

RESEARCH ARTICLE

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Nutrient Patterns and Risk of Breast Cancer among Iranian Women: a Case- Control Study

Samira Sadat Fereidani¹, Hasan Eini-Zinab¹, Zeinab Heidari¹, Saba Jalali¹,
Fateme Sedaghat², Bahram Rashidkhani^{1*}

Abstract

Objective: To explore the role of nutrient patterns in the etiology of breast cancer (BCa) among Iranian women. **Methods:** The study included 134 newly diagnosed cases of BCa and 267 hospitalized controls. A validated semi-quantitative food frequency questionnaire (FFQ) was used to assess dietary intake. Nutrient patterns were obtained using principal component analysis using Varimax rotation and logistic regression was performed to estimate breast cancer risk. **Results:** We identified 4 major nutrient patterns. First was high in consumption of vitamins B1, B2, B3, B5, B6, B9, C, magnesium, iron, carbohydrate, fiber, selenium, zinc, protein, potassium, and calcium. The second nutrient pattern included Vitamins B12, A and cholesterol, while the third featured vitamin D, EPA and DHA. The fourth was characterized by vitamin E, MUFA and saturated fatty acids. After adjusting for age, patterns 1 and 3 were associated with a lower risk of BCa (OR=0.51, 95% CI: 0.33- 0.80, P=0.003, OR=0.64, 95% CI: 0.42- 0.98, P= 0.04 respectively). However, after further adjustment for all confounders in multivariate analysis, the association remained significant only for pattern 1 (OR=0.52, 95% CI: 0.32- 0.82, P=0.006). **Conclusion:** Adherence to a nutrient pattern rich in vitamin B, minerals and fiber is associated with a lower risk of breast cancer.

Keywords: Breast cancer- diet- food- nutrients- factor analysis

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Introduction

Breast cancer (BCa) is the most common cancer among women worldwide (Ghoncheh et al., 2016). Among Iranian women, BCa is the third cause of death and age at the onset of BCa has been decreased about ten years (from 40.0 to 30.0 years) (Naderimagham et al., 2014; Abedi et al., 2016; Vahid et al., 2018). Diet is a recognized modifiable factor associated with most cancers (Kotepui, 2016). Most epidemiological studies on diet and BCa have largely centered on single food items or nutrients. Defining the overall nutrient patterns may be more important in BCa etiology than assessing the effects of nutrients in isolation due to complexity and potential synergistic interactions between individual nutrients and food (Demetriou et al., 2012; Chang et al., 2017). Findings of recent studies on nutrients patterns and BCa showed that patterns which were higher in fruits and vegetables (that contain vitamins and minerals) and lower in animal products (which contain cholesterol and saturated fatty acids) and refined grain have been associated with a reduced risk of breast cancer (Edefonti et al., 2008; Ronco et al., 2010). Studying the links between dietary patterns and BCa is especially relevant for Middle-Eastern

populations because of their mounting prevalence of a particular type of BCa (affected patients are at least a decade younger with more advanced stage of disease at first presentation), and their diets' unique characteristics (Vahid et al., 2018). Therefore, the aim of this study was to detect Iranian women nutrient patterns and its association with breast cancer risk.

Materials and Methods

Study design and sample

We recruited 136 women ≥ 30 year old who were newly (<6 months) diagnosed with histologically confirmed BrCa, at the 2 referral hospitals in Tehran (Iran), between September 2015 and February 2016. Fewer than 8% of subjects approached for the interview refused to participate. The control group consisted of 272 women of similar age who were admitted to the same hospital for a wide spectrum of non-neoplastic diseases that were unrelated to smoking, alcohol abuse, and long-term diet modification. Conditions among controls contained disk disorders, traumas and orthopedic conditions, acute surgical conditions and eye, nose, ear, or skin disorders. Controls were matched to cases on age (within 5 years).

¹Department of Community Nutrition, ²Department of Basic Medical Sciences, Faculty of Nutrition Sciences and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

*For Correspondence: rashidkhani@yahoo.com

7 participants were excluded from the analysis because their reported energy intakes were outside of the range of ± 3 standard deviation (SD) from the mean energy intakes of the population ($n=5$ controls, 2 cases). Data from 134 cases and 267 controls remained in the final analysis. All protocols and procedures of the current study were approved by the Shahid Beheshti Ethics Committee. Written informed consent was obtained from all patients.

Dietary assessment

Subjects' dietary intake during the year prior to diagnosis for cases (or interview for controls) was measured based on interview using a valid and reliable semi-quantitative 168-item food frequency questionnaire (FFQ) (Asghari et al., 2012). Subjects were asked to specify their frequency of consumption for each food item on a daily, weekly, monthly or yearly basis. Intakes were then transformed to daily frequencies and a manual for household measures was used to convert intake frequencies to grams of food intake/day (Ghafarpour et al., 1999). Energy and nutrient content of foods were computed by the USDA and Nutrients Composition of Iranian Foods. The latter was used for foods or food ingredients that were not available in the USDA database (e.g., traditional breads).

Other measurements

Trained dietitians administered all measurements and other questionnaires during the same interview. Weight was measured to the nearest 0.1 kg using a digital scale (Seca, Germany) while subjects were wearing light-weight clothing and no shoes. Height was measured to the nearest 0.5 cm, using a tape meter fixed to a wall. Body mass index (BMI) was then calculated as a ratio of weight (kg) to the square of height (m). Physical activity assessed with a valid and reliable questionnaire (Aadahl and Jorgensen, 2003). Subjects' socio-demographic, lifestyle, and clinical information gathered via questionnaire, including age (years), age at menarche (years), age at first pregnancy (years), miscarriage history (yes, no), number of live births (number), breastfeeding history (month), menopausal status (pre-menopause, post-menopause), education, oral contraceptive pills consumption history, history of hormone replacement therapy (yes, no), history of benign breast diseases (yes, no), family history of cancer (yes, no), breast cancer family history (yes, no), bra wearing during the day (yes, no) and at night (yes, no) (Hsieh and Trichopoulos, 1991; Rios et al., 2016), marital status, smoking (yes, no), supplement intake (yes, no); If yes, the complementary information about dose and frequency, and NSAIDs use (yes, no).

Statistical analysis

Data were analyzed using the statistical software package SPSS version 21. Principal component analysis was applied to determine major nutrient patterns based on the twenty-six predefined nutrients and four explainable factors were kept. The chi-square for Bartlett's test of sphericity was statistically significant ($p < .001$), and the Kaiser-Meyer-Olkin measure of sampling adequacy was a score of 0.85 showing that the

correlation among the nutrients was strong enough for a factor analysis. Varimax rotation was then employed to clarify the factors' structures and make it more easily to describe. The number of meaningful components to retain from the total number of extracted nutrient patterns depended mainly on the assessment of scree plots and components' interpretability (4 nutrients pattern). Factor loadings are correlation coefficients between nutrient and nutrient patterns; a positive loading in a factor shows a direct association with the factor, whereas a negative loading means that the nutrient is inversely associated with the factor. The factor score for each pattern was computed by adding intakes of nutrients weighted by factor loadings, and each participant was then allotted a score for each of the identified patterns. Scores for 4 nutrient patterns identified in this study were then divided into 2 categories (low, high) based on the medians of the controls. In order to compare general characteristics of all participants, t-test and Mann-Whitney test were used for continuous variable with normal and non normal distribution respectively, and Pearson Chi-Square test were used for categorical variables. To compare general characteristics across the median categories of nutrient pattern scores, ANOVA and χ^2 tests were applied as proper. The relationship between major nutrient patterns and breast cancer risk was assessed using logistic regression analysis in different models, controlling for age (years) in model I and for age (years), height (cm), age of first pregnancy (years), cancer family history in first or second-degree relatives (yes/no), use of vitamin D supplement (yes/no) in model II. Results are exhibited as odds ratios and 95% confidence intervals.

Results

Characteristics of the participants by case-control status are shown in Table 1. Cases were slightly older and reported somewhat higher age at first pregnancy and cancer family history (than controls). Controls reported higher intake of vitamin D supplements.

Table 2 shows four major nutrient patterns were extracted using principal component analysis. First nutrient pattern was high in consumption of Vitamins B1, B2, B3, B5, B6, B9, C, Magnesium, Iron, carbohydrate, fiber, Selenium, zinc, protein, potassium, and calcium. Second nutrient pattern included Vitamins B12, A and cholesterol. Third nutrient pattern contained Vitamin D, EPA and DHA, and fourth nutrient pattern consisted of Vitamin E, MUFA fatty acid and saturated fatty acid. Altogether, these factors described 76.98% of the total variance.

Selected characteristics of participants within medians of nutrient pattern scores are shown in Table 3. In comparison with participants in lower median of factor 1, those in higher median were significantly taller ($P = 0.05$). However, we did not find any significant associations for other variables across nutrient pattern scores.

The odds ratios for breast cancer risk across median of nutrient pattern scores are shown in Table 4. In model 1, after adjustment for age, a significant inverse association was observed between factor 1 and 3 and breast cancer

Table 1. Characteristics of Iranian Breast Cancer Cases (134) and Controls (267)

Variables	Cases	Controls	P-value*
Age (year) ¹	49.49 ± 10.68	47.13 ± 10.08	0.03
Weight (kg) ²	73.00 (18.25)	71.30 (17.00)	0.42
Height (cm) ²	158.00 (7.25)	158.00 (7.65)	0.04
BMI (kg/m ²) ²	29.47 (7.30)	28.54 (7.65)	0.11
WHR1	0.90 ± 0.08	0.90 ± 0.10	0.66
Total energy intake(kcal) ²	2467.77 (89.015)	2549.63 (1068.66)	0.07
Physical activity score(MET/hr/day) ²	32.10 (6.15)	31.47 (6.03)	0.64
Menarche age (year) ²	14.00 (2.00)	13.00 (1.00)	0.53
Marriage age (year) ²	19.00 (7)	18.00 (4)	0.06
Breastfeeding time (month) ²	36.0 (53)	44.0 (47.3)	0.08
First pregnancy age (year) ²	20.00 (8)	20.00 (5)	0.04
OCP use ³			0.26
Yes	67 (50)	149 (56)	
No	67 (50)	117 (44)	
Menopausal status ³			0.10
Pre menopause	62 (47)	151 (57)	
Post menopause	72 (53)	114 (43)	
Smoking status ³			1.00
Yes	4 (3)	9 (3)	
No	129 (97)	257 (97)	
Education levels ³			0.31
Illiterate	13 (10)	24 (9)	
Less than diploma	55 (42)	134 (50)	
Diploma and more	62 (48)	108 (41)	
Family cancer history ³			0.03
Yes	41 (31)	55 (21)	
No	93 (69)	211 (79)	
Day bra use ³			0.01
Yes	122 (91)	217 (82)	
No	12 (9)	49 (19)	
Night bra use ³			0.10
Yes	106 (79)	190 (71)	
No	28 (21)	76 (29)	
Vitamin D consumption ³			0.02
Yes	20 (15)	65 (24)	
No	114 (85)	201 (76)	

*p≤0.05 considered as significant. Statistically significant p-values are reported in bold. ¹Mean ± SD was reported for variables with normal distribution. ²Median (IQR) was reported for variables without normal distribution. ³Number (percent) was reported for qualitative variables.

risk (OR=0.51, 95% CI: 0.33- 0.80, P=0.003, OR=0.64, 95% CI: 0.42- 0.98, P= 0.04 respectively). In model 2, after adjustment for age, height, age of first pregnancy, cancer family history and vitamin D supplementation, participants in the higher category of factor 1 showed 48% decrease in risk of breast cancer (OR=0.52, 95% CI: 0.32- 0.82, P=0.006). However, the significant association between factor 3 and BCa was disappeared after further adjustment for all covariates in model 2. No significant association was found between factor 2 and factor 4 and

Table 2. Factor Loading Matrix for the Nutrients Representing the Three Major Nutrient Patterns from Food Frequency Questionnaire data of Cases (134) and Controls (267)

Nutrients	Factor 1	Factor 2	Factor 3	Factor 4
Vitamin B ₁ (mg)	0.928		0.121	0.176
Vitamin B ₉ (µg)	0.887			0.146
Magnesium (mg)	0.886	0.216		
Vitamin B ₃ (mg)	0.869	0.153	0.194	0.112
Fe (mg)	0.864			0.173
Carbohydrates (gr)	0.853	0.108		
Fiber (gr)	0.832	-0.145		
Selenium (µg)	0.818	0.206		0.182
Vitamin B ₆ (mg)	0.818	0.324	0.242	0.166
Zinc (mg)	0.813	0.417		0.140
Protein (gr)	0.794	0.410	0.277	0.226
potassium (mg)	0.792	0.332	0.143	0.123
Vitamin B ₅ (mg)	0.729	0.507	0.186	0.110
Vitamin B ₂ (mg)	0.675	0.618	0.103	0.129
Calcium (mg)	0.609	0.444	0.125	0.203
Vitamin C (mg)	0.572	0.191		
Vitamin B ₁₂ (µg)	0.111	0.878		
Vitamin A (µg)	0.256	0.795		
Cholesterol (mg)	0.222	0.580	0.181	0.252
EPA 20:5 (gr)	0.142		0.982	
Vitamin D (µg)	0.149		0.980	
DHA 22:6 (gr)	0.162		0.973	
Fatty acid-MUFA (gr)	0.276	0.141		0.921
Vitamin E-alpha tocopherol (mg)	0.121			0.916
Fatty acid- saturated (gr)	0.412	0.411	0.128	0.570
Percentage of Variance explained	41.340	13.910	12.450	9.270

risk of breast cancer in all models.

Discussion

In the present study, 4 major nutrient patterns have been identified. Factor 1 included vitamins B1, B2, B3, B5, B6, B9, C, Magnesium, Iron, carbohydrate, fiber, Selenium, zinc, protein, potassium, and calcium. Factor 2 was characterized by vitamins B12, A and cholesterol. Third nutrient pattern contained vitamin D, EPA and DHA, and fourth nutrient pattern consisted of vitamin E, MUFA fatty acid and saturated fatty acid. After adjusting for age, factor 1 and factor 3 were significantly associated with lower risk of BCa. However, this association was only significant between factor 1 and BCa after adjusting for all confounders in multivariate model.

To our knowledge there are only two studies assessing the association between nutrient patterns and breast cancer risk. In first study in Italy, animal product pattern (animal protein, animal fat, saturated fatty acids and cholesterol) and unsaturated fat pattern (vegetable fat, monounsaturated and polyunsaturated fatty acids and vitamin E) were inversely associated with BCa (Edefonti et al., 2008). However, in second study in Uruguay, High-Meat pattern (characterized by high intake of protein, saturated fat,

Table 3. Selected Participants' Characteristics According to Nutrient Pattern Scores in a Case-control Study of Breast Cancer in Iran

Characteristic	Factor 1				Factor 2				Factor 3				Factor 4						
	Mean	SD	Low ¹	High ²	Mean	SD	Low ¹	High ²	Mean	SD	Low ¹	High ²	Mean	SD	Low ¹	High ²	p*		
Age	48.4	10.3	47.2	10.2	48.7	10.5	47.0	9.9	0.09	48.2	10.7	47.5	9.8	0.55	47.5	10.4	48.2	10.1	0.52
Height	158.1	5.4	159.3	7.1	158.8	6.1	158.5	6.3	0.69	158.2	6.4	159.1	5.9	0.19	158.9	6.3	158.4	6.1	0.16
BMI	29.4	5.6	29.3	5.3	29.7	5.6	29.1	5.3	0.34	29.3	5.5	29.5	5.4	0.56	29.2	5.5	29.5	5.5	0.36
Age of first pregnancy	19.9	6.2	20.1	6.1	20.0	6.1	20.0	6.3	0.44	20.6	6.1	19.3	6.2	0.10	19.5	6.4	20.5	5.9	0.20
Parity	2.6	1.7	2.8	1.9	2.9	1.9	2.5	1.6	0.06	2.7	1.8	2.6	1.7	0.70	2.7	1.7	2.7	1.9	0.91
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Menopausal status																			
Premenopausal	115	52	100	56	107	51	108	56	0.36	115	53	100	54	0.92	105	54	110	53	0.92
Postmenopausal	108	48	78	44	85	44	85	44		101	47	85	46		89	46	97	47	
Cancer family history																			
Yes	55	25	41	23	52	25	44	23	0.64	55	26	41	22	0.48	49	25	47	23	0.64
No	168	75	136	77	156	75	148	77		161	74	143	78		145	75	159	77	
OCP use																			
Yes	121	54	95	54	116	56	100	52	0.48	114	53	102	55	0.61	111	57	105	51	0.22
No	102	46	82	46	92	44	92	48		102	47	82	45		83	43	101	49	
Smoking																			
Yes	9	4	4	2	9	4	4	2	0.26	6	3	7	4	0.58	5	3	8	4	0.57
No	213	96	173	98	199	96	187	98		209	97	77	96		189	97	197	96	
Vitamin D supplementation																			
Yes	45	20	40	23	62	152	79	41	1.00	146	79	39	21	1.00	159	77	48	23	0.33
No	178	80	138	77	164	79	44	21		170	79	46	21		157	81	37	19	

Abbreviation, BMI: Body Mass Index, OCP: Oral Contraceptive Pill; ¹lower than median of controls; ²higher than median of controls; *P of comparison between groups, using t-test, Mann-Whitney or Pearson Chi-Square

Table 4. Odds Ratios (95% Confidence Intervals) for Breast Cancer Risk Across Median of Nutrient Pattern Scores*

Scored pattern	Case/control	OR ¹ (95%CI)	OR ² (95%CI)
Factor 1			
Low ³	89/134	1.0 (reference)	1.0 (reference)
High ⁴	45/133	0.51 (0.33- 0.80)	0.52 (0.32- 0.82)
P-value		0.003	0.006
Factor 2			
Low	74/134	1.0 (reference)	1.0 (reference)
High	60/133	0.84 (0.55- 1.28)	0.81 (0.52- 1.27)
P-value		0.43	0.36
Factor3			
Low	82/134	1.0 (reference)	1.0 (reference)
High	52/133	0.64 (0.42- 0.98)	0.66 (0.42- 1.04)
P-value		0.04	0.07
Factor 4			
Low	60/134	1.0 (reference)	1.0 (reference)
High	74/133	1.22 (0.80- 1.86)	0.13 (0.72- 1.77)
P-value		0.33	0.57

¹Adjusted for age; ²Adjusted for age, height, age of first pregnancy, cancer family history and Vitamin D supplement; ³Lower than median of control; ⁴Higher than median of control; *OR were calculated using logistic regression analysis.

monounsaturated fat, linoleic acid, alpha-linolenic acid and cholesterol) was directly associated with BCa risk. However, the second pattern (glucose, fructose, vitamin C and vitamin E) displayed a protective effect (Ronco et al., 2010). The common feature of these two studies and our study is the protective role of vitamin C. In the prospective cohort study among 3405 women with breast cancer, dietary vitamin C intake was inversely associated with breast cancer-specific mortality (HR : 0.74: Ptrend=0.04) (Harris et al., 2013). Vitamin C may prevent cancer cell proliferation through the suppression of H₂O₂ and its ROS products (Frei, 1994; Willcox et al., 2004). Moreover at high doses, vitamin C may also function as a pro-oxidant causing cytotoxicity to cancer cells (Chen et al., 2008; Ullah et al., 2011).

In our study, third nutrient pattern (loaded heavily on Vitamin D, EPA and DHA) was negatively associated with BCa risk. Majority of the vitamin D studies support the inverse association between vitamin D level and BCa risk, and retrospective and prospective epidemiologic studies revealed that vitamin D deficiency is associated with increased breast cancer risk (Atoum and Alzoughool, 2017). Experimental evidence has also mentioned a possible effect of vitamin D on cancer risk. In studies in mice with various cancers, vitamin D has been shown to have some characteristics that might inhibit the development of cancer, including promoting cellular differentiation, stimulating cell death (apoptosis), diminishing cancer cell growth and tumor blood vessel formation (angiogenesis) (Deeb et al., 2007; Thorne and Campbell, 2008). In addition, Marine fatty acids, particularly the long-chain eicosapentaenoic and docosahexaenoic acids, have been consistently shown to inhibit the proliferation of breast cancer cell lines in vitro and to reduce the risk and progression of these tumors in animal experiments (Terry et al., 2003). A meta-analysis of 16 prospective

cohort studies examining marine omega-3 intake suggests a reduction in breast cancer risk when individuals with highest intakes are compared with those with lowest intakes of marine PUFA (EPA, docosapentaenoic acid, and DHA) in the diet (Zheng et al., 2013).

This study has several strengths; firstly this is the first study to evaluate the association of nutrient patterns with BCa in Middle-East. Secondly, considering all demographic and lifestyle confounders and adjusting for these factors in our analysis reduced the probability of residual confounding bias. The third strength of this study was high participation rate (>90%) which reduces the probability of selection bias in the present study.

This study has several limitations. First, individuals with BCa would probably recall their diets differently than controls as a result of their disease status (recall bias). However since we recruited incident cases, the probability of recall bias would be minimized. Second, arbitrary decisions in factor analysis utilized in this study (included determining the number of factors, eigenvalue and method of rotation) could affect the results. Third, the sample size of the current study was relatively small and this could reduce the precision of the study. Finally, As in most case-control studies, a selection bias is also possible. However, Selection bias should be limited, on account of the high participation rate and of the comparable catchment areas of cases and controls.

In conclusion, the first nutrient pattern (comprising mainly antioxidants), and third nutrient pattern (containing vitamin D, EPA and DHA) showed an inverse association with BCa among Iranian women. However, case-control studies may prove an association, but they do not guarantee causation. As a result, these findings need to be rechecked in future prospective studies for etiological purposes to draw more conclusive results.

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