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Association between red blood cell fatty acids composition and risk of esophageal cancer: a hospital-based case-control study

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Abstract

Background & aims: Esophageal cancer (EC) is a tumor type with high invasiveness and poor prognosis, attracting scientists' attention to its pathogenesis and etiology. Given the limited evidence and conflicting findings regarding the association between EC risk and RBC fatty acids, we aimed to evaluate this association.

Methods The study utilized gas chromatography to analyze RBC fatty acids in 158 EC patients and 224 controls. Multivariable conditional logistic regression and restricted cubic spline analysis were employed to assess the association between EC risk and RBC fatty acids, as well as to determine the odds ratio with a 95% confidence interval (OR, 95% CI) for this association.

Results Higher levels of total n-3 polyunsaturated fatty acids (n-3 PUFA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and n-3 index were associated with lower odds of being an EC case [OR_{T3-T1} = 0.22 (0.12–0.41), OR_{T3-T1} = 0.29 (0.15–0.54), OR_{T3-T1} = 0.49 (0.27–0.88), and OR_{T3-T1} = 0.19 (0.09–0.35), respectively]. Total saturated fatty acids (SFA), particularly palmitic acid (C16:0), stearic acid (C18:0), and arachidonic acid (C20:4n-6) in high concentrations, were associated with higher odds of being an EC case [OR_{T1-T3} = 2.02 (1.11–3.70), OR_{T1-T3} = 2.10 (1.15–3.87), OR_{T1-T3} = 2.82 (1.53–5.30), and OR_{T1-T3} = 2.07 (1.12–3.86), respectively]. Total monounsaturated fatty acids (MUFA) and total trans fatty acids (TFA) showed no significant association with EC case status.

Conclusion The different types of RBC fatty acids may significantly influence susceptibility to EC. Higher levels of total n-3 PUFA in RBC, specifically DHA and EPA, were associated with lower odds of being an EC case, while higher levels of C20:4n-6, C18:0, and C16:0 were associated with higher odds.

Keywords Esophagus cancer, Red blood cell, Fatty acids, Saturated fatty acid, N-3 polyunsaturated fatty acids, Case-control study

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Introduction

According to the latest epidemiological analysis of malignancies from GLOBOCAN 2020 data, esophageal cancer (EC) ranks seventh in incidence and sixth in cancer-related mortality worldwide [1]. Asia accounts for approximately 78% of all EC cases, with the majority being esophageal squamous cell carcinoma, and 49% of cases occur in China [2]. The significance of EC as a public health concern has been increasing in China. Diagnosis often occurs at an advanced stage, resulting in an unfavorable prognosis and poor quality of life [3]. To effectively manage EC, developing methods for early detection is crucial. In addition to several well-established risk factors, such as alcohol consumption, smoking, the intake of very hot drinks, and certain genetic traits [4, 5], the critical role of lipids in cancer risk inspired us to investigate the association between specific red blood cell (RBC) metabolites, particularly fatty acids, and EC risk.

In recent years, RBC fatty acids have emerged as a focal point for biomarker screening due to their stability and objectivity in evaluating fatty acid exposure status within the human body [6]. Compared to other blood samples like plasma or serum fatty acids, RBC fatty acids exhibit a longer half-life and lower biological variability, making them a reliable indicator of relatively long-term exposure [7]. Moreover, the composition of RBC fatty acids is influenced by a combination of dietary intake, genetic factors, and metabolic factors [8]. All major fatty acid categories present in the body are detectable in RBC, including polyunsaturated fatty acids (n-3 PUFA and n-6 PUFA), monounsaturated fatty acids (MUFA), saturated fatty acids (SFA), and trans fatty acids (TFA). Alterations in RBC fatty acids have been associated with various diseases, with lower levels of n-3 PUFA associated with an increased risk of cardiovascular disease [9] and possibly neurodegenerative diseases [10]. Several studies have investigated the role of lipid alterations in cancer development, using RBC fatty acids as potential biomarkers for diseases such as oral cancer [11], colorectal cancer [12], lung cancer [13], liver cancer [14], prostate cancer [15], and breast cancer [16]. These studies have revealed interesting differences in the levels of several RBC fatty acids. Building on this evidence, RBC fatty acids may also influence EC susceptibility through inflammation, oxidative stress, and key metabolic pathways. For instance, n-6 polyunsaturated fatty acids (PUFA), such as arachidonic acid (C20:4n-6), may promote inflammation via the cyclooxygenase-2 and lipoxygenase pathways, leading to the production of pro-inflammatory eicosanoids that could enhance tumor progression [17]. In contrast, n-3 PUFA, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), exhibit anti-inflammatory and antioxidant properties, potentially mitigating cancer risk [18]. However, despite these potential biological links, the association between RBC fatty acids and EC risk remains largely unexplored, warranting further investigation.

Therefore, this case-control study aims to evaluate the association between RBC fatty acids and EC risk while elucidating possible connections that may contribute to understanding the underlying etiology of EC beyond known risk factors. Furthermore, this research offers valuable insights for early dietary recommendations and clinical diagnosis.

Methods

Study population and data collection

This case-control study was conducted at The Affiliated Wuxi People's Hospital of Nanjing Medical University between March 2022 and June 2023. Participants included men and women aged 45 to 80 years, residing in Wuxi, who consented to participate in the study. Cases were patients diagnosed with EC via endoscopy and confirmed through histopathological examination. Inclusion criteria for cases were as follows: (1) newly diagnosed primary esophageal squamous cell carcinoma confirmed histologically or cytologically according to the International Classification of Diseases 10th revision [19]; confirmed macroscopic type diagnosis; and (3) Chinese residents who had lived in Wuxi city for at least 10 years. Cases were excluded if they had (1) a second primary, metastasized, or recurrent cancer, or had received prior chemotherapy or radiotherapy; or (2) refused participation or did not complete the questionnaire.

A total of 723 individuals were initially identified as potential esophageal carcinoma cases, among whom 470 (65%) consented to undergo biopsy for histopathologic analysis. The remaining individuals declined biopsy due to financial constraints, fear, anxiety, lack of trust, or concerns about potential bleeding risks. Of the 470 patients who underwent histopathologic examination, 158 were confirmed as having esophageal carcinoma, while the remaining exhibited benign esophageal lesions.

The control group comprised 224 participants who underwent physical examinations at the same hospital's health examination center. These individuals were recruited from two parts. First, each patient was asked to invite at least one healthy individual from their social circle (e.g., family member, neighbor, or friend) [20, 21]. If an invited individual did not respond or was unavailable, additional control participants were recruited from the hospital's health examination center. To ensure the absence of esophageal cancer or precancerous lesions, all control participants underwent endoscopic screening within one month prior to inclusion in the study. The control group was matched to cases based on age (± 5 years) and gender. Exclusion criteria for controls included refusal to participate, incomplete questionnaire

responses, a history of cancer, or a diagnosis of mental disorders [22]. All participants provided signed informed consent. The study protocol was approved by the Ethical Review Board of Wuxi People's Hospital (NO. 23050) and strictly adhered to the ethical principles outlined in the Declaration of Helsinki.

Face-to-face interviews were conducted with cases in the histopathology unit immediately after EC confirmation. Trained interviewers also conducted face-to-face interviews with controls, either in the community or at the hospital. During these interviews, participants provided general demographic information, including weight (kg), height (meter), age (years), gender (male, female), residence (rural, urban), education level (high school and above, primary and middle school, or low), marital status (married, single), family history of cancer (yes, no), history of diabetes (yes, no), occupation (farmer, worker, or other). Smoking status (yes, no): Smokers were defined as those who had smoked at least 10 cigarettes per week continuously for more than six months [23]. Alcohol consumption (yes, no): Alcohol drinkers were referred to those who had at least one drink per week for at least six months [23]. Tea consumption (yes, no): Tea drinkers were defined as those who consumed at least one cup of tea per week for at least six months [24]. Participants who did not meet the criteria of smokers, alcohol drinkers, and tea drinkers were categorized as non-smokers, non-alcohol drinkers, and non-tea drinkers, respectively. Physical activity was categorized as low (0 h/week), moderate (0.5-<4 h/week), and high (≥4 h/week) [25]. Physical activity included work-related tasks (e.g., moving heavy furniture, loading or unloading trucks, or shoveling) or strenuous sports (e.g., jogging, bicycling on hills, tennis, squash, swimming laps, or aerobics) performed for at least 30 min per week, on average [25].

Blood collection

Blood samples were collected following the methodology outlined by Chen et al. [11]. Before any treatments or medication was administered, approximately 5 mL of fasting blood was drawn from each participant into ethylenediaminetetraacetic acid (EDTA) tubes. Blood samples from control participants were collected at the same hospital, ensuring consistency in sample collection procedures between cases and controls. All collected blood samples were centrifuged at 1,500 rpm, 4℃ for 10 min to isolate the RBC, which were subsequently stored at -80℃ until further analysis.

RBC fatty acid analysis

The measurement of RBC fatty acids was conducted following the method reported by Harris et al. [26], with slight adjustments. Fatty acid methyl esters (FAMEs) were derived from RBC using acid transesterification and analyzed via gas chromatography (GC). In brief, a 25 μL sample of erythrocytes was treated with methanol containing 14% boron trifluoride and hexane in succession (250 μL each). The mixture was stirred and heated at 100℃ for 10 min in an aluminum bead bath. After cooling, 250 μL of HPLC-grade water was added, followed by centrifugation at 1500×g for 3 min to separate the layers. A 50 μL portion of the top hexane layer was transferred into a GC vial for analysis. FAMEs were analyzed using an Agilent GC 6890 equipped with a flame ionization detector and an HP-88 column (100 m × 0.2 μm × 0.25 mm, Agilent, Folsom, CA, USA). The injector and detector temperatures were both set to 240℃. Nitrogen was used as the carrier gas, with a split ratio of 10:1. The column temperature program was as follows: initially set at 140 °C for 1 min, then increased to 200 °C at a rate of 10 °C/min over 7 min, followed by a further increase at 1 °C/min to a final temperature of 225 °C, which was maintained for 10 min. Participant samples were processed together as a group, and researchers handling them were blinded to the subjects' characteristics. The coefficients of variation (CVs) for a single day were calculated using standard quality assurance samples and varied from 1.02 to 6.86%. Individual FAMEs were identified by comparing their retention times with those of a 37-FAMEs mixture standard (Sigma, St. Louis, MO, USA), and their levels were expressed as the relative percentages of the total peak area. The n-3 index was defined as the sum of C20:5n-3 and C22:6n-3, expressed as a percentage of RBC fatty acids.

Statistical analysis

The chi-squared test, independent-sample t-test, and the Mann-Whitney *U* test were employed to assess the differences in characteristics between EC patients and the control group, specifically for categorical variables, normally distributed and non-normally distributed variables, respectively. The normality of the data was assessed using the Shapiro-Wilk test. For normally distributed data, the values were expressed as mean ± standard deviation; for skewed or non-normal distributions, the values were presented as median [interquartile range]. We subsequently employed multivariate conditional logistic regression models after adjusting for potential confounding variables (including alcohol consumption, tea consumption, smoking, age, gender, occupation, level of education, residence, body mass index (BMI), marital status, ethnic group, physical activity, family history of cancer, and history of diabetes) to assess the association between EC risk and RBC fatty acids. These adjustments included socioeconomic factors (education, occupation, and residence type), further minimizing selection bias related to differences in participant backgrounds. The odds ratios (ORs) were calculated in the tertile (T) model and continuous

models. Specifically, T1 (the lowest tertile) served as the reference category, and ORs for T2 and T3 (middle and highest tertiles) were calculated relative to this reference. Adjustments were made for all potential confounding factors. To assess linear trends, we included the median of each tertile as a continuous variable in the models. Additionally, we employed the restricted cubic spline (RCS) function to evaluate potential non-linear relationship between EC risk and RBC fatty acids. The RCS functions were based on three knots, with the median of the first tertile serving as the reference point for each fatty acid. All analyses were performed using R software, with statistical significance determined at a p-value of <0.05 using a two-tailed test.

Results

Table 1 summarizes the demographic information for all participants (224 controls and 158 EC patients). A higher percentage of individuals in the case group were found to smoke, drink alcohol, and consume tea compared to the control group. There were no notable differences between the two groups regarding demographic characteristics or lifestyle factors such as BMI, place of residence, gender, ethnicity, age, family history of cancer, marital status, diabetes, and physical activity level.

As shown in Table 2, the RBC fatty acid profiles in cases and controls indicated that palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2n-6), C20:4n-6, oleic acid (C18:1n-9), and C22:6n-3 (DHA) accounted for almost 85% of total fatty acids, while each of the remaining fatty acids contributed less than 5%. Moreover, compared to controls, EC patients showed lower RBC levels of total n-3 PUFA, DHA, EPA, and eicosadienoic acid (C20:2n-6) ($p<0.05$). Total SFA, myristic acid (C14:0), C16:0, C18:0, C20:4n-6, total TFA, as well as elaidic acid (C18:1 trans-9) were higher in EC patients compared to controls ($p<0.05$). Fatty acid indices, such as the n-3

Table 1 The demographic characteristics of the study population

Characteristics		Control (Mean ± SD or %)	Case (Mean ± SD or %)	*P*-value*
		(*n* = 224)	(*n* = 158)	
Age (year)		61.73 ± 5.02	62.03 ± 4.76	0.560
Body Mass Index (BMI)		24.08 ± 3.11	24.39 ± 3.71	0.391
Gender	Male	123 (54.9%)	77 (48.7%)	0.277
	Female	101 (45.1%)	81 (51.3%)	
Ethnic group	Han nationality	199 (88.8%)	146 (92.4%)	0.325
	Other	25 (11.2%)	12 (7.6%)	
Residence	Rural	98 (43.8%)	70 (44.3%)	0.998
	Urban	126 (56.2%)	88 (55.7%)	
Education level	Low	111 (49.6%)	71 (44.9%)	< 0.001
	Primary and middle school	91 (40.6%)	40 (25.3%)	
	High school and above	22 (9.8%)	47 (29.7%)	
Marital status	Married	203 (90.6%)	140 (88.6%)	0.639
	Single	21 (9.4%)	18 (11.4%)	
History of diabetes	No	190 (84.8%)	139 (88%)	0.467
	Yes	34 (15.2%)	19 (12%)	
Family history of cancer	No	178 (79.5%)	132 (83.5%)	0.384
	Yes	46 (20.5%)	26 (16.5%)	
Occupation	Farmer	104 (46.4%)	59 (37.3%)	0.001
	Worker	53 (23.7%)	65 (41.1%)	
	Other	67 (29.9%)	34 (21.5%)	
Physical activity	Low	113 (50.4%)	72 (45.6%)	0.444
	Moderate	48 (21.4%)	32 (20.3%)	
	High	63 (28.1%)	54 (34.2%)	
Smoking status	No	151 (67.4%)	79 (50%)	< 0.001
	Yes	73 (32.6%)	79 (50%)	
Alcohol consumption	No	153 (68.3%)	81 (51.3%)	0.001
	Yes	71 (31.7%)	77 (48.7%)	
Tea consumption	No	174 (77.7%)	83 (52.5%)	< 0.001
	Yes	50 (22.3%)	75 (47.5%)	

- Data are presented as frequency (percentage) for categorical variables, Mean ± SD for continues variables

- Comparison baseline characteristics between two groups (Chi-square test or Independent-sample t-test)

-*Significant difference between two groups ($P<0.05$)

Table 2 RBC fatty acids composition in the study population

RBC fatty acids (%)	Controls ($n=224$)	Cases ($n=158$)	p-value*
	Median (IQR)	Median (IQR)	
SFA	42.56 (41.22 to 43.86)	43.33 (41.55 to 45.20)	< 0.001
C14:0	0.99 (0.34 to 1.26)	1.10 (0.85 to 1.48)	0.001
C16:0	21.14 (20.10 to 22.48)	21.72 (20.61 to 22.56)	0.013
C18:0	18.79 (17.97 to 19.41)	19.21 (18.10 to 20.54)	< 0.001
C20:0	0.35 (0.27 to 0.41)	0.31 (0.26 to 0.40)	0.058
C22:0	0.66 (0.62 to 0.73)	0.65 (0.56 to 0.71)	0.128
C24:0	0.43 (0.31 to 0.79)	0.62 (0.34 to 0.69)	0.060
MUFA	15.53 (14.66 to 16.25)	15.25 (14.24 to 16.03)	0.010
C16:1 n-7	0.28 (0.21 to 0.47)	0.29 (0.22 to 0.44)	0.987
C18:1 n-9	13.41 (12.57 to 14.17)	13.40 (12.61 to 13.90)	0.246
C24:1 n-9	1.70 (1.41 to 2.08)	1.57 (1.28 to 1.90)	0.008
PUFA	41.29 (40.23 to 42.42)	41.22 (39.44 to 42.90)	0.842
n-6 PUFA	33.69 (32.49 to 34.74)	34.26 (32.37 to 35.59)	0.038
C18:2 n-6	12.50 (11.64 to 13.88)	12.89 (11.12 to 14.46)	0.434
C18:3 n-6	0.11 (0.08 to 0.14)	0.11 (0.08 to 0.14)	0.466
C20:2 n-6	0.99 (0.85 to 1.32)	0.93 (0.74 to 1.14)	0.005
C20:3 n-6	1.35 (1.19 to 1.56)	1.29 (1.08 to 1.59)	0.320
C20:4 n-6	14.97 (14.17 to 15.80)	15.66 (14.20 to 15.93)	< 0.001
C22:4 n-6	2.82 (2.42 to 3.23)	2.85 (2.23 to 3.22)	0.513
C22:5 n-6	0.73 (0.60 to 0.87)	0.73 (0.59 to 0.87)	0.718
n-3 PUFA	7.53 (6.99 to 8.36)	7.06 (6.52 to 7.54)	< 0.001
C18:3 n-3	0.48 (0.41 to 0.56)	0.46 (0.40 to 0.54)	0.119
C20:5 n-3	1.60 (1.19 to 1.80)	1.33 (1.08 to 1.67)	0.002
C22:5 n-3	1.50 (1.26 to 1.69)	1.58 (1.27 to 1.68)	0.098
C22:6 n-3	4.12 (3.49 to 4.63)	3.65 (3.36 to 3.97)	< 0.001
TFA	0.42 (0.37 to 0.51)	0.47 (0.39 to 0.53)	0.047
C18:1 n-9t	0.27 (0.23 to 0.33)	0.31 (0.24 to 0.37)	0.009
C18:2 n-6t	0.16 (0.13 to 0.18)	0.16 (0.13 to 0.18)	0.928
FA index			
n-3 index	5.53 (4.94 to 6.26)	5.03 (4.70 to 5.46)	< 0.001
n-6/n-3	4.48 (3.99 to 4.96)	4.82 (4.41 to 5.40)	< 0.001
AA/EPA	9.16 (8.04 to 12.50)	11.45 (8.92 to 14.41)	< 0.001

- RBC: red blood cell, SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, TFA: Trans fatty acid, FA: fatty acid, EPA: C20:5 n-3, DHA: C22:6 n-3, AA: C20:4 n-6, n-6: n-6 PUFA, n-3: n-3 PUFA, n-3 index: C20:5 n-3 + C22:6 n-3 (in percentages of RBC fatty acids)

- IQR, interquartile range. Calculated using a Wilcoxon rank-sum test

- *Significant difference between two groups ($P<0.05$)

index, the n-6/n-3 PUFA ratio, and the AA/EPA ratio, exhibited significant differences ($p<0.05$).

Table 3 shows a significant inverse association between RBC total n-3 PUFA levels and the odds of being an EC case [OR_{T3-T1} = 0.22 (0.12–0.41)] after controlling for alcohol intake, smoking, tea consumption, educational level, age, gender, place of residence, ethnicity, BMI, marital status, history of diabetes, family history of cancer, occupation, and physical activity. DHA and EPA seemed to be the primary contributors to this association [OR_{T3-T1} = 0.29 (0.15–0.54) and OR_{T3-T1} = 0.49 (0.27–0.88) for EPA and DHA, respectively]. C18:2n-6 levels were not significantly correlated with EC case status, whereas higher levels of C20:4n-6 were linked to increased odds. There was a substantial inverse association between RBC C20:2n-6 and dihomo-γ-linolenic acid (C20:3n-6) levels and EC risk [OR_{T3-T1} = 0.37 (0.19–0.69) and OR_{T2-T1} = 0.41 (0.22–0.75), respectively]. However, RBC total SFA, including the most abundant fatty acids, C16:0 and C18:0, were positively associated with higher EC case odds. Total MUFA and TFA levels in RBCs were not significantly associated with EC case status. The n-3 index demonstrated a statistically significant inverse relationship with EC case odds [OR_{T3-T1} = 0.19 (0.09–0.36)]. However, as shown in Table 3, individuals with higher RBC ratios of n-6/n-3 PUFA or AA/EPA had approximately 3.87-fold or 1.97-fold higher odds of being an EC case, respectively. To account for the competitive interaction between n-3 PUFA and C20:4n-6, the regression models were further

Table 3 OR (95% CIs) of esophageal cancer, by tertile of each fatty acid

RBC Fat	Tertiles of RBC fats			*P*-Trend	Continuous
	T_1	T_2	T_3		
SFA:					
C14:0				0.200	1.59 (0.98, 2.61)
Median (%)	0.31	1.03	1.57		
Controls/cases	87/40	71/56	66/62		
Adjusted OR (95% CI)	1	1.61 (0.86, 3.01)	1.54 (0.84, 2.85)		
C16:0				0.017	1.11 (0.97, 1.27)
Median (%)	19.75	21.49	22.96		
Controls/cases	86/42	73/53	65/63		
Adjusted OR (95% CI)	1	1.63 (0.89, 2.99)	2.10 (1.15, 3.87)*		
C18:0				< 0.001	1.40 (1.15, 1.70)
Median (%)	17.81	18.89	20.44		
Controls/cases	82/46	87/39	55/73		
Adjusted OR (95% CI)	1	1.07 (0.58, 2.01)	2.82 (1.53, 5.30)*		
C20:0				0.150	0.21 (0.01, 2.21)
Median (%)	0.25	0.33	0.42		
Controls/cases	69/58	73/54	82/46		
Adjusted OR (95% CI)	1	0.83 (0.46, 1.49)	0.64 (0.34, 1.17)		
C22:0				0.600	0.27 (0.02, 2.87)
Median (%)	0.57	0.65	0.75		
Controls/cases	69/59	80/46	75/53		
Adjusted OR (95% CI)	1	0.81 (0.45, 1.47)	0.84 (0.47, 1.50)		
C24:0				0.120	1.28 (0.85, 1.95)
Median (%)	0.28	0.48	0.98		
Controls/cases	82/45	73/54	69/59		
Adjusted OR (95% CI)	1	1.68 (0.91, 3.12)	1.60 (0.89, 2.89)		
Total SFA				0.021	1.14 (1.03, 1.26)
Median (%)	40.42	42.89	45.02		
Controls/cases	85/43	79/47	60/68		
Adjusted OR (95% CI)	1	1.16 (0.63, 2.16)	2.02 (1.11, 3.70)*		
MUFA:					
C16:1 n-7				0.800	0.27 (0.07, 1.02)
Median (%)	0.20	0.29	0.53		
Controls/cases	81/47	66/60	77/51		
Adjusted OR (95% CI)	1	1.76 (0.95, 3.28)	1.12 (0.59, 2.11)		
C18:1 n-9				0.200	0.85 (0.71, 1.02)
Median (%)	12.05	13.40	14.35		
Controls/cases	76/52	63/64	85/42		
Adjusted OR (95% CI)	1	1.22 (0.67, 2.21)	0.65 (0.35, 1.18)		
C24:1 n-9				0.800	0.54 (0.36, 0.75)
Median (%)	1.27	1.66	2.26		
Controls/cases	68/60	79/49	77/49		
Adjusted OR (95% CI)	1	0.69 (0.38, 1.23)	0.94 (0.51, 1.73)		
Total MUFA				0.200	0.75 (0.64, 0.87)
Median (%)	14.16	15.46	16.59		
Controls/cases	72/57	70/56	82/45		
Adjusted OR (95% CI)	1	1.29 (0.71, 2.33)	0.69 (0.38, 1.27)		
n-6 PUFA:					
C18:2 n-6				0.059	1.07 (0.95, 1.21)
Median (%)	10.81	12.58	14.83		
Controls/cases	70/58	91/36	63/64		
Adjusted OR (95% CI)	1	0.58 (0.31, 1.09)	1.75 (0.96, 3.22)		
C18:3 n-6				0.400	1.22 (0.95, 1.58)

Table 3 (continued)

RBC Fat	Tertiles of RBC fats			*P*-Trend	Continuous
	T_1	T_2	T_3		
Median (%)	0.07	0.11	0.16		
Controls/cases	74/53	80/47	70/58		
Adjusted OR (95% CI)	1	0.80 (0.43, 1.47)	1.28 (0.70, 2.36)		
C20:2 n-6				0.002	0.32 (0.15, 0.68)
Median (%)	0.61	0.97	1.37		
Controls/cases	69/58	64/63	91/37		
Adjusted OR (95% CI)	1	0.97 (0.53, 1.77)	0.37 (0.19, 0.69)*		
C20:3 n-6				> 0.900	0.49 (0.21, 1.09)
Median (%)	1.07	1.34	1.66		
Controls/cases	66/61	93/34	65/63		
Adjusted OR (95% CI)	1	0.41 (0.22, 0.75)*	1.00 (0.55, 1.79)		
C20:4 n-6				0.021	1.48 (1.21, 1.84)
Median (%)	14.03	15.25	16.12		
Controls/cases	82/46	82/45	60/67		
Adjusted OR (95% CI)	1	0.83 (0.45, 1.53)	2.07 (1.12, 3.86)*		
C22:4 n-6				0.600	0.94 (0.66, 1.34)
Median (%)	2.10	2.83	3.43		
Controls/cases	71/56	78/49	75/53		
Adjusted OR (95% CI)	1	0.90 (0.49, 1.64)	1.19 (0.66, 2.17)		
C22:5 n-6				0.800	0.97 (0.26, 3.75)
Median (%)	0.55	0.73	0.91		
Controls/cases	73/55	75/51	76/52		
Adjusted OR (95% CI)	1	0.90 (0.49, 1.62)	1.06 (0.59, 1.93)		
Total n-6 PUFA				0.002	1.16 (1.04, 1.31)
Median (%)	31.71	33.77	35.74		
Controls/cases	74/54	95/32	55/72		
Adjusted OR (95% CI)	1	0.53 (0.28, 1.00)	2.55 (1.39, 4.77)*		
n-3 PUFA:					
C18:3 n-3				0.500	0.18 (0.03, 1.05)
Median (%)	0.39	0.47	0.63		
Controls/cases	66/61	83/45	75/52		
Adjusted OR (95% CI)	1	0.63 (0.35, 1.14)	0.82 (0.45, 1.47)		
C20:5 n-3				0.017	0.57 (0.32, 0.98)
Median (%)	1.00	1.49	1.9		
Controls/cases	64/64	70/56	90/38		
Adjusted OR (95% CI)	1	0.93 (0.51, 1.69)	0.49 (0.27, 0.88)*		
C22:5 n-3				0.120	1.86 (0.89, 3.94)
Median (%)	1.16	1.52	1.77		
Controls/cases	78/50	82/45	64/63		
Adjusted OR (95% CI)	1	0.97 (0.53, 1.77)	1.62 (0.89, 2.96)		
C22:6 n-3				< 0.001	0.61 (0.46, 0.79)
Median (%)	3.20	3.83	4.69		
Controls/cases	66/61	60/68	98/29		
Adjusted OR (95% CI)	1	1.50 (0.84, 2.70)	0.29 (0.15, 0.54)*		
Total n-3 PUFA				< 0.001	0.64 (0.50, 0.80)
Median (%)	6.42	7.32	8.39		
Controls/cases	55/73	72/54	97/31		
Adjusted OR (95% CI)	1	0.58 (0.32, 1.04)	0.22 (0.12, 0.41)*		
TFA:					
C18:1 n-9t				0.018	1.21 (0.96, 1.54)
Median (%)	0.21	0.28	0.37		
Controls/cases	81/48	85/40	58/70		

Table 3 (continued)

RBC Fat	Tertiles of RBC fats			*P*-Trend	Continuous
	T_1	T_2	T_3		
Adjusted OR (95% CI)	1	0.82 (0.44, 1.53)	2.02 (1.13, 3.65)*		
C18:2 n-6t				> 0.900	1.15 (0.90, 1.47)
Median (%)	0.12	0.16	0.19		
Controls/cases	76/51	71/57	77/50		
Adjusted OR (95% CI)	1	1.46 (0.81, 2.65)	0.99 (0.55, 1.81)		
Total TFA				0.056	1.18 (0.93, 1.50)
Median (%)	0.34	0.43	0.58		
Controls/cases	82/45	78/50	64/63		
Adjusted OR (95% CI)	1	1.32 (0.72, 2.41)	1.80 (0.97, 3.32)		
Fatty acid index:					
n-3 index				< 0.001	0.51 (0.38, 0.68)
Median (%)	4.59	5.32	6.29		
Controls/cases	57/70	65/63	102/25		
Adjusted OR (95% CI)	1	0.73 (0.41, 1.30)	0.19 (0.09, 0.35)*		
n-6/n-3				< 0.001	1.74 (1.32, 2.34)
Median (%)	3.93	4.61	5.46		
Controls/cases	96/32	68/57	60/69		
Adjusted OR (95% CI)	1	2.94 (1.57, 5.62)*	3.87 (2.09, 7.34)*		
AA/EPA				0.007	1.07 (1.02, 1.13)
Median (%)	7.83	10.02	15.03		
Controls/cases	89/38	72/55	63/65		
Adjusted OR (95% CI)	1	1.16 (0.53, 2.52)	1.97 (1.05, 3.70)*		

RBC: red blood cell, SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, TFA: Trans fatty acid, EPA: C20:5 n-3, DHA: C22:6 n-3, AA: C20:4 n-6, n-3: n-3 PUFA, n-6: n-6 PUFA, n-3 index: C20:5 n-3 + C22:6 n-3 (in percentages of RBC fatty acids); OR (95%CI) for the logistic regression model adjusted for smoking status, alcohol consumption, tea consumption, education levels, ethnic group, age, BMI, gender,, residence, marital status, history of diabetes, family history of cancer, occupation, and physical activity; * Significant difference between tertiles ($P < 0.05$)

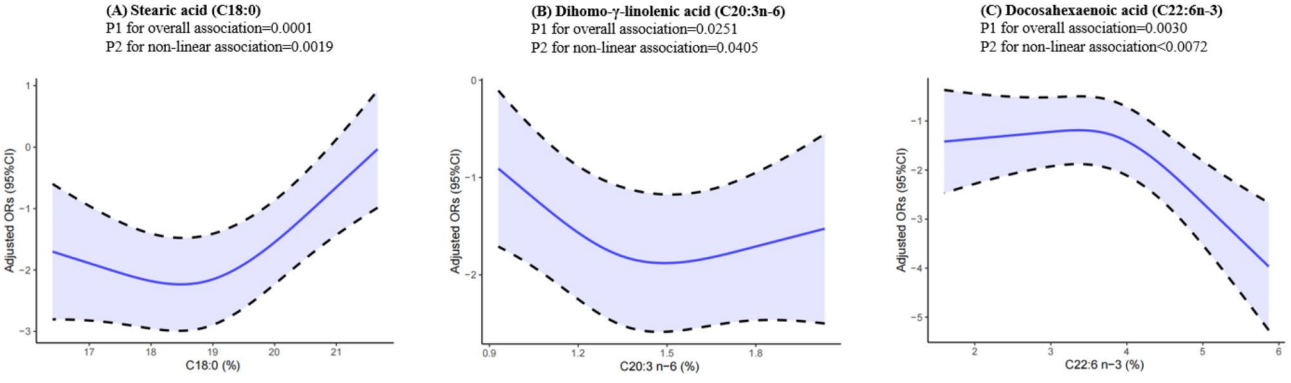

Fig. 1 Potential non-linear associations of some red blood cell fatty acids with odds ratios of EC assessed by restricted cubic spline model. Solid blue line indicated adjusted ORs for EC, and dashed line indicated 95% CIs. Adjustments were made for age, BMI, gender, ethnic group, residence, education level, marital status, history of diabetes, family history of cancer, occupation, physical activity, smoking status, alcohol consumption, and tea consumption; ORs, odds ratios; CIs, confidence intervals

adjusted for n-3 PUFA in the analysis of C20:4n-6 levels and vice versa. The results indicated that after controlling for n-3 PUFA, the association between C20:4n-6 and EC risk remained significant (Table S1). Furthermore, the results from RCS indicated a potential non-linear correlation ($p < 0.05$) between three specific fatty acids and EC risk (Fig. 1), while no significant linear correlation was found with others ($p > 0.05$). All the associations between fatty aicd composition levels in RBC and EC risk are presented in Fig. 2.

Discussion

Although previous epidemiological studies have explored the relationship between plasma fatty acids and EC risk [27, 28], their findings remain inconsistent. RBC fatty acids serve as a more stable biomarker compared to plasma or serum fatty acids, as they are less affected by

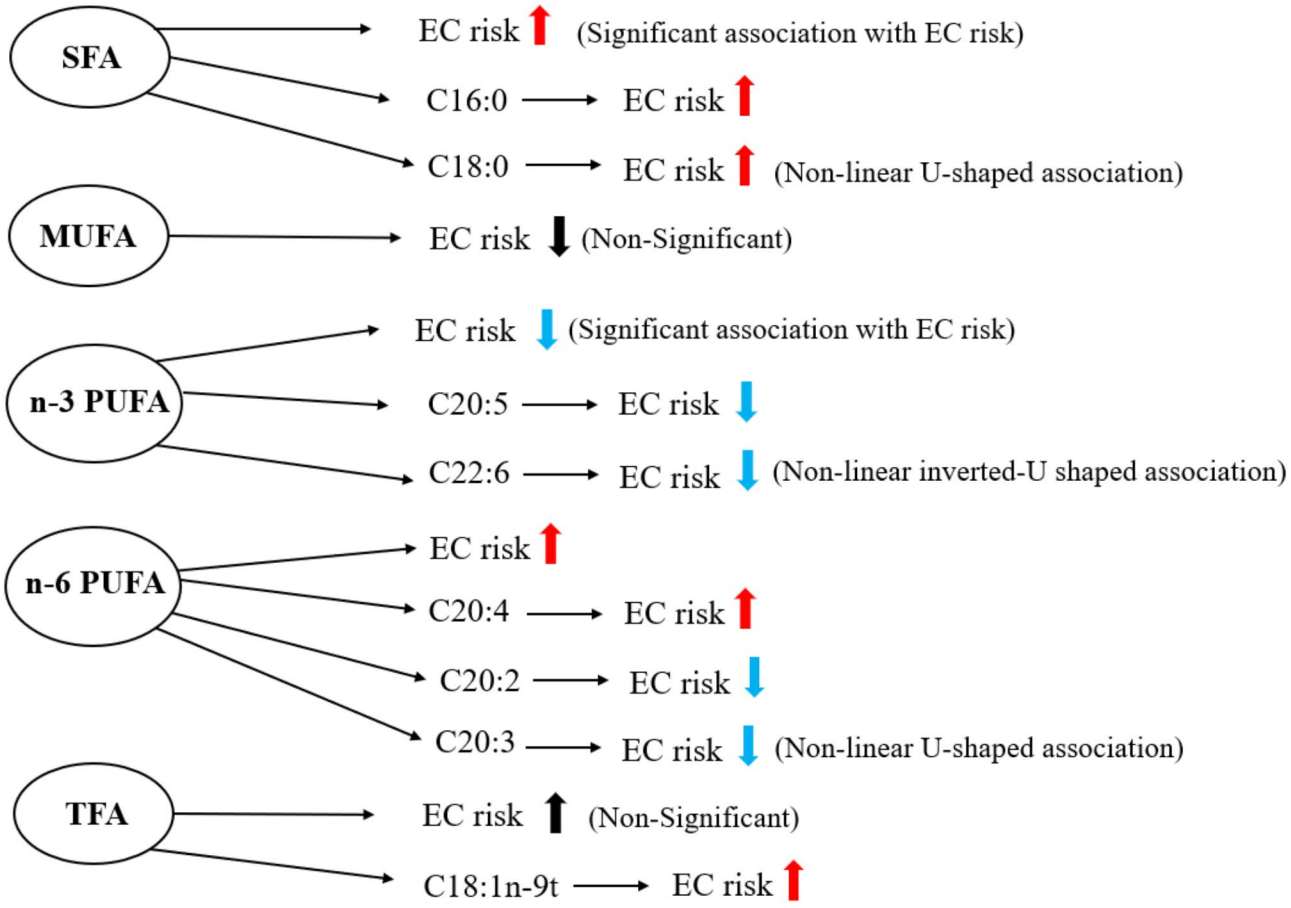

Fig. 2 A glance to all the associations which were found between red blood cell levels of fatty acid groups with the risk of EC

short-term dietary variations and more accurately reflect long-term fatty acid exposure [6]. According to Sun et al. [29], the correlation coefficient between RBC fatty acids and dietary SFA was weak ($r_s \leq 0.20$). Additionally, Nagashree et al. [30] reported that consuming coconut, a source of SFA, for three months had no significant effect on RBC SFA levels. However, our findings showed a positive association between total RBC SFA and higher odds of being an EC case. Notably, elevated C16:0 and C18:0 levels were associated with a two- to three-fold increase in EC risk, with a significant non-linear rising trend (Fig. 1A). These results align with a cross-sectional study by Zemanová et al. [28], which reported a similar positive association between plasma phospholipid SFA levels, particularly C16:0, and EC risk. However, Zuijdgeest-van Leeuwen et al. [27] conducted a case-control analysis of 45 healthy subjects and 35 EC patients and found no significant difference in plasma phospholipid SFA levels between the two groups, whereas plasma cholesterol esters SFA levels were higher in EC patients ($p < 0.05$). The inconsistencies in these findings may be explained by various factors, including differences in sample sizes, blood sample types, and dietary patterns across regions [31, 32].

In contrast, our study did not observe a significant association between total MUFA, C18:1n-9 levels, and EC risk. This finding contradicts Zemanova et al. [28], who reported significantly higher plasma MUFA levels in male EC patients. However, Hajizadeh et al. [33] found that MUFA-rich dietary patterns may reduce the risk of EC in a case-control study. Similarly, Bravi et al. [34] reported that dietary MUFA-rich olive oil was related with lower EC risk. The variability in these results underscores the potential influence of study design, population characteristics, and unaccounted confounding factors. Therefore, caution is warranted when interpreting these findings, and further research is needed to clarify the role of MUFA in EC risk.

Regarding n-6 PUFA, our study revealed a complex relationship. Total n-6 PUFA levels in RBC were positively associated with the increased odds of EC; however, associations between specific n-6 PUFA subtypes and EC risk varied. Higher levels of C20:4n-6 were associated with greater odds of being an EC case [OR_{T1-T3} = 2.07 (1.12–3.86)]. Previous research by Zhi et al. [17]

demonstrated that genes involved in C20:4n-6 metabolism are dysregulated in human esophageal squamous cell carcinoma. Specifically, cyclooxygenase-2, cytosolic phospholipase A2, 5-lipoxygenase, and 12-lipoxygenase were upregulated, while annexin-I and annexin-II were downregulated [17]. These alterations in gene expression suggest that the C20:4n-6 metabolism pathway may contribute to the pathogenesis and progression of esophageal squamous cell carcinoma. Similarly, a Mendelian randomization study by Larsson et al. [35] linked genetically predicted high plasma phospholipid C20:4n-6 levels to an increased EC risk. Meanwhile, higher plasma C20:4n-6 levels were also associated with a higher risk of lung or colorectal cancer [35]. However, a systematic review of observational studies found no association between C20:4n-6 exposure and certain cancers, such as breast or prostate cancer [36]. Additionally, we observed that C20:2n-6 and C20:3n-6 were associated with a significant reduction in EC risk, by approximately 67% and 59%, respectively [OR_{T3-T1}= 0.37 (0.19, 0.69) for C20:3n-6, OR_{T2-T1}= 0.41 (0.22, 0.75) for C20:3n-6]. However, the OR_{T3-T1} for C20:3n-6 did not reach statistical significance, likely due to a non-linear, U-shaped relationship (Fig. 1B). This suggests that moderate levels of C20:3n-6 (T2) may exert a protective effect by limiting the conversion of arachidonic acid into pro-inflammatory eicosanoids while simultaneously promoting the production of anti-inflammatory mediators [37]. At higher levels (T3), this protective effect may diminish, potentially due to metabolic competition or saturation effects [18]. These findings underscore the complexity of fatty acid metabolism and highlight the need for further research to elucidate the underlying mechanisms.

Consistent with previous findings [22], our results further support the protective role of n-3 PUFA against EC. Higher RBC total n-3 PUFA levels, particularly DHA and EPA, were associated with lower odds of being an EC case, with a significant non-linear decreasing trend for DHA (Fig. 1C). These findings align with previous dietary studies [22], such as the NIH-AARP Diet and Health study [38], which linked higher dietary intake of DHA and EPA, mainly from fish, to a reduced EC risk. In addition to epidemiological studies [11, 15, 39], in vivo and in vitro experiments suggest that n-3 PUFA exerts anti-cancer effects through multiple pathways. An in vitro study investigating the effects of n-3 PUFA on carcinogenic keratinized cell lines reported that n-3 PUFA activates the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway via the epidermal growth factor receptor (EGFR), leading to apoptosis and reduced proliferation [40]. Another in vivo study discovered that the antioxidant properties of n-3 PUFA were manifested through activation of the nuclear factor (erythroid-derived 2)-related factor 2 pathway in immortalized mouse Schwann cells [41]. Furthermore, Eltweri et al. [42], demonstrated that the in vitro anti-cancer effects of n-3 PUFA on OE33 and OE19 esophageal cells were associated with the downregulation of p53 and vascular endothelial growth factor (VEGF) in both cell lines. Despite these promising results, some studies have reported no significant association between blood n-3 PUFA and EC risk [27, 28], highlighting the need for further research to confirm these observations.

While total TFA levels were not significantly associated with EC risk, higher RBC C18:1 trans-9 levels were linked to increased odds of being an EC case [OR_{T1-T3} = 2.02 (1.13–3.65)]. Given that C18:1 trans-9 is commonly found in industrially processed foods and has been implicated in cardiovascular diseases and certain cancers [43, 44], this finding underscores the need for dietary regulation of industrial trans fats.

On the other hand, fatty acid indices demonstrated strong associations with EC risk. The n-6/n-3 PUFA ratio and AA/EPA ratio were positively associated with higher odds of EC, whereas the n-3 index showed a significant inverse relationship. These findings are supported by previous research. For example, Wang et al. [45] reported that as RBC n-6 PUFA levels and the n-6/n-3 PUFA ratio increased, obesity risk increased, while high RBC n-3 PUFA levels reduced obesity risk. Lira et al. [46] investigated the association between plasma PUFA and oxidative stress biomarkers in breast cancer, suggesting that n-3 PUFA regulates key processes in breast cancer development through inflammation and oxidation. Simopoulos et al. [47] showed that while eicosanoids derived from AA are bioactive in very small amounts, excessive production can contribute to atheroma and thrombus formation, allergic and inflammatory disorders (particularly in susceptible populations), and increased cell proliferation. Pro-inflammatory eicosanoids are primarily produced from C20:4n-6, whereas anti-inflammatory eicosanoids are more likely to be induced by DHA or EPA [48]. Beyer et al. [49] and Zúñiga-Hernández et al. [50] indicated that DHA, EPA, and their specialized lipid mediators with pro-resolving properties (including maresin, resolvin, and protectin families) modulate inflammatory processes through various biological actions. Specifically, these mediators can inhibit the activation of the NF-κB signaling pathway, thereby reducing the expression of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β [42, 48]. Additionally, DHA and EPA are known to activate PPAR-γ, which suppresses COX-2 and iNOS expression, mitigating inflammation [47, 49, 50]. These mechanisms contribute to the protective role of DHA and EPA against carcinogenesis by reducing oxidative stress, modulating immune responses, and promoting apoptosis in cancer cells. Given these benefits, accumulating evidence suggests that increasing n-3 PUFA intake,

particularly DHA and EPA, while limiting excessive consumption of n-6 PUFA, may be an effective dietary strategy for lowering EC risk.

The current research has several strengths. First, it is the first research to evaluate the association between RBC fatty acids and EC risk, providing novel insights into this potential relationship. Second, RBC samples serve as a reliable and reproducible biomarker for tissue fatty acid composition, with lower biological variability and measurement error compared to plasma and serum fatty acids, which are more susceptible to variations caused by sample handling and storage conditions [6, 51]. Consequently, RBC fatty acid composition offers an objective reflection of long-term dietary intake. Third, to minimize the influence of confounding variables, each case was matched by gender and age, and the statistical analysis was adjusted for multiple potential confounders. Despite these strengths, certain limitations should be acknowledged. As a case-control study, this research is susceptible to reverse causality, preventing the establishment of a definitive cause-and-effect relationship. Additionally, dietary changes resulting from cancer-related symptoms may have influenced RBC fatty acid composition. To confirm these findings and explore potential causal mechanisms, prospective studies are needed. Furthermore, this study did not collect detailed dietary data that could impact RBC fatty acids, potentially introducing residual confounding. Lastly, given the relatively small sample size and the study's focus on a single metropolitan area, further research in larger and more diverse population is necessary to validate these findings.

Conclusion

In conclusion, our study highlights the potential role of RBC fatty acids as biomarkers for understanding the relationship between lipids and EC risk. Specifically, higher RBC levels of total n-3 PUFA, DHA, EPA, the n-3 index, and C20:2n-6 were associated with lower odds of being an EC case, indicating a potential protective effect. Conversely, elevated RBC levels of total SFA, C18:0, C16:0, C20:4n-6, the AA/EPA ratio, and the n-6/n-3 PUFA ratio were associated with higher EC risk. No significant associations were observed between total MUFA, total TFA, and EC risk. These findings contribute to the growing evidence supporting dietary fatty acid modifications as a potential strategy for EC prevention and warrant further investigation in larger, prospective studies.

Abbreviations

AA/C20	4n-6:arachidonic acid
BMI	Body mass index
CVs	Coefficients of variability
DHA/C22	6n-3:docosahexaenoic acid
EDTA	Ethylenediaminetetraacetic acid
EC	Esophageal cancer
EPA/C20	5n-3:eicosapentaenoic acid
EGFR	Epidermal growth factor receptor
ERK1/2	Extracellular signal-regulated kinase 1 and 2
FAMEs	Fatty acid methyl esters
GC	Gas chromatography
MUFA	Monounsaturated fatty acids
OR	Odd ratio
PUFA	Polyunsaturated fatty acids
RBC	Red blood cell
RCS	Restricted cubic spline, SFA: saturated fatty acids
T	Tertile
TFA	Trans fatty acids
VEGF	Vascular endothelial growth factor

Supplementary Information

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Supplementary Material 1

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Author contributions

The list of contributions from each author is as follows: Design and conceptualization were provided by HY and YW; study was conducted by YC. HY and YC were responsible for gathering literature and analyzing data. The text was drafted by YW and HY, with edits contributed by QS and FX; FX and YW supervised the project. The final manuscript was approved by all authors.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Conflict of interest

The authors declare no conflicts of interest.

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