

Vitamin B12, Folate, Homocysteine, Inflammatory Mediators (Interleukin-6, Tumor Necrosis Factor- α and C-Reactive Protein) Levels in Adolescents with Anxiety or Depressive Symptoms

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Purpose: To evaluate the prevalence of abnormal vitamin B12, folate, total homocysteine (tHcy), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), C-reactive protein (CRP) levels, to analyze the relationship between these parameters and the severity of anxiety or depressive symptoms, and to explore the possible factors associated with abnormal levels of these parameters in adolescents with anxiety or depressive symptoms.

Methods: Adolescent (aged 12–18 years) outpatients with anxiety or depressive symptoms were recruited. The patient health questionnaire-9 and generalized anxiety disorder scale-7 were used to measure the severity of depression and anxiety. Serum vitamin B12, folate, tHcy, IL-6, TNF- α , and CRP levels were determined.

Results: 128 subjects were recruited. The prevalence of vitamin B12 and folate deficiency, tHcy, TNF- α , IL-6, and CRP elevation was 8.6%, 10.2%, 25.8%, 14.8%, 21.9%, and 10.2%, respectively, in adolescents with anxiety or depressive symptoms. Lower vitamin B12 levels were correlated with a higher risk of severe anxiety and depressive symptoms. The severity of some symptoms of anxiety or depression were weakly correlated with vitamin B12, folate, tHcy, IL-6, and CRP levels. Vitamin B12, folate, and tHcy levels were not associated with inflammatory mediators. Vitamin B12 deficiency was associated with older age and higher tHcy levels. Folate deficiency was associated with elevated tHcy. Elevated tHcy was associated with lower vitamin B12 and folate levels. IL-6 elevation was associated with elevated CRP and TNF- α . CRP elevation was associated with older age, higher BMI, and current drinking.

Conclusion: Lower vitamin B12 levels were correlated with a higher risk of severe anxiety or depressive symptoms. Weak correlations were observed between the severity of some symptoms of anxiety or depression and vitamin B12, folate, tHcy, IL-6, and CRP levels. Vitamin B12, folate, and tHcy levels were related to each other. IL-6 elevation was associated with elevated CRP and TNF- α . CRP elevation was associated with older age, higher BMI, and current drinking.

Keywords: vitamin B12, adolescents, inflammatory mediators, depression, anxiety

Introduction

Depressive and anxiety disorders are serious and common mental disorders in adolescence. In China, the prevalence of depressive symptoms was estimated as 21.9% in junior secondary school and 24.2% in senior secondary school.¹ Meanwhile, 32.0% Chinese adolescents have anxiety symptoms.² Depressive and anxiety disorders in adolescence may extend into adulthood.³ Depressive disorders in adolescence were associated with poor adult psychiatric and functional outcomes such as higher levels of adult anxiety, illicit drug problems, and worse health, criminal, and social function.⁴ Furthermore, comorbid anxiety and depression is common in children

and adolescents, which leads to a more difficult diagnostic assessment and increased symptom severity.⁵ More severe anxiety and depression symptoms are related to potential consequences such as suicide, self-harm, refusal to eat, poorer treatment outcomes, and treatment resistance.^{6–8} Therefore, it is indispensable to explore modifiable risk factors to decrease the prevalence or severity of depressive and anxiety symptoms in adolescence.

Depressive and anxiety symptoms are related to numerous factors. Diet plays an important role in the occurrence and progress of depression and anxiety.⁹ Vitamin B12 is derived from animal products and folate is mainly from plants. They play a critical role in one-carbon metabolism and DNA, protein, and lipid methylation.¹⁰ The classic manifestations of vitamin B12 or folate deficiencies were hematological abnormalities such as megaloblastic anemia, which can be easily diagnosed by clinicians.¹¹ However, clinicians frequently overlook that vitamin B12 or folate deficiencies may also lead to metabolic disturbance of monoamine, hormones, and neurotransmitters, which have been implicated in depression or anxiety.^{12–14} Vitamin B12 and folate are closely correlated with the metabolism of homocysteine. Methylcobalamin, an activated form of vitamin B12, is a cofactor for methionine synthase which catalyzes the conversion of homocysteine to methionine and 5-methyltetrahydrofolate, a folate derivative, is a substrate.¹⁵ Vitamin B12 or folate deficiencies may elevate circulating total homocysteine (tHcy) level which is a valuable parameter for reflecting Hcy level and the diagnosis of vitamin B12 or folate deficiency.^{16,17} Elevated tHcy may cause oxidative stress, mitochondrial dysfunction, and apoptosis of dopaminergic neurons, which may also induce depression or anxiety.^{18,19} Several observational studies showed that the severity of anxiety and depression symptoms was negatively correlated with vitamin B12 and folate levels, and was positively correlated with tHcy levels in children and adolescents.^{20,21}

In addition, abnormal vitamin B12, folate, or tHcy levels can affect the immune system function.^{17–19} For example, hyperhomocysteinemia or deficiency of vitamin B12 or folate may regulate innate immunity via increasing dendritic cells activation,^{22,23} decreasing cytotoxicity of natural killer cells,²⁴ modulating expression of pro-/anti-inflammatory cytokines.^{25–27} They may also regulate adaptive immunity by changing the percentage of CD4+CD25+ regulatory T cells and CD4+/CD8+ T lymphocytes,^{28,29} and affecting immunoglobulin production.³⁰ Persistent chronic inflammation can dysregulate the hypothalamic-pituitary-adrenal (HPA) axis function, disturb neurotransmitter metabolism, impair neurons, and alter neural activity in the brain regions involved in emotion regulation, which results in anxiety and depression symptoms.^{31–33}

The key mediators of innate and adaptive immunity, called inflammatory mediators, especially tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and C-reactive protein (CRP), play a great role in the pathophysiology of depression and anxiety.³² Evidence supported that patients with depression or anxiety had higher TNF- α , IL-6, or CRP levels, which were positively correlated with the severity of anxiety and depression symptoms.^{34–37} Meanwhile, clinical researches showed that hyperhomocysteinemia or deficiency of vitamin B12 or folate was positively correlated with higher TNF- α , IL-6, or CRP levels in the general population or patients with diabetes, rheumatoid arthritis, and angiographic coronary artery disease.^{25–27,38–41}

However, no studies investigated the association between vitamin B12, folate, tHcy, TNF- α , IL-6, and CRP levels and symptoms of anxiety and depression in adolescent patients. Here, we hypothesized that the severity of anxiety or depression symptoms was negatively associated with vitamin B12 and folate levels, and positively associated with TNF- α , IL-6, CRP, and tHcy levels in adolescents with anxiety or depressive symptoms. Meanwhile, TNF- α , IL-6, and CRP levels were positively associated with tHcy level, and negatively associated with vitamin B12 and folate levels. To test the hypothesis, we first determined the prevalence of abnormal vitamin B12, folate, tHcy, TNF- α , IL-6, and CRP levels in adolescents with anxiety or depression symptoms. Secondly, we assessed the relationship between vitamin B12, folate, tHcy, TNF- α , IL-6, CRP levels and the severity of anxiety or depression symptoms. Finally, we explored the possible factors (eg demographic or clinical features, hemogram parameters) associated with abnormal vitamin B12, folate, tHcy, TNF- α , IL-6, and CRP levels.

Materials and Methods

Subjects

We recruited adolescent (aged 12–18 years) outpatients who presented self-reported anxiety or depressive symptoms assessed with the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) from July 2021 to

August 2022 in the Department of Neurology and Psychiatry, the First Affiliated Hospital of Chongqing Medical University, Chongqing, China.

Subjects were excluded if they had infectious diseases, CRP ≥ 10 mg/L, severe hepatic or renal impairment, tumor, severe aphasia, current pregnancy, psychiatric diagnoses of anorexia nervosa or schizophrenia and other psychotic disorders, a history of severe nervous system diseases (eg dementia, mental retardation, epilepsy), acute or chronic medical illness potentially affecting the HPA axis, hypothalamic-pituitary-thyroid (HPT) axis, systemic immune system (eg autoimmune diseases), or recent use of medications affecting the immune system (eg corticosteroids and nonsteroidal inflammatory drugs, antibiotics and antiviral medications). The study was approved by the Ethical Committee of Chongqing Medical University, in accordance with the principles expressed in the Declaration of Helsinki (approval No.2022-K491). All subjects (adolescents and their parents) provided written informed consent before all the procedures.

A priori power analysis was performed to determine the minimal sample size. Based on the prevalence of severe anxiety in the vitamin B12 < 300 pg/mL group (55.0%, $n = 20$) and in the vitamin B12 ≥ 300 pg/mL group (26.7%, $n = 30$) in the first 50 samples in our preliminary survey, we needed to recruit at least 100 subjects to achieve a power of 0.80 and an alpha of 0.05 in the survey.

Assessment of Anxiety or Depressive Symptoms

An in-person psychological questionnaire was performed. The severity of depressive symptoms was measured with the Chinese version of the patient health questionnaire-9 (PHQ-9), which showed good reliability and validity in Chinese adolescents.⁴² It includes questions about each of the nine DSM-IV criteria for depression in the previous seven days. Each question was scored between 0 (not at all) and 3 (nearly every day). The total scores ranged from 0 to 27, and higher scores indicated greater depressive symptoms. The scores ≥ 20 represented severe depression (SD) and < 20 represented mild depression (MD).⁴³ The Cronbach's alpha coefficient is a credibility rating marker. It is considered acceptable when the Cronbach's alpha coefficient was more than 0.7.⁴⁴ In our study, the Cronbach's alpha coefficient of the PHQ-9 is 0.835.

The severity of anxiety was measured with the Chinese version of the generalized anxiety disorder scale-7 (GAD-7), which showed good reliability and validity in Chinese adolescents.⁴⁵ The scale includes 7 questions to reflect the frequency of anxiety symptoms during the past two weeks. Scores ranged from 0 (not at all) to 3 (nearly every day). The total scores ranged from 0 to 21, and higher scores indicated more severe anxiety. The scores ≥ 15 represented severe anxiety (SA) and < 15 represented mild anxiety (MA).⁴⁶ In our study, the Cronbach's alpha coefficient of GAD-7 is 0.868.

Data Collection

All patients underwent clinical history, neurologic and psychological examination, and complete blood work. Demographic or clinical features included age, gender, body mass index (BMI), course of the disease, parental marriage and education level, smoking, drinking, family history of psychiatric illness, the use of antidepressants or antipsychotics, PHQ-9 and GAD-7.

Blood samples were collected from the antecubital vein after an 8-hour fast (between 2 and 4 PM) and laboratory data were obtained using standard laboratory methods at the Central Laboratory and Endocrine Laboratory of our hospital. Serum vitamin B12 and folate levels were measured with an electro-chemiluminescence method (Unicel DxI 800 Immunoassay System, Beckman Coulter, USA). The tHcy was determined with enzymatic methods in an autoanalyzer (HITACHI 7180, Ichige, Japan). Red blood cells (RBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), hematocrit (HCT), white blood cells (WBC), and platelet count (PLT) were measured with an automatic blood cell counter hematology analyzer (Mindray BC-6600, China). IL-6 and TNF- α were measured with chemiluminescent immunoassay technology (Siemens IMMULITE 1000, Germany). CRP was assessed with immunonephelometry (Beckman Immage 800, USA).

Classification of Vitamin B12, Folate, tHcy, IL-6, TNF- α , CRP Levels

Based on the laboratory reference and previous literature, vitamin B12 levels were classified as deficiency (< 180 pg/mL), margin (180–300 pg/mL), and sufficiency (≥ 300 pg/mL).⁴⁷ Folate levels were classified as deficiency (< 4.0 ng/

mL) and sufficiency (≥ 4.0 ng/mL).⁴⁸ The tHcy levels were classified as severe (> 100 $\mu\text{mol/L}$), moderate (30.1–100.0 $\mu\text{mol/L}$), mild (15.1–30.0 $\mu\text{mol/L}$) hyperhomocysteinemia (HHcy), and normal levels (5.0–15.0 $\mu\text{mol/L}$).⁴⁹ Elevated TNF- α , IL-6, and CRP levels were defined as >8.1 pg/mL, >3.4 pg/mL, and > 3 mg/L, respectively.⁵⁰

Statistical Analysis

The assumption for the normal distribution of variables was made with the Shapiro–Wilk test. Continuous variables with normal distribution were expressed as mean \pm standard deviation (SD). Non-normal distribution of data was expressed as median (interquartile range). Categorical variables were expressed as numbers and percentages. Missing data were handled with full multiple imputation. It generated 20 data sets with 50 iterations to impute missing values of the outcome and covariates based on demographic and clinical features and laboratory data.⁵¹ The significance of the differences between groups was determined by independent sample *t*-test, Chi-square tests (Bonferroni post-hoc correction for pairwise comparison), one-way ANOVA (Welch's ANOVA in case of unequal variances, SNK-q test for pairwise comparison), or Kruskal–Wallis *H*-tests (Bonferroni post-hoc correction for pairwise comparison) according to the type of variables and the number of groups.

The relationship between vitamin B12, folate, tHcy, inflammatory mediators, and the severity of anxiety or depressive symptoms was evaluated with Spearman correlation tests. The degree of correlation was considered as strong ($\text{Rho} \geq 0.7$), moderate ($0.3 < \text{Rho} < 0.7$), or weak ($\text{Rho} \leq 0.3$) after taking significant correlation ($p < 0.05$) values into consideration. Moreover, logistic regression analyses were performed to estimate the odds ratio (OR) of the severity of anxiety or depressive symptoms according to vitamin B12, folate, tHcy, and inflammatory mediators' levels. The above independent variables were analyzed as continuous variables (per standard deviation increase) and categorical variables (deficiency or elevation). Model 1 was the unadjusted model. Model 2 was adjusted for age and gender. The results were expressed as OR with a 95% confidence interval (CI).

Multivariate logistic regression was used to identify factors associated with abnormal vitamin B12, folate, tHcy, and inflammatory mediators levels. Univariate logistic regression was used to screen out variables, and we selected those with overall p -values < 0.1 as candidate variables for the multivariable analysis. The variance inflation factor (VIF) is a marker for assessing multicollinearity. If $\text{VIF} > 10$, the multicollinearity is present and variable(s) were removed, and the multivariate logistic regression was conducted again. Statistical analysis was performed with SPSS 25.0 statistical package. $P < 0.05$ by two-tailed tests was considered significant.

Results

Demographic and Clinical Features and Laboratory Data of the Subjects

We recruited 139 adolescent outpatients with anxiety or depressive symptoms from July 2021 to August 2022 in our Neurology and Psychiatry departments. 11 patients were eventually excluded for $\text{CRP} \geq 10$ mg/L (2 cases), epilepsy (3 cases), abnormal thyroid function (4 cases), and autoimmune diseases (2 cases). Finally, 128 patients (64.1% girls; mean age 15.4 ± 1.6 years) were eligible. [Table S1](#) showed the percentage of subjects with missing data for the continuous and categorical variables, which ranged from 0% (almost all variables) to 3.12% (father's education level and mother's education level).

[Table 1](#) displayed demographic and clinical features and laboratory data of subjects according to the severity of anxiety or depression for 128 subjects which included 71 with mild depression (MD) and mild anxiety (MA), 11 with severe depression (SD) and MA, 14 with MD and severe anxiety (SA), and 32 with SD and SA. Vitamin B12, folate, tHcy, IL-6, TNF- α , and CRP levels were not significantly different in the 4 groups.

Vitamin B12 deficiency and marginal vitamin B12 status patients were 11 (8.6%) and 42 (32.8%), respectively. Folate deficiency was 12 (10.2%) subjects. Mild and moderate HHcy was 29 (22.7%) and 4 (3.1%) subjects, respectively. Elevated TNF- α , IL-6, and CRP were 19 (14.8%), 28 (21.9%), and 13 (10.2%) subjects, respectively. [Figure 1](#) showed the details of vitamin B12, folate, tHcy, TNF- α , IL-6, and CRP levels based on age and gender.

Table 1 Demographic and Clinical Features and Laboratory Data

Variables	MD+MA (N=71)	SD+MA (N=11)	MD+SA (N=14)	SD+SA (N=32)	p
Age (years)	16.0 (15.0, 17.0)	15.0 (14.0, 16.0)	16.0 (13.8, 17.0)	15.0 (14.0, 16.0)	0.29
Gender (% girls)	54.9	90.9	57.1	78.1	0.01 ^a
BMI (kg/m ²)	20.52 (18.52, 22.76)	22.58 (19.81, 24.24)	22.81 (18.95, 23.54)	18.80 (17.66, 20.46)	0.02 ^b
Course of the disease (months)	18.0 (6.0, 36.0)	6.0 (1.0, 27.0)	10.5 (5.5, 30.0)	13.0 (4.5, 24.0)	0.51
Parental marriage N(%)					0.13
Married	60 (84.5)	9 (81.8)	8 (57.1)	27 (84.4)	
Divorced	11 (15.5)	2 (18.2)	6 (42.9)	5 (15.6)	
Father's education level N(%)					0.21
Junior high school or below	38 (55.1)	7 (70.0)	4 (30.8)	20 (62.5)	
Senior high school or college	24 (34.8)	1 (10.0)	8 (61.5)	9 (28.1)	
Bachelor or above	7 (10.1)	3 (20.0)	1 (7.7)	3 (9.4)	
Mother's education level N(%)					0.85
Junior high school or below	42 (60.9)	7 (70.0)	6 (46.2)	18 (56.3)	
Senior high school or college	22 (31.9)	2 (20.0)	5 (38.5)	11 (34.4)	
Bachelor or above	5 (7.2)	1 (10.0)	2 (15.4)	3 (9.4)	
Current smoking N(%)	5 (7.0)	2 (18.2)	2 (14.3)	3 (9.4)	0.42
Current drinking N(%)	5 (7.0)	2 (18.2)	1 (7.1)	4 (12.5)	0.46
Antidepressants or antipsychotics N(%)	24 (33.8)	4 (36.4)	4 (28.6)	11 (34.4)	1.00
Family history of psychiatric illness N(%)	7 (9.9)	0 (0)	0 (0)	3 (9.4)	0.76
RBC (10 ¹² /L)	4.79 (4.48, 5.29)	4.69 (4.46, 5.18)	4.69 (4.41, 5.16)	4.64 (4.41, 4.91)	0.25
Hb (g/L)	141.0 (131.0, 155.0)	139.0 (133.0, 150.0)	139.0 (130.0, 154.0)	134.0 (125.0, 146.8)	0.15
MCV (fL)	89.4 (86.8, 92.8)	90.5 (86.6, 92.3)	88.6 (85.8, 91.3)	88.4 (85.7, 90.8)	0.18
MCHC (g/L)	330 (323, 336)	332 (323, 337)	331 (323, 338)	331 (324, 333)	0.91
HCT (%)	42.90 (39.70, 46.30)	41.20 (40.40, 42.80)	40.95 (39.48, 46.10)	40.30 (37.73, 44.08)	0.10
WBC (10 ⁹ /L)	6.95±1.58	7.33±1.01	6.57±1.73	6.74±1.50	0.59
PLT (10 ⁹ /L)	251 (229, 295)	257 (238, 341)	272 (189, 310)	256 (242, 305)	0.62
Folate (ng/mL)	5.9 (4.6, 7.6)	5.4 (4.7, 6.0)	7.3 (6.1, 9.3)	6.4 (5.1, 7.9)	0.24
Vitamin B12 (pg/mL)	352.0 (227.0, 485.0)	357.0 (312.0, 419.0)	296.4 (239.5, 439.3)	297.0 (212.0, 402.5)	0.55
tHcy (μmol/L)	12.2 (9.4, 15.3)	11.6 (9.4, 13.0)	11.6 (8.9, 14.7)	11.3 (9.2, 15.1)	0.83
IL-6 (pg/mL)	1.5 (0.9, 2.5)	1.3 (0.5, 7.7)	1.4 (0.8, 3.2)	2.0 (1.0, 3.5)	0.92
TNF-α (pg/mL)	5.84 (5.37, 7.11)	6.35 (5.18, 8.79)	6.22 (4.69, 8.24)	6.06 (5.02, 7.28)	0.86
CRP (mg/L)	1.50 (1.25, 2.08)	1.76 (1.37, 2.21)	2.05 (1.20, 2.78)	1.42 (1.09, 2.26)	0.69

Notes: ^aNo significant difference in sex distributions among 4 groups after SNK-q test, ^bNo significant difference in BMI among 4 groups after Bonferroni post-hoc correction.

Abbreviations: MD, mild depression; SD, severe depression; MA, mild anxiety; SA, severe anxiety; BMI, body mass index; CRP, C-reactive protein; Hb, hemoglobin; HCT, hematocrit; IL-6, interleukin-6; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PLT, platelet count; RBC, red blood cells; tHcy, total homocysteine; TNF-α, tumor necrosis factor-α; WBC, white blood cells.

The Relationship Between Vitamin B12, Folate, tHcy, Inflammatory Mediators, and the Severity of Anxiety or Depressive Symptoms

Table 2 displayed the relationship between vitamin B12, folate, tHcy, TNF-α, IL-6, CRP levels and the risk of severe anxiety or depressive symptoms. After adjustment for age and gender, vitamin B12 < 300 pg/mL was associated with an increased risk of severe anxiety symptoms (OR = 2.39, 95% CI = 1.06, 5.35). Moreover, each per standard deviation increase (165 pg/mL) in vitamin B12 levels was associated with a lower risk of severe depressive symptoms (OR = 0.61, 95% CI = 0.38, 0.98). However, the risk of severe anxiety or depressive symptoms was not associated with folate, tHcy, TNF-α, IL-6, and CRP levels.

Correlation Between Vitamin B12, Folate, tHcy Levels and the Severity of Each Symptom of Anxiety or Depression

Vitamin B12 levels were weakly negatively correlated with scores of GAD-7 (Rho = -0.184, *p* = 0.04), not being able to stop or control worrying (Rho = -0.187, *p* = 0.04), worrying too much about different things (Rho = -0.178, *p* = 0.04),

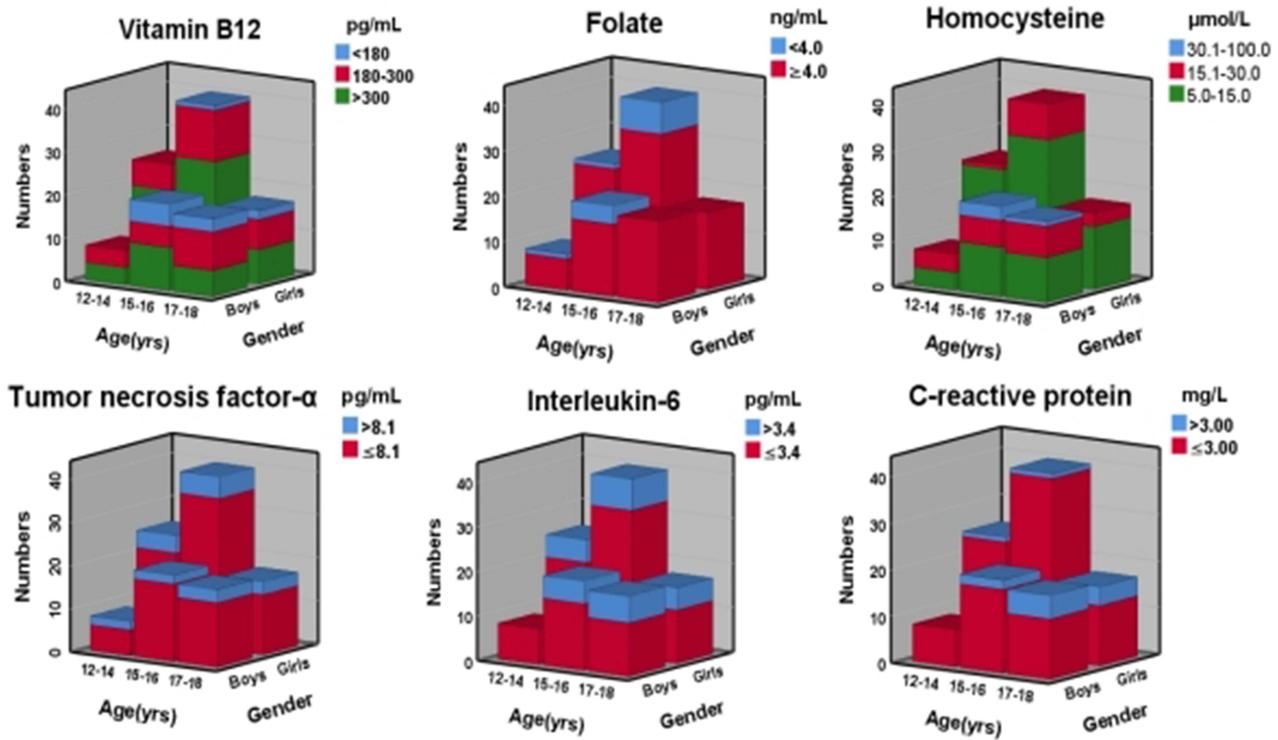


Figure 1 Vitamin B12, folate, tHcy, TNF-α, IL-6 and CRP levels based on age and gender.

trouble relaxing ($Rho = -0.190, p = 0.03$). A weak positive correlation was found between folate levels and scores of not being able to stop or control worrying ($Rho = 0.190, p = 0.03$). A weak negative correlation was observed between tHcy levels and scores of feeling tired or having little energy ($Rho = -0.209, p = 0.02$) (Table 3).

Table 2 Binary Logistic Regression Analysis for Severe Depressive and Anxiety Symptoms

Model	Odds Ratio (95% Confidence Interval)		
	Severe Depression (PHQ-9≥20)	Severe Anxiety (GAD-7≥15)	Severe Depression and Anxiety (PHQ-9≥20 and GAD-7≥15)
Vitamin B12 < 180 pg/mL			
1	0.72 (0.18, 2.87)	1.02 (0.28, 3.69)	1.14 (0.28, 4.58)
2	1.38 (0.31, 6.16)	1.46 (0.38, 5.66)	1.99 (0.44, 8.99)
Vitamin B12 < 300 pg/mL			
1	1.03 (0.49, 2.17)	1.73 (0.83, 3.60)	1.60 (0.71, 3.57)
2	1.55 (0.68, 3.53)	2.39 (1.06, 5.35)*	2.37 (0.97, 5.79)
Vitamin B12 (SD 165 pg/mL)			
1	0.79 (0.53, 1.17)	0.78 (0.53, 1.14)	0.76 (0.49, 1.19)
2	0.61 (0.38, 0.98)*	0.67 (0.43, 1.04)	0.61 (0.36, 1.03)
Folate < 4 ng/mL			
1	0.56 (0.15, 2.16)	1.13 (0.35, 3.67)	0.89 (0.23, 3.46)
2	0.55 (0.14, 2.19)	1.11 (0.34, 3.64)	0.89 (0.22, 3.52)
Folate (SD 3.2ng/mL)			
1	0.98 (0.67, 1.41)	1.04 (0.73, 1.49)	0.98 (0.66, 1.47)
2	0.86 (0.57, 1.27)	1.00 (0.69, 1.45)	0.90 (0.59, 1.39)
Hcy > 15 μmol/L			
1	0.82 (0.35, 1.92)	0.71 (0.30, 1.67)	0.95 (0.38, 2.38)
2	1.41 (0.54, 3.71)	0.88 (0.35, 2.20)	1.44 (0.52, 3.98)

(Continued)

Table 2 (Continued).

Model	Odds Ratio (95% Confidence Interval)		
	Severe Depression (PHQ-9 \geq 20)	Severe Anxiety (GAD-7 \geq 15)	Severe Depression and Anxiety (PHQ-9 \geq 20 and GAD-7 \geq 15)
Hcy (SD 8.5μmol/L)			
1	0.73 (0.43, 1.22)	0.86 (0.57, 1.30)	0.76 (0.43, 1.33)
2	0.94 (0.57, 1.53)	0.95 (0.63, 1.45)	0.91 (0.53, 1.56)
TNF-α > 8.1 pg/mL			
1	1.54 (0.57, 4.16)	1.75 (0.66, 4.69)	1.09 (0.36, 3.29)
2	1.59 (0.56, 4.51)	1.75 (0.65, 4.76)	1.08 (0.35, 3.34)
TNF-α (SD 2.40pg/mL)			
1	1.18 (0.82, 1.70)	1.20 (0.83, 1.72)	1.16 (0.80, 1.69)
2	1.25 (0.83, 1.89)	1.21 (0.82, 1.78)	1.18 (0.79, 1.76)
IL-6 > 3.4 pg/mL			
1	1.38 (0.58, 3.27)	1.20 (0.51, 2.85)	1.27 (0.50, 3.24)
2	1.55 (0.62, 3.86)	1.29 (0.54, 3.10)	1.38 (0.53, 3.62)
IL-6 (SD 3.7pg/mL)			
1	1.16 (0.81, 1.65)	0.92 (0.63, 1.35)	0.92 (0.60, 1.41)
2	1.16 (0.80, 1.69)	0.94 (0.64, 1.38)	0.93 (0.60, 1.43)
CRP > 3 mg/L			
1	1.27 (0.39, 4.13)	1.13 (0.35, 3.67)	1.38 (0.40, 4.83)
2	2.11 (0.56, 7.90)	1.56 (0.45, 5.47)	2.14 (0.55, 8.35)
CRP (SD 1.30mg/L)			
1	1.02 (0.71, 1.47)	1.01 (0.71, 1.46)	1.00 (0.67, 1.49)
2	1.20 (0.80, 1.79)	1.12 (0.76, 1.64)	1.13 (0.73, 1.75)

Notes: *Significantly $p < 0.05$. Model 1: unadjusted. Model 2: adjusted for age and gender.

Abbreviation: SD, Standard Deviation.

In addition, the tHcy levels were moderately negatively correlated with vitamin B12 levels ($Rho = -0.346$, $p < 0.01$) and folate levels ($Rho = -0.611$, $p < 0.01$). Vitamin B12 levels were weakly positively correlated with folate levels ($Rho = 0.179$, $p = 0.04$) (Figure 2).

Correlation Between Inflammatory Mediators and the Severity of Each Symptom of Anxiety or Depression

IL-6 showed a weak positive correlation with fidgeting ($Rho = 0.190$, $p = 0.03$) and annoyed or irritable scores ($Rho = 0.190$, $p = 0.03$). A weak negative correlation was observed between CRP levels and scores of slowly moving or speaking or restless ($Rho = -0.189$, $p = 0.03$). TNF- α levels were not associated with the severity of any symptoms (Table 3). In addition, IL-6 levels were weakly positively correlated with TNF- α levels ($Rho = 0.175$, $p = 0.049$) and CRP levels ($Rho = 0.299$, $p < 0.01$) (Figure 2).

Correlation Between Vitamin B12, Folate, tHcy and Inflammatory Mediators

Table 4 showed vitamin B12, folate, tHcy and inflammatory mediators levels according to demographic and clinical features and laboratory data. Girls, subjects with younger ages, lower tHcy levels had higher vitamin B12 levels. Vitamin B12 levels were higher in adolescents aged 12–14 years compared with adolescents aged 17–18 years after Bonferroni post-hoc correction ($p = 0.03$). Girls with lower Hb, MCV, HCT, tHcy levels and divorced parents had higher folate levels. Boys with higher RBC, Hb, MCHC, HCT levels and lower vitamin B12 and folate levels had higher tHcy levels. Subjects with higher RBC levels had higher TNF- α levels. Subjects with older ages, higher BMI, and higher CRP levels had higher IL-6 levels. IL-6 levels were higher in adolescents aged 17–18 years compared with adolescents aged 12–14 years after Bonferroni post-hoc correction ($p = 0.03$). Subjects with higher BMI and IL-6 levels had higher CRP levels. In addition, CRP levels were higher in adolescents aged 17–18 years compared with adolescents aged 12–14 years ($p = 0.02$), and adolescents aged 15–16 years ($p < 0.01$) after Bonferroni post-hoc correction.

Table 3 Spearman Correlation Analysis Between Vitamin B12, Folate, tHcy, IL-6, TNF- α , CRP Levels and Anxiety or Depressive Symptoms

Variables	Vitamin B12		Folate		tHcy		TNF- α		IL-6		CRP	
	Rho	p	Rho	p	Rho	p	Rho	p	Rho	p	Rho	p
PHQ-9 (total)	-0.092	0.30	0.053	0.55	-0.103	0.25	0.060	0.52	0.084	0.35	-0.040	0.66
Little interest or pleasure in doing things	-0.105	0.24	0.111	0.21	-0.036	0.69	0.111	0.21	-0.049	0.58	-0.019	0.83
Feeling down, depressed, or hopeless	-0.108	0.22	0.036	0.69	-0.041	0.65	0.007	0.94	0.074	0.41	-0.024	0.79
Trouble falling or staying asleep, or sleeping too much	-0.108	0.23	-0.006	0.95	-0.069	0.44	0.049	0.59	0.168	0.06	0.146	0.10
Feeling tired or having little energy	0.029	0.75	0.084	0.35	-0.209	0.02*	0.108	0.22	0.055	0.54	0.083	0.35
Poor appetite or overeating	-0.104	0.24	-0.009	0.92	-0.065	0.46	-0.034	0.70	0.023	0.80	-0.037	0.68
Feeling bad about yourself	-0.101	0.26	0.089	0.32	-0.085	0.34	0.049	0.58	0.054	0.55	-0.086	0.34
Trouble concentrating	-0.140	0.11	0.071	0.43	0.022	0.81	0.087	0.33	0.084	0.34	-0.162	0.07
Moving or speaking slowly or restless	0.053	0.56	0.048	0.59	-0.119	0.18	-0.083	0.35	0.090	0.31	-0.189	0.03*
Death thoughts	-0.030	0.74	-0.002	0.98	-0.083	0.35	0.004	0.96	-0.011	0.90	-0.094	0.29
GAD-7	-0.184	0.04*	0.134	0.13	-0.114	0.20	0.033	0.71	0.093	0.30	-0.004	0.97
Feeling nervous, anxious or on edge	-0.090	0.31	0.165	0.06	-0.137	0.12	0.049	0.59	0.068	0.45	-0.016	0.86
Not being able to stop or control worrying	-0.187	0.04*	0.190	0.03*	-0.104	0.24	-0.005	0.96	-0.024	0.79	-0.057	0.52
Worrying too much about different things	-0.178	0.04*	0.161	0.07	-0.080	0.37	0.035	0.69	-0.048	0.59	-0.008	0.93
Trouble relaxing	-0.190	0.03*	0.010	0.91	0.053	0.55	0.083	0.35	0.025	0.78	0.045	0.61
Being so restless that is hard to sit still	-0.138	0.12	-0.022	0.81	-0.077	0.39	0.130	0.14	0.190	0.03*	0.131	0.14
Becoming easily annoyed or irritable	-0.117	0.19	0.071	0.43	-0.126	0.16	-0.072	0.42	0.190	0.03*	-0.073	0.41
Feeling afraid as if something awful might happen	-0.036	0.69	0.122	0.17	-0.158	0.08	-0.015	0.87	-0.037	0.68	-0.051	0.57
Vitamin B12	1.000	-	0.179	0.04*	-0.346	<0.01*	-0.062	0.48	-0.085	0.34	0.072	0.42
Folate	0.179	0.04*	1.000	-	-0.611	<0.01*	-0.141	0.11	0.033	0.71	-0.032	0.72
tHcy	-0.346	<0.01*	-0.611	<0.01*	1.000	-	0.149	0.09	-0.026	0.77	0.058	0.52
IL-6	-0.085	0.34	0.033	0.71	-0.026	0.77	0.175	0.049*	1.000	-	0.299	<0.01*
TNF- α	-0.062	0.48	-0.141	0.11	0.149	0.09	1.000	-	0.175	0.049*	0.070	0.43
CRP	0.072	0.42	-0.032	0.72	0.058	0.52	0.070	0.43	0.299	<0.01*	1.000	-

Note: *Significantly $p < 0.05$.

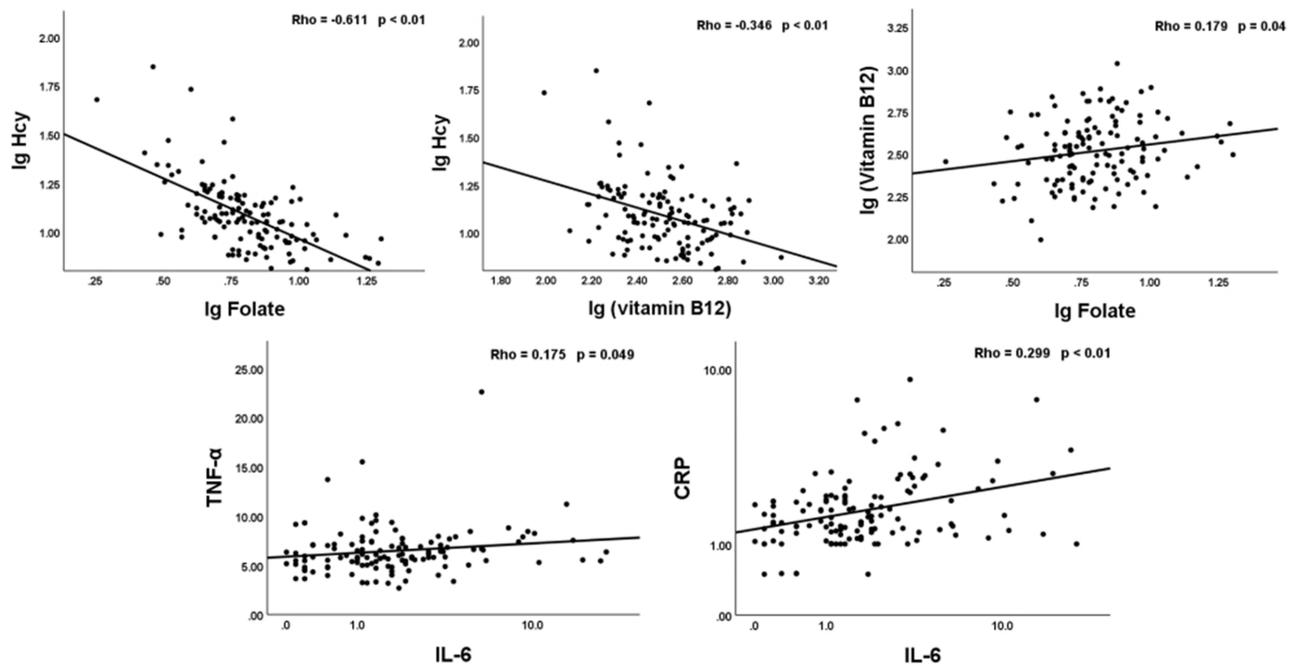


Figure 2 Graphics of significant correlations.

Spearman correlation tests showed that there was no significant association between vitamin B12, folate, tHcy levels and IL-6, TNF- α , CRP levels in all subjects. Considering the different levels of these parameters in subjects of different ages and genders, we analyzed the relationship between them at different ages and gender.

Subgroup analysis showed that folate levels were moderately negatively correlated with CRP levels in subjects aged 13 years or less ($Rho = -0.645$, $p < 0.01$), TNF- α levels were weakly negatively correlated with folate ($Rho = -0.178$, $p = 0.049$) and weakly positively correlated with tHcy levels ($Rho = 0.178$, $p = 0.049$) in subjects aged 13 years or older, tHcy levels were weakly positively correlated with TNF- α levels in subjects aged 14 years or older ($Rho = 0.197$, $p = 0.04$), and vitamin B12, folate, tHcy levels were not significantly associated with inflammatory mediators when boys and girls were respectively analyzed.

Odds Ratio of Abnormal Vitamin B12, Folate, tHcy, TNF- α , IL-6 and CRP Levels

As shown in Table 5, multivariate binary logistic regression analyses demonstrated that vitamin B12 deficiency and vitamin B12 < 300 pg/mL were associated with older age (OR = 1.88, 95% CI = 1.04, 3.40), (OR = 1.35, 95% CI = 1.04, 1.75) and higher tHcy levels (OR = 1.08, 95% CI = 1.03, 1.15), (OR = 1.13, 95% CI = 1.04, 1.23), respectively. Folate deficiency was associated with higher tHcy levels (OR = 1.11, 95% CI = 1.04, 1.19). HHcy was associated with lower vitamin B12 (OR = 1.009, 95% CI = 1.004, 1.014) and folate levels (OR = 2.68, 95% CI = 1.70, 4.23).

IL-6 elevation was associated with higher CRP (OR = 1.65, 95% CI = 1.17, 2.32) and TNF- α levels (OR = 1.19, 95% CI = 1.00, 1.43). CRP elevation was associated with older age (OR = 3.06, 95% CI = 1.41, 6.65), higher BMI (OR = 1.42, 95% CI = 1.16, 1.76) and current drinking (OR = 11.71, 95% CI = 1.61, 85.12).

Discussion

The present study showed that the prevalence of vitamin B12 and folate deficiency, tHcy, TNF- α , IL-6, and CRP elevation was 8.6%, 10.2%, 25.8%, 14.8%, 21.9%, and 10.2%, respectively, in adolescents with anxiety or depressive symptoms. Lower vitamin B12 levels were correlated with a higher risk for severe anxiety and depressive symptoms. The severity of some symptoms of anxiety or depression was weakly correlated with vitamin B12, folate, tHcy, IL-6, and CRP levels, so these results were unlikely to be clinically meaningful. Although vitamin B12, folate, and tHcy levels were not significantly associated with inflammatory mediators, significant correlations were found between folate and

Table 4 Vitamin B12, Folate, tHcy, TNF- α , IL-6, and CRP Levels According to Demographic and Clinical Features and Laboratory Data

Features	Vitamin B12	p	Folate	p	tHcy	p	TNF- α	p	IL-6	p	CRP	p
Age		0.04 ^a		0.70		0.07		0.95		0.03 ^b		<0.01 ^c
12–14y	384.0 (281.5, 492.0)		6.1 (5.0, 7.6)		10.2 (8.7, 12.5)		6.11 (5.20, 7.50)		1.1 (0.4, 2.8)		1.46 (1.23, 1.96)	
15–16y	337.0 (243.8, 419.5)		6.0 (4.4, 8.3)		12.1 (9.4, 16.1)		5.92 (5.10, 7.41)		1.5 (0.9, 2.9)		1.42 (1.11, 2.04)	
17–18y	265.0 (198.0, 402.0)		6.0 (5.1, 7.7)		12.4 (9.7, 15.2)		5.99 (5.44, 7.13)		2.1 (1.1, 5.0)		2.17 (1.38, 3.03)	
Gender		<0.01*		<0.01*		<0.01*		0.13		0.66		0.43
Boys	284.4 (208.8, 361.5)		5.3 (4.5, 6.8)		14.7 (11.7, 17.3)		6.38 (5.50, 7.34)		1.5 (0.7, 3.2)		1.57 (1.27, 2.51)	
Girls	360.0 (264.8, 486.5)		6.6 (5.2, 8.7)		10.2 (8.3, 12.6)		5.80 (4.96, 7.36)		1.7 (1.0, 3.1)		1.55 (1.20, 2.16)	
BMI (Kg/m²)		0.68		0.62		0.65		0.89		0.03*		<0.01*
≤20.34	331.5 (247.5, 420.3)		5.9 (4.7, 8.1)		11.3 (9.2, 15.7)		6.06 (5.10, 7.47)		1.3 (0.5, 2.3)		1.37 (1.02, 1.80)	
>20.34	327.0 (222.0, 456.0)		6.1 (4.9, 7.9)		11.8 (9.4, 14.4)		5.92 (5.43, 7.19)		2.1 (1.1, 3.6)		1.92 (1.40, 2.82)	
Course of the disease		0.48		0.40		0.08		0.91		0.15		0.97
≤ 1 month	312.5 (192.5, 433.3)		5.7 (4.8, 10.6)		12.0 (9.6, 15.7)		5.75 (4.76, 7.40)		1.3 (0.9, 2.9)		1.77 (1.20, 2.25)	
> 1 month and < 6 months	310.5 (215.0, 446.3)		7.0 (5.8, 8.9)		9.3 (7.8, 13.7)		6.58 (5.55, 7.09)		2.3 (1.7, 4.1)		1.41 (1.12, 2.09)	
≥6 months and < 1 year	345.0 (265.0, 394.5)		5.7 (4.5, 7.9)		13.5 (11.3, 16.6)		6.12 (5.47, 7.68)		1.4 (0.9, 3.0)		1.64 (1.19, 2.18)	
1 year- 2 years	361.0 (244.5, 502.0)		6.3 (5.1, 8.1)		11.2 (9.2, 14.5)		5.84 (4.91, 7.15)		1.4 (0.4, 2.9)		1.47 (1.28, 2.10)	
> 2 years	313.5 (210.3, 411.0)		5.7 (4.6, 7.6)		12.3 (9.6, 15.3)		5.82 (5.15, 7.58)		1.5 (0.7, 3.6)		1.66 (1.10, 2.49)	
Parental marriage		0.21		<0.01*		0.21		0.71		0.97		0.45
Married	344.0 (224.0, 472.8)		6.0 (4.7, 7.6)		11.8 (9.4, 15.3)		6.12 (5.15, 7.37)		1.7 (0.9, 3.1)		1.53 (1.19, 2.19)	
Divorced	284.5 (231.3, 377.3)		7.5 (5.7, 9.3)		9.9 (8.4, 14.3)		5.79 (5.41, 6.95)		1.5 (0.7, 3.2)		1.67 (1.29, 2.31)	
Father's education level		0.66		0.63		0.53		0.86		0.20		0.39
Junior high school or below	345.0 (220.5, 480.5)		6.1 (4.8, 8.1)		11.2 (8.7, 15.7)		5.93 (5.17, 7.22)		1.7 (0.6, 3.1)		1.46 (1.19, 2.03)	
Senior high school or college	301.0 (227.8, 439.0)		6.1 (5.1, 8.0)		11.7 (10.2, 14.8)		6.04 (5.24, 7.35)		1.8 (1.1, 4.9)		1.72 (1.17, 2.46)	
Bachelor or above	349.0 (300.0, 390.0)		5.4 (4.6, 8.2)		12.1 (10.9, 13.9)		6.12 (5.18, 7.59)		1.1 (0.9, 2.1)		1.76 (1.53, 2.04)	
Mother's education level		0.70		0.62		0.82		0.78		0.04 ^d		0.09
Junior high school or below	312.0 (220.5, 420.5)		6.4 (4.9, 8.3)		11.2 (9.2, 15.5)		5.93 (5.28, 7.22)		1.5 (0.6, 2.9)		1.46 (1.13, 2.00)	
Senior high school or college	343.5 (247.5, 456.0)		6.0 (5.1, 7.9)		11.7 (9.4, 15.1)		5.96 (5.02, 7.34)		2.3 (1.1, 3.9)		1.83 (1.29, 2.75)	
Bachelor or above	342.0 (235.0, 422.0)		5.4 (4.5, 6.9)		12.3 (10.9, 14.7)		6.33 (5.53, 7.73)		1.1 (0.7, 1.9)		1.78 (1.24, 2.14)	
Current smoking		0.18		0.17		0.31		0.68		0.55		0.16
Yes	288.9 (241.5, 363.3)		5.4 (4.4, 7.1)		12.6 (11.0, 15.6)		5.87 (5.06, 6.30)		2.2 (1.0, 3.6)		1.69 (1.34, 3.25)	
No	337.5 (227.3, 460.0)		6.1 (4.9, 8.1)		11.6 (9.2, 15.1)		6.10 (5.27, 7.37)		1.6 (0.9, 3.0)		1.50 (1.21, 2.15)	
Current drinking		0.73		0.72		0.75		0.38		0.57		0.19
Yes	315.5 (255.8, 379.5)		6.0 (5.1, 6.8)		12.6 (11.0, 15.0)		5.65 (5.02, 6.65)		1.3 (0.8, 2.8)		1.69 (1.28, 4.17)	
No	334.5 (223.3, 460.0)		6.0 (4.9, 8.1)		11.6 (9.2, 15.2)		6.10 (5.31, 7.37)		1.7 (0.9, 3.2)		1.53 (1.21, 2.15)	
Antidepressants or antipsychotics		0.45		0.90		0.46		0.22		0.12		0.73
Yes	338.0 (230.0, 493.0)		5.7 (4.5, 9.4)		12.3 (9.4, 16.3)		5.82 (5.02, 7.00)		1.8 (1.2, 3.7)		1.80 (1.20, 2.35)	
No	316.0 (227.5, 419.5)		6.1 (5.1, 7.8)		11.3 (9.2, 14.8)		6.11 (5.41, 7.36)		1.4 (0.7, 2.9)		1.50 (1.24, 2.05)	
Family history of psychiatric illness		0.12		0.31		0.74		0.60		0.62		0.30
Yes	402.5 (315.8, 565.5)		5.6 (4.1, 7.3)		11.9 (9.2, 14.2)		5.83(4.98, 6.94)		2.3 (0.9, 3.7)		2.04 (1.26, 2.40)	
No	321.0 (225.8, 421.3)		6.1 (4.9, 8.0)		11.7 (9.2, 15.2)		6.04 (5.25, 7.35)		1.5 (0.9, 3.0)		1.50 (1.22, 2.16)	

RBC (10¹²/L)		0.11		0.09		<0.01*		0.02*		0.67		0.16
≤4.71	349.0 (247.5, 469.5)		6.5 (5.1, 8.9)		10.2 (8.2, 13.1)		5.78 (4.86, 6.98)		1.5 (0.9, 3.1)		1.48 (1.10, 2.16)	
>4.71	306.0 (220.0, 404.0)		5.7 (4.6, 7.6)		13.2 (11.1, 16.0)		6.36 (5.50, 7.73)		1.6 (0.9, 3.1)		1.60 (1.30, 2.44)	
Hb (g/L)		0.06		<0.01*		<0.01*		0.31		0.70		0.15
≤139.0	347.0 (249.8, 478.3)		6.8 (5.2, 9.2)		10.0 (8.2, 12.7)		5.79 (4.96, 7.46)		1.5 (0.8, 3.4)		1.48 (1.08, 2.18)	
>139.0	300.0 (219.5, 398.0)		5.7 (4.5, 7.4)		13.3 (11.3, 15.9)		6.17 (5.47, 7.33)		1.7 (0.9, 3.0)		1.61 (1.30, 2.51)	
MCV (fL)		0.12		0.03*		0.20		0.53		0.08		0.74
≤89.0	345.0 (252.0, 491.0)		6.5 (5.3, 8.9)		11.3 (9.2, 14.3)		6.08 (5.37, 7.38)		2.0 (1.0, 3.6)		1.50 (1.19, 2.21)	
>89.0	312.0 (217.0, 419.0)		5.7 (4.5, 7.6)		12.2 (9.4, 15.7)		5.84 (5.02, 7.27)		1.4 (0.7, 2.2)		1.59 (1.27, 2.16)	
MCHC (g/L)		0.43		0.81		0.04*		0.28		0.60		0.31
≤331	337.0 (227.0, 491.0)		6.0 (5.1, 7.6)		11.1 (9.0, 14.7)		5.84 (4.87, 7.38)		1.5 (0.5, 3.2)		1.47 (1.14, 2.14)	
>331	316.0 (238.0, 409.0)		6.0 (4.8, 8.4)		12.2 (10.6, 15.7)		6.33 (5.47, 7.34)		1.6 (1.1, 3.1)		1.59 (1.28, 2.33)	
HCT (%)		0.07		<0.01*		<0.01*		0.07		0.88		0.12
≤42.35	347.0 (254.3, 427.8)		7.0 (5.3, 9.3)		9.7 (8.1, 12.5)		5.78 (4.85, 7.07)		1.6 (0.9, 3.4)		1.47 (1.12, 2.15)	
>42.35	300.0 (220.5, 402.0)		5.6 (4.5, 7.4)		13.3 (11.3, 15.8)		6.35 (5.50, 7.42)		1.6 (0.9, 3.0)		1.63 (1.28, 2.78)	
WBC (10⁹/L)		0.22		0.92		0.05		0.98		0.20		0.07
≤6.74	315.5 (221.3, 419.5)		6.1 (4.9, 7.7)		12.3 (9.6, 15.6)		6.29 (5.31, 7.13)		1.5 (0.7, 2.2)		1.46 (1.13, 1.97)	
>6.74	343.5 (254.3, 488.8)		6.0 (4.9, 8.2)		11.2 (9.1, 13.3)		5.84 (5.17, 7.51)		1.8 (1.0, 3.7)		1.79 (1.26, 2.49)	
PLT (10⁹/L)		0.37		0.95		0.51		0.09		0.47		0.05
≤256	327.0 (226.0, 419.0)		6.0 (5.0, 8.0)		12.2 (9.3, 15.3)		5.80 (5.01, 7.06)		1.6 (0.9, 2.5)		1.46 (1.16, 1.94)	
>256	337.0 (228.0, 508.0)		6.0 (4.9, 7.9)		11.3 (9.2, 14.9)		6.38 (5.44, 7.73)		1.5 (0.9, 3.6)		1.79 (1.30, 2.51)	
Vitamin B12 (pg/mL)		<0.01*		0.35		<0.01*		0.68		0.29		0.71
≤331.5	229.0 (192.3, 284.0)		5.9 (4.7, 7.9)		13.0 (9.8, 16.5)		6.00(5.27, 7.52)		1.7 (1.0, 3.5)		1.47 (1.22, 2.32)	
>331.5	422.0 (384.3, 557.8)		6.0 (5.0, 8.1)		11.2 (8.3, 13.2)		5.96 (5.17, 7.13)		1.5 (0.8, 2.5)		1.63 (1.25, 2.15)	
Folate (ng/mL)		0.19		<0.01*		<0.01*		0.36		0.36		0.84
≤6.0	329.5 (218.5, 415.3)		5.0 (4.2, 5.5)		14.6 (11.5, 17.4)		6.28 (5.18, 7.53)		1.4 (0.7, 3.0)		1.53 (1.21, 2.16)	
>6.0	334.5 (245.3, 486.5)		7.9 (7.1, 9.6)		9.7 (8.3, 12.0)		5.82 (5.25, 7.03)		1.8 (1.0, 3.4)		1.62 (1.24, 2.24)	
tHcy (μmol/L)		<0.01*		<0.01*		<0.01*		0.07		0.95		0.27
≤11.7	391.0 (267.5, 492.0)		7.5 (6.0, 9.4)		9.4 (7.9, 10.9)		5.73 (4.93, 7.06)		1.7 (0.9, 3.4)		1.46 (1.21, 2.03)	
>11.7	284.0 (208.0, 361.0)		5.2 (4.4, 6.2)		15.2 (13.2, 17.5)		6.36 (5.51, 7.45)		1.4 (0.9, 3.0)		1.64 (1.27, 2.30)	
IL-6 (pg/mL)		0.88		0.64		0.41		0.44		<0.01*		0.01*
≤1.6	332.0 (238.0, 419.5)		5.7 (4.9, 8.3)		11.9 (9.4, 15.4)		5.84 (5.06, 7.24)		0.9 (0.3, 1.2)		1.46 (1.08, 2.00)	
>1.6	316.0 (214.0, 526.0)		6.3 (4.9, 7.7)		11.3 (8.8, 14.9)		6.35 (5.44, 7.38)		3.1 (2.1, 5.2)		1.64 (1.27, 2.89)	
TNF-α (pg/mL)		0.78		0.17		0.09		<0.01*		0.05		0.81
≤6.00	331.0 (238.0, 449.0)		6.4 (5.2, 8.4)		11.2 (9.1, 13.9)		5.27 (4.45, 5.70)		1.4 (0.6, 2.4)		1.60 (1.27, 2.15)	
>6.00	338.0 (222.0, 422.0)		5.7 (4.8, 7.6)		12.3 (9.4, 15.6)		7.34 (6.69, 8.43)		1.9 (1.1, 3.8)		1.55 (1.20, 2.36)	
CRP (mg/L)		0.38		0.91		0.63		0.48		0.04*		<0.01*
≤1.57	310.0 (222.0, 419.5)		5.9 (4.7, 8.6)		11.3 (8.9, 15.8)		6.02 (4.89, 7.37)		1.3 (0.5, 2.2)		1.24 (1.00, 1.38)	
>1.57	340.0 (243.0, 488.8)		6.1 (4.9, 7.9)		12.0 (9.5, 14.7)		6.00 (5.46, 7.28)		2.0 (1.0, 3.5)		2.18 (1.88, 2.95)	

Notes: *Significantly $p < 0.05$, 12–14y = 12–14 years old, ^aVitamin B12 levels were higher in adolescents aged 12–14 years compared with adolescents aged 17–18 years after Bonferroni post-hoc correction ($p = 0.03$), ^bIL-6 levels were higher in adolescents aged 17–18 years compared with adolescents aged 12–14 years after Bonferroni post-hoc correction ($p = 0.03$), ^cCRP levels were higher in adolescents aged 17–18 years compared with adolescents aged 12–14 years ($p = 0.02$), and adolescents aged 15–16 years ($p < 0.01$) after Bonferroni post-hoc correction, ^dNo significant difference in IL-6 levels of subjects whose mothers with different education levels after Bonferroni post-hoc correction, the cut-off values were median values of BMI, RBC, Hb, MCV, MCHC, HCT, WBC, PLT, vitamin B12, folate, tHcy, IL-6, TNF- α , and CRP.

Table 5 Multivariate Binary Logistic Regression for Odds of Abnormal Vitamin B12, Folate, Hcy, IL-6, TNF- α and CRP Levels

Variables	Odds Ratio	95% Confidence Interval	p
		Vitamin B12 <180 pg/mL	
Older age	1.88	(1.04, 3.40)	0.04*
Higher tHcy levels	1.08	(1.03, 1.15)	<0.01*
		Vitamin B12 < 300 pg/mL	
Older age	1.35	(1.04, 1.75)	0.02*
Higher tHcy levels	1.13	(1.04, 1.23)	<0.01*
		Folate < 4 ng/mL	
Higher tHcy levels	1.11	(1.04, 1.19)	<0.01*
		Hcy > 15 μmol/L	
Lower vitamin B12 levels	1.009	(1.004, 1.014)	<0.01*
Lower folate levels	2.68	(1.70, 4.23)	<0.01*
		IL-6 > 3.4 pg/mL	
Higher CRP levels	1.65	(1.17, 2.32)	<0.01*
Higher TNF- α levels	1.19	(1.00, 1.43)	0.049*
		TNF-α > 8.1 pg/mL	
Higher MCV levels	0.94	(0.88, 1.00)	0.06
		CRP > 3 mg/L	
Older age	3.06	(1.41, 6.65)	<0.01*
Higher BMI	1.42	(1.16, 1.76)	<0.01*
Current drinking	11.71	(1.61, 85.12)	0.02*
Higher PLT levels	1.02	(0.999, 1.03)	0.06

Note: *Significantly $p < 0.05$.

CRP, between folate and TNF- α , between tHcy and TNF- α in age-subgroup analyses. Vitamin B12 deficiency was associated with older age and higher tHcy levels. Folate deficiency was associated with higher tHcy levels. HHcy was associated with lower vitamin B12 and folate levels. IL-6 elevation was associated with higher CRP and TNF- α levels. CRP elevation was associated with older age, higher BMI, and current drinking.

In 2006, Gao reported the estimated prevalence of vitamin B12 deficiency (serum vitamin B12 < 200 pg/mL) was 4.5% in Chinese children aged 2 to 7 years.⁴⁷ In 2014, a meta-analysis showed that the prevalence of HHcy was 27.5% in the Chinese population aged 3–97 years.⁵² Here, we first reported that the prevalence of vitamin B12 and folate deficiency, tHcy, TNF- α , IL-6, and CRP elevation was 8.6%, 10.2%, 25.8%, 14.8%, 21.9%, and 10.2%, respectively, in the Chinese adolescents with anxiety or depressive symptoms, which was an extension of previous research.

In individuals with depression or anxiety, lower levels of vitamin B12, folate, and higher levels of tHcy were often reported compared with healthy controls.^{21,53} An individually matched case-control study showed that unhealthy eating habits were associated with a higher risk of depression via lower serum levels of folate and vitamin B12.⁵⁴ Our results further supported the negative association between vitamin B12 and the severity of anxiety or depression symptoms. However, no significant association was observed between folate, tHcy and overall severity of anxiety or depression symptoms.

Some studies attempted to explore the possible molecular mechanism on how vitamin B12, folate, and Hcy metabolism are coupled to depressive or anxiety symptoms. A recent study showed that vitamin B12 supplementation reduced the DNA methylation of the Ntrk-2 3'UTR, which increased the expression of Ntrk-2 in vitro. In addition, acute vitamin B12 supplementation reversed the reduction of Ntrk-2 caused by accumulating stressors in the mouse prefrontal cortex.⁵⁵ Ntrk-2 is the gene for tropomyosin/tyrosine receptor kinase B, the receptor for brain-derived neurotrophic factor, which plays an important role in the pathophysiology of depression and the antidepressant response.⁵⁶ Budni showed that folate exerted antidepressant effects with the involvement of N-methyl-D-aspartate receptors and the L-arginine-NO pathway.⁵⁷ Duan suggested that HHcy damages dopaminergic neurons directly via exacerbating methyl-4-phenyl-

1,2,3,6-tetrahydropyridine-induced dopamine depletion or indirectly via exacerbating oxidative stress, resulting in the increased risk of depression.¹⁸ We found that vitamin B12, folate, and tHcy were weakly associated with some symptoms of anxiety or depression symptoms, not all the symptoms. This reminds us that the impact of abnormal vitamin B12, folate, and tHcy levels localized to specific brain regions. Future studies could focus on investigating why vitamin B12, folate, and tHcy were associated with specific anxiety or depression symptoms.

Some studies showed correlations between vitamin B12, folate, tHcy levels and IL-6, TNF- α , CRP levels in general population or patients.^{25,58,59} We also presented correlations between folate and CRP, between folate and TNF- α , between tHcy and TNF- α in age-subgroup analyses. The animal experiment showed that TNF- α levels were higher in the spinal cord of rats 2 months after total gastrectomy (vitamin B12 deficiency) and the TNF- α levels decreased to control levels after treatment with vitamin B12 in totally gastrectomized rats.⁶⁰ IL-6 levels decreased in the cerebrospinal fluid of totally gastrectomized rats and increased after treatment with vitamin B12.⁶¹ The clinical trials suggested that vitamin B12 was negatively correlated with TNF- α among adolescent girls.⁵⁸ CRP elevation was found to be related to higher levels of tHcy in multiple diseases such as type 1 diabetes, rheumatoid arthritis, and angiographic coronary artery disease.^{39–41} However, the exact molecular mechanism between vitamin B12, folate, Hcy and IL-6, TNF- α , CRP remains to be explored.

TNF- α , produced predominantly by macrophages and natural killer cells, may induce anxiety and depressive symptoms by stimulating the HPA axis, endothelial activation, neutrophil activation, and inducing apoptotic cell death.^{32,62–64} IL-6, produced predominantly by macrophages and T cells, may induce anxiety and depressive symptoms by causing HPA axis dysfunction, altering synaptic neurotransmission, and reducing neurotrophic factors.^{65–67} CRP, a nonspecific acute-phase protein, whose production was stimulated by IL-6, was the most widely used to measure low-grade inflammation in psychiatric conditions, especially depression.^{68,69} It was accepted that inflammatory mediators levels were higher in patients with anxiety or depression compared with controls in cross-sectional studies,^{70,71} and subjects with higher inflammatory mediator levels had a higher risk of anxiety or depression in longitudinal studies.^{66,72,73} What's more, alterations in inflammatory mediator levels are associated with antidepressant treatment outcomes and prognosis in interventional studies.^{74,75} In summary, inflammatory mediators may be potential markers for the diagnosis, treatment, and prognosis of anxiety and depression.

The relationship is complex between vitamin B12, folate, tHcy, IL-6, TNF- α , CRP, and anxiety or depressive symptoms, and our research failed to make a causal inference. Firstly, poor appetite or overeating, one of the common depression symptoms, may result in abnormal IL-6, TNF- α , CRP, vitamin B12, folate, and tHcy levels.^{76–78} Secondly, abnormal vitamin B12, folate, and tHcy levels may affect IL-6, TNF- α , and CRP levels and result in anxiety or depression symptoms. Lastly, abnormal IL-6, TNF- α , and CRP levels may lead to the occurrence of anxiety or depression symptoms. Therefore, more high-quality prospective cohort studies and network analyses are needed to explore their relationship in the future.

Our study had some limitations. Firstly, the results cannot be extrapolated to the general population of adolescents with anxiety or depressive symptoms for a small sample size. Secondly, vitamin B12, folate, tHcy, IL-6, TNF- α , and CRP levels were not assessed in a healthy control group. Thirdly, causal inference cannot be performed because we only conducted a cross-sectional study without the conditions of temporal precedence and covariation. Lastly, we cannot access the effect and adverse events of vitamin B12 or folate supplements in adolescents with anxiety or depressive symptoms because we did not collect follow-up data.

Conclusion

Above all, the conclusions of the present study are as follows. Firstly, the prevalence of vitamin B12 and folate deficiency, tHcy, TNF- α , IL-6, and CRP elevation was 8.6%, 10.2%, 25.8%, 14.8%, 21.9%, and 10.2%, respectively, in adolescents with anxiety or depressive symptoms. Secondly, lower vitamin B12 levels were correlated with a higher risk of severe anxiety and depressive symptoms. The severity of some symptoms of anxiety and depression were weakly correlated with vitamin B12, folate, tHcy, IL-6, and CRP levels. Vitamin B12, folate, and tHcy levels were not associated with inflammatory mediators. Lastly, Vitamin B12, folate, and tHcy levels were related to each other. IL-6 elevation was

associated with higher elevated CRP and TNF- α . CRP elevation was associated with older age, higher BMI, and current drinking.

Data Sharing Statement

The data that supports the findings of this study are available from the corresponding author upon reasonable request.

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Disclosure

The authors declared that they have no competing interests.

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