Correlation of several forms of folic acid with endometrial cancer: cross-sectional data from the National Health and Nutrition Examination Surveys (NHANES) 2011-2018

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Abstract

Objective

Endometrial cancer (EC) is a common malignancy of the female reproductive system and although most patients have a good prognosis, 20–30% of patients with advanced disease have a poor prognosis. There are currently no reliable biomarkers for early diagnosis and effective prognostic improvement of the disease. The aim of this study was to explore the effect of folic acid on the occurrence of EC and its clinical application using the National Health and Nutrition Examination Survey (NHANES) database.

Methods

This study included 8,809 female subjects aged ≥ 20 years in the NHANES database from 2011–2018, including 8,738 non-oncology patients and 71 EC patients. Reduced selection bias using 1:1 propensity score matching (PSM) method. Restricted cubic spline (RCS) was plotted to explore the non-linear relationship between different forms of folic acid and EC.

Result

Using data from the NHANES database from 2011–2018 to assess the association between folic acid and the risk of developing EC. The results of the 1:1 ratio propensity score matching (PSM) showed 68 each for EC patients and non-oncology participants. Total serum folate, 5-methyltetrahydrofolate (5-methylTHF), 5-formyltetrahydrofolate (5-formylTHF), tetrahydrofolate (THF) and 5,10-methylenetetrahydrofolate (5,10-methenylTHF) were significantly correlated with EC ($p < 0.05$). In addition, the RCS showed a significant non-linear correlation between THF and 5,10-formyl THF and the risk of developing EC.

Conclusion

The results of this study showed that changes in serum total folate, 5-methylTHF, 5-formylTHF, THF and 5,10-methenylTHF were closely related to EC.

Introduction

Endometrial cancer (EC), a common malignancy of the female reproductive system, is increasing in incidence and mortality every year[1, 2]. In 2020, 417,367 newly diagnosed cases were reported globally, accounting for 2.2% of all newly diagnosed malignant tumors. EC causes 97,370 deaths worldwide, accounting for 1% of all deaths from malignancies[3]. Most EC patients are in the early stages at diagnosis (International Federation of Gynecology and Obstetrics FIGO stage I or II) and have a good

prognosis after surgery only, with a 5-year survival rate of over 90%[4, 5]. For patients in FIGO stages III and IV, it is difficult to apply effective individualized treatment regimens, with 5-year overall survival rates falling to 47–69% and 15–17%[6], and 5-year survival rates for recurrent patients being even lower than 20%[7, 8]. The identification of risk factors for EC and subsequent interventions targeting the risk factors, particularly in high-risk groups, are essential to improve survival in EC patients.

Folic acid (FA) is a water-soluble B vitamin consisting of pterin, p-aminobenzoic acid and glutamic acid[9, 10]. As the body does not have the ability to synthesize folic acid, it must be obtained through diet or supplements[11]. Serum folate levels reflect recent intake, with red blood cell (RBC) folate representing folic acid status over several months. The WHO recommends a threshold value of 340 nmol/L for erythrocyte folate and 10 nmol/L for serum folate[12]. Elevated urinary formyl glutamate excretion and deoxyuridine inhibition tests are also used to assess folic acid status. Elevated homocysteine is a functional indicator of folic acid status[13]. Physiologically folic acid enters cells by endocytosis using cell membrane-associated proteins or the folate receptor (FOLR)[14]. Folic acid is a component and catalyst of fundamental biochemical reactions and plays an important role, particularly in DNA methylation, synthesis and repair[15]. As a result, rapidly proliferating cells such as intestinal cells, haematopoietic cells and tumor cells consume high levels of folic acid to meet their needs for newly synthesized nucleotides and for DNA replication and gene expression[16]. Folic acid deficiency can lead to DNA hypomethylation, as well as hypermethylation of the promoter region of oncogenes, which in turn activates proto-oncogenes and suppresses the expression of oncogenes. Secondly, folic acid deficiency leads to DNA strand breaks, enhanced mutation rates and impaired DNA repair mechanisms. Folic acid deficiency can also inhibit the proliferation of CD8 + T lymphocytes and reduce the body's ability to clear tumor cells[17]. However, excess folic acid can also promote the synthesis of DNA in rapidly proliferating cells, thereby promoting the growth and progression of tumors in situ[18]. One study showed that people with high serum folate concentrations (> 23.61ng/mL) had 4.86 times the risk of colorectal cancer than those with low folate levels (≤ 8.86ng/mL)[19]. There is a lack of evidence for a correlation between high or low levels of folic acid and EC tumor growth.

Using data from the 2011–2018 Continuous National Health and Nutrition Examination Survey (NHANES), this study aimed to examine the association of RBC folate, serum total folate and five serum folate forms, namely 5-methyltetrahydrofolate (5-methylTHF), unmetabolized folic acid (UMFA), 5-formyltetrahydrofolate (5-formylTHF), tetrahydrofolate (THF), 5,10-methylenetetrahydrofolate (5,10-methenylTHF) and pyrazino-s-triazine derivative of 4-α-hydroxy-5-methyltetrahydrofolate (MeFox) with the risk of EC.

Materials and Methods

Research population

The NHANES database (https://wwwn.cdc.gov/nchs/nhanes/) is a population-based cross-sectional survey designed to collect information on the health and nutritional status of adults and children in the U.S. The survey is unique in that it combines interviews and physical examinations. The database includes demographics data, dietary data, examination data, laboratory data, questionnaire data, limited access data. The survey approach uses a complex, multi-stage probability sampling design. The survey results will be used to determine the prevalence of major diseases and risk factors for the diseases. Using information from the NHANES database, this study aimed to investigate the association between erythrocyte folate and different serum folate forms and the risk of EC.

Data from participants in the NHANES database from 2011–2018 were selected for data collection in 2-year cycles: 2011–2012, 2013–2014, 2015–2016 and 2017–2018, including a total of 39,156 participants. 19,848 women were included, excluding those with missing covariate data and folic acid concentration data, resulting in the inclusion of 8,809 participants, including 8,738 non tumor patients and 71 EC patients. A flow chart of the screening process is shown in Fig. 1.

Folic acid concentration determination

Determination of RBC folic acid using a microbiological assay. Total serum folate was determined by liquid chromatography-tandem mass spectrometry, as well as the concentrations of different forms of folic acid in serum, namely 5-methylTHF, UMFA, 5-formylTHF, THF, 5,10-methenylTHF and MeFox.

Malignancy status

Malignancy status was assessed by two consecutive questions: first, if a participant answered "Yes" to the question "Did your doctor or other health professional tell you that you have a malignancy or any type of malignancy?" he/she was classified as having a malignancy. Next, participants will be asked "What kind of malignancy?" and the type of malignancy can be identified.

Covariates

The following potential confounding variables were selected from demographic, examination, laboratory test data, and questionnaire data: age (≤ 44 and > 44 years), race (non-Hispanic white, non-Hispanic black, other), marital status (married, unmarried, other), education level (less than high school, high school or above, other), body mass index (BMI, kg/m2) (≤ 23.85, > 23.85), hypertension (yes, no), diabetes (yes, no), smoking status (< 100 sticks/lifetime, ≥ 100 sticks/lifetime, other and unknown), alcohol drinks (< 14 drinks/past 12 Mos, ≥ 15 drinks/past 12 Mos, other and unknown), age in months at menarche (< 9, 9–18, > 18, hasn't started yet), age at last menstrual period (< 40, 40–55, > 55, other/unknown), taken birth control pills (yes, no, other/unknown), use female hormones (yes, no, other/unknown). BMI was obtained in the Mobile Examination Centre (MEC) and other covariates could be obtained during the interview by means of a standardized questionnaire.

Statistical methods

All analyses in this study were performed using State 26.0 (SPSS Inc., Chicago, IL, USA) and R 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/). All statistical analyses took into account NHANES complex survey design factors, followed NHANES guidelines for analysis and reporting, and were weighted to account for complex sampling designs and to obtain appropriate weights. Categorical variables in the baseline characteristics were described using compositional ratios, and comparisons between groups were made using chi-square tests or exact Fisher tests. Folic acid concentration was a continuous variable and was first tested for normal distribution and variance, with normality expressed as mean ± standard deviation ($\bar{x}$±s) and non-normality expressed as median (quartiles), and comparisons between groups were made by independent samples t-test or rank sum test. Propensity score matching (PSM) has been used extensively in observational studies to reduce selection bias for matching EC patients with non-oncology participants. A 1:1 ratio PSM analysis was used to balance differences between EC patients and non-oncology participants, adjusted for confounding variables including: age, race, marital status, education, BMI, hypertension, diabetes, smoking, alcohol consumption, age at menarche, age at last menstruation, oral contraceptive use and use of hormonal drugs. Propensity matching was performed by the R software, resulting in 68 successful matches. Correlation analysis of different serum folic acid forms and EC prevalence using matched data. In addition, Restricted cubic spline (RCS) with four sections was used to explore the dose-response relationship between different folic acid concentrations and EC. $p < 0.05$ indicates a statistically significant difference.

Results

Participant baseline characteristics

The NHANES database was searched and a total of 8,809 eligible participants were included, including 8,738 non-oncologists and 71 EC patients. Of the EC patients, 91.5% were 44 years of age or older, 32 (45.1%) were non-Hispanic white, and 39.4% were married. There were 47 (66.2%) with hypertensive disorders, 16 (22.5%) with diabetic disorders, 28 (39.4%) under the age of 40 years at menopause, 34 between 40 and 55 years, and 26 (36.6%) on hormonal drugs (Table 1). Some of the variables included in this study were collected in the MEC and were therefore analyzed using the MEC examination weights (WTMEC2YR). The sample weight used in the final analysis was equal to one quarter of the "WTMEC2YR" value, corresponding to the MEC weights for the eight survey years. The final weighted sample of 8809 participants representing 94380076 was analyzed and the weighted results showed statistically significant differences ($p < 0.05$) between the two groups for age, race, marital status, hypertension, age at menopause and use of hormonal medication (Table 2).

TABLE 1 Baseline characteristics of NHANES participants during 2011-2018 (before weighting)

Variables	Total N (%)	Non tumor patients N (%)	EC patients N (%)	*p*-value
Total	8809	8738 99.2	71 0.8	
Age (years)				**<0.001**
≤44	4251(48.3)	4245(48.6)	6(8.5)	
>44	4558 (51.7)	4493 (51.4)	65 (91.5)	
Race				**0.033**
Non-Hispanic white	3025 (34.3)	2993 (34.3)	32 (45.1)	
Non-Hispanic black	2040 (23.2)	2032 (23.3)	8 (11.3)	
Other	3744 (42.5)	3713 (42.5)	31 (43.7)	
Marital status				**<0.001**
Married	4109 (46.6)	4081 (46.7)	28 (39.4)	
Unmarried	1742 (19.8)	1738 (19.9)	4 (5.6)	
Other	2958 (33.6)	2919 (33.4)	39 (54.9)	
Education				**0.005**
Less than high school	1840 (20.9)	1814 (20.8)	26 (36.6)	
High school or above	6965 (79.1)	6920 (79.2)	45 (63.4)	
Other	4 (0.0)	4 (0.0)	0 (0.0)	
BMI kg/m^2				**0.008**
≤23.85	2120(24.0)	2104(24.1)	7(9.9)	
>23.85	6698 (76.0)	6634 (75.9)	64 (90.1)	
Hypertension				**<0.001**
Yes	2974 (33.8)	2927 (33.5)	47 (66.2)	
No	5827 (66.1)	5803 (66.4)	24 (33.8)	
Other/Unknown	8 (0.1)	8 (0.1)	0 (0.0)	
Diabetes				**0.008**
Yes	1017 (11.5)	1001 (11.5)	16 (22.5)	
No	7568 (85.9)	7516 (86.0)	52 (73.2)	
Other/Unknown	224 (2.5)	221 (2.5)	3 (4.2)	

Smoking status				0.402
<Sticks / lifetime	2824 (32.1)	2796 (32.0)	28 (39.4)	
≥Sticks / lifetime	5980 (67.9)	5937 (67.9)	43 (60.6)	
Other/Unknown	5 (0.1)	5 (0.1)	0 (0.0)	
Alcohol drinks				0.256
<14 drinks/past 12 Mos	5042 (57.2)	5008 (57.3)	34 (47.9)	
≥15 drinks/past 12 Mos	14 (0.2)	14 (0.2)	0 (0.0)	
Other/Unknown	3753 (42.6)	3716 (42.5)	37 (52.1)	
Age in months at menarche (years)				0.601
<9	44 (0.5)	44 (0.5)	0 (0.0)	
9-18	7772 (88.2)	7705 (88.2)	67 (94.4)	
>18	27 (0.3)	27 (0.3)	0 (0.0)	

Hasn't started yet	3 (0.0)	3 (0.0)	0 (0.0)	
Other/Unknown	963 (10.9)	959 (11.0)	4 (5.6)	
Age at last menstrual period (years)				**<0.001**
<40	909 (10.3)	881 (10.1)	28 (39.4)	
40-55	2582 (29.3)	2548 (29.2)	34 (47.9)	
>55	176 (2.0)	174 (2.0)	2 (2.8)	
Other/Unknown	5142 (58.4)	5135 (58.8)	7 (9.9)	
Taken birth control pills				0.256
Yes	5268 (59.8)	5229 (59.8)	39 (54.9)	
No	2621 (29.8)	2594 (29.7)	27 (38.0)	
Other/Unknown	920 (10.4)	915 (10.5)	5 (7.0)	
Use female hormones				**<0.001**
Yes	1184 (13.4)	1158 (13.3)	26 (36.6)	
No	6692 (76.0)	6652 (76.1)	40 (56.3)	
Other/Unknown	933 (10.6)	928 (10.6)	5 (7.0)	

TABLE 2 Baseline characteristics of NHANES participants over the period 2011-2018 (weighted)

Variables	Total N (%)	Non tumor patients N (%)	EC patients N (%)	*p*-value
Total	94380076.7	93642489.2	737587.5	
Age (years)				**<0.001**
≤44	47432336.1(50.3)	47359649.4(50.6)	72686.7(9.9)	
>44	46947740.6 (49.7)	46282839.8 (49.4)	664900.8 (90.1)	
Race				**0.033**
Non-Hispanic white	58878519.9 (62.4)	58328668.9 (62.3)	549851.0 (74.5)	
Non-Hispanic black	11821629.3 (12.5)	11782093.3 (12.6)	39536.0 (5.4)	
Other	23679927.5 (25.1)	23531727.0 (25.1)	148200.5 (20.1)	
Marital status				**<0.001**
Married	48671115.6 (51.6)	48338554.6 (51.6)	332561.0 (45.1)	
Unmarried	17391704.8 (18.4)	17372450.8 (18.6)	19254.1 (2.6)	
Other	28317256.2 (30.0)	27931483.8 (29.8)	385772.4 (52.3)	
Education				0.091
Less than high school	13069036.3 (13.8)	12899411.1 (13.8)	169625.2 (23.0)	
High school or above	81280508.4 (86.1)	80712546.1 (86.2)	567962.3 (77.0)	
Other	30532.0 (0.0)	30532.0 (0.0)	0.0 (0.0)	
BMI kg/m^2				0.084
≤23.85	24001076(25.6)	23909380.9(25.5)	91695.2(12.4)	
>23.85	70379000.7 (74.6)	69733108.3 (74.5)	645892.3 (87.6)	

Hypertension				**<0.001**
Yes	28150698.7 (29.8)	27674776.2 (29.6)	475922.4 (64.5)	
No	66142953.4 (70.1)	65881288.3 (70.4)	261665.1 (35.5)	
Other/Unknown	86424.7 (0.1)	86424.7 (0.1)	0.0 (0.0)	
Diabetes				0.184
Yes	8223164.3 (8.7)	8119539.8 (8.7)	103624.5 (14.0)	
No	84188122.8 (89.2)	83584767.9 (89.3)	603354.9 (81.8)	
Other/Unknown	1968789.6 (2.1)	1938181.5 (2.1)	30608.1 (4.1)	
Smoking status				0.657
<Sticks / lifetime	33569494.7 (35.6)	33256396.9 (35.5)	313097.9 (42.4)	
≥Sticks / lifetime	60762218.2 (64.4)	60337728.5 (64.4)	313097.9 (42.4)	
Other/Unknown	48363.8 (0.1)	48363.8 (0.1)	0.0 (0.0)	
Alcohol drinks				0.552
<14 drinks/past 12 Mos	61956909.6 (65.6)	61524908.8 (65.7)	432000.8 (58.6)	
≥15 drinks/past 12 Mos	155683.2 (0.2)	155683.2 (0.2)	0.0 (0.0)	
Other/Unknown	32267483.9 (34.2)	31961897.1 (34.1)	305586.8 (41.4)	
Age in months at menarche (years)				0.459
<9	344614.5 (0.4)	344614.5 (0.4)	0.0 (0.0)	
9-18	85136483.4 (90.2)	84422161.2 (90.2)	714322.2 (96.8)	
>18	197582.6 (0.2)	197582.6 (0.2)	0.0 (0.0)	
Hasn't started yet	31132.4 (0.0)	31132.4 (0.0)	0.0 (0.0)	
Other/Unknown	8670263.8 (9.2)	8646998.5 (9.2)	23265.4 (3.2)	
Age at last menstrual period (years)				**<0.001**
<40	10168652.3	9855680.1 (10.5)	312972.2	

	(10.8)		(42.4)	
40-55	26192027.6 (27.8)	25859242.4 (27.6)	332785.2 (45.1)	
>55	2016252.0 (2.1)	1983431.6 (2.1)	32820.4 (4.4)	
Other/Unknown	56003144.9 (59.3)	55944135.2 (59.7)	59009.7 (8.0)	
Taken birth control pills				0.261
Yes	64280348.8 (68.1)	63789269.9 (68.1)	491078.9 (66.6)	
No	21873222.9 (23.2)	21656661.1 (23.1)	216561.8 (29.4)	
Other/Unknown	8226505.0 (8.7)	8196558.2 (8.8)	29946.8 (4.1)	
Use female hormones				**<0.001**
Yes	15767240.0 (16.7)	15395279.8 (16.4)	371960.2 (50.4)	
No	70256290.2 (74.4)	69920609.6 (74.7)	335680.6 (45.5)	
Other/Unknown	8356546.6 (8.9)	8326599.7 (8.9)	29946.8 (4.1)	

Propensity score matching

To eliminate the effects of confounding factors, a 1:1 match was performed using PSM, as shown in **Figure 2**. 68 each of EC patients and non-oncology participants were included, and the clinical baseline characteristics of all subjects after PSM are shown in **Table 3.** Subsequently, comparison of the different forms of folate with EC showed that total serum folate, 5-methylTHF, 5-formylTHF, THF and 5,10-methenylTHF were significantly correlated with EC (p <0.05) (**Table 4**).

TABLE 3 Baseline characteristics after propensity score matching analysis

Variables	Total N (%)	Non tumor patients N (%)	EC patients N (%)	*P*-value
Total	136	68	68	
Age (years)				0.743
≤44	10(7.4)	4(5.9)	6(8.8)	
44	126 (92.6)	64 (94.1)	62 (91.2)	
Race				0.962
Non-Hispanic white	64 (47.1)	32 (47.1)	32 (47.1)	
Non-Hispanic black	17 (12.5)	9 (13.2)	8 (11.8)	
Other	55 (40.4)	27 (39.7)	28 (41.2)	
Marital status				0.777
Married	58 (42.6)	31 (45.6)	27 (39.7)	
Unmarried	8 (5.9)	4 (5.9)	4 (5.9)	
Other	70 (51.5)	33 (48.5)	37 (54.4)	
Education				0.716
Less than high school	45(33.1)	21(30.9)	24(35.3)	
High school or above	91 (66.9)	47 (69.1)	44 (64.7)	
Other	-	-	-	
BMI kg/m^2				0.762
≤23.85	12(8.8)	5(7.4)	7(10.3)	
23.85	124 (91.2)	63 (92.6)	61 (89.7)	
Hypertension				0.858
Yes	88(64.7)	43(63.2)	45(66.2)	
No	48 (35.3)	25 (36.8)	23 (33.8)	
Other/Unknown	-	-	-	
Diabetes				0.671
Yes	29 (21.3)	13 (19.1)	16 (23.5)	
No	104 (76.5)	54 (79.4)	50 (73.5)	
Other/Unknown	3 (2.2)	1 (1.5)	2 (2.9)	

Smoking status				0.729
Sticks / lifetime	59(43.4)	31(45.6)	28(41.2)	
≥Sticks / lifetime	77 (56.6)	37 (54.4)	40 (58.8)	
Other/Unknown	-	-	-	
Alcohol drinks				1.000
14 drinks/past 12 Mos	67(49.3)	33(48.5)	34(50.0)	
≥15 drinks/past 12 Mos	-	-	-	
Other/Unknown	69 (50.7)	35 (51.5)	34 (50.0)	
Age in months at menarche (years)				1.000
9	-	-	-	
9-18	128(94.1)	64(94.1)	64(94.1)	
18	-	-	-	
Hasn't started yet	-	-	-	
Other/Unknown	8 (5.9)	4 (5.9)	4 (5.9)	
Age at last menstrual period (years)				0.857
40	54 (39.7)	29 (42.6)	25 (36.8)	
40-55	66 (48.5)	32 (47.1)	34 (50.0)	
55	3 (2.2)	1 (1.5)	2 (2.9)	
Other/Unknown	13(9.6)	6 (8.8)	7 (10.3)	
Taken birth control pills				0.940
Yes	79 (58.1)	40 (58.8)	39 (57.4)	
No	48 (35.3)	24 (35.3)	24 (35.3)	
Other/Unknown	9 (6.6)	4 (5.9)	5 (7.4)	
Use female hormones				0.455
Yes	40 (29.4)	17 (25.0)	23 (33.8)	
No	87 (64.0)	47 (69.1)	40 (58.8)	
Other/Unknown	9 (6.6)	4 (5.9)	5 (7.4)	

Table 4 Correlation of different forms of folic acid with EC

Folic acid(nmol/L)	Non tumor patients (n=68)	EC patients (n=68)	*p*-value
RBC folate	1215.00 (967.00, 1830.00)	1150.00 (806.25, 1417.50)	0.077
Serum total folate	47.6 (35.00, 77.00)	36.25 (24.03, 47.65)	**0.001**
5-methylTHF	44.3 (31.40, 74.90)	34.10 (23.20, 43.65)	**0.001**
UMFA	0.83 (0.52, 1.24)	0.66 (0.55, 1.06)	0.210
5-formylTHF	0.21 (0.14, 0.21)	0.14 (0.14, 0.21)	**0.004**
THF	0.96 (0.71, 1.38)	0.77 (0.50, 1.34)	**0.050**
5,10-methenylTHF	0.24 (0.20, 0.24)	0.18 (0.14, 0.24)	**0.005**
MeFox	1.63 (0.96, 2.88)	1.72 (1.15, 2.70)	0.931

Restricted cubic spline

To further test the existence of a correlation between different folic acid concentrations and EC, an RCS visual depiction was used (Fig. 3). The results showed that total serum folate, 5-methylTHF, 5-formylTHF, THF and 5,10-methenylTHF correlated with EC ($p < 0.05$). Among them,5-formylTHF, THF and 5,10-methenylTHF had significant non-linear correlation with EC with *P*-values of 0.005,0.045 and 0.005 for non-linear test respectively. There was no significant non-linear relationship between total serum folate and 5-methylTHF and EC, with *P*-values of 0.524 and 0.562 for the non-linear test respectively. However, the 5-formylTHF data could not be curve plotted. For THF, EC prevalence increased with increasing concentration at concentrations less than 4.172 nmol/L; at concentrations equal to 4.172 nmol/L, the cut-off point (Odds ratio, OR) ≈ 1; at concentrations greater than 4.172 nmol/L, EC prevalence decreased significantly with increasing concentration. For 5,10-methenylTHF, EC prevalence increased with increasing concentrations at concentrations less than 0.219 nmol/L; at concentrations equal to 0.219 nmol/L, the OR of the cut-off point was ≈ 1; after concentrations greater than 0.219 nmol/L, EC prevalence decreased significantly with increasing concentrations.

Discussion

EC is a common malignancy of the female reproductive system, predominantly adenocarcinoma, and the prognosis is influenced by a number of factors[20]. Most patients can be treated by early diagnosis surgery and the overall prognosis is more positive[21]. However, in patients with advanced disease, where treatment options are limited and prognosis is poor, it is important for early identification of high-risk factors associated with poor outcomes[22]. In recent years, studies have found that folic acid plays a key role in tumor growth[23]. The folate receptor mediates the entry of folate into the cytoplasm of human eukaryotic cells and is expressed in elevated amounts in many types of tumors[24]. Folic acid, a small molecule ligand with high affinity for the folate receptor, can specifically recognize folate receptors highly

expressed on tumor cells for targeted delivery[25–27]. Thus, folic acid can be used not only as a predictive marker for tumors, but also as a target for treatment and visualization.

It has been shown that low folate status leads to genomic instability and DNA mutation rates, and affects the methylation patterns of oncogenes[28]. In contrast, increased levels of folic acid accelerate the growth of tumors at the primary site[29]. There is a lack of evidence for a correlation between high or low levels of folic acid and EC tumor growth[30, 31]. This study explored the potential relationship between RBC folate and different serum folate forms and EC by combining and analyzing NHANES data from 2011–2018, including 8,809 participants. Fan et al. showed by a dose-response analysis that each 100 ug/d increase in folic acid intake was associated with a 4.3% reduction in the risk of head and neck squamous cell cancer[32]. Li et al. included nine case-control studies and five cohort studies for a meta-analysis and showed that increased folic acid intake led to a decreased risk of EC (OR = 0.89, 95% CI: 0.76–1.05)[33]. The serum total folate concentration is usually considered to be an indicator of recent folate intake, while RBC folate concentration is considered to be an indicator of long-term folate status. In this study, no correlation was found between RBC folate and EC ($P > 0.05$) and serum total folate showed a negative correlation with EC prevalence. For the different folate isoforms, 5-methylTHF is directly involved in the metabolism of the one-carbon unit as the main circulating form of folate[34]. This study also demonstrated a negative correlation between 5-methylTHF and the incidence of EC.

UMFA may impair folic acid metabolism by inhibiting dihydrofolate reductase and methylenetetrahydrofolate reductase[35–38]. No correlation between UMFA and EC was found in this study. Wei et al. also found that higher levels of 5-methylTHF were associated with a lower risk of developing lung cancer[39]. Yang et al. investigated the association of different serum folate forms (total serum folate, 5-methylTHF, and UMFA) with the prevalence of nonalcoholic fatty liver disease and advanced fibrosis by including NHANES data from 2011–2018.The results showed that total serum folic acid and 5-methylTHF were negatively correlated with the prevalence of nonalcoholic fatty liver disease and advanced fibrosis ($p < 0.05$).The concentration of UMFA was positively correlated with the prevalence of nonalcoholic fatty liver disease and advanced fibrosis ($p < 0.05$)[40]. THF metabolic pathway has long been a target for anti-tumor therapy[41, 42], which is consistent with our research results. MeFox is an oxidation product of 5-methyltetrahydrofolate, and there may be a correlation between the two forms. This study found that there was no correlation between the increase in serum MeFox concentration and EC. In conclusion, using data from a large national representative cohort of American adults, the study found that high levels of folic acid reduced the incidence rate of EC, and that non methyl folic acid affected the incidence of EC in a non-linear mode. This study may provide guidance for the study of folic acid in EC.

The advantage of this study was to use the NHANES database, using standardized measurement methods, to better study the association between RBC folate and different serum folate forms (serum total folate, 5-methylTHF, UMFA, 5-formylTHF, THF, 5,10-methenylTHF and MeFox) and EC by adjusting for potential confounding factors. However, this study also had certain limitations. First, cross-sectional

design limited the ability to assess causal relationships; Second, the study was conducted in American adults receiving folic acid fortification; Third, because the study was exploratory, multiple tests were not considered.

Conclusion

Analysis of NHANES data from 2011–2018 showed that serum total folate, 5-methylTHF, 5-formylTHF, THF and 5,10-methenylTHF in haemorrhage serum were closely related to the occurrence of EC. The incidence of EC decreased with the increase of serum total folate and 5-methylTHF concentrations. THF and 5,10-methenylTHF had significant nonlinear correlations with EC. It was helpful for clinicians to better conduct quantitative treatment for EC patients and improve their prognosis.

Declarations

Author conclusions

Conceptualization, M.Z.; Methodology, R.L.; Validation, S.Z. and X.F; Formal Analysis, L.L.; Data Curation, X.X.; Writing-Original Draft Preparation, M.Z.; Writing – Review & Editing, Y.G. All authors have read and agreed to the published version of the manuscript.

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Ethical approval and informed consent

The study was conducted according to the guidelines of the Declaration of Helsinki. The NHANES database has been approved by the National Center for Health Statistics (NCHS) Research Ethics Review Committee of the United States Centers for Disease Control and Prevention (CDC), and all participants have signed informed consent forms.

Data Availability Statement

All NHANES data for this study are publicly available and can be found here: https://wwwn.cdc.gov/nchs/nhanes.

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Conflicts of Interest

The authors declare no conflict of interest.

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Figures

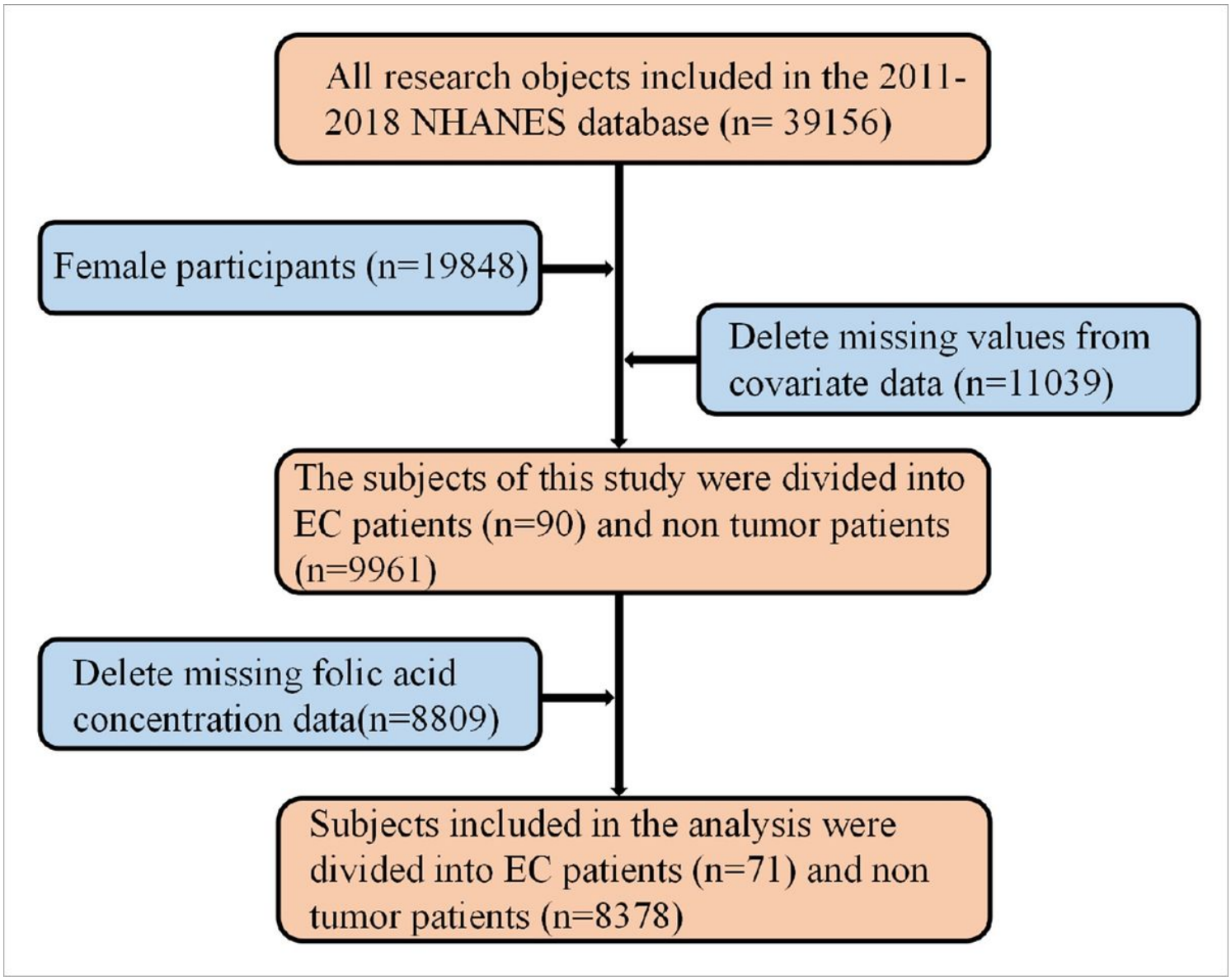

Figure 1

Flowchart

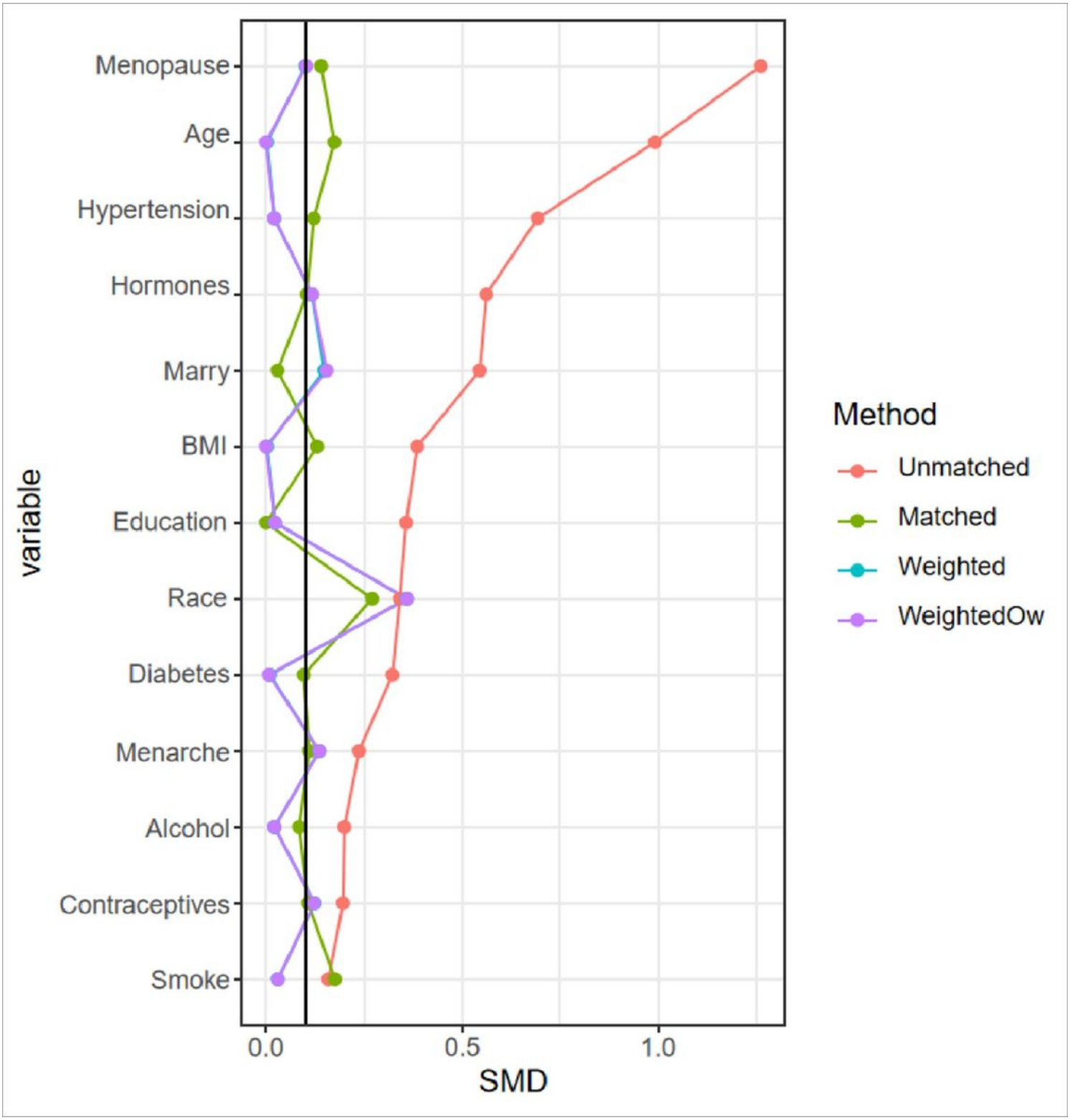

Figure 2

Propensity score matching analysis

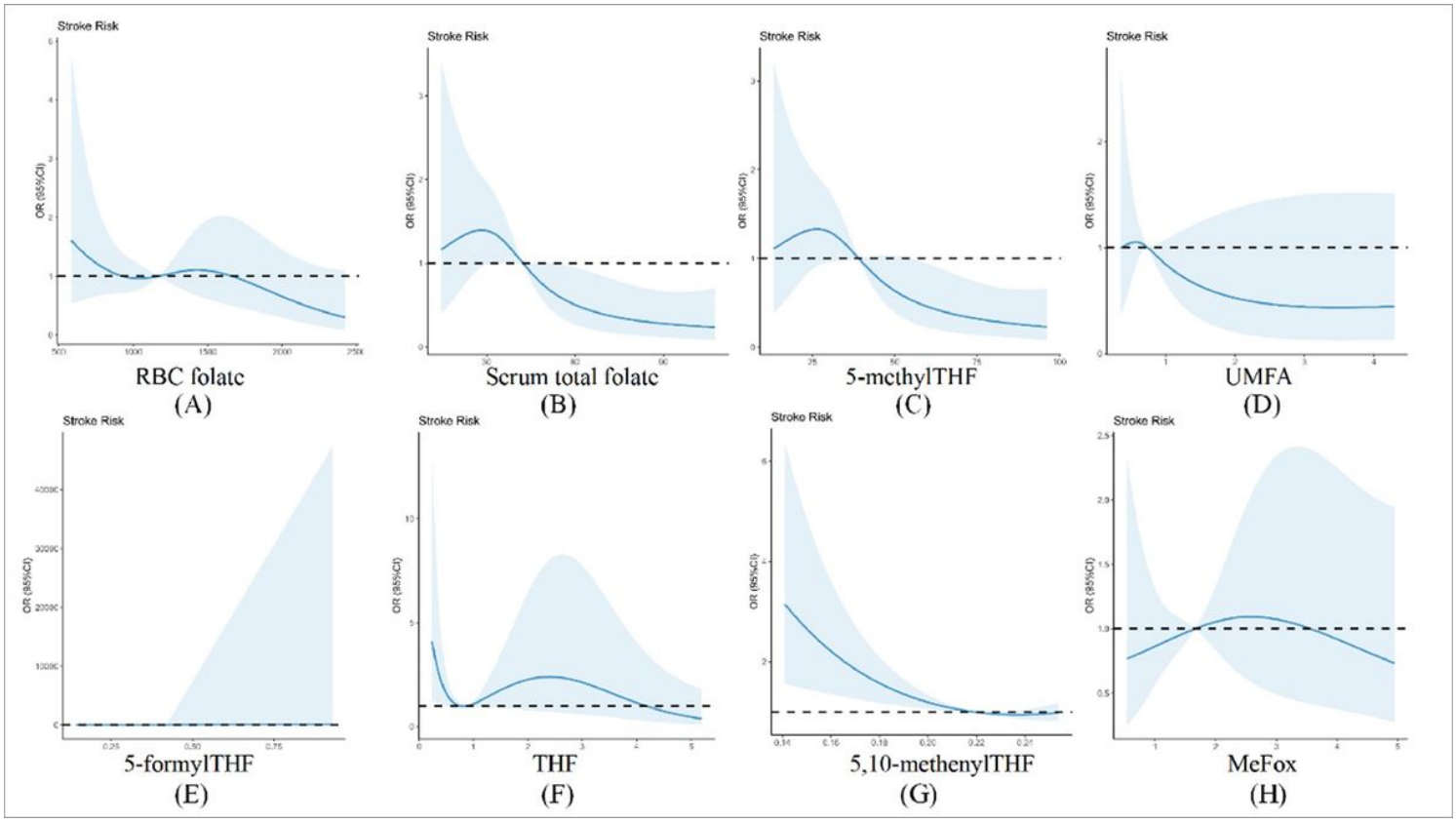

Figure 3

Showing the association between folic acid and EC prevalence using the 4-section RCS