% of tumors have a p53 mutation; therefore, this marker is considered to be one of the most critical tumor suppressor proteins. However, the role of p53 in the occurrence of recurrence remains unclear, although it has been observed in various large-scale studies.[17] Cho et al. found that the immunoreactivity of p53 was significantly higher in recurrent meningiomas than in non-recurrent meningiomas.[3] Nevertheless, several studies have shown no significant association between p53 expression and recurrence in meningiomas.[23,24] Our systematic review and research conducted by Csonka et al. showed that p53 expression plays a significant role in meningioma recurrence. The p53 marker was significantly associated with tumor recurrence, regardless of the tumor histology level, and was also an independent predictor of the incidence of recurrence in WHO Grade I meningiomas.

Multivariate analysis by Sanz et al. demonstrated that cyclooxygenase-2/COX-2, along with MIB-1/Ki-67, served as a significant predictor of recurrence. COX-2 functions as a survival factor under various cellular stress conditions and protects cancer cells from apoptosis by regulating the expression of Bcl-2 family proteins. Furthermore, COX-2 inhibits anoikis or the process of cell death during detachment by activating the PI-3K/Akt pathway. This anti-anoikis effect of COX-2 may contribute to tumor development and progression.[25] Research conducted by Ruiz et al. confirmed that COX-2 levels were significantly higher in the recurrent meningioma group.[19] Additionally, Cello et al. noted that H3K27me3 loss was associated with worse prognoses for patients with meningiomas. H3K27me3 loss was significantly linked to higher-grade meningiomas, male gender, recurrent meningiomas, and the requirement for adjuvant radiation therapy. Therefore, H3K27me3 loss can serve as a robust prognostic marker.[11]

Despite our systematic approach to this review, several limitations should be mentioned regarding our findings and wider evidence-based studies. The final analysis did not include several non-English unpublished studies without sufficient data. Therefore, more well-defined and large-scale prospective studies are required to confirm our findings. Further meta-analysis is required

to obtain an association statistical analysis for a better conclusion.

CONCLUSION

The molecular characteristics of tumors are independent risk factors for recurrence of meningiomas, regardless of the histological grade and resection extent. Proliferative markers such as MIB-1/Ki-67, COX-2, p53, topoisomerase IIα, mitosin, and H3K27me3 were identified as independent variables and reliable predictors of meningioma recurrence. Further research is required to discover additional markers to enhance the understanding of meningioma characteristics for researchers and clinicians.

Data Availability Statement

The data supporting the findings of this review article are available from the cited primary literature sources. No new data were generated or analyzed for this study.

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DECLARATION

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There are no grants and funding sources in this research.

Conflict of Interest

The authors have no conflicts of interest to declare.

Registration Number of Clinical Trial

There is no clinical trial number because this study is not a clinical trial/experimental study.

Author Contributions

All the authors contributed to the conception and design of the study. RAA and DRS prepared the materials, DRS and SAHP review article, DRS, RM, SAHP, MRH, and FS collected the data, and RAA and FS performed the analysis. RAA, SAHP and FS drafted the manuscript. All authors have read and approved the manuscript.

Use of Artificial Intelligence

This study did not use artificial intelligence.

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Effectiveness of a Brain Training Program on the Cognitive Function of Sepsis Survivors: A Randomized Controlled Trial Study

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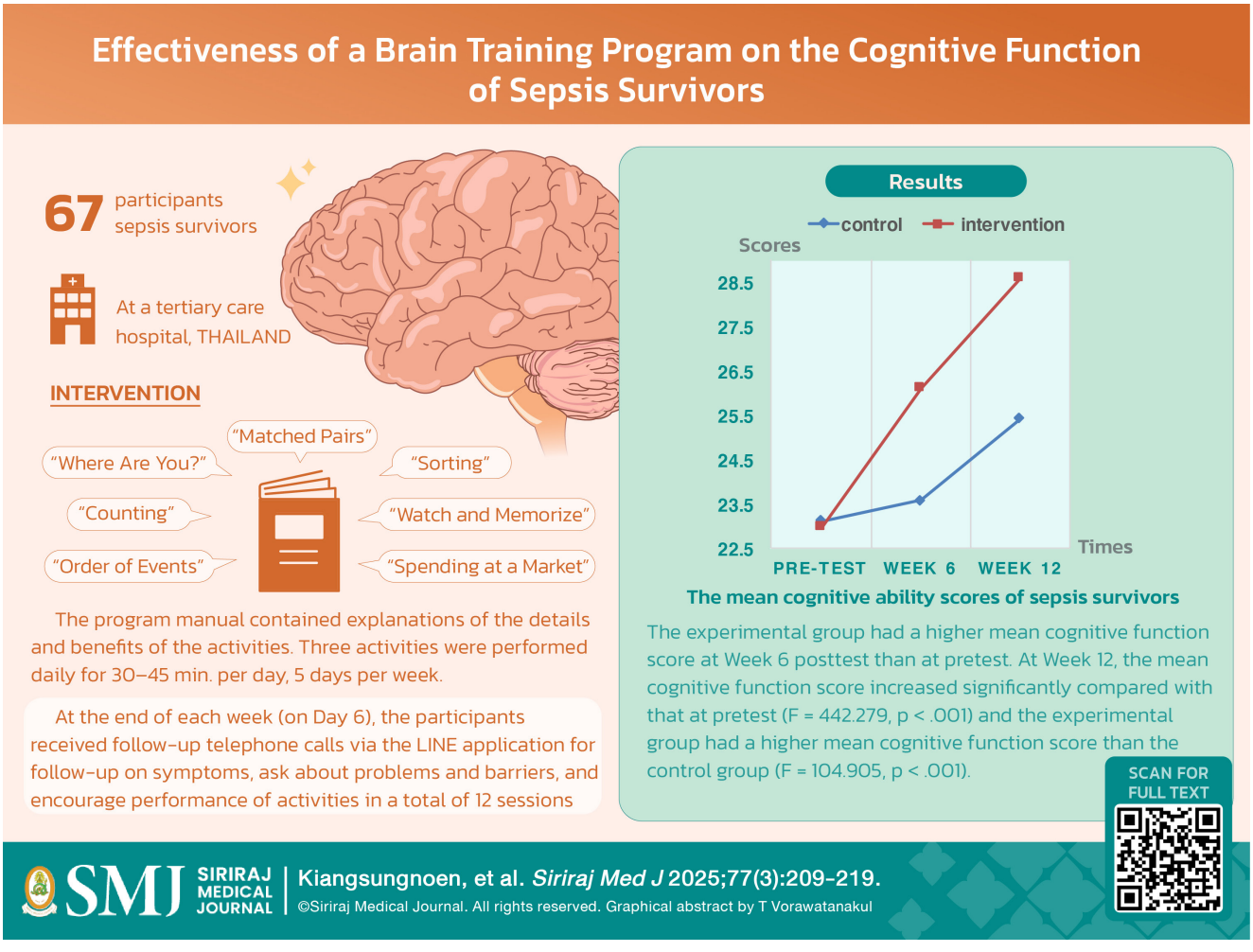

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ABSTRACT

Objective: This study aimed to evaluate the effectiveness of a brain training program designed to enhance the cognitive function of sepsis survivors.

Materials and Methods: We conducted a single-blind randomized controlled trial at a tertiary care hospital involving 67 participants aged over 18 years with participants randomly assigned to two groups, an experimental group (n=33) receiving the brain training program, and a control group (n=34) receiving standard care only. We measured cognitive function at three different time points: Baseline, Week 6, and Week 12, using the Thai Mental State Examination for testing and repeated measure ANOVA for statistical analysis.

Results: The experimental group had a higher mean cognitive function score at Week 6 posttest than at pretest. At Week 12, the mean cognitive function score increased significantly compared with that at pretest ($F = 442.279$, $p < .001$) and the experimental group had a higher mean cognitive function score than the control group ($F = 104.905$, $p < .001$).

Conclusion: The brain training program significantly increased the cognitive function levels of sepsis survivors in 6–12 weeks. The result of this study shows the benefits of a brain training program in increasing cognitive functions. Therefore, such a brain training program should be implemented among sepsis survivors to improve their cognitive functions.

Keywords: Sepsis survivors; brain training program; cognitive functions (Siriraj Med J 2025; 77: 209-219)

INTRODUCTION

Sepsis is a major current public health problem worldwide. A report on the analysis of the global sepsis situation in 2017 revealed that 48.9 million patients have had sepsis and approximately 38 million survive sepsis.[1] According to estimates on sepsis incidence and deaths from 15 countries from 1979 to 2015, 19.4 million patients experience sepsis annually. Although the mortality rate is lower, the number of sepsis survivors increased to 14.1 million patients annually (72.68%).[2] This finding is consistent with a report showing that medical advances in the treatment of sepsis have enabled patients with sepsis and those with severe cases to gain greater access to treatment and care in the intensive care unit, thereby doubling their survival rates.[3,4]

Currently, reports of sepsis survivors are increasing due to rapid access to treatment and increased quality of related clinical treatments; specifically, more stringent screening, concise diagnosis criteria, fast diagnosis, and clearer treatment guidelines have led to an increase in the number of survivors by 40%. After hospital discharge survivors have a mortality rate of 30-50% in 2 years, whereas 60% recover normal function within 1 year.[5-7] Moreover, sepsis survivors usually contract post-infection illnesses called post-sepsis syndrome with similarly increasing incidence and effects causing impaired psychological and physical balance.[8]

Psychological effects usually cause reduced cognitive function, attention deficiency, incoherence, reduced speaking ability and poor executive functions in the long term.[9,10] After hospital discharge, patients experience anxiety and abnormal psychological symptoms. Consequently, depression manifests with physically fatigue, appetite loss, and exhaustion, all of which weaken the immune system and increase the likelihood of severe recurrent infections affecting return to work, quality of life, and family relationships.[6] In these patients, common physical effects include the inability to work independently, reduced ability to perform the activities of daily living such as toileting or bathing and financial management due to muscle fatigue and damaged peripheral nerves. Cognitive impairment is a frequently encountered and significant starting point of psychological and physical effects in patients with post-sepsis syndrome.

The evidence shows that long-term cognitive impairments are commonly observed among sepsis survivors, with up to 50% experiencing neurocognitive impairment.[6,11] Around one in six patients, particularly those recovering from severe sepsis, may develop significant cognitive and physical impairments within the first 72 hours of recovery.[11] These impairments result from systemic inflammation that affects brain function.[6] Additionally, these patients are at higher risk of cognitive deficits such as memory and executive function impairments, which can significantly reduce quality of life and increase the need for rehabilitation.[6,11] Moreover, 6.1% of patients had cognitive impairment before hospitalization compared with 16.7% who had it after hospital discharge. One year after hospital discharge, 25%–45% of patients experience cognitive impairment. Among patients treated in an

intensive care unit because of sepsis, the incidence of cognitive impairment was 79% in the first 3 months and persisted for at least 8 years after hospital discharge.[12]

Sepsis triggers immune system cells to release more high-mobility group box 1 (HMGB1) protein, causing permanent inflammation and neurocognitive impairment, affecting the neuroendocrine system and causing impairments in memory, attention, verbal fluency, and executive functions.[8,13] Although natural recovery from cognitive impairment to normal conditions within 1 year is possible, patients cannot fully recover alone. In the long term, sepsis survivors encounter difficulties in returning to work. Memory, attention, and executive function impairment continue to affect quality of life and family burden.[6] However, despite the lack of direct treatment, patients can recover from cognitive impairment when the brain recovers; otherwise, patients require a long time to recover.

In the literature, cognitive function recovery has become possible through brain training programs focused on cognitive impairment in various areas, particularly attention, memory, and executive functions to treat patients with other brain injuries such as stroke. Currently, research has focused on the effects of cognitive rehabilitation programs for survivors of critical care, with sepsis survivors being a subgroup in some studies. However, there is a lack of research specifically on cognitive rehabilitation programs targeted at patients who have survived sepsis. Brain training programs have been proven to be effective and beneficial for patients. Therefore, to recover brain function, specific methods are needed. By organizing activities for practicing cognitive skills, nurses can help patients recover cognitive function by encouraging and promoting neurological recovery through brain training, which can begin immediately after patients are out of crisis from sepsis and have stable neurological symptoms. Activity models must be clear and repetitive until patients learn how to trigger nerve cell connections and cognitive recovery. Early cognitive function recovery after a crisis from sepsis can, therefore, result in the recovery of more cognitive function.[7,13]

MATERIALS AND METHODS

Study design and participants

This study was a single-blind randomized controlled trial with repeated-measures. Measurements were taken at 3 time points: Baseline, Week 6, and Week 12. This randomized trial was conducted from September 2022 to April 2023. The study was approved by Mahidol University Multi-faculty Cooperative IRB Review COA No. IRB-NS2022/691.2705.

The study population included male and female patients aged ≥18 years who had been diagnosed with sepsis, treated until they were out of crisis, and met the following inclusion criteria: survival after >72 hrs. of sepsis, no 2 of 4 symptoms, presenting symptoms based on the SIRS criteria 2021[14,15]; good consciousness, a TMSE score of 20–25 points (mild to moderate level), no depression (2Q), and co-habiting caregivers who possess a smartphone with internet or Wi-Fi access and ability to participate in the brain training program with patients.

The researcher calculated the sample size using power analysis. The required sample size calculation for repeated measures analysis of variance (ANOVA) tests is a sample of 68 participants (34 per group), with a power (p) of 0.80, a significance level (α) of 0.05, a medium effect size (f) of 0.25, and an attrition rate of 20%. The researcher prepared for the sampling process with the assistance of a research assistant who was not involved in the research project. The research assistant generated numbers 1–68 by using a computer program to randomize assignments. The researcher then used these numbers to designate participants as part of either Group 1 or Group 2. The numbers were then placed in sealed brown envelopes. Group 1 served as the control group and Group 2 as the experimental group. The researcher asked the participants to randomly select an envelope. The researcher then opened each sealed envelope to reveal the assigned number and grouped the participants into either the control group or the experimental group based on the numbers inside the envelope. To prevent dissemination of the program from the experimental group to the control group, the researcher conducted individual teaching sessions in designated rooms to train participants in the experimental group on brain training program usage. Participants in the control group were instructed to maintain a daily journal as part of their activities. Additionally, the researcher explicitly advised participants and their caregivers in both groups to avoid sharing any information or activities received during the study, beyond the standard nursing care provided by ward nurses, with other research participants.

Intervention

Both groups received routine care consisting of collection of demographic data, assessment of consciousness with the Glasgow coma scale, assessment of perception of dates, times, places, and persons, personal hygiene care, and consultation with a physical therapist to assess physical impairment. In patients who had impaired physical function, physical therapists began therapy immediately once vital signs and symptoms were stable.

The experimental group participated in the brain training program based on the literature review.[8,16,17] Before activities, the program stimulated patients' perception by having them meditate and count backward (30, 29, 28, 27…1), The program consisted of the following eight activities: "Where Are You?" "Matched Pairs", "Sorting", "Counting", "Watch and Memorize", "Order of Events", "Spending at a Market", and "Record of Emotions". The program emphasized the development of memory, recall, attention, and executive functional capacity. The program manual contained explanations of the details and benefits of the activities. Three activities were performed daily for 30–45 min. per day, 5 days per week. At the end of each week (on Day 6), the participants received follow-up telephone calls via the LINE application for follow-up on symptoms, ask about problems and barriers, and encourage performance of activities in a total of 12 sessions. The participants were granted 24-hour. access to the researchers for consultation throughout the study period.

The control group used the daily record form prepared by the researcher for the participants to plan activities and follow plans to increase cognitive function over 12 weeks.

The clinical trial protocol was registered with the Thai Clinical Trial Registry No. TCTR20240824003 on 19 September 2022 before enrollment of the first case.

Data collection and outcome

For data collection, the participants completed a demographic data questionnaire consisting of items on gender, age, weight, height, body mass index, marital status, education level, religion, occupation, mean monthly income, treatment rights, caregivers, alcohol consumption, use of the brain recovery program, and activities of daily living. Data on illness and treatment consisted of records of personal illnesses or concomitant diseases and blood test results. The researcher collected data from medical records in person by asking for permission from the participants. The depression level was assessed first. For participants who met the criteria, the TMSE was administered. The researcher evaluated the content validity and reliability of the instruments by consulting a total of five experts. The content validity was 0.85 in a sample of five patients. Using Cronbach's alpha coefficient, the reliability of the TMSE was 0.90 in a sample of 30 patients with similar characteristics. The researcher considered a reliability threshold of 0.80 acceptable. In the present study, the reliability in the sample of 68 participants was 0.90

Statistical analysis

The researchers performed all data analyses by using SPSS Statistics 25, using descriptive statistics to analyze demographic characteristics, as well as the history of illness and treatment. The researchers compared the differences between the experimental and control groups at Baseline by using chi-square tests, Fisher's exact tests, or independent t-tests. The researchers then compared the differences in mean cognitive function scores at Baseline, Week 6, and Week 12 between the experimental and control groups by using repeated measures ANOVA.

RESULTS

Overall, 68 participants completed the baseline assessment. One participant from the control group withdrew from the study. Since including this case did not meet the study's assumptions, the researchers excluded it as an outlier. Therefore, a total of 67 participants completed the study with an experimental group of 34 persons and a control group of 33 persons (Fig 1) The experimental group was composed of 44.1% males and 55.9% females, whereas while the control group was composed of 54.5% males and 45.5% females. The overall mean age was 54.52 years (SD = 9.001) with mean ages of 55.50 (SD = 8.969) and 53.52 (SD = 9.059) years in the experimental and control groups, respectively. Most participants had an elementary level of education (73.5% in the experimental group; 57.6% in the control group). The experimental and control groups had mean BMI of 23.56 (SD = 3.566) and 23.40 (SD = 3.465) Kg/m^2, respectively. Most of the participants in both groups had concomitant diseases (overall = 86.8%; experimental group = 91.2%; control group = 82.4%). The top three concomitant diseases found in the experimental and control groups were hypertension (50% and 32.4%), diabetes (20.6% and 38.2%), and kidney disease (17.6% and 20.6%), respectively. When the researcher compared the two groups by demographics, illness, and treatment data using chi-square statistics or Fisher's exact test and independent t-test, there were no significant differences ($p > .05$) (Table 1).

At Pretest, Week 6 posttest, and Week 12 posttest, in the control group, the researcher analyzed the variability in mean cognitive ability scores across repeated measurements at different time points using one-way repeated measures ANOVA. Preliminary tests for compound symmetry revealed that a failure to meet the assumption. Consequently, the researcher applied the Huynh-Feldt correction method ($p > .75$). The analysis showed significant differences in mean cognitive ability scores across at 3 time points: before the intervention at Week 6, at the conclusion of the study and at Week 12 ($F = 132.048$, $p < .001$). The experimental group met the assumption of compound symmetry, allowing for the use of the Sphericity Assumed method for data interpretation. The results also demonstrated

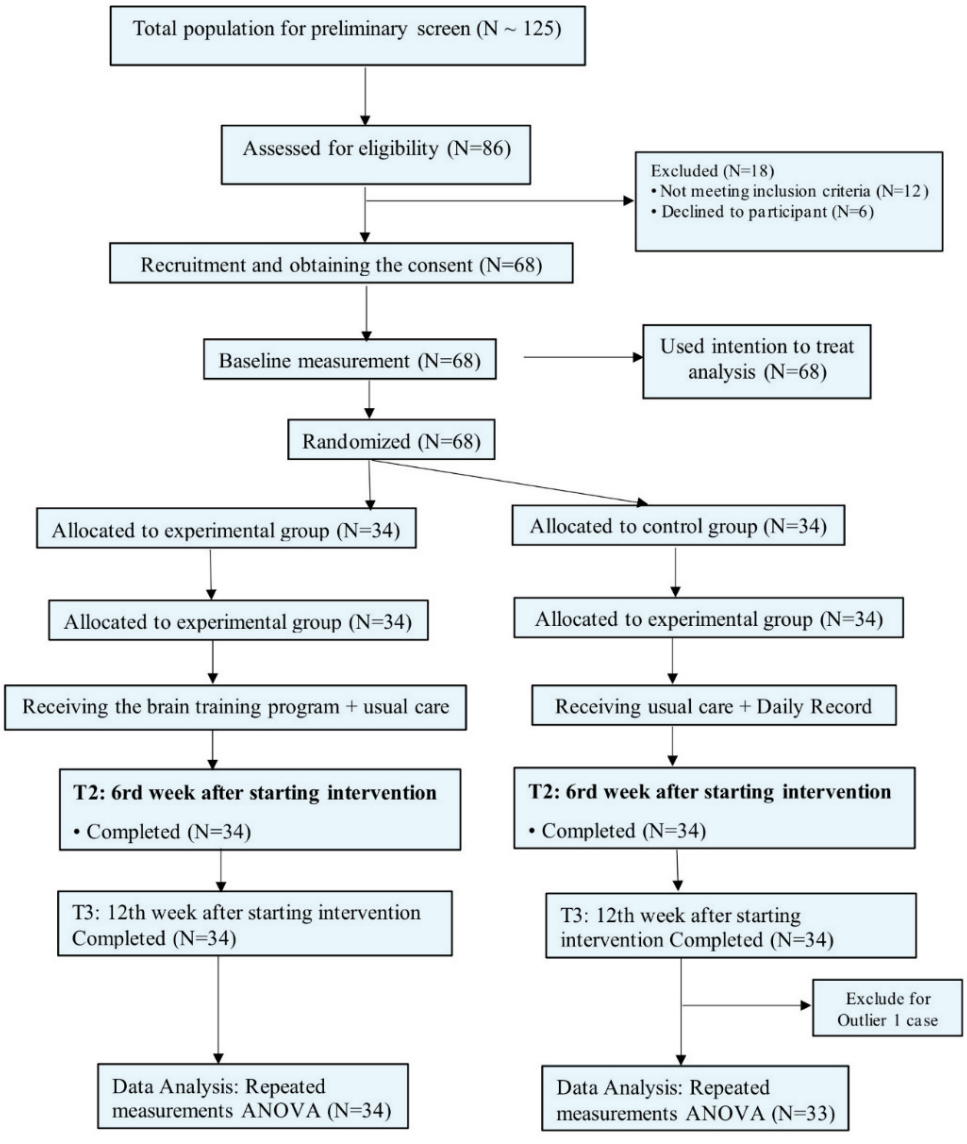

Fig 1. The flow diagram of the study

significant differences in mean cognitive ability scores across at 3 times ($F = 442.279$, $p < .001$).

In comparing the differences between pairs at each time point within the experimental group, the mean scores for cognitive functions differed in the following three pairs: 1) the mean score for cognitive function at Week 6 posttest had a higher score than that at pretest (a differences of 3.14 points); 2) the mean scores for cognitive functions at Week 12 posttest was higher than that at Week 6 (a difference of 2.47 points) 3) and the mean scores for cognitive functions at Week 12 posttest were higher than that at pretest (a difference of 5.61 points), showing a significant difference ($p < .001$) every time. (Table 2) The graph slope for the experimental group was higher than that for the control group. (Fig 2)

In comparing the differences in the mean scores of cognitive functions at each time point between the groups at pretest, Week 6 posttest, and Week 12 posttest, the time effect from Period 1 (pretest) to Period 3 (Week 12 posttest) in the experimental group showed a significant increase the mean scores for cognitive functions ($F = 534.702$, $p < .001$). Furthermore, the time * group interaction was significantly different ($F= 104.905$, $p < .001$). The control group had the same mean scores for cognitive functions, whereas the experimental group had consistently higher mean scores for cognitive functions. (Table 3)

DISCUSSION

Effects of the brain training program on the cognitive functions of sepsis survivors

The findings from this study support its hypothesis. After the brain training program, the experimental group had higher cognitive function levels at Week 6 and

TABLE 1. Demographic characteristics and history of illness and treatment in sepsis survivors (N = 67).

Demographic Characteristics	Experimental (n=34)		Control (n= 33)		$\chi 2$ / *t*-test	P - value
	Number	Percentage	Number	Percentage		
Gender					0.729	0.393
Male	15	44.1	18	54.5		
Female	19	55.9	15	45.5		
Education					-	0.587[F]
Uneducated/						
Primary Level	25	73.5	19	57.6		
High School	7	20.6	10	30.3		
Graduate Diploma	1	2.9	2	6.1		
Bachelor Degrees	1	2.9	2	6.1		
Age (years)					-	0.311[F]
≤ 45	4	11.8	7	21.2		
46-64	29	85.3	23	69.7		
≥ 65	1	2.9	3	9.1		
Mean (SD)	55.50 (8.969)		53.52 (9.059)			
MIN-MAX	33-69		32-66			
BMI (Kg/m^2)					-	0.901[F]
< 18.5	1	2.9	1	3.0		
18.5-22.9	16	47.1	17	51.5		
≥ 23	17	50.0	15	45.5		
Mean (SD)	23.56 (3.566)		23.40 (3.465)			
MIN-MAX	18.07-33.69		18.36-31.11			
Comorbidity					-	0.476[F]
No	3	8.8	6	17.6		
Yes (more than one)	31	91.2	28	82.4		
Hypertension	17	50.0	11	32.4	2.186	0.139
Diabetes	7	20.6	13	38.2	2.550	0.110
Kidney Disease	6	17.6	7	20.6	0.095	0.758
Dyslipidemia	4	11.8	4	11.8		1.000[F]
Cancer	6	17.6	2	5.9		0.259[F]
Gout	1	2.9	1	2.9		1.000[F]
Other	16	47.1	15	44.1	0.059	0.808
Severity of Sepsis						
Mild	17	50	17	51.5	0.901	0.015
Moderate	17	50	16	48.5		

F = Fisher's exact test

TABLE 2. Within-group comparisons of the mean cognitive ability scores of sepsis. Survivors at Pretest and Posttest (Weeks 6 and 12).

Source		Time			F[a]	P-value	Post-hoc[b]
		Pretest	Week 6	Week 12			
Mean Cognitive Ability Scores							
Control	Mean	23.12	23.58	25.42	132.048	<.001*	Pre> wk 6> wk 12
(N=33)	SD	1.408	1.119	1.119			
Experimental	Mean	22.98	26.12	28.59	442.279	<.001*	
(N=34)	SD	1.138	1.149	1.048			

* *p-value* < .001, Pre = Mean cognitive function at Baseline, Week 6 = Mean cognitive at Week 6, Week 12 = Mean cognitive at Week 12, [a] = Huynh-Feldt and Sphericity Assumed method, [b] = Bonferroni

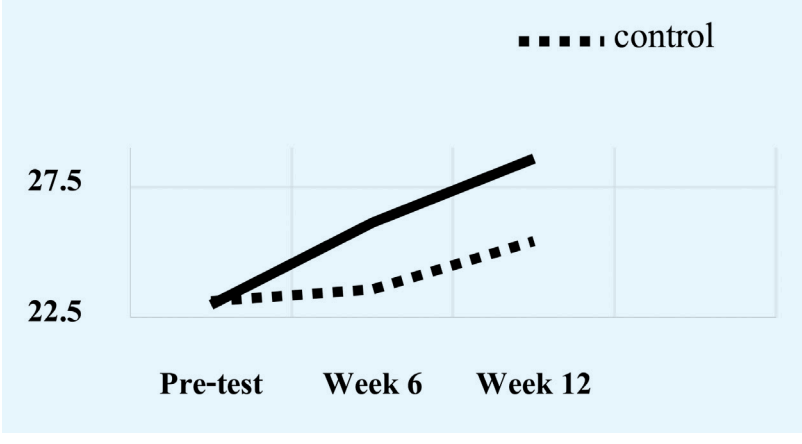

Fig 2. The graph slope for between-group comparisons of the mean cognitive ability scores of sepsis survivors at pretest and posttest (Weeks 6 and 12)

TABLE 3. Between-group comparisons of the mean cognitive ability scores of sepsis survivors at pretest and posttest (Weeks 6 and 12).

Source	SS	df	MS	F[a]	P-value	Partial Eta Squared
Time	524.854	2	262.427	534.702	< .001***	0.892
Error	63.803	130	.491			
Group	173.182	1	173.182	55.633	< .001***	0.461
Error	202341	65	3.113			
Time* Group	102.973	2	51.487	104.905	< .001***	0.617
Error	63.803	130	.491			

*** *p-value* < .001, [a] = Huynh-Feldt method.

Week 12 posttest than at pretest levels; these scores were significantly higher than those of the control group ($p < .01$). The brain training program in the experimental group consisted of meditation training and activities such as "Where Are You?", "Matched Pairs", "Sorting", "Counting", "Watch and Memorize", "Order of Events", "Spending at a Market", and "Record of Emotion", which helped stimulate cognitive function.

Brain training programs for cognitive awareness have not been directly studied in relation to sepsis survivors. However, critically ill patients in ICUs include a significant subgroup of sepsis survivors. Post-ICU cognitive impairment (Post-ICU CD) is a neurological disorder caused by conditions leading to the degeneration of the central nervous system. Memory, logical thinking, attention, visual perception, and overall cognitive functions are commonly impaired in ICU survivors.[18] Epidemiological data suggest that Post-ICU CD occurs at high rates among ICU survivors. A study from China reported an incidence of cognitive dysfunction ranging from 18.2% to 61.6% based on evaluations conducted from 7 days to 24 months after ICU discharge.[18-22] Systematic reviews also highlight the association of Post-ICU CD, particularly in elderly patients with reduced physical functioning.[23] Memory impairment has been identified as the most prominent issue, followed by deficits in processing and attention, and these impairments can persist for up to six years or longer after hospital discharge.[24] A full recovery of pre-ICU cognitive function levels has been achieved by few patients, leaving Post-ICU CD as a lasting challenge affecting daily life.

Zhao et al. implemented a brain intervention plan proposed by Brummel et al. to examine its effects on Post-ICU CD, observing significantly lower rates of cognitive impairment in the intervention group compared to the control group after three months.[25,26] Patients with severe symptoms demonstrated prevention of further decline in cognitive domains such as processing, language, orientation, memory, and vision. Similarly, the RETURN (Returning to Everyday Tasks Utilizing Rehabilitation Networks) reported significant improvements in planning, decision-making, and cognitive processing functions in a study through goal-oriented brain rehabilitation training in general and surgical ICU survivors.[27] Despite these advancements, no standardized rehabilitation protocol has been established for improving cognitive function in sepsis survivors with severe symptoms and cognitive impairment.[28]

The researchers included eight components in the brain interventions designed for this study. It has been shown that brain training improves brain function by promoting the expression of neurotrophic factors in the hippocampus, which supports neuronal recovery and enhances memory and cognitive functions. Improvements in different areas of cognition have been attributed to each intervention component. Following brain intervention training, the intervention group demonstrated significant improvements in cognitive functions such as awareness, processing, memory, attention, language, abstract thinking, and orientation when compared to the control group. These findings suggest that brain rehabilitation training can result in significant improvements in brain function for sepsis survivors.

In the study, the TMSE scores in both groups improved at varying levels after ICU discharge, though cognitive functions such as processing, memory, attention, and abstract thinking initially showed the most significant decline. The researchers observed substantial improvements in brain function and quality of life in the intervention group compared to the control group after three months of brain training. The intervention group had a mean TMSE score of 28.59, which was significantly higher than the score of 25.49 recorded in the control group. These results underscore the therapeutic benefits of early brain training in mitigating cognitive impairment and enhancing quality of life for patients discharged from the ICU.

However, some patients did not fully recover normal brain function after three months of rehabilitation. Consistent with previous findings, the researchers identified older age and multiple underlying conditions as common factors in these patients, noting that older patients with more comorbidities face greater challenges in recovering from Post-ICU CD compared to younger, healthier individuals.

Over the past few decades, psychological and neurobiological studies have demonstrated that mental health profoundly affects brain functions such as memory, attention, language, and cognitive processing. Positive emotions coordinate and optimize brain activity, while negative emotions disrupt and impair it. Since the hospital is designated as the mental health center for Sichuan Province and specializes in psychiatric care, the researchers incorporated mental health evaluations and treatments into the brain rehabilitation protocol to ensure maintenance of a positive outlook by patients. After completing the brain training program, the intervention group demonstrated better quality of life compared to the control group in various aspects, including physical functioning, physical roles, emotional roles, and mental health.[29,30]

Reinforcement from healthcare personnel such

as nurses through the LINE application, which enables monitoring and assessment of performance of activities and communication with the participants, provides social support based on feedback from messages and stickers praising and encouraging the participants to improve activity outcomes. After the researchers saw the participants' performance, the participants were encouraged and supported to keep performing activities. Furthermore, the follow-up telephone calls via the LINE application once weekly throughout the study to inquire in real time about problems and barriers in activities, including general symptoms, encouraged health behavior modification, which is similar to many studies that monitored patients by telephone and found that periodical follow-up telephone calls about activities and consultations improved cooperation among participants and encouraged them to continue the program[26], which further helped make the brain training program more effective.

In this study, the researcher used the concepts of pathophysiology[8] and brain plasticity by Hebb[16] consisting of three mechanisms: (1) Loss of balance in endothelial functions and blood pressure changes resulting in insufficient brain circulation and cerebral ischemia; (2) Increases in acetylcholinesterase activity and reductions in receptor density in the hippocampus causing cholinergic dysfunction, which results in impaired neurotransmission, neurocognitive impairment, and partial memory loss; (3) Microglia and astroglia stimulation worsening inflammation (Interleukins 6 and 12) leading to intrusion to the blood–brain barrier and function loss, which dangerously allows the passage of toxins and thus causes nervous system injury and inflammation. The brain training program emphasizes stimulating the brain to return to activity. The immediate restructuring process of neurons in the brain takes only 1 second. Neurons change shape and coordinate to replace the damaged parts of the brain continuously, which is a unique self-repair characteristic of the brain. Changes to brain structure with neuron connections can occur at all times. Interactions with the environment, including past experiences and new learning, results in the creation of new circuits and nerve connections. According to Hebb,[16] repetitive learning or practice of any activity stimulates dendrites to grow and branch in the cerebral cortex, thereby improving coordination effectiveness among the neurons in the brain, which can restore lost functions.

Among sepsis survivors, brain cell death causes cognitive impairment due to ischemia, causing brain injuries at the cerebral cortex and disrupting nerve cells containing monoamines such as dopamine, norepinephrine, and serotonin. Neurotransmitters communicate and exchange information with the prefrontal cortex. The disruption of such functions reduces neurotransmitter production and signal and information exchange with the prefrontal cortex. Increased acetylcholinesterase activity in the hippocampus causes loss of function and cognitive impairment in the areas of memory, recall, and executive function among sepsis survivors.

The brain training program using the cognitive exercise manual consists of eight activities focused on the development of memory, attention, and executive functions, requiring 30–45 min. per activity, 3 activities per day, 5 days per week over 12 weeks. The program promotes changes in body shape and nervous system coordination to replace the damaged parts of the brain. Brain training programs and cognitive stimulation activities are effective for enhancing cognitive functions by promoting dendritic growth in the prefrontal cortex and strengthening neural connections in the hippocampus, critical for memory and decision-making. Engaging in cognitively demanding tasks, such as solving problems or learning new skills, supports dendritic branching in the prefrontal cortex, which governs executive functions like decision-making and emotional regulation[9] Structured programs like Brain HQ and Lumosity have demonstrated their ability to improve neural connectivity and cognitive performance while slowing cognitive decline, particularly in older adults.[30] These findings highlight the potential of brain training to enhance cognitive resilience and mitigate age-related or disease-related cognitive impairments.

In this study, the control group demonstrated significantly stable cognitive impairment with slight increases in cognitive functions at Week 12 ($p < .05$), possibly because of the natural recovery of neurological and cerebral functions. In other words, in brain injury caused by ischemia and inflammation, connection functions between DNA, molecules, and nerve cells in the brain and electrophysiology triggered the recovery of brain functions by promoting changes in its structure, causing other parts of the brain to develop and function in place of the damaged parts by promoting the growth of new nerve cells, starting from within 3–7 days after injury. The aforementioned processes occur mostly 7–14 days after injury and are nearly complete at 30 days. After an injury, cognitive impairment can persist for up to 6 months. This finding aligns with the results of Qionglan et al.[32] who examined the effects of an early cognitive function recovery program and found that the incidence of cognitive impairment in the experimental group significantly declined after 3 months into the

program ($p < .05$). This study showed that the cognitive function recovery program could prevent loss of executive, language, planning, memory, and attention functions.

One limitation of this study was the lack of a standardized cut-off point for cognitive impairment among sepsis survivors in Thailand. The Thai Mental State Examination (TMSE), a cognitive assessment tool tailored for the Thai population, has been studied across various age groups and educational levels. For example, research on community-dwelling individuals aged 50 and above found median TMSE scores of 27 for literate participants and 23 for illiterate participants. These findings suggest that a TMSE score between 20 and 25 might be considered within the normal range for certain populations.[33] However, this range has not been specifically validated for sepsis survivors, highlighting the need for further investigation in this subgroup. Additionally, the brain training program for cognition in sepsis survivors relied on monitoring participants via the LINE application, which excluded individuals without access to smartphones from participating. Lastly, data collection was limited to sepsis survivors receiving follow-up care at a single urban tertiary hospital in northeastern Thailand. As a result, the findings may not be generalizable to the broader population of sepsis survivors.

Recommendations for implementing the findings

Nursing practice

Nurses and healthcare team members can incorporate the brain training program into the care of sepsis survivors to assess cognitive function and enhance brain stimulation. The program particularly targets cognitive impairments in areas such as attention, memory, and executive function.

Nursing research

Prospective studies on the effectiveness of the brain training program for sepsis survivors across various settings and contexts are recommended. Additionally, studies with follow-up periods of 6 months, 9 months, or over 1 year focusing

CONCLUSIONS

The brain training program improves cognitive function in sepsis survivors over 6 to 12 weeks with focus on attention, memory and executive function. Healthcare teams are encouraged to integrate the program into regular follow-up care to motivate and support behavior adjustments. Further studies in diverse settings and with long-term follow-ups are recommended to comprehensively evaluate the program's effects on brain function, daily activities, and quality of life.

Data Availability Statement

Available for review upon reasonable request.

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DECLARATION

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There are no sources of funding to disclose.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Registration Number of Clinical Trial

TCTR20240824003

Author Contributions

JK was responsible for study design, review literature, development of methodology, data collection, application of statistical techniques to analyze data and interpretation, writing initial and final drafts, and visualization. WP was responsible for conceptualization, study design, development of methodology, data interpretation, discussion, writing review and editing, and supervision. CR assisted with study design, development of methodology, application of statistical techniques to analyze data, writing review and editing, and supervision. YR assisted with the development of methodology, writing review and editing, and supervision. All authors read and approved the final manuscript.

Use of Artificial Intelligence

We confirm that no artificial intelligence (AI) was used in the writing of this work. All content was created solely by human authors.

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Aerosol Bioburden and Antimicrobial Resistance in Orthopaedic Operating Unit in a Tertiary Hospital in Thailand

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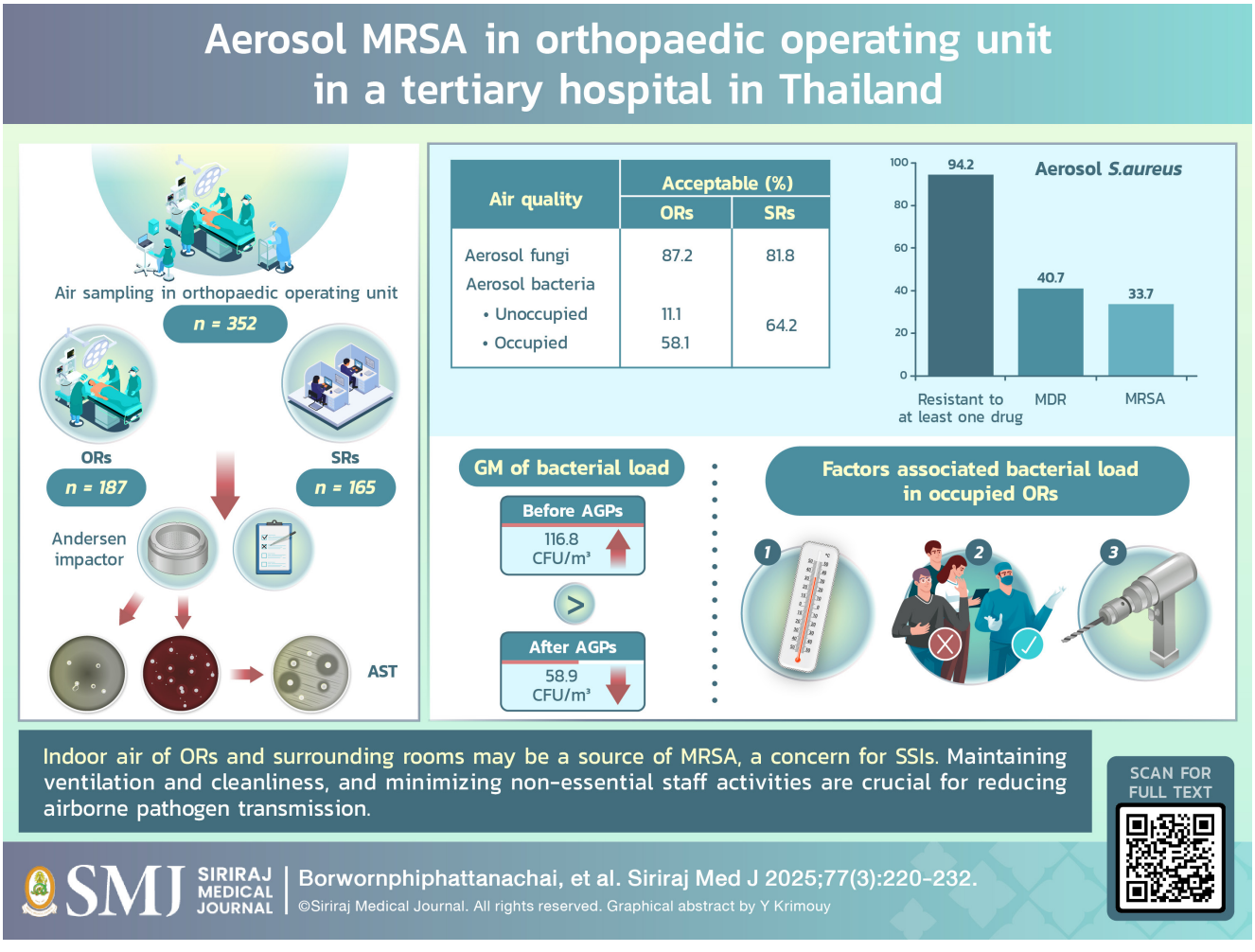

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ABSTRACT

Objective: This study aimed to determine the microbial indoor air quality and factors associated with bacterial air contamination in the orthopaedic operating unit.

Materials and Methods: Conducted in seven operating rooms (ORs) and six surrounding rooms (SRs) in an orthopaedic operating unit. A total of 352 air samples were collected using an Andersen air sampler. Fungal and bacterial counts were determined as air quality indicators. Antimicrobial resistance (AMR) of predominant bacteria and factors influencing microbial air quality were analyzed.

Results: Most air samples in the ORs (87.2%) and SRs (81.8%) contained acceptable fungal counts. However, unoccupied ORs (11.1%), occupied ORs (58.1%), and SRs (64.2%) had fewer samples with acceptable bacterial levels. The geometric mean (GM) bacterial load in the ORs before aerosol-generating procedures (AGPs) was 116.8±1.8 CFU/m^3, higher than after AGPs (58.9±2.1 CFU/m^3). After controlling potentially confounding factors, the factors influencing bacterial loads occupied OR were temperature before AGPs (0.164 CFU/m^3, 95%CI 0.017-0.311, p=0.029), the number of staff after AGPs (0.082 CFU/m^3, 95%CI 0.019-0.144, p=0.011), and using saw/drill device after AGPs (0.701 CFU/m^3, 95%CI -1.326-0.076, p=0.029). Predominant bacteria were Gram-positive cocci (90.8%), of which 20.5% were *S. aureus*. Most *S. aureus* (94.2%) were resistant to at least one drug, with 40.7% being multidrug-resistant. Additionally, 33.7% were methicillin-resistant *S. aureus* (MRSA).

Conclusion: The indoor air of ORs and SRs may be a source of AMR bacteria, particularly MRSA, a concern for surgical site infections. Maintaining ventilation, cleanliness, and minimizing non-essential staff activities are crucial for reducing airborne pathogen transmission.

Keywords: Aerosol *S. aureus*; orthopaedic operating rooms; MRSA; antimicrobial resistance (Siriraj Med J 2025; 77: 220-232)

INTRODUCTION

The indoor air quality of healthcare facilities or medical centres can be affected by numerous biological pollutants, giving rise to concerns about infection control and healthcare workers' health and well-being.[1,2] Biological contaminants, such as bacteria, fungi, and viruses, may be present in a wide range of healthcare environments and also infected patients, and can be transferred through room air, which could cause healthcare-associated infections (HAIs). Generally, exposure to these bioaerosols is associated with severe effects on people with a weakened immune system, not only patients but also healthcare workers and visitors.[2,3]

Surgical site infections (SSIs) are costly HAIs that can involve prolonged hospitalization, readmission, reoperation, and increased diagnostic and medical costs.[4,5] In Thailand, one study found that the reported incidence of SSIs in a university hospital between 2007 and 2016 showed a decreasing trend, with an average incidence of 2.98%; however, severe problems were observed, including significantly increased hospital costs of around US $5,509 and extra hospital stays of 24 days compared with non-SSI patients.[6] SSIs are generally most evident in orthopaedic surgery[7], especially operations with aerosol-generating procedures (AGPs), such as hip and knee arthroplasties[8], hip fracture surgery[9], internal fixation of fractures[10], and spinal surgery.[11] The incidence of orthopaedic SSIs has been reported to be as low as 1.9% and up to 22.7% in different studies, and can cause diverse adverse effects on patients, including health implications, long-term disability, financial loss, and increased mental stress.[4,12] Therefore, SSI prevention is a priority in healthcare facilities, with especially strong attention needed in orthopaedic surgery units, and consequently, SSI prevention is included as a surgery quality indicator in many facilities.[13]

The microbial air quality in the orthopaedic operating room (OR) is considered to represent an associated risk for SSIs, particularly for clean operations.[14-18] One report stated that airborne transmission accounted for 20%–24% of SSIs.[19] The microbes in bioaerosols can fall directly into a wound, or may land on the exposed surfaces of instruments or the staffs' hands, and then may be transferred into the wound.[20] Implementing unidirectional airflow could ensure an acceptable bacterial load and lower the risk of SSIs compared with in the case of turbulent (mixing) airflow in orthopaedic ORs.[21] Many factors can affect the presence, concentrations, and diversity of bioaerosols in hospital environments, including the season, temperature, humidity, working activities, frequency of door closing and opening, behaviors of the surgical staff, and the surgical procedures.[20,22,23] Moreover, microbial contamination on the floor, walls,

and high-touch surfaces in orthopaedic ORs may be important areas of microbial air contamination.[24,25] There is also evidence that airborne particles could be dispersed from staff walking on a contaminated floor in an orthopaedic OR.[26] Many previous studies have reported different varying levels of microbial contamination during surgical activities, consistently finding the lowest values under "at rest" conditions and the highest values under "in operation" conditions. Additionally, the indoor air quality before the beginning of operations reflects the effectiveness of the ventilation system and cleanliness in OR.[18,22] However, the duration of the surgical activities, both pre-incision and post-incision, can increase risk factors for microbial air contamination in OR.[17,25]

The most common microorganisms involved in SSIs after orthopaedic procedures are *Staphylococcus aureus*, including methicillin-resistant *S. aureus*, coagulase-negative Staphylococci (CoNS), including methicillin-resistant CoNS, and *Escherichia coli*. These pathogens are responsible for several SSIs and usually originate from the patient's commensal flora and from exogenous microbial contamination in the air environment of ORs.[27] Moreover, there is much evidence that these aerosol bacteria have the potential for prolonged survival on the surfaces of items, equipment, and in the air in ORs.[24,25,28-30]

In 2018, the Ministry of Public Health of Thailand promoted policies and practices referring to "2P safety goals" (patient safety and personal safety) for ensuring a sustainable healthcare system that corresponds to the World Health Organization's Strategy for Patient Safety.[31] Orthopaedic ORs are important hospital areas that require a clean environment and good hygiene practices for preventing not only serious SSIs but also healthcare workers' exposure to infectious agents that may be found both on inanimate surfaces and equipment and in the indoor air environments occupied by colonized persons or infected patients. As indoor air quality is critical to patient safety in hospitals, the World Health Organization (WHO) recommended maximum guideline values for hospital areas at 100 CFU/m^3 for bacteria and 50 CFU/m^3 for fungi.[32] Microbial air monitoring in orthopaedic ORs and nearby areas is an important control measure for ensuring the safety of both patients and staff. Despite most centers being aware of the issue, the determination of microbial air contamination in orthopaedic ORs is still rarely performed. This study aimed to assess the air microbial load in orthopaedic ORs and SRs in operating units in a tertiary hospital, as well as the burden of antimicrobial-resistant (AMR) aerosol bacteria, and the factors associated with bacterial air contamination in orthopaedic ORs.

MATERIALS AND METHODS

Study design and sampling

This laboratory-based cross-sectional study was conducted with air samples collected in ORs and SRs between July and September 2022 in orthopaedic operating units of a tertiary hospital, in Bangkok, Thailand. For the ORs, 187 air samples were collected from seven selected ORs, with the samples collected in both unoccupied and occupied ORs. In the unoccupied ORs, the air samples were collected before working hours (7.00 am to 8.30 am). In the occupied ORs, the air samples were collected during working hours (9.30 am and 3.00 pm), and were collected before the incisional (before the AGPs) and wound-closing (after the AGPs) stages in each operation. For the SRs, 165 air samples were collected from six types of SRs, namely scrub rooms (SCs), induction rooms (IDs), sterile storeroom (ST), X-ray room (XR), specimen room (SP), and office room (OF). For these, the air samples were collected before working hours between 7.00 am to 8.30 am (unoccupied SRs) and after the AGPs in the ORs (occupied SRs).

The HVAC system in the ORs utilizes high-efficiency particulate air (HEPA) filters, ensuring optimal air quality. These ventilation systems achieve a minimum of 20 air changes per hour (ACH) while providing 100% fresh air. Vertical laminar airflow systems effectively direct particle-free air over the aseptic surgical field. Regular maintenance of air conditioning equipment is performed, and filter units are replaced on time. Each OR shares similar structure designs, featuring two entry points: one from the corridor and another from the scrub room, with an additional door opening to the sterile storeroom. During the workday in ORs, the floor, equipment, and environmental surfaces are cleaned and decontaminated before each case. Throughout surgical procedures, the OR doors remain closed, except when extra equipment is needed or staff members must pass through. Following the last surgical case of the day, routine cleaning is conducted. The SRs are equipped with a turbulent airflow HVAC system providing 6 ACH, which does not utilize HEPA filters.

Air sampling and data collection

Air samples were collected using a single-stage Andersen air sampler (Bio-Stage Single-stage Impactor, SKC, Inc., USA) at a flow rate of 28.3 litres per minute (LPM) for 5 minutes, as recommended in the NIOSH-0800 instructions.[33] To achieve sampling the breathing zone, the air sampler was placed one metre above the floor level and one metre away from the surgical table in the ORs, while in the SRs, the air sampler was placed one

metre above the floor level and at the centre of each room. For each air sampling site, one air sample was collected onto blood agar (BA) for bacterial cultivation, and another onto potato dextrose agar (PDA) for fungal cultivation. The bacterial and fungal sampling plates were incubated at 37 °C for 24–48 hours and at 25 °C for 5–7 days, respectively. Total bacterial and fungal colonies were counted manually, and the number of the microbial count was calculated and reported as colony-forming units per cubic metre (CFU/m^3), as described in a previous study.[34]

Bacterial colonies were presumptively identified by their morphology and Gram stain, and then biochemical tests were conducted for suspected *Staphylococcus* spp., which have been found to be the dominant type. The identified *S. aureus* was subjected to antimicrobial susceptibility tests using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (Oxoid, UK) against eleven antibiotics, with the test organism adjusted to 0.5 McFarland turbidity standards. The susceptibility test results were interpreted according to the Clinical Laboratory Standard Institute (CLSI).[35] *S. aureus* ATCC25923 was used as a control bacteria strain for quality control for the susceptibility tests.

Determination of the factors affecting the bacterial load in the occupied operating rooms

During the air sampling, the air bacterial load-related factors in the orthopaedic ORs were recorded on the sample collecting form. These factors comprised the physical factors (temperature, and relative humidity), operative procedure-related factors (e.g., types of procedure, perioperative activities, size of incision, types of AGPs, operative time of the procedure, type of surgical devices, extra special instruments), number of staff, and frequency of door opening during surgery.

Statistical analyses

IBM SPSS (SPSS Inc., Chicago, USA) was used for all the statistical analyses. Descriptive statistics were employed to explore the physical and microbial air quality, and microbial load in the air samples, and are reported in terms of the percentage, mean ± standard deviation (SD), median, and GM ± geometric standard deviation (GSD). The Mann–Whitney test, independent t-test, and ANOVA were used to compare the air quality and microbial loads among the different sets of data. Univariate and multivariate linear regression analyses were used to analyze the relationship between the bacterial load (continuous variable, measured in CFU/m^3) and the associated factors by controlling for potential confounding variables (temperature, relative humidity, number of staff, frequency of door opening, and operative time as continuous variables; perioperative activities, surgical devices, cloth, size of incision as categorical variables). Statistical significance was set at $p<0.05$.

RESULTS

Air quality in the orthopaedic operating unit

A total of 352 indoor air samples were collected: 187 samples in ORs and 165 samples in SRs. The mean temperature and relative humidity (RH) during air sampling differed between the ORs and SRs, with values of 21.1±1.4 °C and 54.6±4.0% in the ORs, and 22.3±1.1 °C and 57.2±4.7% in the SRs, respectively. The mean temperature in the unoccupied ORs was highest at 23.4±2.0 °C, which was significantly higher than before and after the AGPs ($p<0.05$). Similarly, the mean temperature in the unoccupied SRs (23.0±1.1 °C) was significantly higher than in the occupied SRs (21.9±0.9 °C) ($p<0.05$). Additionally, the mean RH in the unoccupied SRs was significantly lower (56.0±5.7%) than in the occupied SRs (57.8±4.0%) ($p<0.05$). In the overall comparison, the mean air temperature and RH in the ORs were significantly lower than in the SRs ($p<0.05$) (Table 1).

The air quality assessment in the ORs showed that the median aerosol fungal count in the unoccupied ORs, and in the ORs before AGPs and after AGPs were 3.5, 0, and 0 CFU/m^3, respectively. Of these, the percentages of air samples with an acceptable fungal count (<10 CFU/m^3) were 77.8%, 86.3%, and 91.3% in the unoccupied ORs, and in the ORs before AGPs and after AGPs, respectively. Using the bacterial count as an indicator, a significantly higher median bacterial count was evidenced before AGPs (120.1 CFU/m^3) compared with that after AGPs (65.4 CFU/m^3) and in the unoccupied ORs (28.3 CFU/m^3). These indicated there was a lower percentage of acceptable aerosol bacterial counts before AGPs (36.3%) compared with after AGPs (80.0%). However, the lowest percentage of the acceptable bacterial count (11.1%) was detected in the unoccupied ORs, where a lower acceptable level of <10 CFU/m^3 is recommended compared with <100 CFU/m^3 for occupied ORs, as detailed in Table 1.

Regarding the microbial air quality in the SRs, the median fungal count in the unoccupied SRs (21.2 CFU/m^3) was significantly higher than that in the occupied SRs' air samples (14.1 CFU/m^3). The percentages of acceptable fungal counts were 78.2% for the unoccupied SRs and 83.6% for the occupied SRs. According to the aerosol bacterial count assessment, the median bacterial counts in the unoccupied SRs (91.9 CFU/m^3) and occupied SRs (84.8 CFU/m^3) detected at an acceptable level were 60.0% and 66.4%, respectively.

TABLE 1. Air quality measurement in orthopaedic operating units.

Variable/ Air quality indicator	Operating rooms (N= 187)			*p*-value	Surrounding rooms (N=165)		*p*-value
	Unoccupied (n=27)	Occupied Before AGPs (n=80)	After AGPs (n=80)		Unoccupied (n=55)	Occupied (n=110)	
Temperature (ºC)							
Mean (SD)	23.4 (2.0) **	20.9 (0.9)	20.6 (0.8)	<0.001	23.0 (1.1) *	21.9 (0.9)	0.014
MIN-MAX	18.1-25.3	18.5-23.7	18.4-22.7		20.1-25.3	19.4-24.4	
Relative humidity (%)							
Mean (SD)	54.8 (5.1)	54.4 (3.8)	54.8 (3.8)	0.804	56.0 (5.7) *	57.8 (4.0)	0.02
MIN-MAX	47.0-66.0	46.0-65.0	46.0-64.0		48.0-79.0	50.0-69.0	
Bacterial count (CFU/m³)							
Acceptable#, N (%)	3 (11.1)	29 (36.3)	64 (80.0)		33 (60.0)	73 (66.4)	
Median	28.3[a]	120.1[b]	65.4[c]	<0.001	91.9	84.8	0.692
25th percentile	21.2	86.6	35.3		56.5	49.5	
75th percentile	63.6	169.6	97.2		130.7	114.8	
MIN-MAX	7.1-212.0	14.1-515.9	7.1-282.7		7.1-438.2	0-2572	
Fungal count (CFU/m³)							
Acceptable#, N (%)	21 (77.8)	69 (86.3)	73 (91.3)		43 (78.2)	92 (83.6)	
Median	3.5	0.0	0.0	0.157	21.2	14.1	0.033
25th percentile	0.0	0.0	0.0		7.1	7.1	
75th percentile	7.1	7.1	7.1		42.4	28.3	
MIN-MAX	0-63.6	0-215.6	0-148.4		0-212.0	0-848.1	

**ANOVA test, p<0.05 compared between unoccupied, before AGPs, and after AGPs; * t-test, p<0.05 compared unoccupied and occupied; [a,b,c] Mann-Whitney test, p<0.05

#European Union Good Manufacturing Practice (EU GMP) for cleanroom recommended microbial count "acceptable" in unoccupied operating room ≤ 10 CFU/m³ and occupied operating room ≤ 100 CFU/m³ for bacteria and ≤10 CFU/m³ for fungal count, while in surrounding rooms bacterial count ≤ 100 CFU/m³ and fungal count ≤ 50 CFU/m³ for unoccupied and occupied rooms.

Abbreviations: AGPs, aerosol-generating procedures; GM, geometric mean; GSD, geometric standard deviation,

Aerosol microbial load in the ORs

The microbial load in the air samples that were fungal positive (n=55) and bacterial positive (n=160) collected in the occupied ORs was analyzed and the GM of the microbial load was compared for different AGP procedures, as shown in Table 2. The GM of the fungal load in the occupied ORs (before and after AGPs) showed non-significant differences in each surgical procedure (p>0.05). However, the overall GM of the bacterial load in the occupied ORs collected before AGPs (116.8±1.9 CFU/m³) was significantly higher than after AGPs (58.9±2.1 CFU/m³) (p<0.05). Specifically, the GM of the bacterial load in samples collected before AGPs was significantly higher than that after AGPs for each of the AGPs (p<0.05), of which the highest aerosol bacterial load (152.5±1.5 CFU/m³) was found in the air sampled before the spinal laminectomy procedure, as shown in Table 2.

Aerosol microbial load in SRs

The microbial load in the occupied SRs was analyzed and compared between different types of SRs. A total of 87 air samples collected in the occupied SRs were found to be fungal positive with a GM of 20.2 CFU/m³. The highest GM fungal load was observed in the sterile storeroom (44.7±3.4 CFU/m³). In comparison, the GM fungal loads in the sterile storeroom and office room were significantly higher than in the induction

TABLE 2. Microbial load in air sample collected in occupied ORs with different operating procedures.

Microbial load	Total		TKA	SL	ALR	IFI	*P*-value
Fungal load (CFU/m³)							
Before AGPs							
No. of positive samples (%)	30	(100)	8 (26.7)	7 (23.3)	9 (30.0)	6 (20.0)	
GM (GSD)	10.7	(2.4)	7.4 (1.8)	14.5 (4.0)	11.1 (1.8)	11.4 (2.2)	0.532
After AGPs							
No. of positive samples (%)	25	(100)	7 (28.0)	10 (40.0)	5 (20.0)	3 (12.0)	
GM (GSD)	9.7	(2.3)	7.5 (2.5)	8.8 (1.6)	11.2 (2.0)	19.5 (5.8)	0.421
Bacterial load (CFU/m³)							
Before AGPs							
No. of positive samples (%)	80	(100)	20 (25.0)	20 (25.0)	20 (25.0)	20 (25.0)	
GM (GSD)	116.8*	(1.9)	140.9 (1.6)*	152.5 (1.5)*	106.4 (1.6)*	81.5 (2.5)*	0.005
After AGPs							
No. of positive samples (%)	80	(100)	20 (25.0)	20 (25.0)	20 (25.0)	20 (25.0)	
GM (GSD)	58.9	(2.1)	59.6 (2.2)	75.3 (1.9)	61.4 (2.2)	43.7 (1.9)	0.128

* t-test, p<0.05 compared before and after AGPs each procedure

Abbreviations: AGPs, aerosol-generating procedures; GM, geometric mean; GSD, geometric standard deviation; ALR, arthroscopic ligament repair; IFI, internal fixation with implant; SL, spinal laminectomy; TKA, total knee arthroplasty.

rooms, scrub rooms, and x-ray room (p<0.05). Among the bacterial-positive air samples collected in the occupied SRs (n=109), the highest GM bacterial load was observed in the scrub rooms (93.4±2.1 CFU/m³), followed by the sterile storeroom (89.8±2.6 CFU/m³), specimen room (85.9±1.3 CFU/m³), and induction rooms ((77.5±2.0 CFU/m³), with a non-significant statistical difference. However, these rooms contained a significantly higher bacterial load than the X-ray room and office room (p<0.05) (Table 3).

Factors influencing the indoor air bacterial load in the ORs

The independent factors influencing bacterial loads during surgeries were the indoor air temperature before AGPs (0.164 CFU/m³, 95%CI 0.017-0.311, p=0.029), indoor air temperature after AGPs (0.218 CFU/m³, 95%CI 0.011-0.425, p=0.039), number of staff (0.075 CFU/m³, 95%CI 0.006-0.144, p=0.033), used saw/drill devices (0.526 CFU/m³, 95%CI-0.873-0.179, p=0.003), used midas/burr equipment(0.420 CFU/m³, 95%CI 0.031-0.809, p=0.035), and size of incision (0.343 CFU/m³, 95%CI 0.033-0.654, p=0.030). After controlling for potentially confounding factors for the bacterial load in the ORs using multiple linear regression analysis, the result exhibited a statistically significant correlation between the aerosol bacterial loads before AGPs and temperature (R^2=4.8%, p=0.029). Moreover, the aerosol bacterial load after AGPs was correlated with the number of staff, and use of saw/drill devices (coefficient of determination, R^2=15.70% p=0.031) (Table 4).

Dominant aerosol bacterial and antimicrobial resistance in the orthopaedic operating unit

A total of 925 bacterial colonies were isolated and identified from the blood agar air samples. Gram-positive cocci were dominantly observed, accounting for 90.8% of the species (840/925). Of these, CoNS accounted for 49.8% (418/840), followed by *Micrococcus* spp. (29.8%, 250/840), and *S. aureus* (20.5%, 172/840). The antimicrobial susceptibility tests were performed on 172 *S. aureus* isolates that were isolated from the ORs (n=98) and SRs (n=74). The results indicated high rates of AMR among the *S. aureus* isolates, of which, 94.2% (162/172) of the isolates demonstrated resistance to at least one tested antimicrobial agent, with high rates of resistance to erythromycin (73.8%, 127/172) and penicillin (61.0%,105/172), as detailed in Table 5. Furthermore, 40.7% (70/172) and 33.7% (58/172) of the AMR aerosol *S. aureus* were defined as multidrug-resistant strains and methicillin-resistant *S. aureus* (MRSA).

TABLE 3. Microbial load in air sample collected from occupied SRs.

Microbial load	Total	SC	ID	ST	XR	SP	OF	P-value
Fungal load (CFU/m³)								
No. of positive samples (%)	87 (100)	29 (33.3)	22 (25.3)	24 (27.6)	4 (4.6)	4 (4.6)	4 (4.6)	
GM (GSD)	20.2 (2.9)	12.7 (2.2)	15.5 (2.2)	44.7 (3.4)	10.0 (1.5)	24.3 (1.9)	36.6 (3.6)	<0.001
Bacterial load (CFU/m³)								
No.of positive samples (%)	109 (100)	39 (35.8)	27 (24.8)	32 (29.3)	4 (3.7)	3 (2.7)	4 (3.7)	
GM (GSD)	81.8 (2.3)	93.4 (2.1)	77.5 (2.0)	89.8 (2.6)	31.7 (1.4)	85.9 (1.3)	23.4 (2.6)	0.01

Abbreviations: AGPs, aerosol-generating procedures; GM, geometric mean; GSD, geometric standard deviation; ID, induction room; OF, office room; SC, scrub room; SP, specimen room; ST, sterile storeroom; XR, x-ray room

TABLE 4. The factors associated with bacterial load in occupied operating rooms using linear regression analysis.

	Univariate analysis			Multivariate analysis#		
Variables	**Estimate**	**95% CI**	**p-value**	**Estimate**	**95%CI**	**p-value**
Bacterial load of before AGPs (CFU/m³)						
Temperature (ºC)	0.164	0.017-0.311	0.029	0.164	0.017-0.311	0.029*
Relative humidity (%)	0.022	-0.015-0.059	0.244			
General anesthesia	-0.066	-0.348-0.216	0.642			
Regional anesthesia	0.103	-0.204-0.410	0.507			
Number of staff (n)	-0.011	-0.070-0.049	0.724			
Hair removal	-0.262	-0.685-0.161	0.221			
Bacterial load of after AGPs (CFU/m³)						
Temperature (ºC)	0.218	0.011-0.425	0.039	0.177	-0.018-0.372	0.075
Relative humidity (%)	0.032	-0.011-0.075	0.140	0.036	-0.005-0.076	0.087
Number of staff (n)	0.075	0.006-0.144	0.033	0.082	0.019-0.144	0.011*
Saw/drill devices	-0.526	-0.873-0.179	0.003	-0.701	-1.326-0.076	0.029*
Midas/burr equipment	0.420	0.031-0.809	0.035	-0.401	-1.087-0.286	0.248
Bovie	-0.277	-0.951-0.396	0.415			
Irrigation	-0.462	-1.130-0.206	0.173	-0.623	-1.281-0.035	0.063
Fluoroscope	-0.193	-0.521-0.135	0.246			
Frequency of door opening	-0.019	-0.042-0.004	0.098	-0.010	-0.032-0.012	0.387
Operative time (mins)	-0.001	-0.003-0.001	0.355			
Size of incision	0.343	0.033-0.654	0.030	0.256	-0.040-0.552	0.089
Type of gown	0.072	-0.300-0.444	0.701			

#The risk factors with P-value <0.2 by univariate analysis were included into multivariate analysis;
*significant with P-value <0.05
Abbreviations: AGPs, aerosol-generating procedures; CI, confidence interval

TABLE 5. Antimicrobial drug resistance of *S. aureus* isolates from orthopaedic operating unit (n=172).

Antimicrobial agent	PEN	FOX	GEN	ERY	TET	CIP	CLI	LIN	CHL	RIF	SXT
Number of isolates (%)											
Resistance	105 (61.0)	58 (33.7)	8 (4.7)	127 (73.8)	23 (13.4)	14 (8.1)	75 (43.6)	2 (1.2)	7 (4.1)	7 (4.1)	14 (8.1)
Susceptible	67 (39.0)	114 (66.3)	164 (95.3)	45 (26.2)	149 (86.6)	158 (91.9)	97 (56.4)	170 (98.8)	165 (95.9)	165 (95.9)	158 (91.9)

Abbreviations: FOX, cefoxitin; CIP, ciprofloxacin; CHL, chloramphenicol; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; LIN, linezolid; PEN, penicillin; RIF, rifampin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole

DISCUSSION

This study evaluated the aerosol bioburden in ORs and SRs located in operating units of a tertiary hospital using culturable fungus and bacteria as indicators. Because of the budget and time constraints of this study, the total air sample of 187 was determined by randomly collecting, ensuring coverage of all four types of interested AGPs performed in the seven operating rooms in the study location. In the study of aerosol fungus in the ORs, the air contamination was found to have low fungal counts in both the unoccupied ORs (median=3.5 CFU/m^3) and occupied ORs (median=0 CFU/m^3). Of these samples, 87.2% of the total air samples in the ORs had acceptable fungal counts that were considered very clean according to the standard recommended by EU good manufacturing practice (GMP) for cleanrooms (≤ 10 CFU/m^3). Of note, the air fungal count was the highest at 215.6 CFU/m^3 in the air sample collected before the AGPs for a spinal laminectomy procedure. In our observation, this high fungal count may be affected by the activity occurring before the AGPs, such as the more complex patient preparations, use of certain instrumental techniques, and the involvement of a large surgical team for the operation. In addition, the complicated internal fixation with implant procedure resulted in the highest fungal count of 148.4 CFU/m^3 in the air samples collected after the AGPs. However, the aerosol fungal loads during surgeries (before and after the AGPs) of each procedure did not show statistically significant differences. Generally, indoor fungal spores can originate from outdoor air exposure and are uncommonly found in closed and cleaned areas such as OR. In this study, a low fungal load was observed in the ORs, except for occasional high fungal counts in a few air samples collected while company technicians were in the ORs preparing special surgical instruments. The high fungal load observed in this study may not have been affected by routine activities and showed no significant difference before and after AGPs. This observation is evidence to recommend limiting non-healthcare personnel in ORs. In some parts of the SRs, the median fungal count in the unoccupied SRs (21.2 CFU/m^3) was significantly higher than that of the occupied SRs (14.1 CFU/m^3). A high percentage of 81.8% of the total air samples had an acceptable fungal count ≤ 50 CFU/m^3, which is considered very clean according to the standard recommended by EU GMP for cleanrooms. In occupied SRs, it was observed that the GM aerosol fungal load in each SR was lower than that for the unoccupied SRs (data not shown), except for the occupied sterile storeroom, which had the highest fungal load (44.7±3.4 CFU/m^3). The higher aerosol fungal load in the sterile storeroom during working hours may be affected by the greater number of people entering the storeroom compared with before working hours. This study also observed a higher relative humidity (RH) in the occupied sterile storeroom (56.9%) compared with the unoccupied room (54.5%). The higher RH may serve as the optimal fungal growth condition as it was previously reported that the concentration of fungi was correlated with the temperature and humidity.[36] Moreover, the high fungal load in the air samples may be due to the resistance of fungal spores that can survive and grow under stress conditions, such as dehydrated and moisturized ambient air, as well as under UV radiation.[19]

In comparison to fungal contamination, the aerosol bacterial counts in the ORs showed heavier contamination. Only 58.1% of the air samples collected from the occupied ORs had acceptable bacterial levels ≤ 100 CFU/m^3 and could be considered clean according to the standard recommended by EU GMP for cleanrooms. Furthermore, 11.1% of the air samples collected from the unoccupied ORs had a very low acceptable bacterial count of ≤10

CFU/m^3, which is considered very clean according to the standard recommended by the EU GMP for cleanrooms. This study revealed that the overall bacterial load in air samples collected before AGPs was significantly higher than after AGPs. High GM aerosol bacterial loads were observed before the spinal laminectomy procedure (152.5 CFU/m^3), total knee arthroplasty (140.9 CFU/m^3), and arthroscopic ligament repair (106.4 CFU/m^3). These results indicate that the indoor air bacterial load during the pre-incisional period of patient preparation was higher than the standard recommendation (≤100 CFU/m^3). This high bacterial load may be affected by the activity of the surgical teams, in particular their many movements and use of several instruments, especially surgical teams in critical operations. There is evidence that the behaviours of the surgical team during patient preparation before AGPs, such as surgical planning, staff postures, door opening, removing cloth sheets, and handling surgical tools, are associated with increasing the bacteria load in the air.[37] Staff, visitors, and patients are potential sources of aerosol bacteria as they shed the aerosolized bacteria from the skin and respiratory tract via walking, talking, and coughing.[22,38] Crowd traffic during the pre-operation stage can also harm the indoor air quality by interrupting the airflow and ventilation in ORs, potentially resulting in a heavy aerosol bacterial load, as demonstrated in a previous study.[22] This study also investigated the bacterial load after different procedures and found the spinal laminectomy procedure had the highest GM of 75.3 CFU/m^3, followed by arthroscopic ligament repair (61.4 CFU/m^3), total knee arthroplasty (59.6 CFU/m^3), and internal fixation with implantation (43.7 CFU/m^3). During operations, aerosol bacteria may settle down directly on the surgical wound, or surrounding surfaces, which may then be indirectly transmitted to the wound by the surgeon's hands and/or devices.[14] It was noted that the GMs for the bacterial loads after these AGPs were lower than the recommended threshold. In the present study, lower activities and lower numbers of surgical staff were observed during the air sampling after AGPs. This indicated that decreasing staff activities during operation may be an effective measure for lowering the aerosol bacteria for improving patient safety. However, keeping a highly efficient laminar airflow is also a crucial measure for preventing SSIs.[21]

Orthopaedic surgery involves making a large incision into deep tissue or organ space and presents specific concerns for postoperative SSI due the significant factors that must be considered for airborne pollutants that may impact patient safety. Differences in aerosol bacterial loads have been reported to be associated with the highest levels of activity among surgical teams in spinal laminectomy.[25]

Regarding the aerosol bacterial contamination in SRs, 64.2% of the total air samples were acceptable with a bacterial count of ≤100 CFU/m^3, which is considered clean according to the standard recommended by EU GMP for cleanrooms. The lowest bacterial load of 36.1 CFU/m^3 was observed in the unoccupied X-ray room, while the unoccupied specimen room had the highest bacterial load of 189.7 CFU/m^3. The higher aerosol bacterial load in the specimen room before working hours may be affected by its smaller volume than other areas, and need to keep the door closed to protect the aerosolized volatile substances, which results in poor air ventilation and a high RH (59.0%), as also recorded in this study. This study also revealed that the bacterial loads in the occupied scrub rooms (93.4 CFU/m^3) and sterile storeroom (89.9 CFU/m^3) were higher than during no-staff activities in the early morning. There was evidence that the microbial indoor air quality may be affected by external and internal sources, including the numbers of staff, crowds of visitors, polluted outdoor air, and poor ventilation, which can all increase the aerosol bacterial density.[36,39] This study was only a short-term assessment of indoor air quality and aerosol microbial contamination in a closed OR area located in the central city without seasonal factor, that many studies have reported that seasonal variation showed no significant difference in fluctuations of bacterial density in OR.[38,40,41]

This study investigated the factors associated with the indoor air bacterial load using linear regression analysis. The univariate analysis and multivariate analysis showed that the temperature during perioperative periods (before AGPs) had a significant positive correlation with the bacterial load in ORs (p<0.05). This finding agrees with previous studies that reported a correlation between the temperature and indoor air bacterial load in surgical rooms[42], and surgical and medical wards.[43] After adjusting potential confounding factors, the results revealed that the temperature and number of staff were significantly correlated with an increase in bacterial load before and after AGPs, respectively, whereas the use of saw-drill instruments showed significant correlation with a decrease in bacterial load after AGPs. Among these associated factors, several reports have evidenced that the temperature and the number of staff can negatively affect the indoor air bacterial load.[37,42,44] Several staff members and their behaviors, including opening and closing the OR doors, foot traffic, and entering or exiting the room, led to a changed airflow pattern and created pressure differences in ORs, which caused microbial particle contaminants

to enter the ORs.[45] The correlation between an open or deep incision site and the concentrated bacterial load in this study may be due to the requirement for more sewing of tissue and muscle layers, and the need for more assistant staff for these complicated operations.[39] These results confirmed that several staff members in the operation room are at potential risk of indoor air bacterial load. Although saws and drills are surgical devices that generate an aerosol, larger- and medium-sized particles are predominantly produced by oscillating saws and drills, while lesser small-sized particles are generated by oscillating saws (28%–40%), high-speed air-powered drills (17%), and high-speed drills with irrigation (9%).[46,47] In a simulation of AGPs in the OR, the dispersal of body fluid was observed to have the highest particle count during bone cutting. These particles fly up and hover in the air for a short period and then slowly move under the direction of laminar airflow. Ensuring laminar airflow with a HEPA filter may be a crucial control measure for preventing occupational contact with infectious body fluids during AGPs in ORs.[48] According to several studies conducted in operating rooms, a general correlation exists between the total microbial count and the risk of infection. However, there are discrepancies in findings among different studies, which can be attributed to several factors, including the surveillance method (active or passive air sampling), sampling time (during surgery or at rest), ventilation system of the ORs, adequacy of room cleaning, and type of disinfectants used.[48]

This study found that 90.8% of the isolated bacteria present in indoor air environments were Gram-positive bacteria. Of these, 49.8 % were CoNS, which arose from the normal flora of the skin and mucous membranes, and 20.5% were *S. aureus*, which is a common SSI causative bacteria. Gram-positive bacteria possess a thick cell wall and exhibit higher resistance compared to Gram-negative bacteria, enabling them to survive under adverse environmental conditions and in closed environments.[45] Gram-positive bacteria, particularly *Staphylococcus* spp., are more frequently found in hospital indoor air and are considered as a bacterial indicator of indoor air pollution.[34,36] Reasonable explanations for the predominantly widespread nature of Gram-positive bacteria in hospital environments are their resistance to dry conditions and frequent transmission through a variety of reservoirs, including human (skin, nasal cavity, cloth, and boils of healthcare workers, patients, and visitors), and environmental surfaces (high touch surfaces, floors, walls, ceilings, and doors).[49]

The WHO declared *S. aureus* and CoNS species as major contaminants in the air of patients rooms, floors, and other surfaces in ORs. Based on our findings, CoNS was more frequently found as an aerosol than *S. aureus*, which is consistent with a study by Shaw LF *et al.*[37] However, studies in Ethiopia[44], Iran[36], and Nigeria[42] showed that *S. aureus* was predominant, followed by CoNS. *S. aureus* and CoNS are potential pathogens that can cause skin and soft tissue infections and are recognized as important causes of SSIs. Therefore, the presence of these pathogens, particularly *S. aureus*, in OR indoor air could indicate a significant risk of SSI from airborne bacteria. There is evidence that these airborne particles with viable bacteria released from the surgical team members and patients can settle onto surfaces, including surgical wounds and instruments.[18,21,46] Therefore, the OR should be regularly cleaned and disinfected, including all surfaces, after every operation. Integrated hygienic control measures in ORs and the medical operatives are essential, not only wearing personal protective equipment but also following environmental cleaning guidelines, including a hand hygiene regimen, and limiting the number of personnel in the room as these may generate bioaerosols in the indoor air.

According to the reported SSIs causative bacteria after orthopaedic surgeries, *S. aureus* accounts for a high prevalence, including a high level antimicrobial resistance rate.[51] The observed high resistance of *S. aureus* to penicillin (61%) and erythromycin (73.8%) in our study aligns with global trends, possibly attributed to the penicillin-resistant strains.[52] Moreover, this study also found MRSA that exhibited resistance to cefoxitin (58/172, 33.7%) and was distributed in the indoor air in both the ORs and SRs. This finding is at a higher percentage than that reported in another hospital in Thailand, where MRSA was observed in 2.3% of indoor air samples and on 0.5% of surface samples.[53] The frequency of MRSA in the nasal carriage of surgical patients was also reported to be 0.3%–9.4%.[54] In general, MRSA has a lower prevalence in non-human isolates than in human isolates. However, several studies have reported a high prevalence of MRSA among environmental surfaces, and in sand/water samples. A previous study found that 44.7% of *S.aureus* isolated from hospital surfaces were identified as MRSA, that was higher than aerosol isolates observed in this study.[51] In contrast, some studies indicated a low prevalence of MRSA ranging from 0.3%–3.5% in non-human samples of non-hospitals. In global epidemiology, MRSA is classified in the group 2 priority list of antibiotic-resistant bacteria, with varying prevalence rates globally, including 19.1% in Africa, 31.9% in the Americas, 17.4% in the Mediterranean, 67.9% in Europe, 27.3% in Southeast Asia, and 43.2% in the Western

Pacific.[47] Also, the prevalence of MRSA colonizers was revealed to be 12.0% in India, 15.6% in Africa, and 1.6% in Europe[55] among healthcare workers. Several reasons may explain the variation in the prevalence of MRSA in non-human samples, including differences in the sampling period, sample size, sampling site, sampling techniques, isolation method, single enrichment step, frequency of MRSA in different samples, or geographical locations.[56]

The high prevalence of MRSA and the emergence of MDR in OR settings require careful attention and is an alarm that the surveillance of *S. aureus* and MRSA colonizers in surgical patients and healthcare workers, especially surgical teams, should be included in infection control measures. Mastering these methods necessitates a strong focus on hospital hygiene training, including hand hygiene and recommendations for surgical attire (such as shirt, trousers, mask, cap, and gloves), the use of personal protective equipment, and adherence to regulatory requirements.[51] The hospital's standard manual cleaning protocol for OR involves using a combined cleaner and disinfectant.[57] Using portable air cleaning technologies[58], and ultraviolet (UV) light, aims to ensure clean air and reduce viable airborne microorganisms in the OR. However, its disadvantages include high costs, potential gas irritation, and degradation and discoloration of certain surface materials, which may pose risks in the OR environment.[57] Thus, we recommend extending the determination of the susceptibility profile to all bacteria associated with air contamination and developing their genetic characteristics so that we can trace back or identify the MRSA reservoir and their mode of transmission.

CONCLUSION

These findings support that the indoor air in ORs and surrounding areas may serve as potential sources of AMR bacteria, posing a critical concern for SSI as a postoperative complication. The overall bacterial contamination rate in the air within the OR areas suggests ineffective cleaning and decontamination practices, disturbed airflow ventilation, and high activity levels. Maintaining a well-controlled ventilation system, ensuring environmental cleanliness, and minimizing non-essential staff activities are crucial for reducing the risk of airborne transmission of pathogens among surgical patients and healthcare workers. Applying integrated hygienic control measures in operative procedures with high compliance and also the surveillance of MRSA colonizers are essential for ensuring the safety of surgical patients and reducing the risk of SSIs.

Data Availability Statement

The data supporting this study are available upon request from the corresponding author.

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DECLARATION

All authors declare that they have no personal or professional conflict of interest.

Registration Number of Clinical Trial

None

Author Contributions

KB: Project administration, Sample collection and analysis, Data analysis, Writing original draft manuscript and editing. MR: Conceptualization and research design, Manuscript review and criticize. YU: Sample collection and analysis, Manuscript review and editing. JN: Research design, Data analysis, Manuscript review and editing. FU: Conceptualization and research design, Academic and methodology supervision, Manuscript criticism and revision.

Ethical Approval Statement

The present study was approved by the Standard Operating Procedures of Ethical Review Committee for Human Research, Faculty of Public Health, Mahidol University (MUPH 87/2022).

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Newly Developed Operative Instrument for Carpal Tunnel Release: A Cadaveric Study

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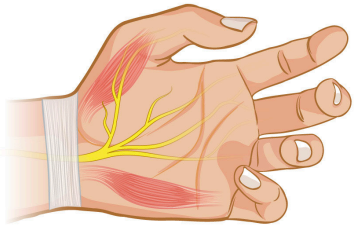
CTR knives mark 2 and 3 for carpal tunnel release

CTR Knives 2 and 3 effectively transected the TCL without causing injury to surrounding structures and preserved, the overlying fascial coverage of the ligament.

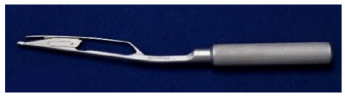


18 fresh cadavers (36 wrists) for CTR Knife 2

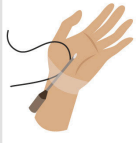
12 fresh cadavers (24 wrists) for CTR Knife 3

All cadavers had no history of previous injury or surgical interventions at their wrists.

CTR knife 2 with a disposable blade no. 15 installed



CTR knife 3 with disposable blade no. 11 installed



Carpal tunnel release operation using limited longitudinal palmar incision

Assess the completeness of transverse carpal ligament (TCL) transection and identify any potential injuries

- CTR Knife 2 achieved a complete cut of TCL for 35 of 36 cadaveric wrists
- CTR Knife 3 had 21 of 24 complete TCL cuts
- No identifiable injury to the neurovascular structures or tendons in the surrounding area.

SCAN FOR FULL TEXT

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ABSTRACT

Objective: This study aims to assess the effectiveness and safety of the newly designed instruments, Carpal tunnel release (CTR) Knives 2 and 3, which use a limited skin incision technique in cadavers.

Materials and Methods: The study utilized 36 wrists from 18 fresh cadavers for CTR Knife 22 and 24 wrists from 12 fresh cadavers for CTR Knife 3. None of the cadavers had a history of previous injury or surgical interventions. A limited longitudinal palmar incision was made for the carpal tunnel release, conducted with the newly developed CTR Knives, which are equipped with a disposable blade number 15 (for CTR Knife 2) or 11 (for CTR Knife 3). To assess the completeness of transverse carpal ligament (TCL) transection, the skin incision was extended proximally to expose and evaluate the released ligament. Meticulous dissection was performed to identify any potential injuries to the neurovascular structures around the surgical area.

Results: CTR Knife 2 achieved a complete cut of TCL for 35 of 36 cadaveric wrists, with only one incomplete cut, while CTR Knife 3 achieved 21 out of 24 complete TCL cuts. The incomplete cuts were likely due to severe thickening of the ligament. Subsequent exploration after the procedure revealed no identifiable injury to the neurovascular structures or tendons in the surrounding area.

Conclusion: The newly developed CTR Knives 2 and 3 effectively transected the TCL without causing injury to surrounding structures and preserved, the overlying fascial coverage of the ligament.

Keywords: Carpal tunnel release; limited incision; instrument; safety; effectiveness; cadaveric study (Siriraj Med J 2025; 77: 233-238)

INTRODUCTION

Carpal tunnel syndrome (CTS), the most prevalent compression neuropathy of the upper extremity,[1] is characterized by numbness, tingling, and weakness in the hand and arm resulting from the compression of the median nerve within the carpal tunnel at the wrist. When conservative treatments such as magnetic stimulation,[2] splinting, medication, and therapy fail to provide relief, surgical interventions are necessary. The standard surgical treatment for CTS is a procedure known as carpal tunnel release (CTR), which involves severing the ligament that forms the roof of the carpal tunnel to relieve pressure on the median nerve. This procedure can be performed using traditional open incisions or minimally invasive techniques such as endoscopic or arthroscopic methods. By releasing pressure on the median nerve, CTR aims to alleviate symptoms and improve hand function.

Carpal tunnel release (CTR) can be performed traditional open incisions or minimally invasive techniques such as endoscopic or arthroscopic approaches. Traditional open CTR often leads to higher post-operative complications, including pillar pain and scar tenderness compared to minimally invasive techniques,[3,4] which are associated with a higher incidence of nerve injury, incomplete release of the carpal ligament, and vascular injury.[5-8] Additionally, there is often a learning curve for surgeons adopting these newer techniques, which can potentially affect surgical outcomes.

A technique using a limited palmar incision has been developed to reduce complications associated with both traditional and minimally invasive carpal tunnel release (CTR), such as pillar pain, painful scarring, and the learning curve for surgeons. Despite these improvements, instances of serious median nerve injury have still been reported.[9] In response, the CTR Knife 1 was developed and has shown promising results in reducing nerve injury complications.[10,11] However, a limitation of this tool is its manufacturing process, particularly the sharpening of the blade, which requires skilled technicians. Additionally, frequent use can dull the blade, leading to ineffective cutting. Therefore, this study aimed to assess the effectiveness of CTR knives 2 and 3 for carpal tunnel releases using a limited incision technique on fresh cadavers.

MATERIALS AND METHODS

Subjects

This proof-of-concept study was carried out at the Department of Orthopaedic Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, between 2020 and 2021. All fresh cadavers aged 18 years or older at the time of death. No specimens showed any signs of previous injuries, wrist surgeries, or congenital defects. The study protocols received approval from the Siriraj Institutional Review Board (SIRB) (Protocol no. 447/2563 (Exempt) for CTR Knife 2 and Protocol no. 890/2563 (Exempt) for CTR Knife 3.

Sample size calculation

This calculation was based on the sample size formula used for estimating the proportion of an infinite population.[12] With an alpha error set at 0.05, $Z_{0.975}$ =1.96, the researcher anticipated that the complete severance of the transverse carpal ligament (TCL) would be at least 90% (P=0.9), with an allowable margin of error at 10% (d=0.1). To maintain a 95% confidence level, a minimum sample size of 35 hands was necessary. Therefore, the study involved the examination of 35 fresh cadaveric wrists (sourced from 18 bodies) for each version of the CTR knives.

CTR Knife 2 and CTR Knife 3

The CTR Knife 2 and 3 (Fig 1) were modified from the original CTR Knife 1.[10,11] The alterations involved replacing the cutting component of CTR Knife 1 with a commercially available blade, specifically blade number 15 for CTR Knife 2 (Fig 1a, 1b) and blade number 11 for CTR Knife 3 (Fig 1c, 1d), with each featuring a locking mechanism. These enhancements facilitate the swift replacement of blades during operations, addressing issues related to decreased sharpness and the necessity for frequent sharpening of the cutting element. Additionally, a protective bar was added to the design to safeguard soft tissues during the removal of the CTR Knives 2 and 3 following the incision of the TCL.

Surgical technique

For consistency, a single orthopaedic surgeon (WP) carried out all carpal tunnel release operations in the present study. A detailed version of the surgical technique used in this procedure has been described in a previous study.[11] In brief, a longitudinal incision of 1.5-2.0 centimeters was made at the intersection of two imaginary lines: one extending from the radial border of the ring finger and the other from Kaplan's cardinal line (Fig 2a). Following this, the palmar aponeurosis was longitudinally incised and retracted to expose the distal border of the TCL. Subsequently, a 0.5-centimeter longitudinal incision was then made on the distal border of the TCL using a No. 15 scalpel blade to expose the median nerve within the carpal tunnel. Penfield dissectors were employed to detach the median nerve from the ligament. Subsequently, the flat blunt tip of the CTR Knife 2 or 3 was carefully inserted under the remaining TCL (Fig 2b), while the wrist was kept slightly extended. Gentle pressure was applied to advance the blade against the ligament until it met resistance at the proximal wrist crease, after which the knife was retracted. Throughout this process, a protective bar was utilized to prevent soft tissue damage, particularly to the fascial layer between the thenar and hypothenar muscles.

Effectiveness and safety of CTR Knife 2 evaluation

After finishing the procedure, the surgeon departed from the operative site to maintain the integrity of the blinding process. Following the procedure, a skin incision was made extending proximally to the proximal wrist crease (Fig 2c) by the hand surgeon to assess the adequacy of the ligament release and to check for potential injuries to structures within the carpal tunnel, including the median

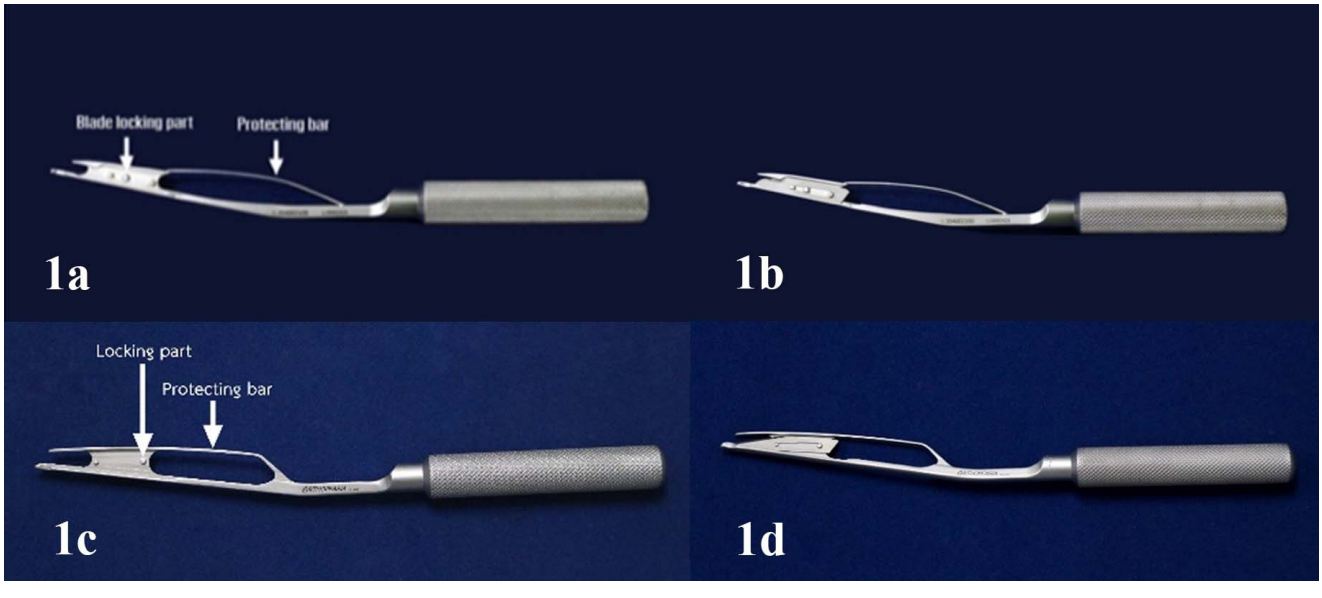

Fig 1. CTR Knives 2 and 3. CTR Knife 2 shows the blade locking part and the protecting bar (1a), and CTR Knife 2 with a disposable blade no. 15 installed (1b). CTR Knife 3 shows the blade locking part and the protecting bar (1c), and CTR Knife 3 with disposable blade no. 11 installed (1d).

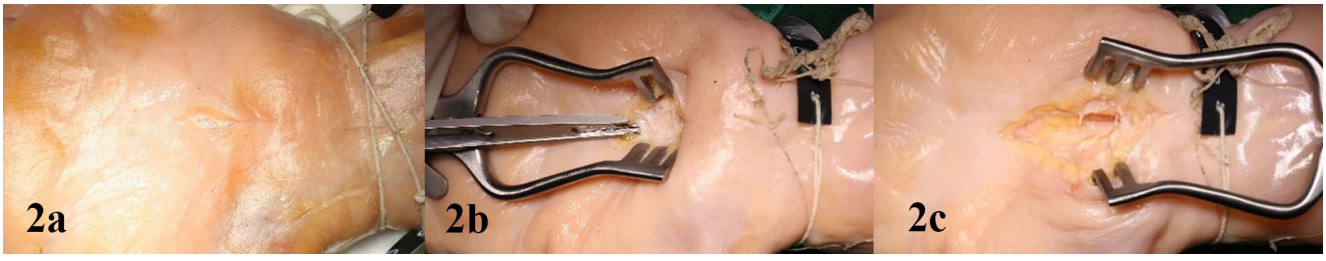

Fig 2. Surgical technique. The incision was made at the convergence point of two theoretical lines: one originating from the radial edge of the ring finger and the other originating from Kaplan's cardinal line. (2a); the insertion of CTR Knife 2 (with blade no.15 installed) to the distal border of the transverse carpal ligament (2b); An extended incision after the procedure to evaluate the effectiveness and safety of CTR Knives (2c).

nerve, the recurrent motor branch of the median nerve, the palmar cutaneous branch of the median nerve, flexor tendons, and the superficial palmar arterial arch. These structures were carefully examined and evaluated under direct visualization from two independent assessors, a hand surgeon, and an experienced research assistant who was trained and involved in CTR knives studies since 2019.

RESULTS

Following a minimally invasive carpal tunnel release surgery using the CTR Knife 2, equipped with blade number 15, a complete transection of the TCL was achieved in 34 out of 35 cadaveric wrists, constituting a success rate of 97.1%. Notably, in one case, incomplete cutting was observed due to pronounced TCL thickening (Fig 2c). When using CTR Knife 3 (equipped with disposable blade number 11), a complete transection of the transverse carpal ligament was achieved in 21 out of 24 cadaveric wrists (87.5%). However, in some cases, obstructions caused by the catching or snagging of the transverse carpal ligament within the narrow, acute-angled niche between the protective bar and the blade, were encountered during the cutting process. During the decompression maneuver, a tactile sensation of obstruction was perceived. Upon further inspection, it was evident that obstructions occurred at this specific niche area and required the application of increased force pressure against the transverse carpal ligament to overcome the impediment. However, no obstructive sensation was observed within the gap between the protective bar and blade number 15.

Throughout the procedure, the integrity of the fascia between the thenar and hypothenar muscles were preserved. Subsequent exploration encompassed assessment of the median nerve, its motor branch, the palmar cutaneous branch of the median nerve, tendons, and associated vessels, revealing no indications of neurovascular injury from both CTR Knife 2 and 3.

DISCUSSION

The effectiveness and safety of CTR Knives 2 and 3 in performing carpal tunnel release procedures using a limited incision surgical technique was demonstrated in our study. The rate of incomplete cutting of the transverse carpal retinaculum (TCR) was comparable between the previously studied CTR Knives 1,[10] 2 and 3 (current study). There were no incomplete cuts in 36 attempts with Knife 1, one incomplete cut in 35 attempts with CTR Knife 2, and three incomplete cuts in 24 attempts with CTR Knife 3. However, no model of CTR knives could detect potential injuries to the surrounding neurovascular structures adjacent to the operative field.

Several instruments, including the retinaculotome,[13] carpal tunnel tome,[9] and Knifelight,[14] have been developed and used for limited incision carpal tunnel release procedures. A major concern with these approaches is the increased risk of nerve and blood vessels injuries due to limited visibility and anatomical variations in the TCL among patients. Studies have shown that the Paine retinaculotome effectively releases the TCL through a limited incision approach, yielding satisfactory results.[13,15,16] However, both the Paine retinaculotome and CTR Knife 1 have experienced issues with blade dullness of the cutting edge after multiple uses, which affects sharpness and cutting ability.

The carpal tunnel tome, while effective, has a high cost which is a significant drawback. It requires the use of several specialized and complex instruments, including an elevator for the TCL, a nerve protection instrument, and a disposable carpal tunnel tome, which can be expensive. Additionally, there have been reports of complications where the median nerve was lacerated during the procedure.[9]

One issue with the Knifelight device is with the plastic skids that sandwich the cutting blade, which are prone to breaking under the increased force required to transect the TCL. Furthermore, the Knifelight is a costly disposable device, and its technical complexity poses challenges for surgeons during its use.

Given the successful application of CTR Knife 1 in both cadaveric studies and clinical patients,[10,11] we strongly recommend adopting a similar surgical technique. This approach enables a complete transection of the TCL with a single cut while preserving the fascial integrity between the thenar and hypothenar muscular fasciae. Ongoing clinical trials evaluating CTR Knives 2 and 3 should demonstrate reduced tissue trauma and an absence of neurovascular injuries when compared to existing techniques or instruments used in TCR.

Our study, conducted using fresh cadaveric specimens, has certain limitations inherent to its design. First, certain procedural aspects may deviate from those encountered in live patient scenarios. For instance, the administration of local anesthesia could potentially induce anatomical alterations around the injection site, and employing a tourniquet might lead to vascular congestion in the hand. Consequently, these factors could result in variations in the incidence of neurovascular injuries in living patients. Second, this study did not explore common complications associated with open carpal tunnel release, such as pillar pain, painful scarring, or postoperative infection rates. These complications are intended to be addressed in future research involving patients diagnosed with carpal tunnel syndrome who undergo future carpal tunnel release procedures. The third limitation of this study was our inability to achieve the initially planned sample size of 35 cadavers for CTR Knife 3. Despite calculations indicating this number would suffice, logistical constraints resulted in the inclusion of only 24 cadavers in the study. The primary reason for this limitation was the unavailability of additional cadavers during the designated study period. While efforts were made to adhere to the predetermined sample size, practical constraints necessitated adjustment of the sample size. Consequently, the reduced sample size may impact the generalizability of our findings. Future studies with larger sample sizes are required to validate the results obtained in this study.

CONCLUSION

The recently developed CTR Knife 2, a specialized tool for limited incision carpal tunnel release procedures, has proven to be both effective and safe. Nonetheless, further investigation through clinical trials is necessary to assess its suitability for widespread clinical application.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available due to privacy or ethical restrictions.

ACKNOWLEDGMENTS

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DECLARATION

Grants and Funding Information

This project is not funded by any external sources.

Conflict of Interest

WP is the patent owner of the CTR knives 2 and 3. Other authors have no relationships, conditions, or circumstances that present a potential conflict of interest.

Registration Number of Clinical Trial

This study utilized cadaver for research. As it does not involve living human participants, clinical trial registration was not required.

Author Contributions

W.P. : conceptualisation, project administration, methodology, funding acquisition, supervision, investigation, data curation, formal analysis, visualisation, validation, writing-original draft preparation, review & editing. N.P. : conceptualisation, methodology, investigation, writing – review & editing. P.C. : conceptualisation, methodology, investigation, formal analysis, writing –review and editing, corresponding author.

Use of Artificial Intelligence

Artificial Intelligence tool was not used in this manuscript.

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Platelet Rich Plasma as a Potential Treatment for Melasma: A Review

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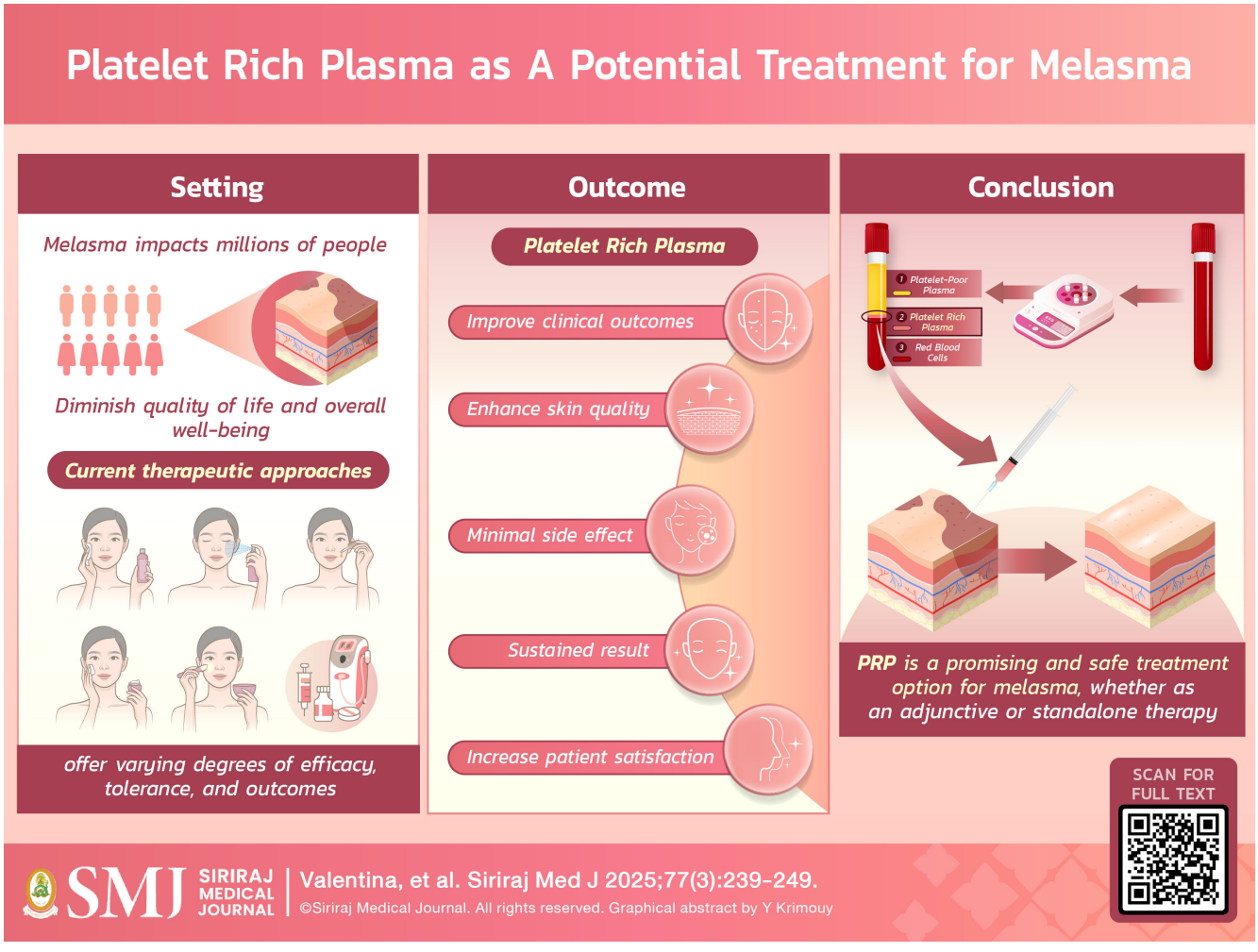

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ABSTRACT

Melasma impacts millions of individuals globally. It is characterized by hyperpigmented macules that predominantly affect the centrofacial region. Although not medically dangerous, melasma can significantly diminish quality of life and overall well-being. Current therapeutic approaches offer varying degrees of efficacy, tolerance, and outcomes, underscoring the need for further research to identify treatments that are both effective and safe. Platelet-rich plasma (PRP), an autologous plasma enriched with a high concentration of platelets, has gained attention in the medical field for its regenerative properties and favorable benefit-risk profile. In dermatology and aesthetic medicine, PRP has demonstrated efficacy in applications such as wound healing, skin rejuvenation, alopecia, acne scarring, and, more recently, pigmentation disorders. This review explores the potential of PRP as a treatment modality for melasma, suggesting that PRP, whether used as an adjunctive or standalone therapy, may significantly enhance treatment outcomes. Nevertheless, despite promising evidence supporting its use, further research is required to establish robust biomolecular mechanisms and evaluate the long-term safety and efficacy of PRP in managing melasma.

Keywords: Melasma; melasma treatment; platelet-rich plasma (PRP) (Siriraj Med J 2025; 77: 239-249)

INTRODUCTION

Melasma is a formidable cosmetic issue and ranks among the three most prevalent skin issues in medical aesthetic practice, alongside acne and wrinkles. While melasma is not a dangerous condition, it can considerably affect an individual's physical appearance, resulting in emotional and psychosocial distress associated with embarrassment, frustration, low self-esteem, and interpersonal interactions, ultimately diminishing quality of life.[1–7] Melasma is characterized by irregular brown macules symmetrically distributed on sun-exposed areas of the body, particularly on the face. It is a common reason for seeking dermatological care, primarily affecting women (especially during the menacme). Managing melasma is notably challenging in dermatology, as many treatment approaches frequently yield variable outcomes that fall short of patient expectations.[8] Melasma treatments vary in efficacy and often present issues such as irritation, post-inflammatory hyperpigmentation, and rebound hyperpigmentation[9], highlighting the need for adjunctive or novel alternative therapies.

Platelet-rich plasma (PRP) is a procedure that uses centrifuged blood with a high concentration of platelets in a small plasma volume.[2,10] PRP is a regenerative treatment that remains under investigation. This therapy has garnered significant attention in the medical field due to its favorable benefit-risk profile. The application of PRP has shown promising results for individuals unresponsive to conventional therapies. Numerous skin conditions progress over time and necessitate extended treatment; however, managing these conditions can be challenging due to significant adverse effects, suboptimal therapeutic responses, and high recurrence rates. PRP may serve as a promising treatment option for such complex skin disorders.

As a novel therapeutic approach, PRP has demonstrated potential in treating various skin and cosmetic conditions, including alopecia, wound healing, skin rejuvenation, and acne scarring.[11] Recent studies have indicated positive outcomes for PRP therapy in managing skin hyperpigmentation, especially in individuals with melasma. However, the understanding of PRP's therapeutic efficacy in melasma treatment remains limited. This review aims to examine the efficacy and mechanism of PRP as an alternative and adjunctive therapy for treating melasma.

Melasma

Melasma is a prevalent chronic skin hyperpigmentation that impacts a significant proportion of the global population.[11–13] Melasma presents as brownish macules with uneven borders, symmetrically located on sun-exposed areas of the body, predominantly on the centrofacial areas of the forehead, cheeks, nose, philtrum, and chin.[1–3,6,9,11,12,14–19] Melasma predominantly occurs in women with dark hair, brown eyes, and dark skin. Its prevalence reaches 75% in pregnant women and typically manifests throughout their reproductive years.[20] A population-based study in 2010 reported pigmentation issues as a leading cause of skin treatment requests, affecting 23.6% of men and 29.9% of women.[1] In Southeast Asia, 40% of women seek dermatological care for melasma, with a female-to-male ratio of 9:1 and onset occurring between the ages of 20 and 30. Individuals at a higher risk include those of reproductive age, pregnant individuals, and those with Fitzpatrick skin types III-IV.[6,15,17]

The pathogenesis of melasma is intricate, multifaceted, and not completely elucidated. Melasma results from dysregulation in melanogenesis, with contributing factors such as sun exposure, hormonal fluctuations during pregnancy, use of oral contraceptives and other steroids, hormone replacement therapy, photosensitizing cosmetics and medications, antiseizure therapy, and genetic predisposition.[1,3,4,6,9,11,12,14,16,17,20–22] Melasma is also associated with vascular factors, inflammation, and skin barrier dysfunction.[19] Histopathological studies have shown increased dermal vascularity, basement membrane disruption, higher melanocyte count, increased melanosome, solar elastosis, mild inflammatory cell infiltration, and greater melanin deposition in the dermis and/or epidermis of melasma-affected skin.[3,9,20] Sun exposure is the primary catalyst for melasma, as it stimulates melanogenic activity, upregulating melanin synthesis and its transfer to keratinocytes, leading to increased eumelanin deposition in the epidermis.[1,15,23]

Melasma is diagnosed clinically. Wood's lamp examination can ascertain the distribution of melanin pigment in the dermis or epidermis to assess the type of melasma (epidermal, dermal, or mixed).[20] Dermoscopic evaluation can assess the intensity of melanin pigmentation and the regularity of pigment network, which may suggest the location and density of melanin pigment deposition. It can also be utilized to evaluate the severity of melasma.[1,20] The Melasma Area and Severity Index (MASI) and modified MASI (mMASI) score are standard scales for evaluating the extent and severity of facial melasma.[1] In contrast, the Melasma Quality of Life scale (MELASQOL) assesses the impact of melasma on patients' quality of life.[7]

The management of melasma poses challenges for clinicians and patients. Supportive treatment often begins with avoiding sun exposure or applying sunscreen to mitigate disease progression.[14,23] However, effective treatment necessitates active intervention. Numerous melanogenesis inhibitors have been developed, although many raise significant toxicity concerns and common skin adverse effects, including erythema, dry skin, irritation, desquamation, and hypopigmentation.[8,15,24] Available treatment options for melasma include topical depigmenting agents, such as hydroquinone, kojic acid, glycolic acid, azelaic acid, retinoids, corticosteroids, arbutin, and niacinamide; oral therapies such as tranexamic acid, melatonin, cysteamine, and glutathione; chemical peels; and laser and light therapies.[8,9,11,14,15,25] Current therapeutic approaches exhibit varying degrees of efficacy, leading to inconsistent and predominantly poor outcomes, varying side effects, and a significant recurrence rate post-therapy cessation.[8,11,13,22,23,26]

Platelet-Rich Plasma (PRP)

PRP is a biological product characterized by a small volume of autologous plasma with a platelet concentration three to seven times higher than that of whole blood[2,16], achieved through centrifugation and platelet suspension.[9,11,14,27] Typically, blood comprises approximately 94% red blood cells (RBCs), 6% platelets, and 1% white blood cells. PRP preparation alters the ratio of RBCs to platelets, resulting in a composition of 95% platelets and 5% RBCs.[28] The optimal platelet concentration for effective PRP therapy in skin treatments is 1-1.5 million platelets/μL.[10]

Platelets are small cellular fragments derived from megakaryocytes and contain two types of storage granules: alpha granules and dense granules.[10] Alpha granules are crucial for PRP therapy due to their high concentration of growth factors, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), and fibroblast growth factor (FGF)[10,15] which are instrumental in mediating various mechanisms such as cell differentiation, proliferation, and regeneration. Meanwhile, dense granules contain ADP, ATP, calcium, serotonin, and glutamate, which contribute significantly to the therapeutic benefits of this treatment.[10] Within 10 minutes following PRP injection, 70% of the growth factors in alpha granules are secreted, with at least 95% released within one hour. For up to seven days, platelets continue producing and releasing supplementary growth factors.[10,29]

The clinical applications of PRP have expanded across multiple medical fields. PRP is frequently used in plastic surgery, particularly for treating chronic wounds, ulcers, and burns.[30] PRP has emerged as a promising therapeutic method in aesthetic and dermatological medicine in recent years, demonstrating effective outcomes in wound healing, alopecia with or without scarring, skin rejuvenation, acne scars, and pigmentation disorders.[3,10,15,16,27,28,30] Research indicates that PRP enhances skin quality and increases collagen and elastic fiber production, as it stimulates the proliferation of human dermal fibroblasts and boosts type I collagen synthesis.[28] The ability to stimulate collagen synthesis, reduce recovery time, and yield lasting results renders PRP a compelling therapeutic alternative in cosmetic dermatology.[31]

PRP therapy exhibits an enhanced safety profile due to its autologous nature.[10,14] Adverse effects of PRP are infrequent and minor, including localized pain, infection, skin discoloration, allergic reactions, and thrombus formation.[25] Absolute contraindications include severe thrombocytopenia, platelet dysfunction, unstable

hemodynamics, sepsis, and localized infection at the injection site. Relative contraindications include NSAID administration within 48 hours preceding treatment, glucocorticoid injections within two weeks prior, recent illness or fever, cancer, anemia with hemoglobin below 10 g/dL, moderate thrombocytopenia, and tobacco use.[10]

Currently, there is no global consensus or standardized protocol for optimal PRP preparation.[16,32] PRP preparation typically commences with the collection of 10 to 60 cc of venous blood, which is subsequently transferred into a tube containing dextrose citrate acid or sodium citrate to inhibit platelet activation, degranulation, and premature release of effector molecules. A first centrifugation separates the RBCs from the plasma, after which the yellow-colored plasma supernatant is extracted and subjected to a second centrifugation to isolate plasma rich in platelets and leukocytes from platelet-poor plasma. Following centrifugation, two-thirds of the supernatant plasma is discarded, and the remaining plasma containing a platelet pellet is classified as PRP. The final product typically has a platelet concentration of approximately 1 million/mL, two to eight times higher than whole blood.[15,27,29,32]

PRP as a treatment option for melasma

PRP is an innovative treatment approach for melasma, with numerous case reports and studies have demonstrated its efficacy. Research indicates that PRP, whether used in combination with other treatments or as an independent therapy, is associated with notable clinical improvement in patients with melasma, leading to high patient satisfaction.[15] Patients undergoing PRP treatment achieve a more balanced complexion and improved skin quality, including reduced wrinkles, enhanced elasticity, and increased moisture.[9] Moreover, compared to other melasma therapies, PRP treatment results in fewer adverse effects and reduced pigmentation rebound.[3,16,21,33]

In 2014, Cayrili et al.[30] reported the advantageous application of PRP as an alternative treatment for melasma, with over 80% reduction in epidermal hyperpigmentation in a patient with centrofacial melasma following three biweekly PRP sessions, with the initial objective of skin rejuvenation. Furthermore, no melasma recurrence was observed up to six months post-treatment. Yew et al.[12] found that intralesional PRP as an adjunctive therapy reduced pigmentation in two cases of melasma unresponsive to conventional treatments. Administered over two sessions at four-week intervals, alongside monthly Q-switched Nd:YAG 1064 nm laser treatments and daily topical alpha arbutin, PRP was associated with a reduction in mMASI scores. Garg et al.[35] documented improvement in a case of recalcitrant melasma unresponsive to multiple treatments, including topical depigmentation agents (e.g., topical steroids, tretinoin, hydroquinone, kojic acid, and arbutin), oral tranexamic acid, and chemical peels. Six intradermal PRP sessions achieved clinical improvement and a lowered MASI score, with no recurrence over a three-month follow-up. Recent reports by Wulandari et al.[18] and Shahraki et al.[31] further indicated positive responses in melasma patients treated with microneedling-PRP characterized by brighter skin and significant reductions in brown patches. In other words, PRP can reduce pigmentation and revitalize the skin, enhancing the patient's overall appearance.

Sirithanabadeekul et al.[9] conducted a randomized, split-face, placebo-controlled trial using PRP as an alternative treatment for melasma. Four sessions of intradermal PRP with two-weeks interval significantly improved melasma within six weeks, as evidenced by significantly lower mMASI score, decreased melanin levels, increased patient satisfaction, and reduced wrinkles. These findings are consistent with those of Tuknayat et al.[21] and Rout et al.[6], who observed significant melasma improvements following three intradermal PRP sessions at four-week intervals, with minimal side effects and no recurrence over a three-month follow-up. Rout et al.[6] reported a 77% reduction in mMASI in mild melasma, a 52% reduction in moderate melasma, and a 50% reduction in severe melasma. The improvement of pigmentation depended on skin type, gender, and the type and pattern of melasma. In addition, patients experienced significant improvement in skin quality and reduced wrinkles.[21] Similarly, González-Ojeda et al.[3] reported that three intradermal PRP sessions at 15-day intervals resulted in a significant reduction in the intensity and extent of hyperpigmentation, as assessed by the MASI score, along with improvements in patients' self-perception and quality of life as measured by MELASQOL.

Hofny et al.[11] documented the prospective therapeutic efficacy of PRP as an alternative treatment for melasma, employing two different techniques: microneedling with a dermapen and intradermal microinjection with microneedles. Both techniques resulted in notable improvements in melasma patients, as evidenced by a significant decrease in MASI and mMASI scores following three PRP sessions at four-week intervals. The findings are consistent with a randomized clinical trial by Boparai et al.[26], which demonstrated improvements in melasma following three microneedling sessions with PRP administered every three weeks. No significant adverse effects or recurrence were observed up to 18 weeks post-treatment. A study

TABLE 1. Studies on the use of PRP for melasma treatment.

Study design	Study group and Methods	PRP preparation	Outcomes	Side effects
Tuknayat et al. (2021)[21] An open-labeled prospective therapeutic trial involving 40 patients with melasma.	Intradermal PRP (0.1 ml/cm²) Treatment was conducted over 3 sessions at a month interval.	10 ml of venous blood. 1st centrifugation: 1,600 rpm for 10 minutes. 2nd centrifugation: 4,000 rpm for 10 minutes.	There was a significant reduction in mMASI score at the end of the study (from 13.7 to 6.258, with mean reduction of 54.5%). >90% of patients were satisfied with the treatment results.	There were no serious side effects except xerosis (35%) and pruritus (25%). No recurrence was observed in patients during the 3-month follow-up period.
González-Ojeda et al. (2022)[3] A self-controlled clinical trial on 20 female patients with melasma.	Intradermal PRP Treatment was performed over 3 sessions with 15-day intervals.	Venous blood was collected into a tube containing 3.8% sodium citrate. Centrifugation: 2,500 rpm for 11 minutes. Activation by adding 0.1 units of 10% calcium chloride (CaCl2) for every 1ml of PRP.	There was a significant regression of hyperpigmentation in intensity and extension based on MASI score (from 15.5 ± 8.4 to 9.5 ± 7.2, p=0.001). There was a significant improvement in self-perception and quality of life, as indicated by MELASQOL score (from 42 ± 14.8 to 16.6 ± 7.2, p=0.008).	No local or regional complications were reported.
Sirithanabadeekul et al. (2020)[9] A randomized split-face, single-blinded prospective trial on 10 female patients with bilateral mixed-type melasma.	• PRP side: intradermal PRP (0,1ml/cm²) • Other side (control): intradermal normal saline Treatment consisted of 4 sessions, administered every 2 weeks.	13.5ml venous blood and 1.5ml citrate dextrose A were mixed. Centrifugation: 3,200 rpm for 4 minutes.	Intradermal PRP demonstrated a significant improvement in both mMASI score (p=0.042) and melanin levels (p=0.038) at week 6. There was 28.9% improvement on PRP side, while control side showed 9% improvement. Melanin index values showed no statistically significant difference between the two sides, although a trend toward reduced pigmentation was observed on the PRP side.	Mild side effects, including bruising, were observed, all of which resolved spontaneously within a few days.
Boparai et al. (2020)[26] A randomized clinical trial involving 30 patients with facial melasma.	Microneedling with dermaroller + topical PRP Treatment was performed over 3 sessions at 3-week intervals.	Venous blood was collected into a tube containing sodium citrate. 1st centrifugation: 1,500 rpm for 10 minutes. 2nd centrifugation: 4,000 rpm for 10 minutes. Activation by adding 0.1ml CaCl2 to 1ml PRP.	There was a significant decrease in MASI score (from 12.73 to 6.09, p<0.05) at week 18. Reduction in MASI score was noted starting from week 3. A total of 10%, 30%, and 60% of patients showed improvement of <25%, 25-<50%, and 50-<75%, respectively.	No serious side effects except transient mild erythema in 80% of patients after procedure. No recurrence of melasma was observed during the follow-up period, which extended up to 18 weeks.

TABLE 1. Studies on the use of PRP for melasma treatment. (Continue)

Study design	Study group and Methods	PRP preparation	Outcomes	Side effects
Hofny et al. (2019)[11] A randomized clinical trial on 23 adults Egyptian melasma patients with Fitzpatrick skin types III-IV.	• Right side of the face: micro-needling with dermapen + topical PRP • Left side of face: intradermal PRP Treatment was conducted over 3 sessions at 4-week intervals	10 mL of venous blood was collected into a tube containing ethylenediamine-tetraacetic acid (EDTA). 1st centrifugation: 160 g for 10 minutes. 2nd centrifugation: 400 g for 10 minutes. Activation by adding 1 ml of 3% CaCl2 to 1.5 ml of PRP.	There was a significant decrease in MASI (from 11.86 ± 5.25 to 6.96 ± 4.82, with 34.8% significant to excellent improvement) and mMASI scores (from 5.71 ± 2.56 to 2.90 ± 2.05, with 47.8% significant to excellent improvement) on both sides of the face following treatment (p<0.000). However, no significant difference was found when comparing the two sides.	Most patients experienced greater pain on the left side than on the right side of the face. All patients reported less downtime (in swelling, redness, and soreness) on the left side of the face compared to the right side following the procedure.
Panda et al. (2022)[13] A randomized prospective comparative study with 60 participants diagnosed with melasma.	• Group A: micro-needling with dermaroller only • Group B: Micro-needling with dermaroller + topical PRP Treatment was performed over 3 sessions at a month interval.	5 ml of venous blood was collected into a tube containing anticoagulant. 1st centrifugation: 1,500 rpm for 10 minutes. 2nd centrifugation: 3,000 rpm for 20 minutes.	There was a significant reduction in MASI scores in both groups (Group A from 10.2 ± 6.2 to 7.6 ± 5.4, with 62.5% moderate improvement, p=0.001 vs Group B from 10.6 ± 5.9 to 4.9 ± 3.5, with 37% moderate improvement, 59.2% significant improvement, 3.7% excellent improvement, p=0.0001). Microneedling + PRP had a superior effect due to higher MASI score reduction and better patient satisfaction than microneedling alone.	The side effects were transient and generally well tolerated, including mild pain during the procedure, along with mild erythema and localized edema, which resolved within 48-72 hours. Notably, Group B reported a shorter recovery time in terms of redness and swelling.
Gharib et al. (2021)[33] A single-center clinical trial involving 26 patients with melasma.	• Group 1: micro-needling + topical PRP • Group 2: micro-needling + topical tranexamic acid (TXA) 4mg/ml Both treatments were conducted over 4 sessions.	10 ml of venous blood was collected into a tube containing acid citrate dextrose. 1st centrifugation: 1,500 rpm for 10 minutes. 2nd centrifugation: 3,700 rpm for 10 minutes. Activation by adding calcium gluconate to PRP in a 1:9 ratio.	There was a statistically significant difference between the two groups (p<0.017). Microneedling + PRP gave better results in MASI score (from 6.48 ± 3.37 to 3.17 ± 2.05, with 50% improvement) than microneedling + TXA (from 9.06 ± 2.95 to 5.23 ± 3.51, with 42% improvement).	• PRP group: pain (100%), erythema (46.15%), post-inflammatory hyperpigmentation (PIH, 7.69%) • TXA group: pain (84.62%), erythema (53.46%), PIH (15.38%) There were no significant differences between the two groups regarding side effects.
Mumtaz et al. (2021)[23] Non-randomized controlled trial on 64 patients with melasma.	• Group A: intradermal PRP 1ml • Group B: intradermal TXA 4mg/ml Both treatments were administered over 3 sessions at 4-week intervals.	15-20 ml of venous blood was collected into a tube containing sodium citrate. 1st centrifugation: 1,500 rpm for 10 minutes. 2nd centrifugation: 4,000 rpm for 10 minutes. Activation by adding 0.1 ml CaCl2 to 1 ml PRP.	Intradermal PRP showed significantly better results than intradermal TXA at 4 weeks (p=0.01), 12 weeks (p=0.0001), and 24 weeks (MASI score decreased from 29.84 ± 5.14 to 8.72 ± 3.40 in PRP group vs 29.56 ± 4.39 to 14.97 ± 4.33 in TXA group, p=0.02).	No specific side effects of the treatment were reported.

TABLE 1. Studies on the use of PRP for melasma treatment. (Continue)

Study design	Study group and Methods	PRP preparation	Outcomes	Side effects
Abd Elraouf et al. (2023)[14] A randomized split-face prospective comparative study on 40 facial melasma patients with Fitzpatrick skin types III-IV.	• Right side of the face: Intradermal TXA 4 mg/ml was injected at a dose of 0.05 ml per injection point with a distance of 1cm per point. • Left side of the face: intradermal PRP 1 ml per session Both treatments were performed over 3 sessions at 4-week intervals.	10 ml of venous blood was collected into a 3.2% sodium citrate tube. 1st centrifugation: 3,000 rpm for 7 minutes. 2nd centrifugation: 4,000 rpm for 5 minutes. Activation by adding 0.1 ml CaCl2 to 0.9 ml PRP.	There was a significant decrease in mMASI score of both groups (p<0.001), but the percentage decrease on the PRP side was higher than on the TXA side (53.66 ± 11.27% vs 45.67 ± 8.10%).	• TXA side: pain (62.5%), erythema (55%) • PRP side: pain (7.5%), erythema (32.5%) There was no statistically significant difference between the two groups regarding side effects.
Gamea et al. (2022)[22] A randomized comparative study involving 40 female patients with melasma.	• Group A: Topical TXA 5% in liposome-based cream applied twice daily for 12 weeks • Group B: Topical TXA 5% combined with 4 sessions of intradermal PRP every 3 weeks	10-15 ml of venous blood was collected into a tube containing sodium citrate. 1st centrifugation: 2,000 rpm for 3 minutes. 2nd centrifugation: 5,000 rpm for 5 minutes. Activation by adding 0.1ml CaCl2 to 1ml of PRP.	Both groups exhibited significant improvement in mMASI scores (p<0.001); however, the treatment response was significantly greater in Group B than Group A (35% good to excellent response vs 20% good to excellent response, p=0.024). Patient satisfaction was notably higher in Group B compared to Group A, with the difference reaching statistical significance (p=0.029).	• Group A: rebound pigmentation (10%) • Group B: rebound pigmentation (5%), moderate pain during PRP injection (60%), transient erythema <24 hours after injection (50%)
Tawanwongsri et al. (2024)[5] A randomized prospective investigator-blinded controlled trial on 26 patients with mixed-type melasma.	• Group A: intradermal PRP (0.1 ml/cm^2) conducted over 3 sessions with 4 weeks interval • Group B: intradermal PRP + oral TXA 500 mg/day for 12 weeks	16ml of venous blood was collected into a tube containing acid citrate dextrose and gel. Centrifugation: 3,200 rpm for 10 minutes.	There was a significant decrease in mMASI score in both groups (group A from 4.3 to 3.6 vs group B from 6.4 to 3.6), but the median change was significantly higher in group B than in group A (2.90 vs 0.90, p=0.006).	15.4% of patients experienced transient erythema and swelling, which resolved within 4 hours, along with mild pain during injection. In Group B, 1 patient experienced transient mild gastrointestinal discomfort during the 1st week of oral TXA administration, with no subsequent symptoms reported.

TABLE 1. Studies on the use of PRP for melasma treatment. (Continue)

Study design	Study group and Methods	PRP preparation	Outcomes	Side effects
Rout et al. (2023)[17] A randomized comparative split-face prospective study on 20 female patients with Fitzpatrick skin types IV-V who had mixed-resistant melasma and bilateral facial involvement.	• Facial Side A: Intradermal PRP (0.1 ml/cm²) every 2 weeks for 7 sessions • Facial Side B: 1064 nm Q-switched Nd-YAG laser administered weekly for 12 weeks	No details on the PRP preparation procedure were provided.	Hemi mMASI score on PRP side decreased from 7.52 to 3.05, while on laser side decreased from 7.67 to 5.43. PRP administration showed significant improvement in pigmentation within 12 weeks of treatment.	Some patients experienced mild redness and burning post-procedure which resolved within a few days. PRP side has lower incidence compared to laser side. PRP side has lower relapse rate of melasma after 3 months compared to the laser side.
Adel et al. (2021)[34] A randomized prospective split-face study involving 20 Egyptian female patients with refractory melasma.	• Right side of the face: intradermal PRP + intense pulsed light (IPL) • Left side of face: intradermal PRP only Treatment was performed over 4 sessions at 2-week intervals.	8 ml of venous blood was drawn and centrifuged. 1.5 ml of PRP was injected intradermally into the melasma area using the papule method.	There was a significant decrease in MASI score after treatment ($p<0.05$), but there was no statistically significant difference between the two groups ($p>0.05$).	The side effects were minimal, temporary, and well tolerated.

by Panda et al.[13] yielded comparable results, concluding that microneedling followed by topical application of PRP effectively treated melasma, leading to decreased MASI scores, higher patient satisfaction, and sustained MASI score reductions three months post-treatment.

Nada et al.[36] conducted a case-control study comparing two melasma treatment approaches: topical hydroquinone (HQ) 2% administered for nine weeks and intradermal PRP administered in four sessions at three-week intervals. At week 13, the PRP group exhibited a mean MASI score reduction of 54.79%, whereas the HQ group demonstrated a reduction of 24.52%. This indicated that PRP may serve as a more alternative to standard melasma therapies. A split-face study by Rout et al.[17] compared the efficacy of intradermal PRP administered biweekly over seven sessions with weekly 1064 nm Nd:YAG Qs laser treatment for 12 weeks in patients with mixed resistant melasma. The results showed significant pigmentation improvement and a lower relapse rate on the PRP-treated side three months post-treatment compared to the Nd-YAG Qs laser side. These findings suggested PRP may serve as a primary and maintenance therapy for mixed-resistant melasma.

Recent studies indicate that PRP significantly outperforms tranexamic acid (TXA) in treating melasma, particularly in the long-term.[4,14,23,33,37] This indicates PRP's potential to surpass conventional therapies. According to Mumtaz et al.[23], PRP demonstrated statistically significant outcomes by week 12. In split-face studies[4,14], TXA 4 mg/ml was administered intradermally on the right side of the face, while PRP was injected intradermally on the left side. After 12 weeks, a statistically significant reduction in mMASI scores was observed on both sides, but the percentage reduction was greater on the PRP side than the TXA side without notable side effects. Research comparing the benefits of microneedling combined with PRP and microneedling combined with TXA[33] indicated that melasma patients receiving microneedling-PRP had superior improvement compared to those treated with microneedling-TXA. Consequently, without contraindications to PRP administration, PRP may be a practical option for treating melasma.

Bikash et al.[38] and Tekam et al.[39] evaluated the efficacy of PRP combined with HQ comparing it to the gold standard of HQ alone. It was concluded that the combination of microinjection/microneedling PRP with topical HQ 4% enhanced melasma treatment efficacy. Gamea et al.[22] evaluated the effectiveness of topical TXA 5% versus its combination with intradermal PRP administered every three weeks for 12 weeks. Both groups exhibited a notable decrease in mMASI scores following therapy, leading to a recommendation of PRP as a safe adjunctive therapy to enhance the effectiveness of TXA in treating melasma. Tawanwongsri et al.[5] evaluated the efficacy and safety of combined PRP and oral TXA against standalone PRP, finding that after 12 weeks, the improvement in mMASI scores was more significant in the group receiving three intradermal PRP sessions at four-week intervals alongside oral TXA at a dosage of 500mg/day for 12 weeks. No significant adverse effects were observed, except for mild and tolerable gastrointestinal symptoms. Zhang et al.[40] confirmed that combining intradermal PRP and oral TXA can enhance therapy efficacy and reduce the risk of melasma recurrence for up to six months post-treatment. Adel et al.[34] investigated the effectiveness of PRP injection alone administered over four sessions at two-week intervals and its combination with intense pulsed light (IPL) in patients with refractory melasma. A notable reduction in melasma scores was observed after six weeks of PRP treatment, although no statistically significant difference was identified between the two groups concerning mMASI scores and patient satisfaction.

Mechanisms of PRP action on melasma

PRP has shown notable potential as a treatment for melasma. However, the exact mechanism responsible for its therapeutic effects remains inadequately comprehended and is only tentatively hypothesized.[6] The therapeutic efficacy of PRP relies on the premise that the degranulation of alpha granules following platelet activation results in the release of multiple growth factors, including EGF, TGF-β, and PDGF. These factors bind to specific receptors on various cells, initiating signal transduction pathways that lead to gene expression and the release of proteins involved in melanogenesis and tissue repair.[2,11,16,21] Two primary processes underlying the effects of PRP on melasma include suppressing melanin synthesis facilitated by TGF-β1 and EGF and enhancing skin volume facilitated by PDGF.[10,11,13,14]

Transcriptional examination of skin samples from melasma patients revealed the upregulation of numerous genes associated with melanin formation, including microphthalmia-associated transcription factor (MITF), tyrosinase, and tyrosinase related protein (TYRP).[16] A randomized clinical trial by Hofny et al.[2] reported a significant reduction in TGF-β protein expression in the skin lesions of melasma patients compared to healthy skin, potentially attributable to UV exposure, which is linked to the suppression or cessation of TGF-β production at transcriptional and translational levels. Conversely, PRP treatment can elevate TGF-β protein expression to levels nearly equivalent to those of healthy skin, correlating with significant clinical improvement. These findings indicate that alterations in TGF-β protein expression in the skin lesions of melasma patients corroborate its involvement in the pathogenesis of the disorder and possess therapeutic implications.

The TGF-β family regulates various cellular activities in the skin, including cell proliferation, differentiation, and melanogenesis.[2,12] TGF-β is a critical growth factor for melasma treatment as it modulates melanocyte pigment synthesis.[6,17] Prior research has demonstrated that TGF-β1 can limit melanin production by directly suppressing the expression of paired-box homeotic gene 3 (PAX3), which encodes a transcription factor crucial for melanocyte proliferation and/or survival, and by downregulating MITF, which is crucial for the transcriptional regulation of tyrosinase, TYRP1, and TYRP2.[2,5,10,21,25] Conversely, another study revealed that TGF-β1 can reduce melanogenesis through delayed extracellular signal-regulated kinase (ERK) activation.[5] TGF-β1 strongly inhibits the MITF promoter's transcriptional activity, thus decreasing MITF expression and consequently inhibiting tyrosinase gene transcription.[2,10,15,17,30] The formation of eumelanin and reduction of pigmentation can be diminished by lowering the expression of tyrosinase and other enzymes involved in melanin biosynthesis.

Prior research has demonstrated that melasma is a melanocytic disorder and a photoaging skin disease. Ultraviolet exposure elevates the levels of MMP-2 and MMP-9, leading to the degradation of collagen types IV and VI in the skin and resulting in basement membrane damage, thereby facilitating the infiltration of melanocytes and melanin into the dermis.[16] Consequently, conventional therapy targeting melanosome or melanocyte activity may prove inadequate for treating this condition. On the other hand, PRP induces a pigmentary lightening effect by promoting basement membrane repair facilitated by laminin, collagen IV, and tenascin, which are stimulated by TGF-β1 produced upon PRP activation, thereby inhibiting the migration of melanocytes and melanin into the dermis.[5,10,11,13,16,22]

EGF is widely used in cosmetic formulations for skin

lightening, wound healing, and reducing post-inflammatory hyperpigmentation from lasers or UV exposure.[25] EGF can influence the activity of pro-inflammatory mediators released by damaged keratinocytes, such as prostaglandin-E2 (PGE2), which stimulates melanogenic activity in the skin by regulating melanocyte dendrite formation, proliferation, and tyrosinase expression. EGF can limit melanogenesis by suppressing PGE2 expression and activating the ERK pathway, thereby reducing tyrosinase enzyme activity and ultimately decreasing melanin synthesis.[5,10,21,23] The improvement in pigmentation following PRP treatment is also attributed to increased skin volume induced by PDGF stimulation. PDGF plays a critical role in angiogenesis, collagen production, and the formation of extracellular matrix component, particularly hyaluronic acid, which enhances skin tone and volume, leading to a radiant complexion.[5,10,11,16,21,25,30]

The synergy of bioactive compounds present in PRP improves pigmentation in patients with melasma. PRP possesses bacteriostatic, anti-inflammatory, and reparative properties that rectify the aberrant hyperpigmentation metabolism associated with melasma.[40] Growth factors, fibrin, and leukocytes present in PRP can modulate and restore the overall architecture of the skin layer, enhance skin barrier function, re-establish microcirculation, reduce hyperpigmentation, and stimulate collagen synthesis and epidermal regeneration to enhance skin quality and texture. They can also minimize the risk of re-pigmentation in the area.[6,17,31,40] Thus, the therapeutic efficacy of PRP is thought to be associated with the restoration of aberrant pigment metabolism and numerous reparative actions that address compromised skin-barrier integrity, inflammation, and vascular alterations contributing to the etiology of melasma.

CONCLUSION

Platelet-rich plasma (PRP) is emerging as a promising and safe treatment option for melasma, potentially serving as either an adjunctive or alternative therapy. When used as a first-line treatment, PRP has shown the ability to significantly improve clinical outcomes and enhance skin quality, with minimal adverse effects and sustained results. Combining PRP with other melasma therapies may further reduce hyperpigmentation and increase patient satisfaction. However, individual responses to PRP can vary, and multiple sessions may be required to achieve optimal results. PRP's mechanism of action is believed to involve the growth factors in alpha granules, which may suppress melanin production while promoting skin volume. Nevertheless, further research is needed to fully understand the efficacy of PRP in treating melasma. Specifically, biomolecular studies and clinical trials are essential to determine optimal treatment protocols and assess the long-term safety and efficacy of PRP therapy.

Data Availability Statement

The data supporting the findings of this review are available within the article.

DECLARATION

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Conflict of Interest

The authors have declared that there are no conflicts of interest.

Registration Number of Clinical Trial

None

Author Contributions

Conceptualized the study and wrote the main manuscript text, S.V.; Reviewed, revised, and approved the final manuscript, D.A.A.S.L. All authors have read and agreed to the final version of the manuscript.

Use of Artificial Intelligence

None

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