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PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330
Int J Hum Genet, 25(1): 42-50 (2025)
DOI: 10.31901/24566322.2025/25.01.905

Study on the Impact of Timing of Surgery Under General Anesthesia on Postoperative Sleep Quality

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KEYWORDS Daytime Surgery. General Anesthesia. Melatonin. Night Surgery. Sleep Quality

ABSTRACT Patients undergoing elective laparoscopic abdominal surgery were enrolled from January to December 2022 and randomly assigned 1:1 to daytime surgery (8:00 to 12:00 hours) and nighttime surgery (18:00 to 22:00 hours). Sleep quality was assessed using the Pittsburgh Sleep Scale (PSQI), and melatonin concentrations were measured in urine. The dose of remifentanil and propofol in the daytime group was higher than that in the night group, and the difference was statistically significant ($P<0.05$). Compared with the daytime group, PSQI in the night group was higher at 3 days after surgery, and urine melatonin concentration was lower at 1 and 3 days after surgery ($P<0.05$). Compared to the daytime group, the nighttime group also showed a significant increase in SpO2, MAP, and HR levels. Compared to the daytime group, the nighttime group showed a statistically significant decrease in the occurrence rate of difficulty falling asleep, early awakening, feeling unwell after waking up, interrupted sleep, and urinary retention.

INTRODUCTION

Due to poor physical function, low tolerance to anesthesia, and multiple diseases, patients with this condition have relatively higher anesthesia reactions than young patients after anesthesia, which can easily lead to adverse reactions such as restlessness and insomnia after anesthesia (Cui et al. 2023). Sleep disorders are a relatively common adverse reaction after general anesthesia in the elderly (Gao et al. 2023). The mechanism of such symptoms is complex and related to the patient's surgical stress, illness, unfamiliar environment, psychological pressure, insomnia, neurasthenia, and other factors. Once postoperative insomnia occurs, it not only increases the patient's mental stress, but also affects their physiological function, ultimately affecting their recovery. Therefore, sleep support for patients needs to be based on nursing interventions (Guo et al. 2023; Huang et al. 2023; Chung et al. 2023).

This could have different impacts on postoperative recovery and sleep quality. Sleep deprivation is commonly observed in postoperative patients, particularly elderly individuals (Luo et al. 2023). Patients who undergo thoracoscopic surgery experience intense early postoperative pain stimulation, which reduces their comfort and increases the likelihood of sleep deprivation. Conversely, sleep deprivation exacerbates hyperalgesia, creating a vicious cycle that contributes to complications such as neuroendocrine and cardiovascular disorders, thereby impeding patient recovery (Qiu et al. 2023). Postoperative pain and anesthesia-related factors can both influence sleep deprivation following surgery. Additionally, the inflammatory response triggered by surgical stress plays a crucial role in postoperative sleep deprivation.

Spinal anesthesia, due to its simplicity and rapid onset of action, has been widely used in clinical practice (Yan et al. 2023). However, although it suppresses the central nervous system, patients remain conscious during the procedure, which can exacerbate their fear of surgery and lead to adverse reactions such as postoperative sleep disturbances (Yu et al. 2022). In contrast, general anesthesia effectively blocks nociceptive input from surgical procedures, reducing the stress response associated with it (Zheng et al. 2022). Furthermore, the anesthetic state shares many similarities with physiological sleep, alleviating sleep pressure caused by abnormal conditions and aiding in the treatment of chronic insomnia.

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General anesthesia, also known as general anaesthesia, involves the administration of anesthetic drugs via inhalation through the respiratory tract or through intravenous or intramuscular injection into the bloodstream (Deljou et al. 2023).

To ensure the safety of the surgery and reduce the patient's surgical stress, invasive surgeries currently require anesthesia, with general anesthesia being one of the main types of anesthesia. The occurrence of insomnia can greatly affect the patient's postoperative outcome and physical and mental state, and may lead to immune and neurological problems. Improving the patient's insomnia status is of great significance. The postoperative elderly population has poor physical condition, and long-term use of sedatives carries risks. Therefore, good intervention is needed to improve patients' sleep (Cao et al. 2023). Clinically, this is characterised by loss of consciousness, general analgesia, amnesia, reflex inhibition, and skeletal muscle relaxation (Kim et al. 2022).

Propofol has the functions and effects of rapid onset, short action time and quick recovery. The possible reason may be that propofol mainly works by dilating venous vessels, increasing vascular volume, further reducing vascular resistance, and thus exerting its effect (Moody et al. 2021). Fentanyl is a potent opioid analgesic with the characteristics of fast onset and strong analgesic effect. It is a commonly used analgesic drug in patient-controlled intravenous analgesia (PCIA) (Prior et al. 2022).

Objectives

This study aims to compare the effects of the timing of general anesthesia on the demand for anesthetic drugs and postoperative sleep quality.

MATERIAL AND METHODS

Objectives and Methods

This study enrolled patients who underwent selective laparoscopic abdominal surgery at the researchers' hospital from January to December 2022, including cholecystectomy, appendectomy, oophorectomy, and total anesthesia hysterectomy. The inclusion criteria were that the participants must be of age between 18 and 65, and with a physical condition classified as American Society of Anesthesiologists (ASA) grade I or II. The exclusion criteria were for participants with a presence of sleep disorders, pain syndromes, cardiovascular diseases, sleep apnea syndrome, psychiatric disorders, unwillingness to provide informed consent, or patients with language communication barriers. This study complied with the Helsinki Declaration, and all participants in the trial signed written informed consent forms.

Standardised Anesthesia

Patients were randomly assigned in a 1:1 ratio to either the daytime surgery group or the nighttime surgery group using a computer-generated random number table. All surgeries in the daytime group started between 8:00 and ended before 12:00 hours, while surgeries in the nighttime group started between 18:00 and ended before 22:00 hours. Neither the patients, surgeons, nor the attending anesthesiologists were aware of the group assignment. The attending anesthesiologist also administered intravenous fluids during the surgery to maintain intraoperative hemodynamic stability based on the patient's weight, duration of surgery, intraoperative blood loss, and surgical trauma.

Upon entering the operating room, routine monitoring including electrocardiography, heart rate (HR), non-invasive blood pressure (NIBP), and peripheral oxygen saturation (SpO2) was performed. General anesthesia was induced with propofol (2.0 mg/kg), fentanyl (0.2 μg/kg), and rocuronium (0.15 mg/kg). Endotracheal intubation was performed 3 minutes later, followed by mechanical ventilation to maintain end-tidal carbon dioxide (PETCO2) between 35-45 mmHg. Anesthesia maintenance was achieved by continuous infusion of propofol (50-100 μg/kg/min) and remifentanil (0.15-0.2 μg/kg/min). Patients inhaled a gas mixture of 50 percent oxygen and 2 percent air at a flow rate of 50 L/minute, with intermittent administration of rocuronium (0.05 mg/kg) for muscle relaxation. No other opioids were administered during the procedure. The bispectral index (BIS), a multifactorial electroencephalogram parameter specifically designed to measure the effects of anesthetics on the hypnotic state of the brain, was used to assess the depth of anesthesia. The anesthesiologist adjusted the infusion rates of remifentanil and propofol to maintain the BIS monitor (Aspect Medical System, Newton, MA) between 40 and 55. Prophylactic administration of ondansetron (0.3 mg) was given at the end of the surgery. After the surgery, patients were transferred to single-patient rooms and postoperative monitoring of HR, NIBP, and SpO2 was performed.

Outcome Measures

The quality of sleep and melatonin concentration in patients were evaluated on the day before surgery, the first day after surgery, and the third day after surgery. The Pittsburgh Sleep Quality Index (PSQI) was used to assess the quality of sleep, with higher scores indicating poorer sleep quality. The concentration of melatonin in morning urine samples was measured using the enzyme-linked immunosorbent assay (ELISA) method, following the instructions provided in the kit. The intra- and inter-assay coefficients of variation for the kit were less than 15 percent. The total melatonin content was calculated based on the patients' nocturnal urine volume, and the average nocturnal urine volume was also calculated.

Statistical Analysis

Statistical analysis was performed using SPSS version 23.0. Age, weight, anesthesia time, remifentanil dose, propofol dose, PSQI scores, and melatonin concentration were presented as mean ± standard deviation (x ±s) for continuous variables. The t-test was used to compare the differences in continuous variables between the daytime and nighttime groups, and paired t-tests were used to compare the changes in PSQI scores and melatonin concentration within each group. Gender was presented as counts and percentages (n(%)), and the chi-square test was used to compare the differences between the two groups. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Comparison of General Information and Anesthesia Use Between Two Groups

This study included a total of 90 patients, with 45 in each group. After the completion of the study, a total of 80 patients were included for statistical analysis. The daytime group had 39 patients (6 excluded) and the nighttime group had 41 patients (4 excluded). The reasons for exclusion were patients not retaining morning urine or the retained samples not meeting the requirements. There were no statistically significant differences in age, weight, and gender between the daytime and nighttime groups. However, the daytime group had a shorter anesthesia time compared to the nighttime group, and the doses of remifentanil and propofol were higher in the daytime group, with statistically significant differences ($P<0.05$), as shown in Table 1.

Comparison of Sleep Status on the First Night After Surgery Between Two Groups of Patients

The AIS scores at 20:00, 22:00, 0:00, 2:00, 4:00 and 6:00 hours on the first night after surgery in the nighttime group were lower than those in the daytime group, and these differences were statistically significant ($P<0.05$, Table 2).

Table 1: Comparison of general conditions and anesthetic use between two groups

Characteristic	*Daytime group (n=39)*	*Nighttime group(n=41)*	*P value*
Age (year)	40.89± 6.83	43.01± 8.46	0.223
Weight (kg)	58.80± 6.88	58.97± 7.01	0.916
Male (n(%))	19(48.7)	20(51.3)	0.823
Female (n(%))	20 (48.8)	21 (51.2)	0.831
Duration of anesthesia (minutes)	111.00±19.68	119.48±11.69	0.021
Remifentanil dosage (¼g/h/kg)	10.09± 0.76	7.99± 0.40	<0.001
Propofol dosage (mg/h/kg)	5.75± 0.66	4.22± 0.44	<0.001

Table 2: Comparison of sleep status on the first night after surgery between two groups of patients

Group	*AIS score*					
	20:00	22:00	00:00	02:00	04:00	06:00
Daytime group	2.17±0.09	3.07±0.19	3.11±0.08	3.18±0.19	2.31±0.07	2.19±0.04
Night group	2.05±0.07*	3.01±0.11	3.02±0.07	3.05±0.07*	2.19±0.05*	2.04±0.05*

Compared with the hundred day group; $^{*}p<0.05$ versus Daytime group

Comparison of Sedation and Anesthesia Recovery Between Two Groups of Patients

The patients in the nighttime group had significantly better scores on various indicators of clinical sedation and anesthesia recovery compared to the patients in the daytime group. The intergroup differences were statistically significant (P<0.05, Table 3).

Comparison of Melatonin Concentration and Sleep Quality Between Two Groups at Different Time Points

Compared to the daytime group, the nighttime group had higher PSQI scores on the 3rd day after surgery and lower melatonin concentration on the 1st and 3rd days after surgery (P<0.05, Table 4), with no other statistical differences observed. Regardless of whether it was in the daytime or nighttime group, there were significant differences in higher PSQI scores and lower melatonin concentration on the 1st and 3rd days after surgery compared to the day before surgery (P<0.05, Table 4).

Comparison of Hemodynamic Indicators Between Two Groups of Patients Before and On The First Night After Surgery

After the surgery, there was a significant increase in SpO2, MAP, and HR levels in the daytime group (P<0.05, Table 5). Compared to the daytime group, the nighttime group also showed a significant increase in SpO2, MAP, and HR levels (P<0.05, Table 4).

Comparison of Postoperative Adverse Reactions Between Two Groups of Patients

Compared to the daytime group, the nighttime group showed a statistically significant decrease in the occurrence rate of difficulty falling asleep, early awakening, feeling unwell after

Table 3: Comparison of sedation and anesthesia recovery between two groups of patients

Group	*Sedation status (Ramsay Score)*		*Wake-up time*	*Extubation time*
	15 minutes after removing tube	*0 minutes after 3 removing tube*		
Daytime group	2.31±0.32	2.81±0.36	6.83±0.81	8.81±0.85
Nighttime group	1.69±0.29*	1.98±0.41*	7.51±0.91*	9.62±0.74*

Compared with the hundred day group; $^{*}p<0.05$ versus Daytime group

Table 4: Comparison of melatonin concentration and sleep quality between two groups at different time points

Index	*Group*	*1 day before surgery*	*1 day after surgery*	*3 day after surgery*
PSQI	Daytime	3.36±0.65	7.75±2.26^{b}	4.53±0.93^{ab}
	Night	3.49±0.58	8.06±2.10^{b}	5.49±1.38^{b}
Melatonin (ng/kg)	Daytime	8.84±2.17	4.41±1.33^{ab}	6.45±1.98^{ab}
	Night	8.22±1.84	3.42±0.94^{b}	5.22±1.88^{b}

Compared with the hundred day group; P<0.05.

Table 5: Comparison of hemodynamic indicators between two groups of patients before and on the first night after surgery

Group	SpO2(%)		MAP (mm Hg)		HR (times/min)	
	Preoperative	Postoperative	Preoperative	Postoperative	Preoperative	Postoperative
Daytime	98.14±0.62	98.26±0.33$^{\#}$	81.38±7.23	86.06±7.52$^{\#}$	71.63±8.95	75.36± 9.98$^{\#}$
Night	97.88±0.46	98.91±0.51$^{\#}$	81.26±7.12	89.61±8.14$^{*,\#}$	72.14±9.11	77.63±10.14$^{*,\#}$

HR: Heart rate; MAP: mean arterial pressure; SPO2: oxygen saturation; $^{*}p<0.05$ versus Daytime group; $^{\#}p<0.05$ versus Preoperative

waking up, Interrupted sleep, and urinary retention (P<0.05, Table 6).

DISCUSSION

The scientific anesthesia method is the foundation for the smooth progress of surgery. Related studies suggest that a reasonable anesthesia plan should ensure both appropriate anesthesia depth and surgical safety for patients. Surgical stress response can cause changes in the patient's hemodynamics, increase the burden on the patient's organs, induce ischemic injury, and may lead to difficulties in postoperative recovery and cognitive dysfunction. Regional nerve block is a technique of blocking nerve transmission signals by injecting local anesthetic drugs. Combined with general anesthesia, it can provide precise analgesia to the surgical area and reduce the dosage of general anesthesia drugs, thereby reducing the time for postoperative recovery and lowering the incidence of complications.

Postoperative sleep deprivation can impair patients' physical function recovery, increase the risk of delirium and cognitive impairment, and have adverse effects on postoperative outcomes (Yildiz et al. 2023). After thoracoscopic lung resection surgery, patients experience intense pain, reduced comfort, especially in elderly individuals who may have lower physical function and lower pain tolerance, making them more susceptible to sleep deprivation and experiencing more severe negative consequences (Zheng et al. 2022). Patients undergoing general anesthesia surgery are prone to abnormal function of the hypothalamic thermoregulatory centre due to trauma, anesthesia drugs, and other factors, which can disrupt the body's heat production and dissipation mechanisms, leading to a sustained decrease in body temperature. Once hypothermia occurs, it will directly interfere with the normal metabolism of anesthetic drugs in the body, prolonging the time required for wakefulness. At the same time, patients undergoing general anesthesia surgery generally have varying degrees of sleep disorders after surgery, mainly manifested as insufficient sleep depth, difficulty falling asleep, and easy awakening at night. Patients with severe symptoms may even be unable to fall asleep all night, which seriously hinders the smooth recovery after surgery (Hou et al. 2022; Huang et al. 2023). General anesthesia, often administered through methods such as intramuscular or intravenous injection and inhalation, temporarily inhibits the central nervous system of patients (Zhu et al. 2023). This effectively reduces intraoperative pain sensation and minimises the likelihood of surgical stress response, ensuring the smooth progress of surgical treatment. Research suggests that various factors, including anesthesia duration, anesthetic drugs, and depth of anesthesia, are interconnected. Rapid elimination of anesthetic drugs from the body postoperatively can pro-

Table 6: Comparison of postoperative adverse reactions between two groups of patients [n (%)]

Group	DFA	EA	FUAWU	IS	UR
Daytime	8 (19.51)*	11 (26.83)*	6 (10.26)*	7 (17.95)*	7 (17.95)*
Nighttime	5 (12.82)	4 (10.26)	3 (7.69)	5 (12.82)	3 (7.69)

DFA: Difficulty falling asleep; EA: Early awakening; FUAWU: Feeling unwell after waking up; IS: Interrupted sleep; UR: Urinary retention; $^{*}p<0.05$ versus Daytime group mote patient recovery speed and reduce the occurrence of adverse reactions.

In recent years, sleep disorders have received increasing attention, and the relationship between sleep and anesthesia has also received more and more clinical attention. Relevant studies have shown that the inner part of the hypothalamic preoptic area and some neurotransmitters are closely related to sleep quality. At the same time, anesthetic drugs can activate these neural networks and exert similar effects to sleep. Low head and high foot during laparoscopic hysterectomy surgery can increase the anterior and posterior load on the patient's heart, resulting in more significant fluctuations in hemodynamic parameters, especially in older patients. Therefore, the selection of anesthetic drugs is particularly important for laparoscopic hysterectomy patients.

Patients often require general anesthesia, but some patients may experience postoperative sleep disorders, anxiety, or depression due to their varying sensitivity to anesthetic drugs. Research has shown that postoperative sleep disorders not only affect patients' postoperative recovery process, but may also exacerbate psychological problems, which in turn can worsen sleep disorders and prolong hospitalisation time. Therefore, optimising perioperative anesthesia management, improving postoperative sleep quality and mental health, can help promote the postoperative rehabilitation process. The sleep state is mainly manifested as a decrease in the body's consciousness and reaction ability. Good sleep quality is an important foundation for ensuring survival and health. Postoperative sleep mainly refers to postoperative sleep quality disorders or abnormal behaviours during postoperative sleep, which can also be manifested as rhythmic alternation disorder between sleep and wakefulness. This may lead to emotional apathy in patients after surgery, and in severe cases, delirium, which can trigger negative emotions such as anxiety and depression, thereby affecting postoperative recovery. The use of anesthetics is one of the influencing factors for postoperative sleep disorders. Among them, propofol is a common intravenous anesthetic, while remdesilam is a new type of analgesic hypnotic drug mainly used for anesthesia induction and maintenance. Additionally, the study also found patients undergoing surgery at night required lower total doses of propofol and remifentanil compared to those undergoing surgery in the morning. These findings suggest that daytime surgery may necessitate higher doses of anesthetic drugs compared to nighttime surgery, possibly due to circadian rhythms. The severity of postoperative sleep disturbances appeared to be greater after nighttime surgery, which may be related to melatonin secretion.

Good sleep is an important physiological process that preserves body energy and promotes rapid recovery for patients. However, studies have shown that 64.9 percent of adult patients may experience varying degrees of postoperative sleep disturbances (POSD). Perioperative factors such as preoperative anxiety, surgical trauma, postoperative inflammatory response, pain, use of sedatives, and medical environment (including noise, medical intervention, and light intensity) are the main causes of POSD. For patients undergoing laparoscopic hysterectomy, anesthesia intervention with propofol significantly improves their sleep quality. The possible reason for this may be that propofol mainly exerts sedative and hypnotic functions by effectively increasing the binding of gamma aminobutyric acid to postsynaptic GABAA receptors in the nervous system. Therefore, patients may experience a state of consciousness similar to sleep after anesthesia. In addition, propofol can effectively intervene in the sleep wake cycle of patients with sleep disorders, thereby achieving the goal of reshaping the sleep wake cycle. Therefore, the improvement of sleep quality in patients after anesthesia is more effective

Postoperative sleep disturbances are a common complication following surgical treatment (Song et al. 2019). Patients often experience reduced nighttime sleep duration, frequent awakenings, and poor sleep quality after clinical intervention. Research indicates that such symptoms can lead to nitrogen balance disturbances, decreased immune function, and lowered resistance in the patient's body, impacting their postoperative recovery. In severe cases, patients may also develop anxiety and depression (Li et al. 2021). Through various administration routes, general anesthesia effectively inhibits the central nervous system, reducing neural reflexes and psychological stress during surgery (Yu et al. 2022). Additionally, anesthesia has minimal impact on nerve damage, and postoperatively, the drugs are gradually metabolised and eliminated from the patient's body through the gastrointestinal system, allowing the restoration of normal reflexes and consciousness (Song et al. 2020). At present, relying solely on fentanyl for intravenous patient-controlled analgesia is not very effective. The combination of various intravenous drugs for postoperative analgesia has gradually become mainstream.

Based on this study, the medications used were short-acting and rapidly metabolised. Therefore, in this study, the impact of these two anesthetic agents on postoperative sleep quality was minimal. The data from this study indicated that patients undergoing nighttime surgery required lower total doses of propofol and remifentanil compared to those undergoing morning surgery. This is consistent with previous findings that circadian rhythms, which generate a day-night cycle in all organisms, can influence the pharmacological sensitivity. Additionally, the data from this study showed that both groups of patients experienced severe sleep disturbances after surgery, which is in line with previous research findings. General anesthesia exacerbated sleep disturbances on the night of surgery.

In this study, the AIS scores at 20:00, 22:00, 0:00, 2:00, 4:00 and 6:00 hours on the first night after surgery in the nighttime group were lower than those in the daytime group. The patients in the nighttime group had significantly better scores on various indicators of clinical sedation and anesthesia recovery compared to the patients in the daytime group. Compared to the daytime group, the nighttime group had higher PSQI scores on the 3rd day after surgery and lower melatonin concentration on the 1st and 3rd days after surgery ($P<0.05$), with no other statistical differences observed. Regardless of whether it was in the daytime or nighttime group, there were significant differences in higher PSQI scores and lower melatonin concentration on the 1st and 3rd days after surgery compared to the day before surgery. After the surgery, there was a significant increase in SpO2, MAP, and HR levels in the daytime group ($P<0.05$). Compared to the daytime group, the nighttime group also showed a significant increase in SpO2, MAP, and HR levels. Compared to the daytime group, the nighttime group showed a statistically significant decrease in the occurrence rate of difficulty falling asleep, early awakening, feeling unwell after waking up, interrupted sleep, and urinary retention.

Melatonin, an indole hormone produced by the pineal gland, regulates sleep through the melatonin projection pathway from the suprachiasmatic nucleus to the nucleus of the optic tract (Heida et al. 2020).

The present study found that postoperative sleep disturbances were more severe after nighttime surgery compared to morning surgery (Mihara et al. 2015). This could be explained by the possibility that general anesthetics were administered at relatively higher doses during the night, and propofol may be more effective during nighttime compared to daytime. Possible mechanisms are as follows. Firstly, propofol is primarily anesthetic through the activation of GABAA receptors, which are known to be increased during the night and play a crucial role in sleep regulation. Therefore, general anesthetics such as propofol that activate GABAA receptors may have a better effect during the night compared to the day. Secondly, melatonin, which plays a significant role in regulating sleep, can also modulate GABAA receptors, leading to a significant reduction in total propofol dose during the night (Dispersyn et al. 2010). Melatonin secretion exhibits a clear circadian rhythm, with the highest levels typically occurring in the early morning (Faghihian et al. 2018).

There are several limitations that should be noted in this. Firstly, data was only collected on the night before surgery, which may not fully reflect the patients' normal sleep patterns. Secondly, despite efforts to mitigate factors that could potentially affect postoperative sleep quality, the light, noise, or interruptions caused by nighttime nursing care may unavoidably have a negative impact on sleep quality. Thirdly, only short-term postoperative sleep was measured, and data on the long-term effects of general anesthesia on sleep could not be obtained.

CONCLUSION

There may be an association between general anesthesia and circadian rhythms. Patients undergoing surgery at night require lower doses of anesthesia compared to those undergoing surgery during the day. However, postoperative sleep disturbances are more pronounced in patients who undergo nighttime surgery, possibly due to alterations in melatonin secretion.

RECOMMENDATIONS

Daytime surgery requires higher doses of narcotic drugs than night surgery, which may be related to circadian rhythm. The degree of postoperative sleep disturbance is greater in the evening than in the morning, which may be related to the secretion of melatonin.

DECLARATIONS, ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committee of the 901st Hospital Of the Joint Logistics Support Force of PLA.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data underlying this paper will be shared on reasonable request to the corresponding author.

FUNDING

Not applicable.

COMPETING INTERESTS

None.

AUTHOR CONTRIBUTIONS

Zhang Yuwei: Project conceptualisation, investigation and data analysis.

Zhang Yuwei and Liu Tao: Data collection, analysis and methodology development.

Liu Tao and Wang Cheng: Investigation and methodology development.

All authors have made important contributions to the revision and approval of the final manuscript.

ACKNOWLEDGEMENTS

Not applicable.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Paper received for publication in November, 2024
Paper accepted for publication in March, 2025