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Research article

Serum molecules of the folate-driven one-carbon cycle as indicators of depressive tendencies in the elderly

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ABSTRACT

Aims: The folate-driven one-carbon (1C) cycle plays a significant role in the occurrence and development of depression. This study aimed to examine the potential of important molecules of the folate-driven 1C cycle as biomarkers for depressive tendency.
Methods: Ninety-five serum samples from older adults (age >60 years) were collected for this study. We quantified the concentrations of key metabolites and coenzymes of the folate-driven 1C cycle using Ultra-Performance Liquid Chromatography coupled with Tandem Mass Spectrometry (UPLC-MS/MS) and familiar clinical liver and kidney indicators in serum. Based on the differences in Hamilton Depression Rating Scale (HAMD)-17 scores, we compared the concentrations of measured molecules between elderly individuals with low and high levels of depression defined as HAMD-17 scores of 0–7 and 8–24, respectively. We also analyzed the concentration ranges of these molecules reflecting the level of depressive tendencies in the cohort.
Results: Our results showed significant variations in serum folate concentrations, SAM (S-adenosylmethionine), TBA (total bile acid), and SAM/SAH (S-enosylhomocysteine) ratios in elderly individuals with different HAMD-17 scores. Serum folate concentrations below 15.5 nmol/L and SAM/SAH ratios below 13.0 exhibited elevated levels of depressive tendency experienced among the participants.
Conclusions: The concentrations of serum folate, SAM, TBA, and SAM/SAH ratios might be used as indicators of depressive tendencies in the elderly population. A serum folate concentration of 15.5 nmol/L and a SAM/SAH ratio of 13.0 might be critical thresholds for indicating depressive tendencies in the Chinese elderly population.

1. Introduction

With the rapid increase in the global elderly population, addressing aging and its associated mental disorders has become imperative. Because elderly individuals are more likely to experience adverse life events such as bereavement, social isolation,

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financial hardship, and deteriorating physical health, these stressors can exacerbate depressive symptoms, leading to a higher prevalence of depression in this demographic [1]. However, depression in the elderly can be difficult to identify, as their symptoms may differ from those of younger people. For some elderly individuals with depression, sadness is not the primary symptom, instead, they may feel more numbness or lack of interest in activities. Elderly people, particularly those influenced by Confucian culture in China, are less likely to discuss their feelings, which adds to the challenge of studying depression in this population.

Vitamins play a crucial role in maintaining health, and their deficiencies can be linked to symptoms of mental illness. B vitamins are essential for the methylation cycle, monoamine oxidase production, DNA synthesis, and the repair and maintenance of phospholipids, with vitamins B1, B2, B3, B6, B9, and B12 being particularly important for neuronal function [2,3]. Vitamin B2 (riboflavin) and Vitamin B6 (pyridoxine), acting as a coenzyme in the synthesis of various neurotransmitters, are important for mood regulation and cognitive function [4]. Patients with depression often have lower levels of vitamin B6 [4]. Moreover, studies have demonstrated that supplementing B vitamins (vitamins B1, B2, and B6) in elderly patients with depression can lead to significant improvements in both depressive symptoms and cognitive function [2].

Folate (vitamin B9) significantly impacts the cardiovascular and cerebrovascular endothelial systems [5], the hematological system [6], and the nervous system functions [7]. Folate is closely related to one-carbon (1C) metabolism, jointly producing methyl groups and regulating methylation-related processes. Folate is critical for the development and function of the nervous system, particularly during early pregnancy, influencing neural development and placental growth [8]. Folate deficiency is common in patients with epilepsy, neurological and psychiatric issues, and the elderly. People deficient in folate exhibit psychological symptoms such as apathy, fatigue, insomnia, irritability, forgetfulness, and difficulty concentrating, which are common manifestations of functional cognitive loss and depression [9]. A meta-analysis revealed that serum folate levels in patients with depression are lower compared to those with individuals without depression [10]. However, the literature has been inconsistent with regard to different populations. Another meta-analysis focusing on elderly men and women showed that low folate levels are associated with an increased risk of depression, with no significant association in elderly men [11].

Serum metabolite concentrations vary among different populations. There are notable racial differences in metabolic characteristics between the Chinese population and other groups, and age significantly impacts human metabolism [12]. Firstly, the C136T and C677T variant of the MTHFR gene is distributed differently across races [13], and C677T is more common in East Asian populations [14], and is associated with folate and HCY (homocysteine) levels in the one-carbon metabolism pathway [15]. Secondly, dietary habits significantly affect metabolite levels. Traditional Chinese diets differ significantly from Western diets, impacting the levels of essential nutrients and metabolites in the body. Additionally, the elderly in China often adhere to traditional dietary habits, using herbs and dietary supplements to maintain health, which is significantly different from the elderly in Western countries. These cultural habits not only affect the choice and use of dietary supplements but may also lead to differences in metabolite levels among populations. Hence, our hypothesis is that the range of concentrations of important folate-derived 1C cycle molecules in the serum of the elderly Chinese population differs from those in people of different ages and racial backgrounds, warranting further investigation.

The folate-driven 1C cycle is a critical metabolic pathway in the body, responsible for transferring and utilizing one-carbon units involved in the synthesis and methylation of DNA, RNA, proteins, and phospholipids [16,17]. In this process, vitamins B2, B6, B9, and B12, along with SAM, SAH, and HCY, interact closely, forming a complex metabolic network that ensures cellular function and health [18,19]. Folate, through its derivative tetrahydrofolate (THF), plays a vital role in carrying, transferring, and utilizing one-carbon units. Vitamin B6, in its active form pyridoxal-5′-phosphate (PLP), acts as a coenzyme assisting serine hydroxymethyltransferase (SHMT) in converting serine to glycine and transferring a one-carbon unit to THF, forming 5,10-methylenetetrahydrofolate (5, 10-MTHF). Under the influence of vitamin B2 (in its coenzyme form FAD) and methylenetetrahydrofolate reductase (MTHFR), 5, 10-MTHF is converted to 5-methyltetrahydrofolate (5-MTHF). 5-MTHF, with the assistance of methionine synthase (MS) and its coenzyme vitamin B12, transfers a methyl group to HCY, forming methionine (MET). Subsequently, MET is converted to SAM. Following methylation reactions, SAM generates SAH. SAH is then hydrolyzed to HCY and adenosine. HCY can be remethylated to methionine through the remethylation pathway or converted to cystathionine and cysteine via the transsulfuration pathway, with vitamin B6 acting as a coenzyme for cystathionine synthase.

Catechol-O-methyltransferase (COMT) catalyzes the transfer of a methyl group from SAM to the hydroxyl groups on the catechol nucleus, thereby regulating the activity and metabolism of neurotransmitters such as dopamine, epinephrine, and norepinephrine [20,21]. Several clinical trials support the efficacy of SAM in the treatment of depression. When used as a monotherapy or adjunctive therapy, SAM has been shown to significantly reduce depression scores [22]. It is particularly well-tolerated and effective in patients with major depressive disorder (MDD) who have not responded to traditional antidepressants, including selective serotonin reuptake inhibitors [22]. As a byproduct of SAM methylation reactions, the accumulation of SAH inhibits the activity of histone methyltransferases [23]. Additionally, although the causal relationship remains unclear, elevated levels of homocysteine (HCY) have been linked to depression under chronic stress and are associated with depressive symptoms [24].

In this study, we measure and analyze some important molecules in the folate-driven 1C cycle as potential biomarkers for depressive tendencies. By examining the serum levels of these molecules in the Chinese elderly population, we aim to gain insights into their potential links to depressive tendencies, thereby facilitating early detection and treatment of depression.

2. Materials and methods

2.1. Research subjects

From February 2021 to September 2023, 95 individuals undergoing physical examination and completing depression scale

assessments at the Geriatrics Department of Xuzhou Medical University Affiliated Hospital provided residual serum samples. Among them, 67 were male and 28 were female. Inclusion criteria were as follows: (1) Age ≥60 years; (2) Hamilton Depression Rating Scale (HAMD-17) score ≤24. Exclusion criteria included: (1) Any affective disorders; (2) Neurodegenerative disorders; (3) Use of B vitamin supplements or antibiotics in the past two months. As our samples were not sourced from psychiatric hospitals, none of the participants had received any mood stabilizers, ensuring that our findings were not influenced by medications affecting the brain. All assessments and procedures in this study were conducted in accordance with the guidelines of the Declaration of Helsinki. The study received approval from the Ethics Committee of Xuzhou Medical University Affiliated Hospital (Approval No: XYFY2022-KL053-01).

2.2. Serum biochemical laboratory assessment

Height and weight were measured using standard equipment on the day of appointment. HAMD scores were provided by the Geriatrics Department of Xuzhou Medical University Affiliated Hospital, which was approved in 2023 as the Jiangsu Provincial Guidance Center for the Prevention and Treatment of Cognitive Disorders in the Elderly. Clinical parameters and participant characteristics are shown in Table 1. Fasting venous blood (5 mL) was collected in anticoagulant tubes. Whole blood was centrifuged to obtain the supernatant and assess liver and kidney indicators, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), total bilirubin (TBIL), direct bilirubin (DBIL), total bile acid (TBA), cholinesterase (CHE), blood urea nitrogen (BUN), creatinine (CRE), uric acid (UA), cystatin C (CysC), fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and bicarbonate concentration (HCO_3^-). The remaining serum was aliquoted into multiple 200 μL vials and stored at −80 °C for assessing folate-derived metabolic cycle indicators.

2.3. Assessment of serum molecules of the folate-driven 1C cycle

High-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to measure B vitamin levels in serum and plasma [25,26]. This method is highly selective and sensitive. An ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) platform (Waters Xevo-TQS), and MassLynx workstation (Waters, version 4.1) were used to determine the concentrations of key metabolites and coenzymes of the folate-driven 1C cycle.

Specific steps included serum pretreatment with protein precipitation. Standard solutions were prepared by accurately weighing standard substances and diluting them with methanol. Mixed internal standard solutions were added to each sample and standard to correct for matrix effects. Chromatographic separation was performed using an ACQUITY UPLC HSS T3 2.1 × 100 mm 1.8 μm column, with mobile phase A being 0.1 % formic acid in water and mobile phase B being 100 % methanol. The flow rate for chromatographic separation was 0.2 mL/min, and the column temperature was maintained at 30 °C. LC-MS/MS utilized an electrospray ionization (ESI) source and multiple reaction monitoring (MRM) mode for quantification. Standard substances (VB2, VB6, VB9, SAM, SAH, HCY) were purchased from Cambridge Isotope Laboratories, Inc. Isotope-labeled internal standards (vitamin B2-13c4,15N2, vitamin B6-d3, vitamin B9-d4, S-adenosylmethionine-d3 sulfate, S-adenosylhomocysteine-d4) were obtained from TRC.

This method was validated according to CLSI C62-A standards [27]. The limits of detection and quantification for vitamin B2, vitamin B6, vitamin B9, SAM, SAH, and HCY are shown in Table 2. The linear correlation coefficient (R) was greater than 0.995, and the intra- and inter-day precision was less than or equal to 10 %, with a spike recovery rate of 85–115 %.

2.4. Statistical analysis

Statistical analysis was conducted using SPSS 27.0 (SPSS Inc., Chicago, USA). After normality testing with the One-Sample Kolmogorov-Smirnov Test, correlation analysis was performed using Pearson or Spearman methods based on data distribution. ANCOVA was used to control for confounding variables. Based on HAMD-17 scores, the elderly population was divided into two groups: 0–7

Table 1
Clinical and folate metabolic characteristics of study population are summarized (Mean ± standard deviation, SD).

Demographic Profile	Entire Elderly population (N = 95	Elderly Males (N = 67)	Elderly Females (N = 28)
Age (years)	69.55 ± 4.21	69.40 ± 4.50	69.89 ± 3.49
BMI (kg/m2)	24.89 ± 3.14	25.00 ± 3.03	24.63 ± 3.43
HAMD-17	6.87 ± 4.89	7.10 ± 5.05	6.32 ± 4.51
VB2 (ug/L)	16.43 ± 11.56	16.45 ± 12.15	16.38 ± 10.21
VB6 (ug/L)	4.78 ± 6.59	4.62 ± 5.70	5.16 ± 8.45
Folate (nmol/L)	26.83 ± 17.84	27.48 ± 16.57	25.26 ± 20.81
SAM (umol/L)	1.88 ± 0.74	1.88 ± 074	1.86 ± 0.75
SAH (umol/L)	0.10 ± 0.04	0.10 ± 0.04	0.11 ± 0.03
SAM/SAH	19.31 ± 8.47	20.19 ± 9.21	17.22 ± 6.00
HCY (umol/L)	15.61 ± 14.54	15.742 ± 14.40	15.30 ± 15.15
TBA (umol/L)	5.87 ± 3.97	5.89 ± 3.76	5.80 ± 4.51
BUN (mmol/L)	5.76 ± 1.80	6.04 ± 1.86	5.04 ± 1.46

Legend: BMI = weight (kg)/height (m) ^ 2, HAMD-17 = Hamilton Depression Rating Scale-17, VB2 = vitamin B2, VB6 = vitamin B6, SAM=Sadenosylmethionine, SAH=S-adenosylhomocysteine, HCY = homocysteine, TBA = total bile acid, BUN = blood urea nitrogen.

Table 2
Performance indicators for the determination of concentrations of folate-driven one-carbon cycle metabolites using UPLC-MS/MS (Waters Xevo-TQS).

	Limit of Detection (LOD)	Limit of Quantitation (LOQ)	Intra-assay Coefficient of Variation	Inter-assay Coefficient of Variation
	(nmol/L)	(nmol/L)	(CV)(%)	(CV)(%)
VB2	0.05	0.15	4.8	7.5
VB6	0.04	0.12	4.6	6.7
Folate	0.06	0.18	4.5	6.9
SAM	0.03	0.09	4.3	5.7
SAH	0.04	0.12	4.8	6.9
HCY	0.05	0.15	4.5	6.8

Legend: VB2 = vitamin B2, VB6 = vitamin B6, SAM=S-adenosylmethionine, SAH=S-adenosylhomocysteine, HCY = homocysteine.

points and 8–24 points, with independent sample t-tests conducted separately for elderly males and females. We sorted concentrations of important substances in the folate-driven 1C cycle and related pathways, age, IBM, and SAM/SAH ratios based on our experimental data. The elderly population, elderly males, and elderly females were then divided into two (50 % each), three (25 %, 50 %, 25 %), or four groups (25 % each). Independent sample t-tests or ANOVA were used for statistical analysis.

3. Results

3.1. Lower levels of depressive tendencies in Chinese women aged 71 and above

Analysis of confounding variables found that gender, age, and BMI status did not significantly impact levels of depressive tendencies in the entire elderly population and elderly males. However, age showed a statistically significant difference in elderly females. In the sample of elderly Chinese women, the Spearman correlation coefficient between age and HAMD-17 scores was −0.406, indicating a negative correlation with two-tailed significance at $P < 0.05$. Women aged 71 and above had significantly lower HAMD-17 scores compared to the 63–70 age group, with statistical significance at $P < 0.05$ (Fig. 1).

3.2. Serum concentrations of the folate-driven 1C cycle molecules and common biochemical indicators in elderly individuals with varying levels of depressive tendencies

Independent sample t-tests revealed that elderly individuals with lower levels of depressive tendencies (HAMD-17 score 0–7) had significantly higher concentrations of folate, SAM, TBA, and SAM/SAH ratios compared to those with higher levels of depressive tendencies (HAMD-17 score 8–24), with statistical significance at $P < 0.05$ (Fig. 2). Among elderly males with varying levels of depressive tendencies, significant differences in vitamin B2, folate, SAM, BUN concentrations, and SAM/SAH ratios were observed ($P < 0.05$) (Fig. 3). Lower levels of depressive tendencies in elderly males were associated with higher folate, BUN concentrations, and SAM/SAH ratios but lower vitamin B2 concentrations, with statistical significance at $P < 0.05$. Additionally, BUN concentrations in elderly females with lower levels of depressive tendencies were significantly lower than those with higher levels of depressive tendencies ($P < 0.0001$) (Fig. 4).

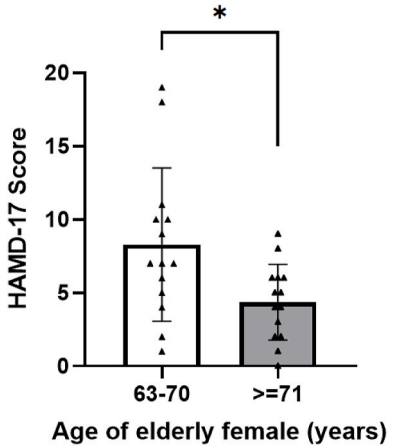

Fig. 1. HAMD-17 scores of elderly females. The values are presented as mean ± SD. *$p < 0.05$.

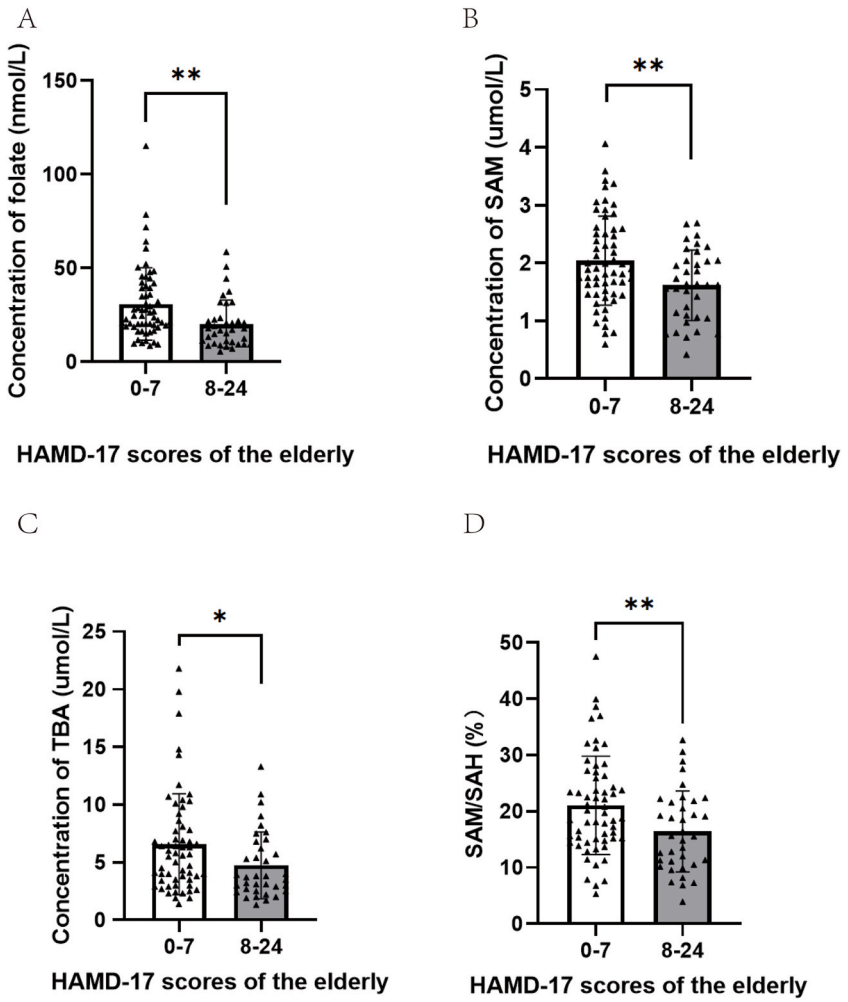

Fig. 2. Serum concentrations of folate, SAM, TBA, and SAM/SAH in the entire elderly population. The values are presented as mean ± SD. *$p < 0.05$, **$p < 0.01$.

3.3. Variations in levels of depressive tendencies among the elderly with different concentrations of measured molecules

Analysis revealed that a serum folate concentration of <15.5 nmol/L could serve as an indicator of depressive tendencies. Elderly individuals with folate concentrations below 15.5 nmol/L had significantly higher HAMD-17 scores compared to those with concentrations above 15.5 nmol/L ($P < 0.05$) (Fig. 5A). In elderly males, folate concentrations below 15.5 nmol/L were associated with significantly higher HAMD-17 scores compared to those above 24.5 nmol/L ($P < 0.05$) (Fig. 5B). In elderly females, folate concentrations below 15.5 nmol/L also resulted in significantly higher HAMD-17 scores compared to those above 15.5 nmol/L ($P < 0.01$) (Fig. 5C). These findings suggest that a serum folate concentration of 15.5 nmol/L may be a critical threshold for reflecting depressive tendencies in the elderly.

When SAM/SAH ratios were below 13.0, levels of depressive tendencies in the elderly were higher compared to those with ratios between 13.2 and 18.1 and above 23.6 ($P < 0.05$), with no significant difference compared to the 18.3–23.4 group (Fig. 6A). In elderly males, SAM/SAH ratios below 13.0 were associated with increased levels of depressive tendencies compared to ratios above 26.0 ($P < 0.05$) (Fig. 6B). Due to the small sample size of elderly females with SAM/SAH ratios below 13.0, further investigation was not possible, but in the entire elderly population and elderly male subgroup, SAM/SAH ratios of 13.0 may be another critical threshold for reflecting depressive tendencies. Additionally, in elderly males, SAM concentrations above 2.30 μmol/L were associated with lower levels of depressive tendencies compared to concentrations below 1.40 μmol/L ($P < 0.05$) (Fig. 6C).

4. Discussion

Folate (vitamin B9) deficiency is associated with decreased levels of serotonin, dopamine, and norepinephrine, contributing to neurochemical susceptibility to depression [7]. A systematic review and meta-analysis found that individuals with depression have

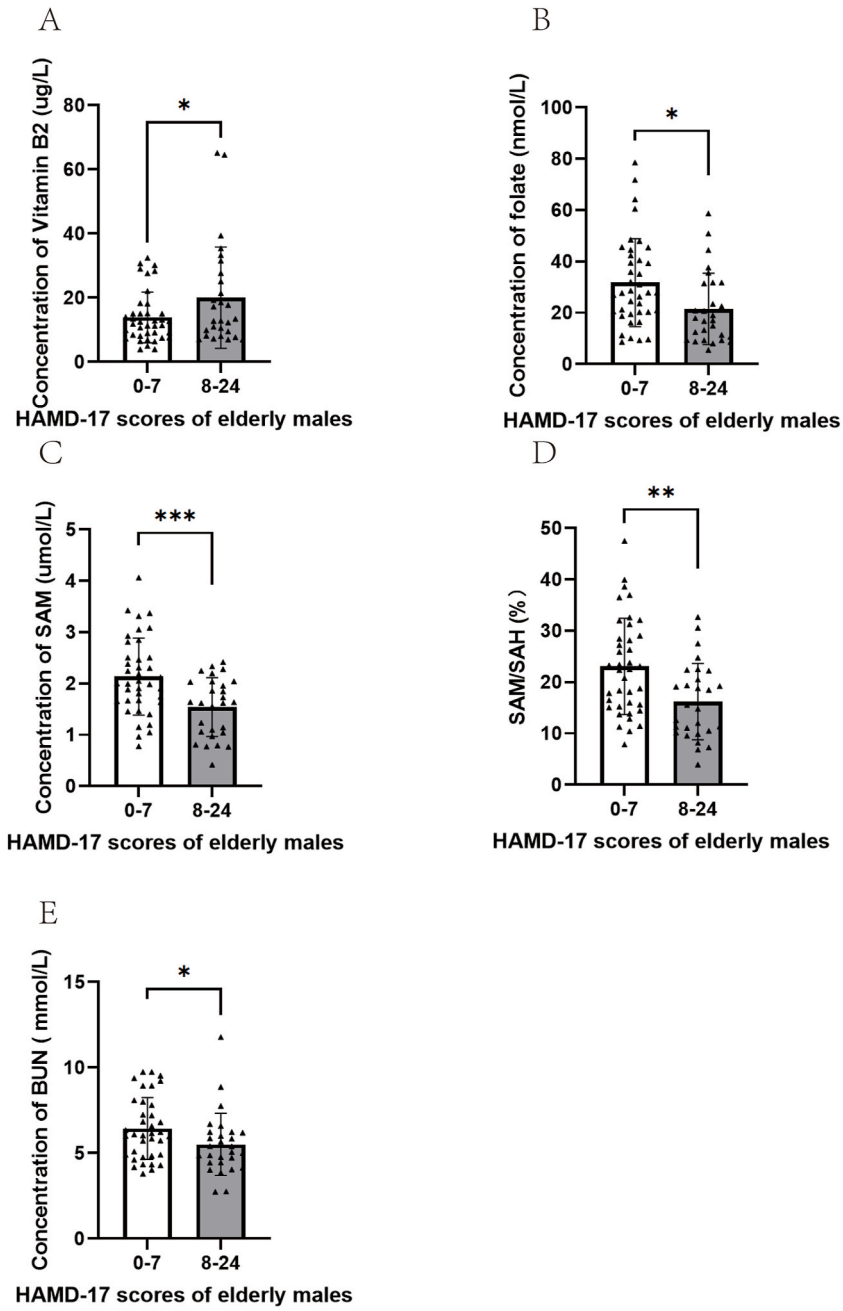

Fig. 3. Serum concentrations of vitamin B2, folate, SAM, BUN, and SAM/SAH in elderly males. The values are presented as mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

significantly lower serum folate levels compared to those without depression, and folate supplementation can enhance the efficacy of antidepressant medications [10]. In rats subjected to life stress, folate prevented depression-like behaviors induced by early life stress, while omega-3 and folate prevented depression-like behaviors induced by later life stress [28]. In dietary supplementation with dexamethasone-fed mice, lipid peroxidation decreased, and antioxidant levels increased [29].

Clinically, the Hamilton Depression Rating Scale (HAMD-17) is often used to assess the severity of depressive symptoms. A common standard is that patients are usually considered not clinically depressed when HAMD-17 scores are below 8. Our results indicate that folate, SAM, TBA concentrations, and SAM/SAH ratios are significantly lower in the entire elderly population and elderly males with higher levels of depressive tendencies. Our findings strongly suggest that a serum folate concentration of 15.5 nmol/L may be a critical threshold for reflecting depressive tendencies in the elderly. Serum folate concentrations below this threshold indicate a higher tendency for depression, supporting previous research findings. It is noteworthy that excessive folate intake may not always be safe for

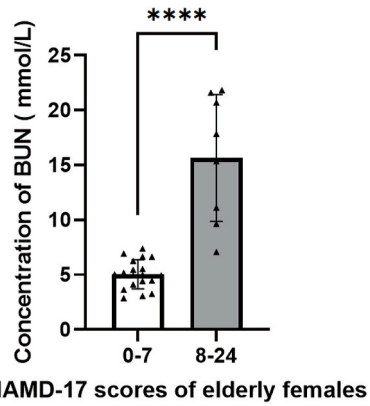

Fig. 4. Serum concentration of BUN in elderly females. The values are presented as mean ± SD. ****$p < 0.0001$.

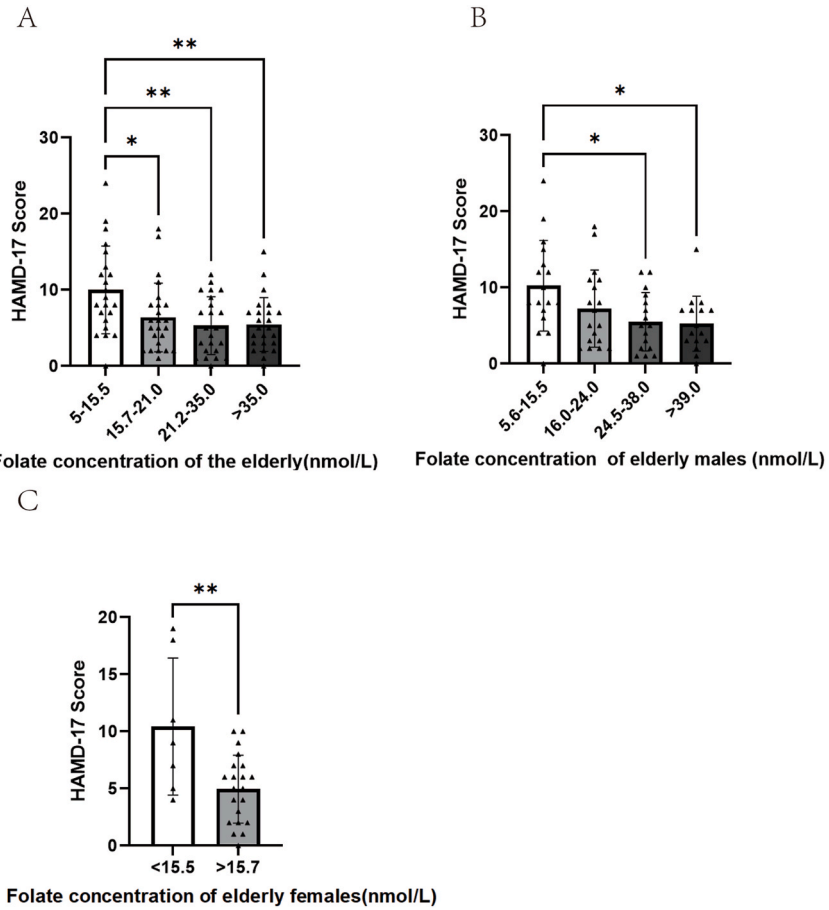

Fig. 5. HAMD-17 scores according to different serum folate concentrations in the entire elderly population (A), elderly males (B), and elderly females (C). *$p < 0.05$, **$p < 0.01$.

individuals with different ages, races, or genetic backgrounds [30,31].

Serum SAM concentrations are typically lower in depressed patients compared to healthy individuals, a phenomenon reflected in our study. SAM serves as a methyl donor in the body, participating in various biochemical reactions, including the synthesis of neurotransmitters such as dopamine, norepinephrine, and serotonin [32]. A clinical trial assessing the efficacy of SAM in treating depression showed greater treatment effects in males than females, suggesting that lower average SAM concentration in males may be a contributing factor [33].

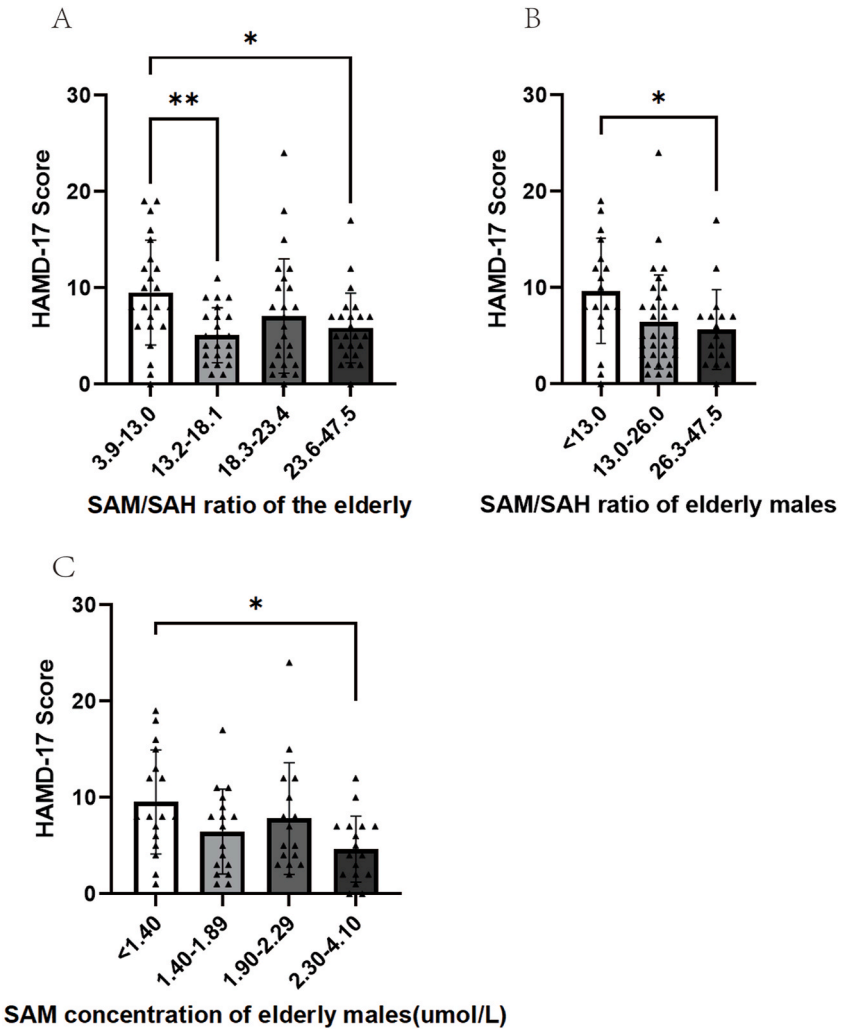

Fig. 6. HAMD-17 scores according to different SAM/SAH ratios in the entire elderly population (A), elderly males (B), and elderly females (C). *$p < 0.05$, **$p < 0.01$.

The SAM/SAH ratio of 13.0 may be another critical threshold for reflecting depressive tendencies in the elderly. Our results show that SAM/SAH ratios lower than 13.0 are associated with a higher tendency for depression in both the entire elderly population and elderly male subgroup. This finding is consistent with a study based on 10 years of data from a Japanese community-dwelling elderly population, which indicated that increased serum SAM/SAH ratios significantly reduce the risk of dementia or death [34]. Current treatment options for children with autism spectrum disorder involve the use of oral or injected methylcobalamin (mB12), which leads to reduced SAH levels and a higher SAM/SAH ratio [35]. Our results show that an increased SAM/SAH ratio correlates with reduced depressive tendencies in the entire elderly population and elderly male subgroup and that a ratio above 26.3 may improve the level of depressive tendencies.

Additionally, our study found that TBA concentrations are lower in the group with high levels of depressive tendencies among the entire elderly population. Research indicates that bile acid levels are typically reduced in patients with bipolar disorder and depression [6]. Further studies on bile acid metabolism profiles are underway. Another intriguing phenomenon, currently unexplained, is that BUN concentrations significantly increase in elderly females with high level of depressive tendencies ($p < 0.0001$), whereas the opposite is true in elderly males ($P < 0.05$), with no correlation found in the entire elderly population.

This study was not longitudinal, which limits the discussion of the causal relationships between biochemical markers and depressive tendencies. Because of the difficulty in collecting cerebrospinal fluid, we used peripheral serum to detect molecular levels. The number of serum samples used for metabolic researches remains limited, especially among elderly women. Depressive symptoms vary significantly between individuals. Our study did not include individuals from other age groups and ethnicities. A significant strength of this study is that none of the participants used antidepressants during the study period. By identifying biochemical markers associated with depressive tendencies, this study may promote early detection and mental health intervention in the elderly population.

5. Conclusion

In conclusion, our study suggests that serum concentrations of folate, SAM, TBA, and SAM/SAH ratios may serve as indicators of depressive tendencies in the Chinese elderly population. A serum folate concentration of 15.5 nmol/L and a SAM/SAH ratio of 13.0 might be critical thresholds for indicating depressive tendencies in the Chinese elderly population.

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author H. Lu. The data are not publicly available due to the ongoing writing of subsequent papers.

CRediT authorship contribution statement

Ye Xiong: Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Guoyiran Zhou:** Writing – review & editing, Writing – original draft, Investigation, Data curation. **Lingxin Zhou:** Visualization, Software, Methodology. **Mengyuan Guo:** Resources, Investigation, Data curation, Conceptualization. **Hailong Lu:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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