Cite as: Liu C, Li XX, Bi YL et al. Relationship of cerebrospinal fluid choline acetyltransferase and cholinesterase with postoperative delirium in elderly patients [J]. Chin J Clin Res, 2024, 37(4):510-513.
DOI: 10.13429/j.cnki.cjcr.2024.04.005

Relationship of cerebrospinal fluid choline acetyltransferase and cholinesterase with postoperative delirium in elderly patients

LIU Chen, LI Xiaoxuan, BI Yanlin , LIN Xu, WANG Bin, QI Feng*
Department of Anesthesiology, Qilu Hospital of Shandong University, Jinan, Shandong 250012, China
Corresponding author: QI Feng, E-mail: 198962001111@sdu.edu.cn

Abstract: Objective To detect the concentrations of choline acetyltransferase (ChAT) and cholinesterase (ChE) in cerebrospinal fluid of elderly patients and explore their relationship with postoperative delirium (POD). **Methods** One hundred and eighty-three patients who underwent total knee/total hip arthroplasty at Qingdao Municipal Hospital from September 2018 to August 2019 were included. All patients underwent total knee or total hip arthroplasty, using combined spinal-epidural anesthesia. After subarachnoid puncture, 2 mL of cerebrospinal fluid (CSF) was extracted. Enzyme linked immunosorbent assay (ELISA) was used to detect the concentration of ChAT, ChE, tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) in CSF, and spectrophotometry was used to detect the activity of ChAT and ChE in CSF. The occurrence of POD was assessed using the Confusion Assessment of Method (CAM) after surgery. **Results** Among 183 patients, 157 cases without POD were as non-POD group, and 26 cases with POD were as POD group, with an incidence rate of 14.21%. In the POD group, the preoperative CSF ChE concentration [(31.99 ± 2.78) *vs* (40.89 ± 4.19) ng/mL, t=13.915, P<0.01] significantly reduced, while ChAT [(1.26 ± 0.12) *vs* (0.86 ± 0.15) ng/mL, t=12.920, P<0.01], TNF-α [(33.98 ± 5.02) *vs* (16.98 ± 3.41) pg/mL, t=14.467, P<0.01], and IL-6 [(17.19±1.32) *vs* (12.29±1.64) pg/mL, t=16.643, P<0.01] significantly increased (P<0.05) compared to the non-POD group. In the POD group, the preoperative CSF ChE activity significantly reduced [(4.28 ± 0.52) vs (5.25 ± 0.26) u/mL, t=9.321, P<0.01], while ChAT activity significantly increased [(0.36±0.05) vs (0.27 ± 0.03) u/mL, t=8.916, P<0.01] compared to the non-POD group. **Conclusion** The high activity state of ChAT and low activity ChE in CSF are related to the occurrence of POD in elderly patients, with cholinergic degradation closely related to its occurrence.

Keywords: Cerebrospinal fluid; Cholinesterase; Choline acetyltransferase; Postoperative delirium; Elderly; Arthroplasty

Postoperative delirium (POD) is a common and serious postoperative complication, characterized by central nervous system dysfunction in elderly patients, leading to higher mortality and financial burdens [1-3]. With the aging of population, the number of elderly patients undergoing total knee arthroplasty / total hip arthroplasty (TKA/THA) is increasing, with a higher risk of POD in patients undergoing joint replacement surgery [4-9].

The pathogenesis of POD is not yet fully understood, and there is a lack of identified biomarker to predict its occurrence [10]. The dynamic equilibrium of cholinergic anti-inflammatory pathways is maintained by choline acetyltransferase (ChAT) and cholinesterase (ChE), and may regulate important behaviors such as learning and cognition [11-12]. Preliminary research has suggested that the anti-inflammatory properties of hippocampal cholinergic are crucial in the development of POD and may contribute to degenerative changes in central cholinergic nerve function [13-14]. Furthermore, individuals with lower serum ChE activity are more likely to develop POD [15], and biomarkers in cerebrospinal fluid (CSF) have more diagnostic value than in plasma [16]. Therefore, this study aims to investigate the relationship between levels of ChAT, ChE in the CSF and POD in elderly patients, providing new targets and possible basis for future prevention.

1 Materials and methods

1.1 General information

Ethical approval was obtained prior to the experiment (2018Linshen Y No. 110) and all patients were informed of the relevant risks and the necessity for research. Two hundred and three patients with TKA/THA in Qingdao Municipal Hospital from September 2018 to August 2019 were selected.

1.1.1 Inclusion criteria
(1) Aged 65-85 years;
(2) Weighed 50-80 kg;
(3) Under combined spinal and epidural anesthesia;

(4) The American Society of Anesthesiologists (ASA) classification of I-III.

1.1.2 Exclusion criteria
(1) Interference in different educational levels;
(2) Score of pre-operative mini-mental state examination (MMSE) < 23;
(3) History of serious neurological and/or mental illness;
(4) Personal history of substance use disorders (drug, medicine and steroids);
(5) Combined with hepatic encephalopathy (HE) III-IV;
(6) Patients who underwent major surgery recently;
(7) Severe vision or hearing impairment;
(8) Abnormal preoperative blood test values, indicators such as coagulation function.

1.2 Anesthesia methods

Preoperative fasting and no medication records were maintained. All patients were under combined spinal and epidural anesthesia with L_{3-4} interspace. After successful anesthesia, 2.0-2.5 mL 0.5% ropivacaine was injected, and the block level was T_{8-10} after patients being positioned at lying-flat position. Patient-controlled intravenous analgesia (PCIA) was used after the operation [10 mg butorphanol+5 mg tropisetron+ physiological saline dilution to 100 mL, visual analogue scale (VAS) score <5]. After a lumbar puncture into the subarachnoid space, the CSF was collected before injection and frozen at -80 °C after centrifugation.

1.3 Observation indicators

Concentrations of ChAT, ChE, tumor necrosis factor (TNF)-α and interleukin (IL)-6 levels in CSF were determined by ELISA. TNF-α kit Lot: L190109552, IL-6 kit Lot: L190117600, ChE kit Lot: L190321385, ChAT kit Lot: L190321380. Spectrophotometry was used to detect the ChAT and ChE activities. ChAT activity assay kit: A079-2, ChE activity assay kit: A026.

The confusion assessment method (CAM) was used to assess the development of POD in patients. The CAM consists of four dimensions: aacute changes in mental state; inattention; disorganized thinking; and altered level of consciousness. POD can be diagnosed if the patient develops the first two and is accompanied by either of the latter two.

1.4 Sample Size Calculation Method

PASS 11.0 software was used to analyze the required sample size. The expected sensitivity was defined as 0.9 and the allowed error was 0.05. The expected specificity was defined as 0.5, and the allowed error of the specific was 0.05. α was defined as 0.05, set to one-sided. The loss ratio was 0.1 and the calculated sample size was 203. According to the exclusion criteria, 183 patients were eventually included.

1.5 Statistical methods

SPSS 25.0 software was used for statistical analysis. Normal distribution of measurement data was expressed as $\bar{x} \pm s$, and a two-sample t-tests was used for comparison. The enumeration data was expressed as case (%) and checked with chi-square test. P <0.05 was statistically significant.

2 Results

2.1 Comparison of general and surgical indicators between two groups

POD developed in 26 of 183 patients, with an incidence of 14.21%. There was no statistically significant difference in the general data between POD group and non-POD group (P >0.05). **[Table 1]**

2.2 Comparison of ChE, ChAT, IL-6 and TNF-α levels in preoperative CSF between two groups

Compared with non-POD groups, ChE levels decreased, while ChAT, IL-6 and TNF-α levels increased of preoperative CSF in POD group, with statistically significant difference (P <0.01). **[Table 2]**

2.3 Comparison of ChE and ChAT activities in CSF between two groups

Compared with non-POD groups, ChE activity in CSF decreased, while ChAT activity in CSF increased in POD group, with statistically significant difference (P <0.01). **[Table 3]**

Tab.1 Comparison of general data and surgical indicators between two groups

Indicators	POD (n=26)	Non-POD (n=157)	t/χ^2 value	P value
Age (years, $\bar{x} \pm s$)	70.3±4.2	69.1±6.5	1.233	0.224
Gender (case, male/female)	10/16	73/84	0.581	0.446
Weight (kg, $\bar{x} \pm s$)	67.8±8.0	68.4±7.0	0.397	0.692
Operation time (min, $\bar{x} \pm s$)	122.6±21.4	119.0±19.5	0.860	0.391
Bleeding volume (mL, $\bar{x} \pm s$)	170.8±60.7	172.2±50.4	0.193	0.847

Pre-operative MMSE (score, $\bar{x}$±s)	28.2±1.3	28.6±1.1	1.672	0.096

Tab.2 Comparison of ChE, ChAT, IL-6 and TNF-α levels in the CSF before operation ($\bar{x}$±s)

Group	Case	ChE (ng/ mL)	ChAT (ng/ mL)	TNF-α (pg/ mL)	IL-6 (pg/ mL)
POD group	26	31.99±2.78	1.26±0.12	17.19±1.32	33.98±5.02
Non-POD group	157	40.89±4.19	0.86±0.15	12.29±1.64	16.98±3.41
***t* value**		13.915	12.920	14.467	16.643
***P* value**		<0.001	<0.001	<0.001	<0.001

Tab.3 Comparison of ChE and ChAT activities between two groups ($\bar{x}$±s)

Group	Case	ChE activity	ChAT activity
POD group	26	4.28±0.52	0.36±0.05
Non-POD group	157	5.25±0.26	0.27±0.03
***t* value**		9.321	8.916
***P* value**		<0.001	<0.001

3 Discussion

In this study, elderly patients undergoing TKA/THA were included, and combined spinal and epidural anesthesia was used. None of the patients received preoperative medication, and PCIA was used for postoperative analgesia. These interventions aimed to maintain intraoperative stability and minimize the interference of other factors on POD.

The specific pathogenesis of POD remains unclear, and cholinergic theory has been a hot research topic in recent years. The cholinergic anti-inflammatory pathway, as a significant neural circuit, promotes the release of acetylcholine following vagus nerve stimulation. The interaction between α-7 subunits of acetylcholine and nicotinic acetylcholine receptors (α7nAChR) plays a role in preventing Alzheimer's disease (AD) [17]. Acetylcholine levels achieve a dynamic equilibrium in adults. ChAT levels maintains stable in plasma and CSF, in conjunction with acetylcholinesterase (AChE) and butyrylcholinesterase (BubChE). The ratio of ChAT to AChE activities was found to be elevated in AD patients [18].

Related studies suggested that cholinergic neurodegeneration was involved in the process of AD, and cerebrospinal fluid analysis demonstrated an increased incidence of POD in AD patients postoperatively [19]. Research indicated that the degeneration in central cholinergic function was closely linked to the development of POD. Experimental findings in elderly mice demonstrated the degeneration of hippocampal cholinergic system and cognitive impairment, highlighting the role of central cholinergic neuronal degeneration in accelerating POD advancement. Furthermore, preoperative assessments in POD patients revealed a significant reduction in plasma ChE activity levels, suggesting changes in ChE levels as a potential biomarker for POD in quiet settings [15]. While CSF analysis of ChE has more diagnostic value than plasma [16]. Thus, exploring the correlation between cerebrospinal fluid cholinergic biomarkers and POD is clinically important and diagnostically valuable.

Additionally, studies have identified cholinergic anti-inflammatory pathways as a key neural mechanism involving the release of acetylcholine by the vagus nerve to inhibit inflammatory cytokines like IL-6 and TNF-α [19]. These inflammatory cytokines can breach the blood-brain barrier and infiltrate the central nervous system from surrounding organs [20]. Microglia activation can trigger the development of a variety of diseases, leading to the creation of pathogenic environments in early POD [21].

The study found that the incidence of POD in elderly patients after TKA/THA was approximately 14.21%. Compared with the non-POD group, the preoperative concentration and activity of ChE in CSF in the POD group were significantly reduced. Conversely, the concentration and activity of ChAT showed a significant increase, indicating that central cholinergic neuronal degeneration played a crucial role in the development of POD. Additionally, IL-6 and TNF-α levels in preoperative CSF were significantly elevated in the POD group, suggesting that inflammatory factors were associated with

POD. Bosancic *et al* [22] investigated a subgroup of patients from the BioCog study and showed that POD developed in 52 of 127 elderly patients undergoing abdominal surgery. There was an association between their AChE activity and POD. A prospective and observational study conducted by single center trials in a European academic hospital [23] found that reduced AChE activity in 114 patients undergoing cardiac surgery may be associated with the development of POD. The results of the present study are in line with them, suggesting that high ChAT activity and low ChE activity could serve as predictive markers for the development of POD prior to surgery.

In conclusion, high ChAT activity and low ChE activity in CSF are associated with the development of POD in patients, highlighting the association between degradation of cholinergic system and POD pathogenesis.

Conflict of Interest: None

References

[1] Sumner M, Deng C, Evered L, et al. Processed electroencephalography-guided general anaesthesia to reduce postoperative delirium: a systematic review and meta-analysis[J]. Br J Anaesth, 2023, 130(2): e243-e253.

[2] Leung JM, Rojas JC, Tang C, et al. Presence of preoperative neurodegeneration biofluid markers in patients with postoperative delirium. Anesthesiology. 2023;139(4):432-443.

[3]Yan E, Veitch M, Saripella A, et al. Association between postoperative delirium and adverse outcomes in older surgical patients: a systematic review and meta-analysis.[J] Clin Anesth, 2023, 90:111221.

[4]Zhao J, Liang G, Hong K, et al. Risk factors for postoperative delirium following total hip or knee arthroplasty: A meta-analysis. Front Psychol, 2022,13:993136.

[5]Zhou Q, Zhou X, Zhang Y, et al. Predictors of postoperative delirium in elderly patients following total hip and knee arthroplasty: a systematic review and meta-analysis. BMC Musculoskelet Disord, 2021, 22(1):945.

[6] Meyer M, Götz J, Parik L, et al. Postoperative delirium is a risk factor for complications and poor outcome after total hip and knee arthroplasty[J]. Acta Orthop, 2021, 92: 695-700.

[7] Zai J,Liu YH,Song XC,et al.Serum vitamin D level in elderly women with postoperative delirium after hip fracture surgery[J]. Chin J Clin Res, 2023,36(4):615-618. **[In Chinese]**

[8] Wang JZ, Zhang DY, Chen HX,et al. Correlation between intraoperative regional cerebraloxy gensaturation and postoperative deliriumin elderly patients undergoing thoracotomy [J]. Chin J Clin Res, 2023,36(1):76-80. **[In Chinese]**

[9]Shin HJ, Woo Nam S,et al. Postoperative delirium after dexmedetomidine versus dropofol dedation in healthy older adults undergoing orthopedic lower limb surgery with spinal anesthesia: a randomized controlled trial[J]. Anesthesiology, 2023,138(2):164-171.

[10]Bosancic Z, Spies CD, Müller A, et al. Association of cholinesterase activities and POD in older adult abdominal surgical patients. BMC Anesthesiol. 2022;22(1):293.

[11] Yamanashi T, Nagao T, Wahba NE, et al. DNA methylation in the inflammatory genes after neurosurgery and diagnostic ability of post-operative delirium[J]. Transl Psychiatry, 2021, 11: 627.

[12] Parrish WR, Rosas-Ballina M, Gallowitsch-Puerta M, et al. Modulation of TNF release by choline requires α7 subunit nicotinic acetylcholine receptor-mediated signaling[J].Mol Med, 2008, 14(9/10): 567-574.

[13] Li XX,Wang T,Dong R,et al.Changes in expression of hippocampal long non-coding RNA and bioinformatics analysis in mice with perioperative neurocognitive disorders[J]. Chin J Anesthesiol, 2019, 39(3): 304-308.

[14] Xu H, Chen LK, Zhang X, et al. Central cholinergic neuronal degeneration promotes the development of postoperative cognitive dysfunction[J]. Lab Investig, 2019, 99(7): 1078-1088.

[15] Cerejeira J, Batista P, Nogueira V, et al. Low preoperative plasma cholinesterase activity as a risk marker of postoperative delirium in elderly patients[J]. Age Ageing, 2011, 40(5): 621-626.

[16] Olsson B, Lautner R, Andreasson U, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis[J]. Lancet Neurol, 2016, 15(7): 673-684.

[17] Vijayaraghavan S, Karami A, Aeinehband S, et al. Regulated extracellular choline acetyltransferase activity- the plausible missing link of the distant action of acetylcholine in the cholinergic anti-inflammatory pathway[J]. PLoS One, 2013, 8(6): e65936.

[18] Saijo K, Glass CK. Microglial cell origin and phenotypes in health and disease[J]. Nat Rev Immunol, 2011, 11(11): 775-787.

[19] Evered L, Silbert B, Scott DA, et al. Cerebrospinal fluid biomarker for Alzheimer disease predicts postoperative cognitive dysfunction[J]. Surv Anesthesiol, 2016, 60(5): 195-196.

[20] Wang B, Li SY, Cao XP, et al. Blood-brain barrier disruption leads to postoperative cognitive dysfunction[J]. Curr Neurovascular Res, 2018, 14(4): 359-367.

[21] Hirsch J, Vacas S, Terrando N, et al. Perioperative cerebrospinal fluid and plasma inflammatory markers after orthopedic surgery[J]. J Neuroinflammation, 2016, 13(1): 211.

[22]Bosancic Z, Spies CD, Müller A,et al. Association of cholinesterase activities and POD in older adult abdominal surgical patients[J]. BMC Anesthesiol,2022,22(1):293.

[23]Adam EH, Haas V, Lindau S, et al. Cholinesterase alterations in delirium after cardiosurgery: a German monocentric prospective study[J]. BMJ Open,2020,10(1):e031212.

Submission received: 2023-07-18 / **Revised**:2023-09-20

·论　著·

脑脊液胆碱乙酰转移酶及胆碱酯酶与老年患者术后谵妄的关系

刘晨[1]，　李肖璇[2]，　毕燕琳[3]，　林旭[3]，　王彬[3]，　齐峰[1]

1. 山东大学齐鲁医院麻醉科，山东 济南 250012；

2. 青岛市妇女儿童医院麻醉科，山东 青岛 266000；

3. 青岛市市立医院麻醉科，山东 青岛 266000

摘要：目的　检测老年患者脑脊液胆碱乙酰转移酶（ChAT）及胆碱酯酶（ChE）浓度，探讨其与术后谵妄（POD）的关系。**方法**　纳入 2018 年 9 月至 2019 年 8 月于青岛市市立医院行全膝/全髋关节置换术的患者 183 例。全部患者实施全膝或全髋关节置换术，采用腰硬联合麻醉，在蛛网膜下腔穿刺完毕之后，提取脑脊液 2 mL。使用酶联免疫吸附试验法检测脑脊液 ChAT、ChE、肿瘤坏死因子-α（TNF-α）和白细胞介素 6（IL-6）的浓度；使用分光光度法检测脑脊液 ChAT、ChE 的活性。手术后采用意识模糊评估法（CAM）评定 POD 的发生情况。**结果**　术后 72 h 内 183 例患者，未发生 POD 157 例（非 POD 组），发生 POD 26 例（POD 组），发生率为 14.21%。与非 POD 组相比，POD 组术前脑脊液 ChE 浓度［（31.99±2.78）*vs*（40.89±4.19）ng/mL，$t=13.915$，$P<0.01$］明显降低，ChAT［（1.26±0.12）*vs*（0.86±0.15）ng/mL，$t=12.920$，$P<0.01$］、TNF-α［（17.19±1.32）*vs*（12.29±1.64）pg/mL，$t=14.467$，$P<0.01$］、IL-6［（33.98±5.02）*vs*（16.98±3.41）pg/ mL，$t=16.643$，$P<0.01$］浓度明显升高。与非 POD 组相比，POD 组术前脑脊液 ChE 活性明显降低［（4.28±0.52）*vs*（5.25±0.26）u/mL，$t=9.321$，$P<0.01$］，ChAT 活性明显升高［（0.36±0.05）*vs*（0.27±0.03）u/mL，$t=8.916$，$P<0.01$］。**结论**　脑脊液中的 ChAT 高活性、ChE 低活性与老年患者 POD 的发生有关，其中胆碱能的退化与其发生密切相关。

关键词：脑脊液；胆碱酯酶；胆碱乙酰转移酶；术后谵妄；老年；关节置换术

中图分类号：R614　**文献标识码：**A　**文章编号：**1674-8182（2024）04-0510-04

Relationship of cerebrospinal fluid choline acetyltransferase and cholinesterase with postoperative delirium in elderly patients

LIU Chen*, LI Xiaoxuan, BI Yanlin, LIN Xu, WANG Bin, QI Feng

** Department of Anesthesiology, Qilu Hospital of Shandong University, Jinan, Shandong 250012, China*

Corresponding author: QI Feng, E-mail: 198962001111@sdu.edu.cn

Abstract: Objective　To detect the concentrations of choline acetyltransferase (ChAT) and cholinesterase (ChE) in cerebrospinal fluid of elderly patients and explore their relationship with postoperative delirium (POD). **Methods**　One hundred and eighty-three patients who underwent total knee/total hip arthroplasty at Qingdao Municipal Hospital from September 2018 to August 2019 were included. All patients underwent total knee or total hip arthroplasty, using combined spinal-epidural anesthesia. After subarachnoid puncture, 2 mL of cerebrospinal fluid (CSF) was extracted. Enzyme-linked immunosorbent assay was used to detect the concentration of ChAT, ChE, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in CSF, and spectrophotometry was used to detect the activity of ChAT and ChE in CSF. The occurrence of POD was assessed using the Confusion Assessment of Method (CAM) after surgery. **Results**　Among 183 patients, 157 cases without POD were as non-POD group and 26 cases with POD were as POD group, with an incidence rate of 14.21%. In the POD group, the preoperative CSF ChE concentration [(31.99±2.78) *vs* (40.89±4.19)

DOI: 10.13429/j.cnki.cjcr.2024.04.005

通信作者：齐峰，E-mail：19896001111@sdu.edu.cn

出版日期：2024-04-20

QR code for English version

ng/mL, t=13.915, P<0.01] significantly reduced, while ChAT [(1.26±0.12) *vs* (0.86±0.15) ng/mL, t=12.920, P<0.01], TNF-α[(17.19±1.32) *vs* (12.29±1.64) pg/mL, t=14.467, P<0.01], and IL-6[(33.98±5.02) *vs* (16.98±3.41) pg/mL, t=16.643, P<0.01] significantly increased compared to the non-POD group. In the POD group, the preoperative CSF ChE activity significantly reduced [(4.28±0.52) *vs* (5.25±0.26) u/mL, t=9.321, P<0.01], while ChAT activity significantly increased [(0.36±0.05) *vs* (0.27±0.03) u/mL, t=8.916, P<0.01] compared to the non-POD group. **Conclusion** The high activity of ChAT and low activity of ChE in CSF are related to the occurrence of POD in elderly patients, with cholinergic degradation closely related to its occurrence.

Keywords: Cerebrospinal fluid; Cholinesterase; Choline acetyltransferase; Postoperative delirium; Elderly; Arthroplasty

术后谵妄(postoperative delirium, POD)是老年患者常见的以中枢神经系统功能障碍为主的术后严重并发症,可引起较高的死亡率,产生较多的经济负担[1-3]。随着人口老龄化,目前老年患者接受全膝关节置换术(total knee arthroplasty, TKA)和全髋关节置换术(total hip arthroplasty, THA)的人数逐渐增加,而接受关节置换手术患者以及其他手术的老年患者POD 的风险较高[4-9]。

POD 的发病机制仍未完全清楚,尚缺乏有效的生物标志物来区分有无 POD 的发生[10]。胆碱能抗炎通路的动态平衡由胆碱乙酰转移酶(choline acetyltransferase, ChAT)与胆碱酯酶(cholinesterase, ChE)维持,并且可能调控学习、认知等重要行为[11-12]。前期研究发现,海马胆碱能的抗炎作用对 POD 的发生和发展起着至关重要的作用[13],而且还可能会导致中枢胆碱能神经的退化性改变[14]。此外,如患有POD 的患者血液中存在低活性 ChE,其更容易被诊断出 POD[15],而脑脊液中的生物标记物比血浆中更具备诊断价值[16]。因此本研究拟探讨脑脊液 ChAT及 ChE 与老年患者 POD 的关系,为今后预防患者POD 找出新的作用靶点和可能的依据。

1　资料与方法

1.1　一般资料　本研究在实验前获得伦理委员会的批准(2018 临审 Y 字第 110 号),所有的患者均告知相关风险性及研究的必要性。选择 2018 年 9 月至2019 年 8 月于青岛市市立医院行 TKA/THA 的患者203 例,均采用腰硬联合麻醉,患者体重 50~80 kg,年龄 65~85 岁,美国麻醉医师协会(ASA)分级为Ⅰ~Ⅲ级。排除了受教育程度的干扰,并且排除了以下情况:(1) 术前进行简易精神状态量表(mini-mental state examination, MMSE)评分,<23 分;(2) 严重神经和/或精神疾病等基础病史的患者;(3) 吸毒和/或药物滥用史等个人史,大量应用类固醇类激素等;(4) 合并有Ⅲ~Ⅳ期的肝性脑病患者;(5) 近期接受重大手术的患者;(6) 严重的视力或听力等障碍;(7) 术前血液检验指标异常,如凝血功能。

1.2　麻醉方法　术前无任何用药记录,严格禁饮禁食。TKA/THA 术均实施腰硬联合麻醉,选用 $L_{3\sim4}$间隙,麻醉成功后注射 0.5%罗哌卡因 2.0~2.5 mL,患者平躺后平面控制为 $T_{8\sim10}$。术后应用患者静脉自控镇痛(patient-controlled intravenous analgesia, PCIA)[布托啡诺 10 mg+托烷司琼 5 mg+生理盐水稀释至 100 mL,视觉模拟评分(VAS)<5 分]。蛛网膜下腔间隙穿刺满意后,在注射药物之前收集脑脊液 2 mL,离心后于-80 ℃冷冻保存。

1.3　观察指标　使用酶联免疫吸附试验检测脑脊液 ChAT、ChE、肿瘤坏死因子-α(tumor necrosis factor-α, TNF-α)和白细胞介素-6(interleukin-6, IL-6)的浓度。TNF-α 浓度试剂盒(批号:L190109552),IL-6 浓度试剂盒(批号:L190117600),ChE 浓度试剂盒(批号:L190321385), ChAT 浓度试剂盒(批号:L190321380)。使用分光光度法检测脑脊液 ChAT、ChE 的活性,ChAT 活性试剂盒货号 A079-2,ChE 活性试剂盒货号 A026。手术后采用意识模糊评估法(confusion assessment method, CAM)评定患者 POD 发生情况,CAM 包含:精神状态的急性改变、注意力不集中、思维无序、意识水平改变,若患者发生前两项,并伴发后两项中的任意一项即可诊断 POD。

1.4　样本量计算方法　使用 PASS 11.0 软件对所需的样本量进行分析,预期的敏感度被定义为 0.9,允许的误差被定义为 0.05,而预期的特异性被定义为 0.5,特异性的允许误差被定义为 0.05,α 被定义为 0.05,设为单侧,失访比 0.1,计算样本量为 203,根据排除标准,最终纳入患者 183 例。

1.5　统计学方法　使用 SPSS 25.0 软件对研究数据进行统计学分析。遵循正态分布的计量资料以 $\bar{x}\pm s$ 表示,使用两独立样本 t 检验比较;计数资料以例表示,使用四格表 χ^2 检验。P <0.05 为差异有统计学意义。

2　结　果

2.1　两组一般情况和手术情况的比较　183 例患者

术后 72 h 内发生 POD 26 例，发生率为 14.21%；未发生 POD 157 例。结果 POD 组和非 POD 组患者的一般情况比较差异无统计学意义（P>0.05）。见表 1。

2.2　两组术前脑脊液 ChE、ChAT、IL-6 和 TNF-α 浓度的比较　与非 POD 组相比，POD 组术前脑脊液 ChE 浓度明显降低，ChAT、IL-6、TNF-α 浓度明显升高，差异有显著统计学意义（P<0.01）。见表 2。

2.3　两组术前脑脊液 ChE 及 ChAT 活性的比较　与非 POD 组相比，POD 组术前脑脊液 ChE 活性明显降低，ChAT 活性明显升高，差异有统计学意义（P<0.05）。见表 3。

表 1　两组一般情况和手术各指标的比较

Tab. 1　Comparison of general conditions and surgical indicators between two groups

指标	POD 组（n=26）	非 POD 组（n=157）	t/χ^2 值	P 值
年龄（岁，$\bar{x}\pm s$）	70.3±4.2	69.1±6.5	1.233	0.224
性别（男/女，例）	10/16	73/84	0.581	0.446
体重（kg，$\bar{x}\pm s$）	67.8±8.0	68.4±7.0	0.397	0.692
手术时间（min，$\bar{x}\pm s$）	122.6±21.4	119.0±19.5	0.860	0.391
失血量（mL，$\bar{x}\pm s$）	170.8±60.7	172.2±50.4	0.193	0.847
术前 MMSE（分，$\bar{x}\pm s$）	28.2±1.3	28.6±1.1	1.672	0.096

表 2　两组术前脑脊液 ChE、ChAT、IL-6 和 TNF-α 浓度的比较　（$\bar{x}\pm s$）

Tab. 2　Comparison of the concentrations of ChE, ChAT, IL-6 and TNF-α in the cerebrospinal fluid before operation between two groups　（$\bar{x}\pm s$）

组别	例数	ChE（ng/mL）	ChAT（ng/mL）	TNF-α（pg/mL）	IL-6（pg/mL）
POD 组	26	31.99±2.78	1.26±0.12	17.19±1.32	33.98±5.02
非 POD 组	157	40.89±4.19	0.86±0.15	12.29±1.64	16.98±3.41
t 值		13.915	12.920	14.467	16.643
P 值		<0.001	<0.001	<0.001	<0.001

表 3　两组术前脑脊液 ChE 及 ChAT 活性的比较　（u/mL，$\bar{x}\pm s$）

Tab. 3　Comparison of ChE and ChAT activities in the cerebrospinal fluid before operation between two groups　（u/mL，$\bar{x}\pm s$）

组别	例数	ChE 活性	ChAT 活性
POD 组	26	4.28±0.52	0.36±0.05
非 POD 组	157	5.25±0.26	0.27±0.03
t 值		9.321	8.916
P 值		<0.001	<0.001

3　讨　论

本研究选择了接受 TKA/THA 的老年患者为研究对象，手术麻醉采用腰硬联合阻滞麻醉；所有患者没有使用术前药物并使用了 PCIA 来进行术后镇痛。这些干预可让患者保持术中平稳状态并减少其他因素对 POD 的影响。

目前 POD 具体的发病机制尚不明确，而胆碱能学说为近年来的研究热点。胆碱能抗炎通路作为重要的神经回路，具有刺激迷走神经后释放乙酰胆碱的机制，并且乙酰胆碱和烟碱型乙酰胆碱受体（nicotinic acetylcholine receptors，α7nAChR）的 α-7 亚基具备相互作用，可阻碍阿尔茨海默病（Alzheimer's disease，AD）发生[17]。乙酰胆碱水平在成年人中具备动态平衡，而 ChAT 在人血浆和脑脊液中与相反作用的乙酰胆碱酯酶（acetylcholinesterase，AChE）和丁酰胆碱酯酶（butyrylcholinesterase，BuChE）共同维持稳态乙酰胆碱水平，而 AD 患者的 ChAT/ChE 比率增高[18]。相关研究表明胆碱能神经系统退化可能参与 AD 过程，脑脊液分析证明 AD 患者术后可能使 POD 发生率增加[19]。许多研究表示，中枢胆碱能神经的退化与 POD 发生有着密切关系。有学者在实验动物中发现，手术后的老年小鼠海马中枢胆碱能神经发生退化并产生认知障碍，证明了中枢胆碱能神经退变会加速 POD 的进展。并且，POD 患者术前检测发现血浆中的 ChE 活性明显减少，同样证明了在安静情况下 ChE 的变化可作为 POD 发生的生物标记物[15]，然而脑脊液检测 ChE 比在血浆中更具备诊断意义[16]，因此探讨脑脊液胆碱能生物标记物与 POD 的相关性具有重要临床意义。

相关研究发现，胆碱能抗炎通路是一种重要的神经机制，它可以促使迷走神经的传导机制释放乙酰胆碱，从而阻止 IL-6、TNF-α 等炎性因子的活动[19]。这些炎性因子具有穿越血脑屏障的能力，也可以从脑室周围的器官入侵人类的中枢神经系统[20]。在激活小胶质细胞后的活动可以在 POD 发展初期就被用来促成多种疾病的发展，导致致病环境的产生[21]。

本研究认为，老年患者在接受 TKA/THA 后的 POD 发生率约 14.21%。与非 POD 组相比，POD 组术前脑脊液 ChE 浓度及活性明显降低，ChAT 浓度和活性显著增加，这表明中枢胆碱能神经系统的退化对于 POD 的发生和发展起着重要作用；与非 POD 组相比，POD 组术前脑脊液 IL-6、TNF-α 浓度明显升高，证明炎性因子参与 POD 的进程。Bosancic 等[22]对 BioCog 研究患者中的一个亚群进行调查，结果显示 127 例腹部手术老年患者中 52 例患者发生 POD，其 AChE 活性与 POD 之间存在关联。一家欧洲学术医院单中心进行的前瞻性观察性研究发现，114 例心脏手术患者的 AChE 活性降低可能与 POD 的发生有关[23]。本研究结果与其符合，提示高活性 ChAT 及

低活性 ChE 可在术前预测 POD 的发生发展。

综上所述,脑脊液中的 ChAT 高活性状态、ChE 低活性状态与患者 POD 的发生有关,其中胆碱能神经功能的退化与其发生密切相关。

利益冲突　无

参考文献

[1] Sumner M, Deng C, Evered L, et al. Processed electroencephalography-guided general anaesthesia to reduce postoperative delirium: a systematic review and meta-analysis[J]. Br J Anaesth, 2023, 130(2): e243-e253.

[2] Leung JM, Rojas JC, Tang C, et al. Presence of preoperative neurodegeneration biofluid markers in patients with postoperative delirium [J]. Anesthesiology, 2023, 139(4): 432-443.

[3] Yan E, Veitch M, Saripella A, et al. Association between postoperative delirium and adverse outcomes in older surgical patients: a systematic review and meta-analysis [J]. J Clin Anesth, 2023, 90: 111221.

[4] Zhao J, Liang G, Hong K, et al. Risk factors for postoperative delirium following total hip or knee arthroplasty: meta-analysis[J]. Front Psychol, 2022, 13: 993136.

[5] Zhou Q, Zhou X, Zhang Y, et al. Predictors of postoperative delirium in elderly patients following total hip and knee arthroplasty: a systematic review and meta-analysis [J]. BMC Musculoskelet Disord, 2021, 22(1): 945.

[6] Meyer M, Götz J, Parik L, et al. Postoperative delirium is a risk factor for complications and poor outcome after total hip and knee arthroplasty[J]. Acta Orthop, 2021, 92: 695-700.

[7] 翟娟,刘艳红,宋星辰,等.高龄女性髋部骨折术后谵妄患者血清维生素 D 水平变化及意义[J].中国临床研究,2023,36(4):615-618.
Zai J, Liu YH, Song XC, et al. Serum vitamin D level in elderly women with postoperative delirium after hip fracture surgery[J]. Chin J Clin Res, 2023, 36(4): 615-618.

[8] 王甲正,张东莹,陈慧霞,等.老年开胸手术患者术中脑氧饱和度与术后谵妄的相关性[J].中国临床研究,2023,36(1):76-80.
Wang JZ, Zhang DY, Chen HX, et al. Correlation between intraoperative regional cerebraloxygen saturation and postoperative delirium in elderly patients undergoing thoracotomy[J]. Chin J Clin Res, 2023, 36(1): 76-80.

[9] Shin HJ, Woo Nam S,et al. Postoperative delirium after dexmedetomidine versus dropofol dedation in healthy older adults undergoing orthopedic lower limb surgery with spinal anesthesia: a randomized controlled trial[J]. Anesthesiology, 2023, 138(2): 164-171.

[10] Bosancic Z, Spies CD, Müller A, et al. Association of cholinesterase activities and POD in older adult abdominal surgical patients. BMC Anesthesiol. 2022, 22(1): 293.

[11] Yamanashi T, Nagao T, Wahba NE, et al. DNA methylation in the inflammatory genes after neurosurgery and diagnostic ability of postoperative delirium[J]. Transl Psychiatry, 2021, 11: 627.

[12] Parrish WR, Rosas-Ballina M, Gallowitsch-Puerta M, et al. Modulation of TNF release by choline requires α7 subunit nicotinic acetylcholine receptor-mediated signaling[J]. Mol Med, 2008, 14(9/10): 567-574.

[13] 李晓晓,王彬,董瑞,等.围术期神经认知障碍小鼠海马长链非编码 RNA 表达的变化及生物信息学分析[J].中华麻醉学杂志,2019,39(3):304-308.
Li XX, Wang T, Dong R, et al. Changes in expression of hippocampal long non-coding RNA and bioinformatics analysis in mice with perioperative neurocognitive disorders [J]. Chin J Anesthesiol, 2019, 39(3): 304-308.

[14] Xu H, Chen LK, Zhang X, et al. Central cholinergic neuronal degeneration promotes the development of postoperative cognitive dysfunction[J]. Lab Investig, 2019, 99(7): 1078-1088.

[15] Cerejeira J, Batista P, Nogueira V, et al. Low preoperative plasma cholinesterase activity as a risk marker of postoperative delirium in elderly patients[J]. Age Ageing, 2011, 40(5): 621-626.

[16] Olsson B, Lautner R, Andreasson U, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis[J]. Lancet Neurol, 2016, 15(7): 673-684.

[17] Vijayaraghavan S, Karami A, Aeinehband S, et al. Regulated extracellular choline acetyltransferase activity- the plausible missing link of the distant action of acetylcholine in the cholinergic anti-inflammatory pathway[J]. PLoS One, 2013, 8(6): e65936.

[18] Saijo K, Glass CK. Microglial cell origin and phenotypes in health and disease[J]. Nat Rev Immunol, 2011, 11(11): 775-787.

[19] Evered L, Silbert B, Scott DA, et al. Cerebrospinal fluid biomarker for Alzheimer disease predicts postoperative cognitive dysfunction [J]. Surv Anesthesiol, 2016, 60(5): 195-196.

[20] Wang B, Li SY, Cao XP, et al. Blood-brain barrier disruption leads to postoperative cognitive dysfunction[J]. Curr Neurovascular Res, 2018, 14(4): 359-367.

[21] Hirsch J, Vacas S, Terrando N, et al. Perioperative cerebrospinal fluid and plasma inflammatory markers after orthopedic surgery[J]. J Neuroinflammation, 2016, 13(1): 211.

[22] Bosancic Z, Spies CD, Müller A,et al. Association of cholinesterase activities and POD in older adult abdominal surgical patients[J]. BMC Anesthesiol, 2022, 22(1): 293.

[23] Adam EH, Haas V, Lindau S, et al. Cholinesterase alterations in delirium after cardiosurgery: a German monocentric prospective study[J]. BMJ Open, 2020, 10(1): e031212.

收稿日期:2023-07-18　**修回日期**:2023-09-20　**编辑**:王娜娜