BMJ Open

Association between homocysteine, vitamin B_{12}, folic acid and erectile dysfunction: a cross-sectional study in China

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To cite: Chen Y, Li J, Li T, *et al*. Association between homocysteine, vitamin B_{12}, folic acid and erectile dysfunction: a cross-sectional study in China. *BMJ Open* 2019;**9**:e023003. doi:10.1136/bmjopen-2018-023003

► Prepublication history and additional material for this paper are available online. To view please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2018-023003).

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Received 29 March 2018
Revised 29 November 2018
Accepted 30 November 2018

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ABSTRACT

Objectives Erectile dysfunction (ED) affects up to 53.4% of men aged 30–80 years. In this study, we aimed to examine the association between homocysteine (HCY), vitamin B_{12} (B12), folic acid (FA) and ED.

Design Cross-sectional study.

Setting Guangxi, China.

Participants A total of 1381 participants who completed questionnaires were included, between September 2009 and December 2009.

Measures ED was evaluated by the International Index of Erectile Function scores. Also, the values of HCY, B12 and FA were acquired. Then, regression and between-group analyses were performed.

Results No association between FA and ED was found. Significant correlations between HCY and ED were found—the relationships between these two parameters were most notable in men aged over 60 years and in men living alone (bachelors or bachelorhood). B12 levels were higher in men with ED (718.53±234.37 pg/mL vs 688.74±229.68, p=0.015). Using multinomial logistic regression analyses, B12 levels were related to mild ED (multivariate adjusted analysis: OR 1.620, 95% CI 1.141 to 2.300, p=0.007), especially among men aged 40–49 years (OR 2.907, 95% CI 1.402 to 6.026, p=0.004).

Conclusions We report, for the first time, a relationship between B12 levels and ED. We also found specific cohorts of men for whom the relationship between HCY levels and ED is most prominent. Further studies are required to elucidate the mechanisms underlying these relationships—these may ultimately result in new therapies for ED.

Strengths and limitations of this study

- Our study is a cross-sectional study, mainly based on the large Fangchenggang Area Male Health and Examination Survey project, including a total of 4303 men.
- This study includes comprehensive analyses of baseline, linear and logistic regression, and multinomial logistic regression.
- According to the changes in the homocysteine (HCY), vitamin B_{12} (B12) and folic acid (FA) levels, and the order of erectile dysfunction (ED) severity, we investigated the associations between HCY, B12, FA and ED.
- The study also took into consideration the effects of age, marital status and educational status.
- Nevertheless, as a cross-sectional analysis, the exact mechanisms of the relationship between HCY, B12, FA and ED cannot be clearly defined.

INTRODUCTION

Erectile dysfunction (ED) is defined as the inability to acquire and maintain satisfying sexual intercourse with a sufficient erection, affecting up to 53.4% of men aged 30 to 80 years.[1] The morbidity increases sharply among men over 40 years of age.[2 3] It has been estimated that the prevalence of ED will reach 322 million worldwide by the year 2025.[4]

Various factors including smoking, hypertension and hyperlipidaemia have been identified to influence the development of ED. Among these factors, the vascular component is dominant.[5 6] Moreover, ED may be one of the indicators of cardiovascular disease (CVD).[7] Homocysteine (HCY), a CVD-associated factor, was recently defined as an independent risk factor for ED.[8 9] HCY is a thiol-containing amino acid, mainly from methionine, with two steps of transformation. First, methionine is catalysed to form *S*-adenosylmethionine (SAM) by the enzyme SAM synthase. As a major methyl group donor for various methylation reactions, SAM is mainly transformed into *S*-adenosylhomocysteine (SAH) after loss of the methyl group. In the second step, SAH is hydrolysed by SAH hydrolase to form HCY and adenosine. Biologically, HCY is involved in two pathways, including remethylation (RM) and transsulfuration (TS). In the RM pathway, HCY regenerates methionine by methylenetetrahydrofolate reductase with cofactors of folic acid (FA) and vitamin B_{12} (B12). In the TS pathway,

HCY is catalysed by the cystathione-β-synthase and γ-cystathionase.[10 11]

FA and B12, as the cofactors of HCY, have also been identified to be associated with ED.[12] However, limited studies have been focused on the relevance of their levels to ED. On the basis of previous studies, we hypothesised that there are likely associations between HCY, B12, FA and ED. In order to comprehensively investigate the exact association between HCY, B12, FA and ED, our study is conducted based on the Fangchenggang Area Male Health and Examination Survey (FAMHES) project. Our study may pave the way to the treatment of ED on the basis of the balance among HCY, B12 and FA.

METHODS AND MATERIALS

Population and data collection

FAMHES is a population-based project, which was mainly performed to investigate environmental and genetic factors, as well as their inter-relations. From September 2009 to December 2009, 4303 men coming for routine physical examination at the Medical Center in Fangchenggang First People's Hospital were enrolled. Then, 3593 participants responded for further interviews (response rate=83.5%).[13] No distinct differences were detected between the men who participated in the interviews and those who did not.

All participants were asked to provide blood samples between 8:00 and 11:00, after fasting for at least 8 hours (overnight). Then, these blood samples were transported to the Department of Clinical Laboratory at the First Affiliated Hospital of Guangxi Medical University in Nanning within 2–3 hours, where they were centrifuged within 15–25 min and stored at −80°C. Serum B12 and FA were detected with electrochemiluminescence immunoassays, while serum HCY was measured with enzymatic cycling methods.

Then, all the participants were invited to complete a comprehensive questionnaire. This process was performed by the trained investigators using a standardised protocol with a face-to-face interview. Essential information (eg, age, sex, smoking and drinking) was collected, and complete physical examinations (eg, height, weight, waistline and hipline) were performed. Smoking status and alcohol consumption were defined as Yes or No. Marital status was classified into living together (married or cohabitation without marriage) and alone (bachelors or bachelorhood). Meanwhile, according to the years of education, three groups could be defined (0–6 years, primary education; 7–12 years, intermediate education; and ≥13 years, superior education). In the physical examination, body weight with thin clothing and height without shoes were measured. Then, body mass index (BMI) was calculated with the formula of weight/(height)^2. Waist circumference was measured at the midpoint between the inferior costal margin and the superior iliac crest in the midaxillary line. The hipline was defined as the maximum circumference over the buttocks. The waist:hip ratio (WHR) was calculated as waist circumference/hipline. These processes above including initial examination (including height, weight, waistline and hipline), further interviews (essential information, such as age, sex, smoking and drinking, etc) and blood collection were performed on the same days coherently. The flow of participants' collection is shown in online supplementary figure S1.

Patient and public involvement

Patients and the public were not involved in the development of the research question and design or recruitment of this study.

ED definition and grouping

In this study, the International Index of Erectile Function (IIEF-5) was applied to define ED.[14] The IIEF-5 system has five questions, which mainly cover the conditions of erection confidence, erection firmness, maintenance ability, maintenance frequency and satisfaction, with the scores ranging from 5 to 25. Each question has six selections. According to the orders of answers, the scores are defined as 0–5. Then, participants can be divided into ED (IIEF-5 ≤21) and non-ED (IIEF-5 >21) groups. According to the symptoms, ED can also be classified into five groups: none (IIEF-5 score 22–25), mild (17–21), moderate (12–16) and severe symptoms (5–11).[13 15] In addition, HCY level can also be divided into normal (HCY 5–15 μmol/L) and hyperhomocysteinaemia (HCY >15 μmol/L).[16]

Participant screening

In order to acquire the eligible participants for this study, we developed rigorous exclusion criteria: (1) incomplete data for the individual information and IIEF-5 score; (2) incomplete data for HCY, B12 and FA or refused to provide the blood samples; (3) with diseases such as CVDs, inflammatory/immune diseases and kinds of cancers, which might influence the levels of HCY, B12 and FA (online supplementary table S1); and (4) currently taking drugs that might affect HCY, B12 and FA levels, such as vitamins, antidiabetic medicines, non-steroidal anti-inflammatory drugs, antibiotics, cimetidine or glucocorticoids (online supplementary table S1). Then, 1381 participants were included for further analyses. The flow for screening the eligible participants is shown in online supplementary figure S2.

Statistical analysis

Before analysis, HCY, B12 and FA levels were tested for Gaussian distribution with the Shapiro-Wilks test. If data were not Gaussian in distribution, they were logarithmically transformed in order to ensure the approximate Gaussian distribution. Based on the 22 IIEF-5 scores, two groups were defined (ED and non-ED), and Student's t-test and χ^2 test were applied in the baseline analysis. Then, linear and logistic regression analyses were used, with the IIEF-5 scores and binary variable (ED or non-ED) as the dependent factors, respectively. Three adjusted models were

Table 1 Characteristics of the eligible participants in the analysis

	ED	Non-ED	P value
N	688	693	
Age, years	37.99±10.75	34.18±8.47	<0.001*
BMI, kg/m^2	23.27±3.26	23.37±3.48	0.591*
WHR	0.88±0.06	0.88±0.06	0.253*
HCY, µmol/L	14.97±4.11	15.34±11.09	0.524*
Normal HCY	392 (56.98%)	433 (62.48%)	
Hyperhomocysteinaemia	296 (43.02%)	260 (37.52%)	0.037*
B12, pg/mL	718.53±234.37	688.74±229.68	0.015*
FA, ng/mL	9.56±2.72	9.89±11.28	0.594*
Smoke			
Yes	392 (56.98%)	385 (55.56%)	
No	296 (43.02%)	308 (44.44%)	0.594†
Drink			
Yes	586 (85.17%)	617 (89.03%)	
No	102 (14.83%)	76 (10.97%)	0.032†
Marital status‡			
Live together	595 (86.48%)	578 (83.41%)	
Alone	93 (13.52%)	115 (16.59%)	0.110†
Educational status§			
Primary	19 (2.76%)	5 (0.72%)	
Intermediate	488 (70.93%)	411 (59.39%)	
Superior	181 (26.31%)	276 (39.89%)	<0.001†

Normal HCY: 5–15 µmol/L; hyperhomocysteinaemia: >15 µmol/L.
*Student's t-test.
†χ^2 test.
‡Marital status was classified into living together (married or cohabitation without marriage) and alone (bachelors or bachelorhood).
§One participant without the information of educational status in the non-ED group.
BMI, body mass index; B12, vitamin B_{12}; ED, erectile dysfunction; FA, folic acid; HCY, homocysteine; WHR, waist:hip ratio.

used: unadjusted, age-adjusted and multivariate adjusted. In the multivariate-adjusted model, the covariates were as follows: age, smoking status, alcohol consumption, BMI and WHR. Among them, BMI and WHR are the indexes applied to estimate obesity. However, BMI tends to evaluate body fatness but has a weak ability to differentiate fatness as central or visceral.[17] Alternatively, WHR is said to be more effective in reflecting the visceral fat and central adiposity but is not suitable for an estimation of body fat.[17 18] Additionally, the predictive effects of BMI and WHR in diseases are different.[19 20] So, in our study, these two obesity indexes were treated as the covariates.

Then, multinomial logistic regression analysis was also performed to discover the potential association between HCY, B12, FA and ED, along with the order of severity of ED or the changes in the HCY, B12 and FA levels quartile (Q1<25%, 25%≤Q2≤50%, 50%<Q3≤75%, Q4>75%). Additionally, considering the non-negligible influences of age on the risk of ED, we also grouped the participants on the basis of age (<40, 40–49, 50–59 and ≥60 years). The Bernoulli correction was applied, with the significant threshold of p value <0.0125 (=0.05/4 tests) for multinomial logistic regression analysis. Additionally, according to the groups of marital status and educational status, logistic regression analyses were also conducted. In these analyses, the missing data were deleted. All statistical tests were two-tailed, which were performed with SPSS V.16.0 software. The threshold for significance was p value <0.05.

RESULTS

In the baseline analysis, based on IIEF-5, the ED and non-ED groups were defined. In line with previous studies, the age of the ED group (37.99±10.75 years) was older than the non-ED group (34.18±8.47 years, p<0.001). Meanwhile, B12 levels were significantly higher in the ED group (p=0.015). However, no significant difference was shown for HCY levels, and the proportion of hyperhomocysteinaemia was higher in the ED group (43.02%) than that in the non-ED group (37.52%, p=0.037). In addition, the proportion of alcohol consumption (p=0.032) and educational status

Table 2 Linear regression analyses for the ED and HCY, B12 and FA

	Unadjusted			Age adjusted			Multivariate adjusted		
	β	95% CI	P value	β	95% CI	P value	β	95% CI	P value
IIEF-5									
HCY	−0.202	−1.080 to 0.676	0.651	0.139	−0.732 to 1.009	0.755	0.084	−0.787 to 0.956	0.850
Binary HCY	−0.338	−0.817 to 0.142	0.167	−0.146	−0.622 to 0.330	0.548	−0.186	−0.663 to 0.291	0.444
B12	0.048	−0.600 to 0.696	0.885	0.212	−0.428 to 0.852	0.515	0.404	−0.259 to 1.068	0.232
FA	0.112	−0.668 to 0.891	0.779	0.388	−0.384 to 1.160	0.986	0.324	−0.496 to 1.145	0.438

IIEF-5 scores were the dependent factor for the linear regression analysis. Multivariate adjusted: age, BMI, WHR, smoke and drink. Binary HCY: normal HCY (5–15 µmol/L); hyperhomocysteinaemia (>15 µmol/L).
BMI, body mass index; B12, vitamin B_{12}; ED, erectile dysfunction; FA, folic acid; HCY, homocysteine; IIEF-5, International Index of Erectile Function; WHR, waist:hip ratio.

(p<0.001) were also identified to have a statistically significant difference in the two groups (table 1).

Signal for the association between HCY and ED

While we discovered no significant association between HCY levels and ED in the comprehensive analyses (tables 2–5), a slight association of HCY with ED was observed in the participants grouped by age, especially in the old men (age ≥60) (online supplementary table S2). Similar relevance was confirmed in marital status (alone, unadjusted severe ED: OR 4.385, 95% CI 1.070 to 17.974, p=0.040; age-adjusted severe ED: OR 5.085, 95% CI 1.195 to 21.636, p=0.028) (online supplementary table S3).

In the latter analysis, HCY was divided into normal (HCY 5–15 µmol/L) and hyperhomocysteinaemia (HCY >15 µmol/L). The significant association between HCY and ED seemed to be more prominent in men living alone (age-adjusted severe ED: OR 2.448, 95% CI 1.046 to 5.733, p=0.039) (online supplementary table S3).

B12 level is significantly associated with ED

To investigate the association between ED and B12, we applied linear and logistic regression analyses, resulting in no significant association for B12 in the linear regression analysis (in which IIEF-5 scores were treated as the dependent factor). For the binary logistic regression (the status of ED evaluated by IIEF-5 was treated as the dependent factor), B12 was identified to be associated with ED in the unadjusted model (OR 1.438, 95% CI 1.070 to 1.933, p=0.016). However, the association signal diminished in other adjusted models (table 3). We next investigated the relationship between B12 and ED based on the severity grades of ED. Interestingly, the positive correlation between B12 and ED was further confirmed, especially among men with mild ED (unadjusted: OR 1.694, 95% CI 1.207 to 2.376, p=0.002; age-adjusted: OR 1.596, 95% CI 1.135 to 2.244, p=0.007; multivariate adjusted: OR 1.620, 95% CI 1.141 to 2.300, p=0.007) (table 4). Subsequently, the levels of B12 were divided into quartiles. The result showed that B12 might be significantly associated with ED, especially at the higher levels (unadjusted: Q2: OR 0.917, p=0.569; Q3: OR 0.988, p=0.939; Q4: OR 1.452, p=0.015; and p for trend <0.001) (table 5).

After adjusting age for the above analyses, the significant association between B12 and ED diminished (tables 2 and 3 and table 5), suggesting that age cannot be excluded while investigating the relationship between B12 and ED. We thus grouped the participants into four age groups (ages <40, 40–49, 50–59 and ≥60 years). Our results showed that the significant correlations between B12 and mild ED (IIEF-5=17–21) mainly presented in the age group 40–49 years (OR 2.907, 95% CI 1.402 to 6.026, p=0.004) (online supplementary table S2).

Our baseline analysis discovered different proportions of educational status in the ED and non-ED groups. In

Table 3 Binary regression analyses for ED and HCY, B12 and FA

	Unadjusted			Age adjusted			Multivariate adjusted		
Binary	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
HCY	1.137	0.766 to 1.687	0.524	0.959	0.641 to 1.435	0.839	0.986	0.658 to 1.479	0.986
Binary HCY	1.258	1.014 to 1.560	0.037	1.151	0.923 to 1.435	0.212	1.174	0.940 to 1.466	0.157
B12	1.438	1.070 to 1.933	0.016	1.338	0.992 to 1.805	0.057	1.311	0.961 to 1.788	0.087
FA	1.100	0.775 to 1.561	0.594	0.956	0.668 to 1.367	0.804	1.041	0.710 to 1.527	0.835

In the binary regression analysis, the ED status (ED: IIEF-5 ≤21; non-ED: IIEF-5 >21) was treated as the dependent factor. Multivariate adjusted: age, BMI, WHR, smoke and drink. Binary HCY: normal HCY (5–15 µmol/L); hyperhomocysteinaemia (>15 µmol/L).
BMI, body mass index; B12, vitamin B_{12}; ED, erectile dysfunction; FA, folic acid; HCY, homocysteine; IIEF-5, International Index of Erectile Function; WHR, waist:hip ratio.

Table 4 Multinomial logistic regression for the association between ED and HCY, B12 and FA

	HCY			Binary HCY			B12			FA		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
ED—unadjusted												
None (IIEF-5=22–25)	1	1	1	1	1	1	1	1	1	1	1	1
Mild (17–21)	1.081	0.695 to 1.681	0.730	1.246	0.980 to 1.583	0.072	1.694	1.207 to 2.376	0.002	1.180	0.801 to 1.740	0.402
Moderate (12–16)	1.258	0.649 to 2.439	0.497	1.298	0.896 to 1.880	0.168	1.187	0.715 to 1.972	0.508	0.960	0.519 to 1.776	0.896
Severe (5–11)	1.259	0.562 to 2.820	0.575	1.258	0.799 to 1.980	0.322	0.891	0.496 to 1.599	0.698	0.923	0.434 to 1.963	0.834
ED—age adjusted												
None (IIEF-5=22–25)	1	1	1	1	1	1	1	1	1	1	1	1
Mild (17–21)	0.948	0.607 to 1.480	0.814	1.166	0.914 to 1.486	0.217	1.596	1.135 to 2.244	0.007	1.052	0.710 to 1.558	0.800
Moderate (12–16)	0.929	0.455 to 1.898	0.840	1.095	0.747 to 1.605	0.643	1.409	0.635 to 1.733	0.851	0.762	0.404 to 1.437	0.401
Severe (5–11)	1.068	0.467 to 2.443	0.876	1.150	0.726 to 1.820	0.552	0.839	0.471 to 1.495	0.551	0.794	0.370 to 1.705	0.554
ED—multivariate adjusted												
None (IIEF-5=22–25)	1	1	1	1	1	1	1	1	1	1	1	1
Mild (17–21)	0.973	0.621 to 1.524	0.904	1.188	0.929 to 1.517	0.169	1.620	1.141 to 2.300	0.007	1.184	0.775 to 1.808	0.435
Moderate (12–16)	0.965	0.472 to 1.971	0.922	1.119	0.762 to 1.643	0.567	0.972	0.576 to 1.640	0.915	0.777	0.401 to 1.507	0.456
Severe (5–11)	1.132	0.491 to 2.613	0.771	1.187	0.748 to 1.885	0.467	0.717	0.388 to 1.324	0.288	0.821	0.368 to 1.831	0.631

Binary HCY: normal HCY (5–15 µmol/L); hyperhomocysteinaemia (>15 µmol/L). The categorical dependent variables were the various ED groups, based on the IIEF-5. The symptoms of ED were divided into None (IIEF-5=22–25), Mild (17–21), Moderate (12–16) and Severe (5–11). The None group (22–25) was treated as the reference. Multivariate adjusted: age, BMI, WHR, smoke and drink.
BMI, body mass index; B12, vitamin B_{12}; ED, erectile dysfunction; FA, folic acid; HCY, homocysteine; IIEF-5, International Index of Erectile Function; WHR, waist:hip ratio.

Table 5 Association between HCY, B12, FA and ED along with the increased levels of these indexes

	Unadjusted			Age adjusted			Multivariate adjusted		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
HCY									
Q1	1	1	1	1	1	1	1	1	1
Q2	1.130	0.838 to 1.525	0.422	1.022	0.753 to 1.387	0.887	1.012	0.744 to 1.377	0.938
Q3	1.292	0.958 to 1.743	0.094	1.187	0.875 to 1.610	0.271	1.210	0.890 to 1.646	0.224
Q4	1.276	0.946 to 1.720	0.110	1.091	0.803 to 1.483	0.578	1.103	0.810 to 1.502	0.534
B12									
Q1	1	1	1	1	1	1	1	1	1
Q2	0.917	0.680 to 1.236	0.569	0.893	0.659 to 1.209	0.464	0.899	0.662 to 1.221	0.496
Q3	0.988	0.733 to 1.333	0.939	0.972	0.717 to 1.316	0.853	0.986	0.726 to 1.338	0.927
Q4	1.452	1.076 to 1.961	0.015	1.299	0.955 to 1.765	0.095	1.286	0.945 to 1.752	0.110
FA									
Q1	1	1	1	1	1	1	1	1	1
Q2	1.313	0.974 to 1.770	0.074	1.325	0.978 to 1.795	0.069	1.332	0.981 to 1.811	0.067
Q3	1.300	0.963 to 1.755	0.086	1.243	0.916 to 1.687	0.163	1.286	0.944 to 1.751	0.111
Q4	1.198	0.888 to 1.616	0.236	1.094	0.806 to 1.487	0.564	1.116	0.819 to 1.522	0.487

In the multinomial logistic regression, the levels of HCY, B12 and FA were divided into quartiles (Q1<25%, 25%≤Q2≤50%, 50%<Q3≤75%, Q4>75%), which were treated as the categorical dependent variables. Q1 was the reference. As a binary categorical variable, the ED was put as the 'Factors'. Multivariate adjusted: age, BMI, WHR, smoke and drink.
BMI, body mass index; B12, vitamin B_{12}; ED, erectile dysfunction; FA, folic acid; HCY, homocysteine; WHR, waist:hip ratio.

order to discuss the influences of marital and educational status in the relevance to ED and B12, we further performed between-group analyses. Similar to previous results, B12 was also identified to be associated with mild ED, even after multivariate adjustment (marital status, living together: OR 1.501, 95% CI 1.035 to 2.175, p=0.032; alone: OR 3.449, 95% CI 1.113 to 10.692, p=0.032; and educational status, intermediate: OR 1.858, 95% CI 1.214 to 2.845, p=0.004) (online supplementary table S3).

DISCUSSION

ED is a common disorder affecting a large number of men.[1–4] Recent studies suggest HCY may be an independent risk factor for ED.[8 9] In order to test this association, we conducted the current study based on the larger population-based FAMHES project. We confirmed that HCY is significantly associated with ED, especially severe ED. Moreover, B12 may also be relevant to mild ED. In contrast, we observed no significant association between FA and ED in our study.

HCY was reported to be associated with many diseases and health conditions, such as psychological disorders,[21 22] lipid profiles,[23] renal impairment[24] and inflammatory/immune factors.[25] Moreover, HCY is also identified to be a useful marker for CVD.[26 27] Meanwhile, ED could be a potentially predictive factor for cardiovascular and other chronic diseases.[28] Based on the relevance, it was assumed that HCY might be a risk factor for ED.[8 9] Consistent with this, we revealed that HCY was significantly associated with ED, especially severe ED. The main mechanism might be that HCY could influence endothelial dysfunction and nitric oxide (NO) diffusion. Previously, in vitro and in vivo studies discovered that HCY could be a toxin for the vasculature by inducing endothelial dysfunction.[29] Additionally, NO is mainly involved in vascular dilation, smooth muscle relaxation (including genitourinary smooth muscle) and permitting penile erection.[30 31] Studies showed that increased HCY could inhibit NO synthase, probably influencing the production of NO, and the development of ED.[32] So, on the basis of these relevance, we could understand the risk effect of HCY on ED. Additionally, the status of living alone for men would also influence this association, hinting the pathogenesis of psychological factors for ED.

B12 is also known as cobalamin. Similar to FA, it is an important cofactor in methionine synthesis and HCY metabolism.[33] Although previous studies identified that FA might be a potential protective factor for ED,[34] no significant association has been detected. In contrast to B12, HCY has been found to protect against ED.[35] Our study also identifies the potential association between B12 and ED, though ED tends to have high levels of B12 (ED: 718.53±234.37, non-ED: 688.74±229.68, p=0.015). Meanwhile, the significant association between B12 and ED was more prominent for mild ED at higher B12 levels. There are two possible explanations. First, our results suggest that the function of B12 in ED might be

dose dependent. Excessive B12 levels would increase the risk of mild ED with some unclear mechanisms. Second, increased B12 might provide negative feedback for this disease. At the beginning of the disease, defence mechanisms are triggered. As a potential protective factor, the absorption of B12 is enhanced. Combining the limited reports, our study can also propose that B12 is significantly associated with ED. As for the exact effects of B12 on ED, further studies are needed, which might pave the way for the treatment of ED with B12 in the future.

Limitations

Our study verified the previous conclusions that HCY could increase the risk of ED. However, some limitations still need to be noted: (1) this study is a cross-sectional analysis, which just reflects the status of specific time points and populations; (2) there are limited numbers of participants with primary educational status. So, the results need to be examined further; (3) although we have identified a significant association between B12 and ED, the exact mechanisms and effects were unclear until now; and (4) after multiple testing, no positive association can be detected, suggesting that our results might be unstable. So, further studies will be needed.

CONCLUSIONS

ED is one of the most common male diseases. This study was conducted in order to discover the functions of HCY, B12 and FA in ED. Our results confirmed the positive correlations of HCY and ED. Meanwhile, B12 was also likely to be significantly associated with ED. Further studies with larger cohorts of participants should be focused on the potential mechanisms and therapeutic effects of B12 on ED.

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Contributors YC, JieL, ZM and JC participated in participants' collection, field investigation, design, writing and modification of all the paper. YC and JieL took part in the statistical analysis. ZM and JC provided important advice for this paper. TL, JiaL, JinL and G-HW provide efforts in the processes of modification.

Funding This study was funded by National Natural Science Foundation of China (81770759, 81370857), and Innovation Project of Guangxi Graduate Education (YCBZ2017037) .

Competing interests None declared.

Ethics approval This study was approved by the Medical Ethics Committee of Guangxi Medical University.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement The data for this study are available in the supplementary materials. Further questions could be sent to ZM (zengnanmo@hotmail.com) and JC (chengjiwen1977@foxmail.com).

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