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Negative associations between folate and bacterial vaginosis in the NHANES 2001 to 2004

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Abstract

Background Bacterial vaginosis (BV) is one of the most common infections among women of reproductive age and accounts for 15–50% of infections globally. The role played by folate in the pathogenesis and progression of BV is poorly understood. The aim of this study was to investigate the association between serum folate, red blood cell (RBC) folate, and BV in American women.

Methods 1,954 participants from the 2001-2004 National Health and Nutrition Examination Survey (NHANES) program were included in this study. Multiple logistic regression was used to analyze the association between serum folate, RBC folate, and BV, and covariates including race, age, education level, and body mass index were used to construct adjusted models. Stratified analysis was used to explore the stability of the above associations in different populations.

Results In the present cross-sectional study, we found that serum folate and RBC folate were inversely associated with the risk of BV. In the fully adjusted model, the risk of BV was reduced by 35% (OR=0.65, 95% CI: 0.51~0.83, p=0.0007) in the highest serum folate group and 32% (OR=0.68, 95% CI: 0.53~0.87, p=0.0023) in the highest RBC folate group compared to the lowest group.

Conclusions The results of this study indicated that serum folate and RBC folate were inversely associated with the risk of BV folate supplementation may play an important role in the prevention and management of BV.

Keywords Serum folate, Red blood cell (RBC) folate, Bacterial vaginosis, NHANES, Health

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Introduction

Bacterial vaginosis (BV) is a syndrome caused by mixed infection of *Gardnerella vaginalis* and some anaerobic bacteria that causes an imbalance in the vaginal microbiome, resulting in increased vaginal discharge, fishy vaginal odour, itching, and burning of the vulva. Women with BV infection are at increased risk of certain sexually transmitted diseases (e.g., HIV, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and HSV-2), complications after gynaecological surgery, pregnancy complications, and recurrence of BV [1, 2]. BV also increases the risk of HIV transmission to their sexual partners [3].

Treatment is usually effective, but it is prone to recur after cessation of medications [4–7]. Prevalence rates of BV are varied considerable from the geographic regions of the world, within the same country, and even within the same population, ranging from 4 to 75%, depending on the population studied, with an intermediate level in the USA (29%) [8, 9].

Theoretically, susceptibility to BV may be increased by factors that compromise the immune system, including folate deficiency. An American study found that the folate antagonist methotrexate, used to treat autoimmune and chronic inflammatory diseases, inhibits thymidine synthesis and induces apoptosis of activated T lymphocytes [10]. Weinstein's studies have shown that low serum and red blood cell folate are associated with an increased risk of invasive cervical cancer, but no studies have demonstrated the effects of serum folate and RBC folate on BV [11]. Therefore, the aim of this study was to investigate the association of serum folate and folate with the risk of BV and to further explore folic acid supplementation as an intervention strategy to reduce the risk of BV.

Materials and methods

Data sources

Source of our datas were selected from The National Health and Nutrition Examination Survey (NHANES) with survey cycle from 2001-2002 and 2003-2004, a program of studies intended for assessing the health and nutritional status of noninstitutionalized U.S. citizens [12, 13]. Missing data on BV status, serum folate, and RBC folate ($n=18,628$) were first excluded, and then other variables including age, race, education level, BMI, uric acid, serum vitamin B12, total cholesterol, HDL-cholesterol, calcium, and physical activity ($n=579$) were dislodged, 1,954 female participants fit the selection criteria were included in our analysis. A flowchart of the screening process is depicted in Fig. 1. Datas from this study are accessible through publicly available NHANES data files.

Determination of serum folate concentrations

The serum folate concentration was measured using the Bio-Rad Laboratories' Quantitative Phase II Folate Radioassay Kit. The method was to combine the serum sample with 125I-folate in a liquor consisting of dithiothreitol (DTT) and cyanide. The mixture was heated to collect to devitalize folate-binding protein from endogenesis. During heating, DTT stabilized reduced folate and its analogues. The mixture was cooled and bound to the affinity-purified porcine inherent factor with immobilization and folinic acid binding protein, and the pH of the reactants was adjusted until it reached 9.2. Then, the mixture was reacted at room temperature for one hour.

Endogenous folate and marked folate compete on a relative concentration basis for a limited binding site. After centrifugation and decantation, the reaction mixtures were analysed. Marked and unmarked folate bound to the immobilized protein was deposited as particles at the bottom of the test tube. Unbound folate in the supernatant was cast off, and the radioactivity from the granules was calculated. The criteria curve was drawn adopting a precalibrated folate/B12 standard in the human serum albumin base. Participants' serum folate concentrations were calculated from standard curves.

Determination of RBC folate concentrations

Procedure for the RBC folate that samples were first diluted 1:11 with 1 g/dL ascorbic acid in an aqueous solution. Then, an incubation period of 90 min followed before the assay or immediately frozen to hemolyze RBC for subsequent assay. By both methods, the endogenous folate conjugate hydrolyzed the conjugated pteryla polyglutamate before the assay. Samples and the protein diluent, human serum albumin, were diluted 1:2 to give matrices similar to the standard and serum samples.

Diagnosis of BV

BV measurement procedures can be found easily in NHANES documentation [14]. Self-collected vaginal swabs were received from participants aged 16–49 in the Mobile Examination Center. Subsequently, these swabs were applied to pH paper and attached to a glass slide, which was Gram-stained and evaluated by NHANES personnel at their central laboratory according to Nugent's criteria. The Nugent scoring system interprets Gram-stained vaginal smears according to the morphological type of bacteria, reflecting the overall characteristics of the vaginal flora [15]. The slides were scanned for 964 d under a microscope with a low

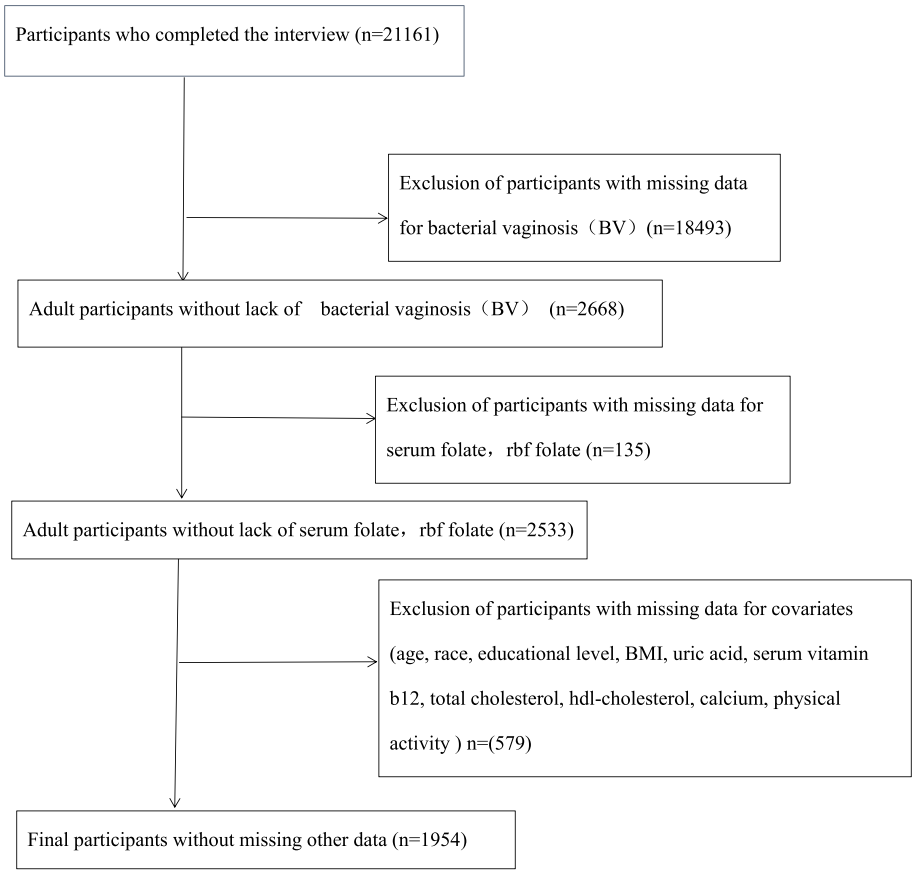

Fig. 1 The flow chart of the study

magnification objective to locate clusters of epithelial cells. BV outcome was recognized as positive when the score was graded from 7 to 10, and a score of 0 to 6 was considered normal [15]. We omitted women without Nugent scoring system results.

Covariates

As the results may be influenced by multiple factors, we selected participants' age, education, race, BMI, serum vitamin B12 [16], uric acid [17], total cholesterol [18, 19], lipoprotein cholesterol, calcium [19], marital status [20, 21], and physical activity as potential covariates for this study. Classification of Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, and other races (including Multi-Racial) was used to describe the race. Education was categorized into lower than high school, high school graduate, or higher than high school. BMI was calculated based on height and weight. Marital status was rated as married, widowed, divorced, separated, never married, and living with a partner. Physical activity was classified by activity level as no activity, moderate activity, vigorous activity, and both. Specific information on serum vitamin B12, uric acid, total cholesterol, lipoprotein cholesterol, and calcium content was extracted from NHANES laboratory test data.

Statistical analysis

R Statistical Package (The R Foundation; http://www.r-project.org; version 3.6.3) and Empower Stats (www.empowerstats.net, X&Y solutions, Inc. Boston, Massachusetts) for our data analyses to perform data processing. When describing the research population, we represent continuous variables with the mean and standard deviation and classified variables with weighted percentages (%) in descriptive analysis. For statistical significance, the $\chi 2$ test and the Kruskal–Wallis test were used separately for categorical variables and continuous variables. OR values reflect the correlation between clinical outcome and exposure [22]. To determine the relationship between serum folate and RBC folate and the incidence of BV, we conducted stratified analysis and multiple logistic regression analysis and fitted smooth curves. We calculated a 95% confidence interval. All statistical analyses in this study were statistically significant when $p < 0.05$.

Results

Baseline characteristics of the study participants

Two cycles of NHANES, 2001–2002 and 2003–2004, were utilized in this study. 21,161 potentially eligible participants were screened; of these, those who completed both the interview and the MEC exam were included in our study. Participants with missing data on BV ($n=18,493$) and serum folate and RBC folate concentrations ($n=135$) were excluded. After further excluding those with missing data on critical covariates, 1,954 participants were enrolled in our analysis. Figure 1 depicts the flowchart of the exclusion criteria. Based on BV, the descriptive characteristics of the participants are listed in Table 1. Compared to those who were negative for BV, participants with positive BV were more likely to be non-Hispanic black, never married, had lower BMI, had lower serum folate, RBC folate, HDL-cholesterol, calcium, higher uric acid, serum vitamin B12, received less than high school education, and took part in both moderate and vigorous physical activity. No statistically significant differences were detected in age or total cholesterol ($p>0.05$).

Table 1 Baseline characteristics of participants ($N=1954$)

Characteristic	Bacterial vaginosis(BV)		
	Negative(Nugent-BV ≤ 6)	Positive(Nugent-BV ≥ 7)	*P*-value
N	1088	866	
Age (year), Mean ± SD	29.37 ± 10.23	29.52 ± 10.58	0.755
BMI (kg/m^2)[a], Mean ± SD	27.18 ± 6.88	28.75 ± 7.69	< 0.001
Folate, serum (ng/ml), Median (Min–Max)	11.80 (2.00–689.00)	10.10 (2.10–61.40)	0.003
Folate, rbc (ng/ml rbc), Mean ± SD	282.06 ± 126.96	247.98 ± 99.34	< 0.001
Uric acid (mg/dl), Mean ± SD	4.29 ± 0.98	4.49 ± 1.11	< 0.001
Vitamin b12, serum (pg/ml), Median (Min–Max)	437.00 (106.00–4031.00)	467.00 (87.00–34,197.00)	0.005
Total cholesterol (mg/dl), Mean ± SD	189.02 ± 39.37	187.59 ± 43.38	0.215
Hdl-cholesterol (mg/dl), Mean ± SD	57.78 ± 15.35	55.36 ± 15.73	< 0.001
Calcium (mg), Median (Min–Max)	0.00 (0.00–8400.00)	0.00 (0.00–8000.00)	< 0.001
Race			< 0.001
Mexican American	262 (24.08%)	241 (27.83%)	
Other Hispanic	39 (3.58%)	39 (4.50%)	
Non-Hispanic White	544 (50.00%)	240 (27.71%)	
Non-Hispanic Black	202 (18.57%)	314 (36.26%)	
Other Race—Including Multi-Racial	41 (3.77%)	32 (3.70%)	
Education level			< 0.001
< High school	372 (34.19%)	358 (41.34%)	
High school	216 (19.85%)	198 (22.86%)	
> High school	500 (45.96%)	310 (35.80%)	
Marital			< 0.001
Married	493 (45.31%)	272 (31.41%)	
Widowed	9 (0.83%)	9 (1.04%)	
Divorced	56 (5.15%)	67 (7.74%)	
Separated	21 (1.93%)	46 (5.31%)	
Never married	442 (40.62%)	394 (45.50%)	
Living with partner	67 (6.16%)	78 (9.01%)	
Physical activity			< 0.001
No	183 (16.82%)	207 (23.90%)	
Both	305 (28.03%)	259 (29.91%)	
Moderate	226 (20.77%)	159 (18.36%)	
Vigorous	374 (34.38%)	241 (27.83%)	

[a] BMI was calculated as the body weight in kilograms divided by the square of the height in meters

P value: if it is a continuous variable, it is obtained by Kruskal Wallis rank sum test. If the theoretical number of counting variables is less than 10, it is obtained by Fisher exact probability test

Table 2 Association between serum folate and BV

	Crude Model		Model 1		Model 2	
Outcome	**OR (95%CI)**	***P*-value**	**OR (95%CI)**	***P*-value**	**OR (95%CI)**	***P*-value**
Serum folate	0.96 (0.94, 0.97)	< 0.0001	0.98 (0.96, 0.99)	0.0041	0.98 (0.97, 1.00)	0.0460
Serum folate (trigonometry)						
T1	Reference (0)		Reference (0)		Reference (0)	
T2	0.59 (0.47, 0.74)	< 0.0001	0.68 (0.54, 0.85)	0.0009	0.69 (0.55, 0.87)	0.0020
T3	0.45 (0.36, 0.57)	< 0.0001	0.60 (0.47, 0.76)	< 0.0001	0.65 (0.51, 0.83)	0.0007
P for trend	< 0.0001		< 0.0001		0.0006	

Non-adjusted model adjusted for None

Model I adjusted for Age, Educational level, Race, BMI

Model II adjusted for the following: Model I + serum vitamin b12; uric acid; total cholesterol; HDL cholesterol; calcium; marital; and physical activity

Association between serum folate and BV

Table 2 shows the association between the three groups of serum folate levels and BV. The crude model is an unadjusted model with no adjustments made for any variables. Adjusted model 1 was an incompletely adjusted model, adjusting mainly for demographic variables, including age, race, education level, and BMI. Adjusted model 2 was a fully adjusted model, with all included covariates adjusted. The group with the highest serum folate levels had the lowest risk of BV compared to the remaining two groups, with a 35% risk reduction in incidence compared to the low-level group, which was statistically significant ($p = 0.0007$). A negative association between serum folate levels and the incidence of BV was stable in all three models. Smoothing curves were used to visualise the association between serum folate levels and the incidence of BV, and Fig. 2 depicts the results.

Association between RBC folate and BV

Table 3 shows the association between the three groups of RBC folate levels and BV, adjusting the model to be consistent with Table 2. The group with the highest RBC folate levels had the lowest risk of BV compared to the remaining two groups, with a 32% risk reduction in

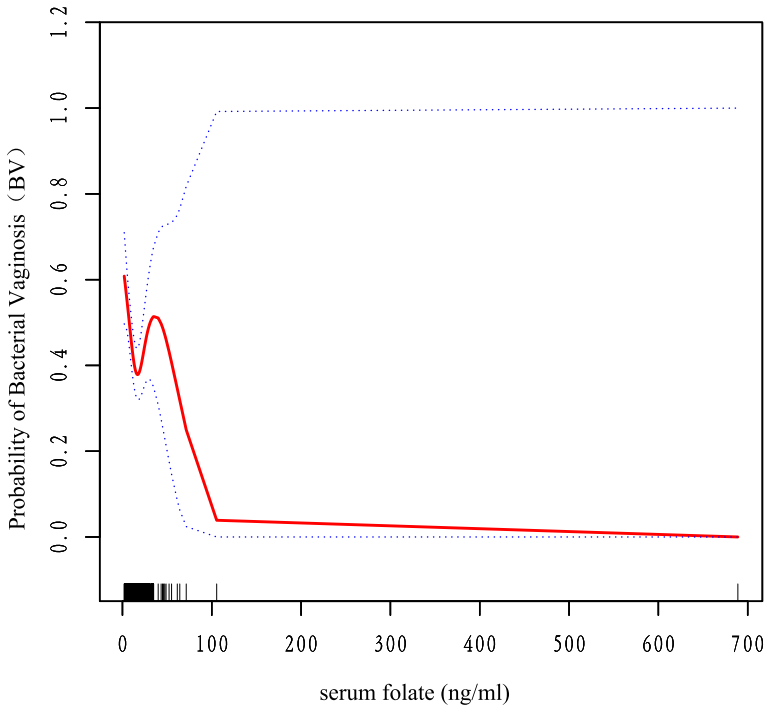

Fig. 2 Correlation between serum folate and BV

Table 3 Association between RBC folate and BV

	Crude Model		Model 1		Model 2	
Outcome	**OR (95%CI)**	***P*-value**	**OR (95%CI)**	***P*-value**	**OR (95%CI)**	***P*-value**
Rbc folate	1.00 (1.00, 1.00)	< 0.0001	1.00 (1.00, 1.00)	0.0041	1.00 (1.00, 1.00)	0.0460
Rbc folate (trigonometry)						
T1	Reference (0)		Reference (0)		Reference (0)	
T2	0.58 (0.46, 0.72)	< 0.0001	0.67 (0.53, 0.84)	0.0006	0.69 (0.55, 0.88)	0.0021
T3	0.47 (0.38, 0.59)	< 0.0001	0.61 (0.48, 0.78)	< 0.0001	0.68 (0.53, 0.87)	0.0023
P for trend	< 0.0001		< 0.0001		0.0019	

Non-adjusted model adjusted for None

Model I adjusted for Age, Educational level, Race, BMI

Model II adjusted for the following: Model I + serum vitamin b12; uric acid; total cholesterol; HDL cholesterol; calcium; marital; and physical activity

incidence compared to the low-level group, which was statistically significant ($p=0.0023$) and consistent with the above results. A negative association between RBC folate levels and the incidence of BV was found to be stable in all three models. Smoothing curves were used to visualize the association between RBC folate levels and the incidence of BV, and the results are shown in Fig. 3.

Stratification analysis between serum folate and BV

As shown in Fig. 4, stratified analyses were conducted for age, race, education level, BMI, marital status, physical activity, and relevant biochemical indicators such as uric acid, vitamin B12, HDL-cholesterol, and total cholesterol. The results of the stratified analysis showed that although the OR values fluctuated across the subgroups, the direction of the results was mostly consistent (OR < 1), indicating that the results of this study were stable and sensitive. Additionally, high heterogeneity was observed in terms of race, BMI, education, and marital status. However, it was relatively stable in the age stratification.

Stratification analysis between RBC folate and BV

As shown in Fig. 5, the stratified analysis revealed greater fluctuations in OR values across subgroups compared to Fig. 4. However, the directions of the results were all

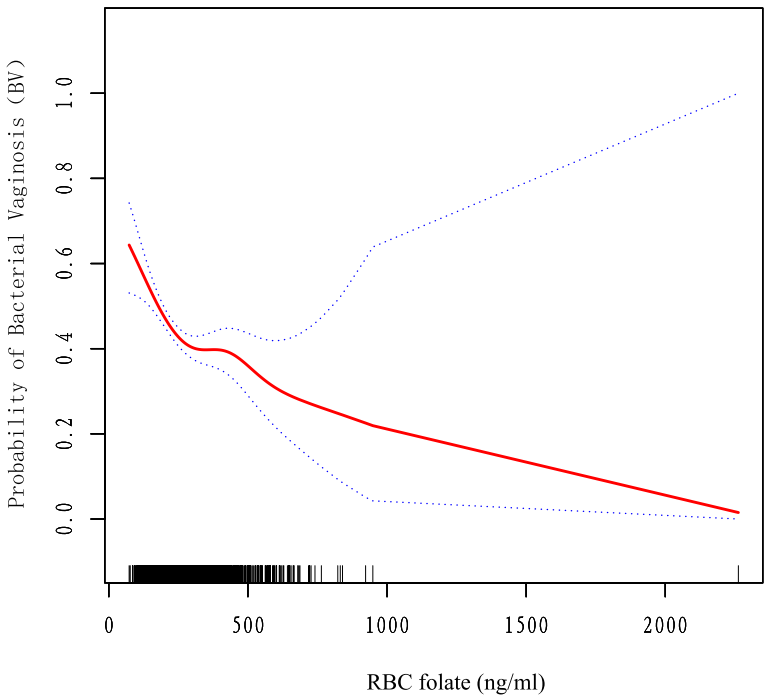

Fig. 3 Correlation between RBC folate and BV

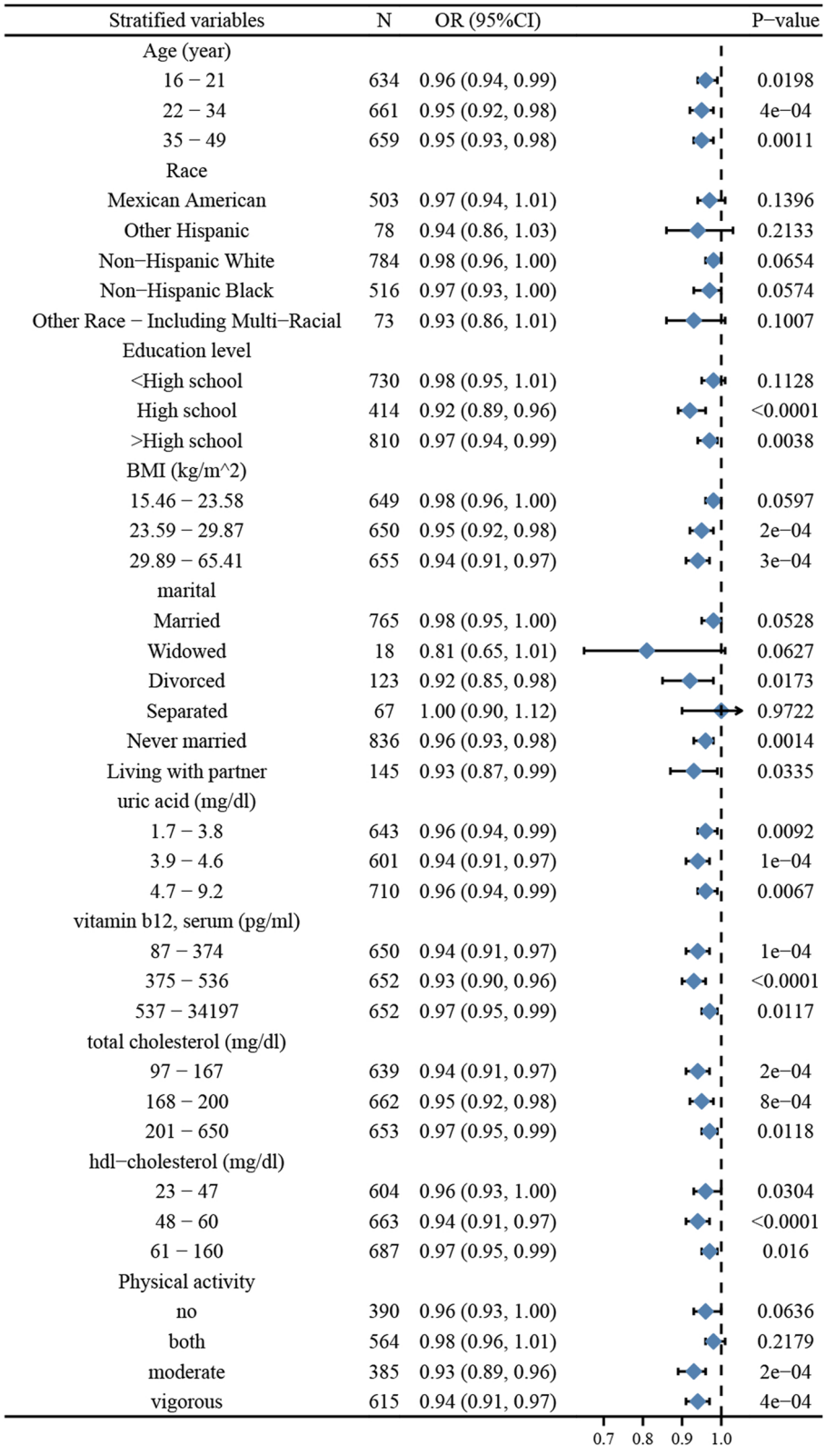

Fig. 4 Stratification analysis between serum folate and BV

Stratified variables	N	OR (95%CI)	P−value
Age (year)			
16 − 21	634	0.92 (0.84, 1.01)	0.0722
22 − 34	661	0.84 (0.78, 0.91)	<0.0001
35 − 49	659	0.84 (0.78, 0.91)	<0.0001
Race			
Mexican American	503	0.92 (0.84, 1.00)	0.0599
Other Hispanic	78	0.85 (0.65, 1.10)	0.2191
Non−Hispanic White	784	0.95 (0.89, 1.02)	0.1781
Non−Hispanic Black	516	0.81 (0.72, 0.90)	2e−04
Other Race − Including Multi−Racial	73	0.89 (0.72, 1.09)	0.2453
Education level			
<High school	730	0.90 (0.83, 0.98)	0.0099
High school	414	0.81 (0.73, 0.90)	1e−04
>High school	810	0.87 (0.82, 0.93)	<0.0001
BMI (kg/m^2)			
15.46 − 23.58	649	0.90 (0.83, 0.99)	0.0236
23.59 − 29.87	650	0.81 (0.74, 0.87)	<0.0001
29.89 − 65.41	655	0.86 (0.80, 0.93)	<0.0001
marital			
Married	765	0.88 (0.82, 0.94)	2e−04
Widowed	18	0.75 (0.49, 1.13)	0.1693
Divorced	123	0.77 (0.63, 0.94)	0.0099
Separated	67	0.89 (0.70, 1.14)	0.3586
Never married	836	0.90 (0.83, 0.98)	0.0129
Living with partner	145	0.87 (0.74, 1.02)	0.0796
uric acid (mg/dl)			
1.7 − 3.8	643	0.85 (0.78, 0.92)	<0.0001
3.9 − 4.6	601	0.80 (0.73, 0.87)	<0.0001
4.7 − 9.2	710	0.92 (0.85, 0.99)	0.0204
vitamin b12, serum (pg/ml)			
87 − 374	650	0.86 (0.80, 0.93)	3e−04
375 − 536	652	0.80 (0.73, 0.88)	<0.0001
537 − 34197	652	0.89 (0.82, 0.96)	0.002
total cholesterol (mg/dl)			
97 − 167	639	0.84 (0.77, 0.91)	<0.0001
168 − 200	662	0.88 (0.81, 0.95)	0.0018
201 − 650	653	0.86 (0.80, 0.93)	<0.0001
hdl−cholesterol (mg/dl)			
23 − 47	604	0.92 (0.85, 1.00)	0.0511
48 − 60	663	0.80 (0.73, 0.87)	<0.0001
61 − 160	687	0.87 (0.81, 0.94)	2e−04
Physical activity			
no	390	0.77 (0.69, 0.87)	<0.0001
both	564	0.90 (0.84, 0.98)	0.0092
moderate	385	0.89 (0.80, 0.99)	0.0286
vigorous	615	0.86 (0.79, 0.93)	3e−04

Fig. 5 Stratification analysis between RBC folate and BV

equally consistent (OR < 1), indicating that the results of this study remained stable and sensitive. Nevertheless, a high degree of heterogeneity was also found between the different strata of factors, and results were mostly significant.

Discussion

The cross-sectional analysis was carried out using two consolidated datasets of NHANES surveys from 2001 to 2004. Based on the outcomes of different models, a significant negative correlation was found between RBC folate concentrations and BV, as well as between serum folate concentrations and BV.

According past researches, this is the first study is to assess the relationship between folate and BV. Only Anne L. Dunlop et al. [23] supposed that folic acid deficiency is closely related to BV during pregnancy. The results showed that with the increase in RBC folate, the incidence of BV decreased significantly. This is consistent with the research results of Neggers YH et al. [19] and Dunlop AL et al. [23], which confirmed that folate was negatively correlated with BV. The risk of BV decreased with the increase in serum folate and RBC folate, indicating that serum folate and RBC folate may affect the occurrence of BV. However, it remains unclear whether the exact mechanism of BV is affected by folic acid.

Common general understanding that supposed immune factors may play a vital role in the pathogenesis of BV [19, 24, 25]. Studies have revealed that folic acid intake might improve immune function [26], thereby reducing the risk of severe BV [19]. Meanwhile, the capacity of folic acid is to improve indirectly mucosal immune of the female subgenital through intestinal immune regulation [27].

Mucosal immunity of the female subgenital system plays an innate immune role through neutrophils, dendritic cells, natural killer cells, and other common innate immune cells [28]. The capacity of folic acid is to maintain or enhance the cellular activity of NK cells [29]. In contrast, the role of NK cells is to enhance innate nonspecific immune function, thereby improving human immune function and reducing the incidence of BV.

In addition to playing a barrier role in the innate immune response, vaginal mucosa also mediates the adaptive immune response of vaginal mucosa through IgG and secretory IgA (sIgA) [28, 30]. The ability of sIgA is to enhance the activity of neutrophils that secrete chemokines that attract macrophages, T cells, and DCs and inhibit proteolysis [31, 32]. Part of the IgG that plays an immune role is locally secreted by the vaginal mucosa, and part of it is transferred to the genital tract mucosa through the circulatory system to play a role. The role of CD8+ T cells is to promote the Th1-cell-mediated immune response to intracellular pathogens [28]. The specific immune function of folic acid is to support and promote the Th1-mediated immune response and to play a significant role in antibody production and metabolism. Its advantage is facilitating the adaptive immune response of the body and reducing the incidence of BV [29].

For the assessment of folate levels, the National Pathology Alliance benchmarking review in the UK [33] and Christopher-John L, Farrell et al. [34] recommend measurement of serum folate, whereas traditionally, studies of folate status have used serum/plasma or RBC folate, both of which are widely available in the laboratory. Among them, RBC folate is favored by many clinicians and is a more labor-intensive test [35]. Therefore, serum folate and RBC folate concentrations were used as research indicators. The results showed that serum folate and RBC folate concentrations were negatively correlated with the risk of BV, because high serum folate and RBC folate concentrations indicated high folate levels in the body, as high folate levels help to enhance the body's immune response, thereby reducing the risk of BV.

In vitro experiments in quantity and epidemiological investigations have shown that the causes of BV are very complex. No research has revealed the connection between serum or RBC folic acid and the risk of BV. Therefore, further research on the association between serum folate and RBC folate and the risk of BV will play a positive role in the treatment, prevention, and reduction of the recurrence of BV. The aim of this study was to further research the relationship between serum or RBC folic acid, and BV risk and to display the study results more comprehensively and intuitively by adjusting the methods of confounding, hierarchical analysis, multiple regression analysis, curve fitting, etc. The findings that serum or RBC folate concentrations are negatively correlated with BV provided biological rationality for the relationship between people's nutritional status and BV and could be used to guide clinical prevention, treatment, and prognosis.

However, there are limitations to the current study. First, as a cross-sectional observational study, it is difficult to determine the temporal relationship of antecedents and consequences. The results may be influenced by other unmeasured variable quantities even after multiple adjustments. Second, although many samples were used, datas were collected from 2001 to 2004. Therefore, when extrapolating to today's environment, there may be some bias It is necessary to consider biases caused by the passage of time.

Conclusions

In conclusion, our study show that both serum folate and RBC folate are negatively associated with BV. Increasing the amount of serum folate and RBC folate in the body may boost our immune function and thereby reduce the risk of BV.

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Authors' contributions
Conceptualization, XG, XY, TT and JL; Data curation, TT, JL, HK and WZ; Writing review and editing, TT, JL, RL, YT, YW, XF, and ZM; Supervision, XG and XY. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
Original data generated and analyzed during this study are included in this published article or in the data repositories listed in References. The dataset supporting the conclusions of this article is available in the NHANES repository, https://www.cdc.gov/nchs/nhanes/index.htm.

Declarations

Ethics approval and consent to participate
The authors are liable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the study are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All protocols for the NHANES were approved by the CDC's National Center for Health Statistics Institutional Research Ethics Review Board. All participants provided written informed consent. All additional materials are available at https://www.cdc.gov/nchs/nhanes/about_nhanes.htm. All information from the NHANES program is available and free for the public, so the agreement of the medical ethics committee board was not necessary.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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