British Journal of Nutrition (2020), 124, 832–843 doi:10.1017/S0007114520001737

Dietary patterns and relative expression levels of *PPAR-γ*, *VEGF-A* and *HIF-1α* genes in benign breast diseases: case–control and consecutive case-series designs

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(Submitted 28 April 2020 – Accepted 7 May 2020 – First published online 14 May 2020)

Abstract

We aimed to study dietary patterns in association with the relative expression levels of *PPAR-γ*, vascular endothelial growth factor-A (*VEGF-A*) and hypoxia-inducible factor-1α (*HIF-1α*) in women with benign breast disease (BBD). The study design was combinative, included a case-series and case–control compartments. Initially, eligible BBD patients (*n* 77, aged 19–52 years old) were recruited at Nour-Nejat hospital, Tabriz, Iran (2012–2014). A hospital-based group of healthy controls was matched for age (*n* 231, aged 20–63 years old) and sex. Dietary data were collected using a valid 136-item FFQ. Principal component analysis generated two main components (Kaiser–Meyer–Olkin = 0·684), including a Healthy pattern (whole bread, fruits, vegetables, vegetable oils, legumes, spices, seafood, low-fat meat, skinless poultry, low-fat dairy products, nuts and seeds) and a Western pattern (starchy foods, high-fat meat and poultry, high-fat dairy products, hydrogenated fat, fast food, salt and sweets). High adherence to the Western pattern increased the risk of BBD (OR_{adj} 5·59; 95 % CI 2·06, 15·10; $P < 0·01$), whereas high intake of the Healthy pattern was associated with a 74 % lower risk of BBD (95 % CI 0·08, 0·81; $P < 0·05$). In the BBD population, the Western pattern was correlated with over-expression of *HIF-1α* (r_{adj} 0·309, $P < 0·05$). There were inverse correlations between the Healthy pattern and expressions of *PPAR-γ* (r_{adj} −0·338, $P < 0·05$), *HIF-1α* (r_{adj} −0·340, $P < 0·05$) and *VEGF-A* (r_{adj} −0·286, $P < 0·05$). In conclusion, new findings suggested that the Healthy pattern was associated inversely with the risk of BBD, and this could be correlated with down-regulation of *PPAR-γ*, *VEGF-A* and *HIF-1α* genes, which might hold promise to preclude BBD of malignant pathological transformation.

Key words: **Benign breast disease: Dietary patterns: *PPAR-γ*: Vascular endothelial growth factor-A: Hypoxia-inducible factor-1α**

Benign breast diseases (BBD) are prominent pathological indicators of increased risk of breast cancer development[1]. BBD is a group of breast diseases that usually emerge in the reproductive age of women[2] and consists of multiple histological sub-types of non-proliferative diseases, proliferative diseases without atypia (raised risk of breast cancer: 1·3- to 1·9-fold) and atypical proliferative diseases (raised risk of breast cancer: 3·9- to 13-fold)[3]. The aetiology of BBD is multi-factorial,

Abbreviations: BBD, benign breast disease; ERK, extracellular signal-regulated kinase; *HIF-1α*, hypoxia-inducible factor-1α; MEK1, mitogen-activated protein kinase-1; PCA, principal component analysis; *VEGF-A*, vascular endothelial growth factor-A.

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resulting mainly from a complex interplay of hereditary and environmental risk factors[4]. Several lines of epidemiological evidence described the significant contributing role of dietary factors to BBD incidence in different populations[2,3,5,6]. Galván-Portillo *et al.*[2] suggested that eating fruits (citrus and non-citrus), dietary sources of lignans and dairy products might decrease the risk of BBD. It has been shown that dietary carotenoid intake in adolescents is correlated with a lower risk of BBD[6]. Adult fat intake is associated with the development of BBD[3]. Moreover, adherence to Dietary Approaches to Stop Hypertension (DASH) or Healthy/Mediterranean patterns might inversely be associated with breast cancer risk[7,8]. Zhang *et al.*[9] demonstrated that adherence to 'vegetable-fruit-soy-milk-poultry-fish' dietary pattern might reduce the risk of breast cancer. However, Western diet is one of the most important dietary patterns enhancing the risk of breast cancer[8,10,11]. Studies usually use two main approaches to evaluate the nutritional status of subjects with the use of diet; they either use single nutrient intake data or determine the dietary pattern, which is an estimate of the individual's whole diet[12]. People usually eat a combination of foods; therefore, evaluating the diet based on dietary patterns can provide many informative data in association with the risk assessment for public health concerns[13]. Genetic factors are fundamentally involved in the initiation of pathological transformation and make breast cells susceptible to rapid growth, thus promoting breast tumorigenesis[4]. Some tumour-associated biological events are necessary to be dysregulated to enable the cancer cells to sprout out of the benign tissue[14]. Angiogenesis is an essential process for providing circulation for rapidly growing cells[14]. The *hypoxia-inducible factor-1α* (*HIF-1α*) is modulated by cellular hypoxia and consequently could promote tumour development[15]. Hypoxia is a tumour-promoting condition induced by fast metabolism of growing cells[16]. *HIF-1α* is known as a tumorigenic factor and is correlated with higher tumour grade, involving breast cancer invasion and metastasis to the lymph nodes[17]. In a study by Gary *et al.*[18], microvessel density, which reflects angiogenesis in the tumour tissue, increased the rate of progression of BBD to breast cancer. Thus, this might have an important role in the transformation of phyllodes tumours at all stages. Subjects with benign prostatic hyperplasia, who had undergone prostatic surgery, had over-expression of *HIF-1α* in dissected tissue specimens[19]. *HIF1-α* is a well-known activator of the *vascular endothelial growth factor (VEGF)* gene at hypoxia[20]. *VEGF* is subsequently released from human breast cancer cells and promotes angiogenesis, lymphangiogenesis, endothelial proliferation, permeability of the vessel and formation of new vessels[21]. Ławicki *et al.*[22] reported that serum *VEGF* could be a predictor of breast cancer, particularly in the early stages of malignancy. *PPAR* is one of the most important transcriptional factors that regulates gene expression. *PPAR-γ* plays controversial roles in cells, serving as a tumorigenic and anti-tumorigenic factor, depending on the cell type and concentration of *PPAR-γ* ligands[23]. *HIF-1α* could interfere in the expression of *PPAR-γ* gene[24]. Moreover, high levels of *PPAR-γ* stimulate angiogenesis in carcinoma through increasing *VEGF* expression[25]. Dietary PUFA modulate *PPAR-γ* and affect signalling pathways related to cell proliferation[26,27]. *PPAR-γ* is responsible for the overall regulation of insulin sensitivity and glucose and lipid homeostasis[28]. Hence, this is a field of nutri-genomics to explore food parameters in association with the transcription of genes involved in the initiation of carcinogenesis[12]. Therefore, the objective of the present research was to explore the relationship between dietary patterns and relative expression levels of *PPAR-γ*, *VEGF-A* and *HIF-1α* in BBD patients.

Materials and methods

Study population

The present study was conducted in two sets including a primary consecutive case series followed by a case–control compartment. The importance of conducting case–control analyses (cases *n* 77, controls *n* 231) was to find out and identify the common dietary pattern which could associate with BBD risk. A pilot design, part of a consecutive case series, ran in a population of women newly diagnosed with BBD with no malignancy background, conducted to explore the associations between pre-determined dietary patterns and fold changes in expression in *PPAR-γ*, *VEGF-A* and *HIF-1α* among BBD population. Similar studies in BBD are few[29], and using a conventional estimation of sample size based on the previous data seems inevitable, therefore the formula of comparing proportions in pilot studies considered according to the protocol provided by Viechtbauer *et al.*[30]. Finally, seventy-seven patients with BBD were recruited for the pilot design. Another reason for a few missing in BBD group was concerned to inadequate extraction of total mRNA. Seventy-seven BBD patients (median age 38, 19–52 years old; 37·17 (SD 7·36)) whose disease was confirmed by ultrasound imaging results were recruited in Nour-Nejat hospital, Tabriz, Iran, from 2012 to 2014. The patients with BBD did not undergo mastectomy or surgical procedure before being included in the study. Eligibility criteria consisted of confirmed diagnosis of fibroadenoma (*n* 11) and fibrocystic lesions (*n* 66), a written informed consent form and no history of malignancy. On enrolment, the patient had at most a 1 year history of diagnosis of BBD. Exclusion criteria for the cases were smoking, lactation, pregnancy, acute and chronic illnesses (including renal or liver malfunction, CVD, hyperthyroidism and other hormone-related disorders, type 1 diabetes, hypoglycaemia and polycystic ovary syndrome), history of other benign lesions, gastrointestinal inflammatory diseases (gastritis, inflammatory bowel syndrome and peptic ulcer), using medicines like anticoagulants (aspirin), glucocorticoids and methotrexate, and any positive medical history of chemo-, radio and/or hormonal therapy. The controls were healthy women who were neither hospitalised at the moment of the interview nor diagnosed with any neoplasm (BBD and malignancy) and were matched with cases for age (±5 years) and region. Sample size required to meet the pre-assumption of factor analysis in case–control design to reveal the major dietary patterns as follows: (1) at least 100–200 according to MacCallum *et al.*[31] and Hair *et al.*[32] and (2) 300 according to Comrey & Lee[33]. In case–control design of the present study, 231 healthy women were individually matched for age (±5 years) and region in a ratio of 1:3 (case: control) to improve the power of analysis. Following the

hospital-based sampling of the control group, after ethical considerations, eligible healthy subjects were interviewed at Nour-Nejat hospital, Tabriz, Iran. Inclusion criteria of control were having a healthy history based on medical subjective information, no history of any neoplasm and completing a consent form. The exclusion criteria for control were considered as follows: having pregnancy and breast-feeding at enrolment, smoking, having acute and chronic illnesses (including renal or liver malfunction, CVD, hyperthyroidism and other hormone-related disorders, type 1 diabetes, hypoglycaemia and polycystic ovary syndrome), history of malignancies, medical history of chemo-, radio-, and/or hormonal therapy, any history of benign lesions, gastrointestinal inflammatory diseases (gastritis, inflammatory bowel syndrome, and peptic ulcer), and using medicines such as anticoagulants (aspirin), glucocorticoids and methotrexate. A dietitian completed the questionnaires through face-to-face interviews for each woman individually after receiving all the relevant information and completing an informed consent form. The general information was collected by means of a demographic questionnaire (including age at the time of diagnosis, menarche, menopause, and first pregnancy; number of pregnancies; history of abortion; use of different dietary supplements; hormonal-based treatment; history of treatment with chemo-, radiation- and hormonal therapy, breast and ovarian surgery); and a lifestyle questionnaire (history of smoking, alcohol intake and physical activity level). The family history of malignancies of each participant was reviewed using pedigree analysis[34].

Ethics approval and consent to participate

The ethical considerations were described to each participant, and then a written consent form was obtained prior to the enrolment. The research protocol, including methodology, study subjects, sample size, data collection and all the relevant ethical considerations, were reviewed and approved by the Ethics Committee of Tabriz University of Medical Sciences (Ethics No: IR.TBZMED.REC.1394·806).

Dietary assessment

Dietary intake was assessed using a detailed interviewer-administered FFQ consisting of 136 food items. The biomarker-based validity of the dietary assessment questionnaire (FFQ) was previously got approval and published for Iranian women with primary breast cancer[35–38]. This FFQ was previously validated for food groups including grains, vegetables, fruits and dairy products among Iranian women with primary breast cancer[35,38]. For BBD patients, the frequency of food intake was asked in the last year before the diagnosis of BBD. For controls, if her diet was not changed, the FFQ was completed according to the habitual diet in the last year before the interview. The timelines used to ascertain the frequency of food items were daily, weekly, monthly and yearly. Fixed portion size was used to quantify each food item, which was different among foods. However, showing different household utensils was helpful to improve the recalling accuracy. In addition, a collection of colour photographs was also utilised. The alternative portion size was then converted to the original one. Nutritionist software IV version 3.5.2 was applied to calculate the total energy (kJ/d) and other nutrients.

RNA extraction and quantitative real-time PCR

Total mRNA extraction was carried out using the phenol–chloroform protocol (CinnaGen) on the whole blood. Phenol (1 ml) was added to the same volume of blood after clearance of lysed erythrocyte. Chloroform (1 ml) was added and centrifuged at 12 000 ***g*** at 4°C for 10 min. The supernatant was transferred to another tube; 2-propanol (500 µl) was added and centrifugation was repeated under the same conditions. Thereafter, the supernatant was removed carefully, and the precipitated pellet containing mRNA was washed twice using 75 % ethanol. After air-drying under a clean hood, the pellet was dissolved in diethyl pyrocarbonate-treated water. Nanodrop ND-1000 was used to measure the mRNA concentration. Complementary DNA (cDNA) was synthesised using the Prime Script™ RT reagent kit (Perfect Real Time) based on the manufacturer's protocol. A total volume of 20 µl of the reaction mixture contained 10 µl of master-mix SYBR Green (Takara), 1·0 µl of each primer, PCR-grade distilled water and template cDNA (mean 2 µg/ml). Fold change of gene expression was calculated using the cycle threshold (Ct) measured by quantitative real-time-PCR by means of a Roche Light Cycler 96 system. The nucleotide sequence of the primers is presented in the online Supplementary Table S1. The expression levels of the genes of interest were calculated using $2^{-\Delta\Delta Ct}$ equation[39]. The *hypoxanthine-guanine phosphoribosyltransferase* gene was applied as an internal normalising control.

Statistical analysis

Data were analysed using SPSS statistical software, version 16.0. A box plot was utilised to detect the outliers. Normality of quantitative variables was assessed using the Kolmogorov–Smirnov test. Descriptive statistics were represented in mean (SD), median values and frequencies. Independent-sample *t* test was utilised to compare the means of continuous variables between the two groups. Comparison of proportions was performed using either the χ^2 test or Fisher's exact test. Principal component factor analysis was carried out using the orthogonal approach (Varimax procedure) with Kaiser's normalisation to derive the dietary patterns according to the classification of individual food items in FFQ in nineteen food groups based on their composition and culinary usage. Crude intake (absolute amount) as well as residual intake of food groups were both used in the principal component analyses (PCA)[40]. Significance Bartlett's test of sphericity and the Kaiser–Meyer–Olkin measure of sampling adequacy greater than 0·6 were used to verify the appropriateness of the PCA[32]. Interpretability of the factors, eigenvalues (>1·5) and the scree plot were considered to determine the number of patterns to retain. Greater values of each factor loading were considered to correspond the food group to that pattern[32]. The sum of factor scores for each subject for each estimated dietary pattern was computed by multiplying the corresponding factor loading and actual intake of that food group[32]. Median-based stratifications regarding factor scores among controls were generated for each dietary pattern. Factor scores

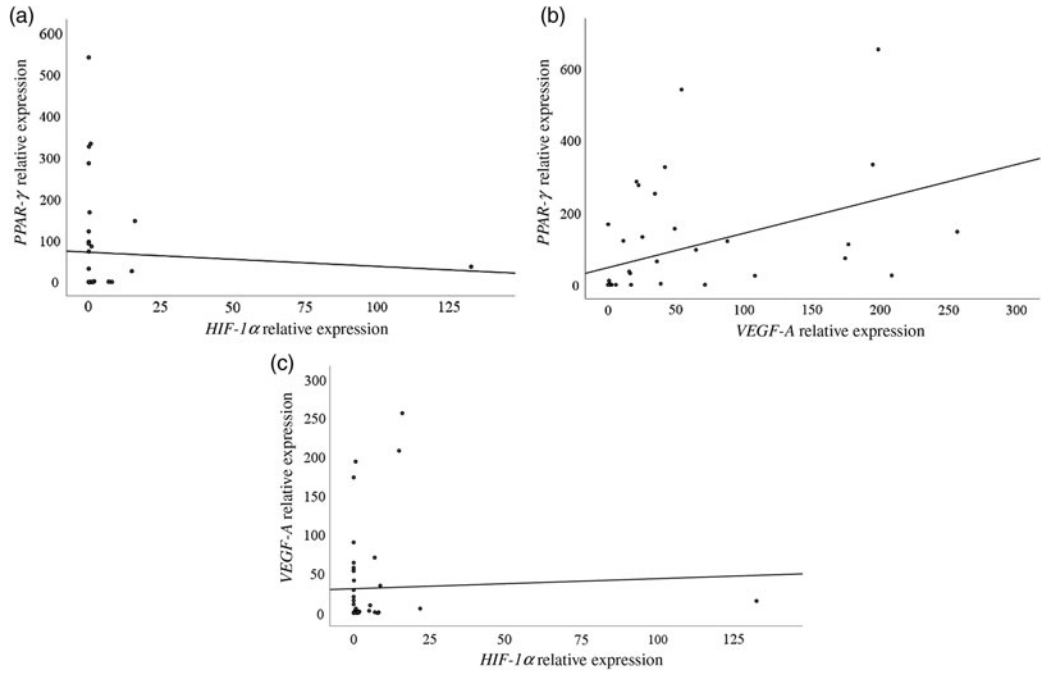

Fig. 1. Pearson's correlation coefficient values show relative expression levels of studied genes (*n* 77). (a) *r* −0·061, *P* = 0·730; (b) *r* 0·442*, *P* = 0·002; (c) *r* 0·042, *P* = 0·782. * *P* < 0·05 considered statistically significant.

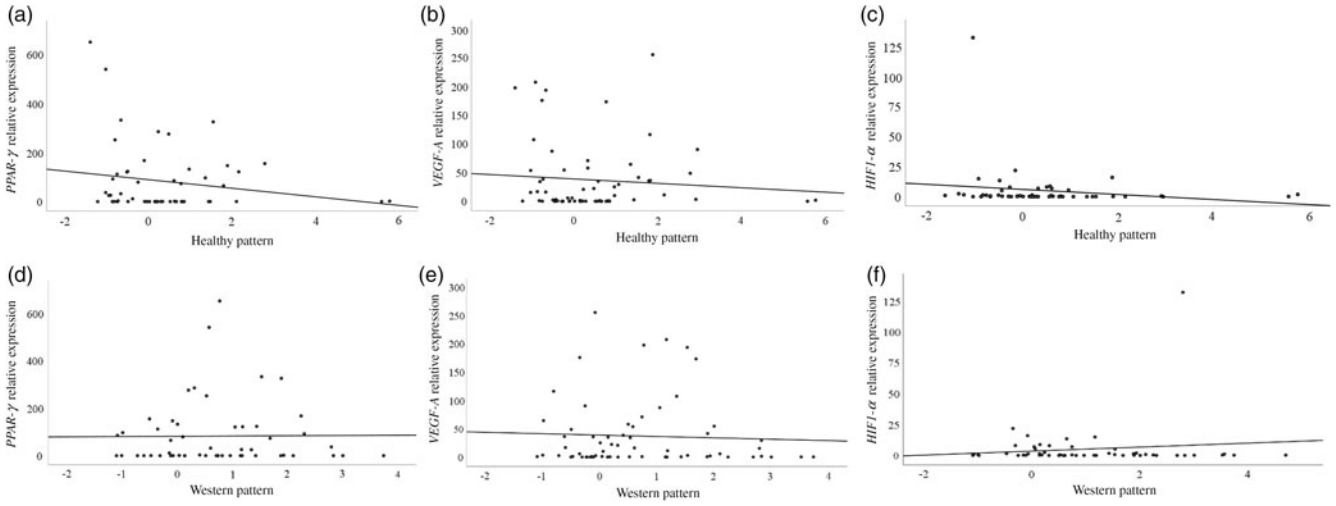

Fig. 2. Pearson's correlation coefficient values represent relative expression levels in association with Healthy and Western dietary patterns (*n* 77). (a) Adjusted for protein (g/d), soluble fibre (g/d), caffeine (mg/d) and plasma levels of insulin growth factor binding protein-3 (mg/l); (b) adjusted for dietary fibre (g/d); (c) adjusted for energy (kJ/d), protein (g/d), caffeine (mg/d), waist circumference (cm) and BMI (kg/m^2); (d) adjusted for energy intake (kJ/d); (e) adjusted for frequency of pregnancy; (f) adjusted for carbohydrate (g/d), crude fibre (g/d), height (cm) and age (years). (a) *r* −0·183, *P* = 0·190 r_{adj} −0·338*, *P* = 0·018; (b) *r* −0·088, *P* = 0·488 r_{adj} −0·286*, *P* = 0·023; (c) *r* −0·165, *P* = 0·243 r_{adj} −0·340*, *P* = 0·024; (d) r 0·009, *P* = 0·947 r_{adj} 0·064, *P* = 0·654; (e) *r* −0·048, *P* = 0·708 r_{adj} −0·080, *P* = 0·549; (f) *r* 0·117, *P* = 0·407 r_{adj} 0·309*, *P* = 0·037. * *P* < 0·05 considered statistically significant.

greater than or equal to the median values of each pattern were labelled as 'high' to define high adherence to the determined dietary pattern, otherwise score was fall in a category less than the median value was defined as 'low' expressing less adherence to the dietary pattern. In the case–control design, the OR and 95 % CI of BBD were determined using logistic regression analysis in crude (unadjusted) and multivariate (adjusted) models to control the covariates (independent variables) (Table 3).

In the case series, logistic regression analysis was used to explore the associations between the identified dietary patterns as the independent variable and the expression status of the studied genes (dependent variable). The median value of expression level of a gene was considered as a cut-off point (Tables 4 and 5). Scatter plots were used to illustrate the correlations between (1) expression levels of the studied genes (Fig. 1) and (2) identified patterns in correlation with relative expression

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of the studied genes (Fig. 2). The '*r*' from Pearson's partial correlation was presented for each scatter plot in crude and adjusted models. A *P* value <0·05 was assumed as statistically significant.

Results

General information, anthropometric and dietetic characteristics of BBD patients (*n* 77) as well as the frequency of pregnancy, breast-feeding, family history of breast cancer and consumed supplements across the median values of relative expression levels of genes of interest are shown in Table 1. Women in the lower category of *PPAR-γ* and *VEGF-A* (fold change in expression) had lower intake of dietary fat than patients in the other categories (non-statistically significant). On the other hand, BBD patients who had lower expression of *HIF-1α* consumed more macronutrients and also they had more breast-fed children than the other categories. Dietary fat ($P<0·05$) and the number of lactations ($P<0·05$) were significantly different between subgroups of *HIF-1α*. Fold changes in expression of *VEGF-A* were statistically different in dichotomous groups of *PPAR-γ* ($P<0·01$) (Table 1). In other words, *PPAR-γ* expression level was significantly associated with *VEGF-A* expression level (r 0·442, $P<0·05$) (Fig. 1).

The PCA was conducted using two different sets of input variables, absolute intakes (g/d) and residual intakes (energy-adjusted), which showed different factor loadings. Primary PCA, in which absolute intakes were used, had shown greater Kaiser–Meyer–Olkin values and generate meaningful dietary patterns to interpret dietary factors rather than energy-adjusted PCA (Table 2). In this case, two major dietary patterns were identified based on the whole study population (cases and controls) using PCA, which can explain 25·38 % of the variances (Table 2). The χ^2 for Bartlett's test of sphericity was 564·1 ($P<0·001$), and the Kaiser–Meyer–Olkin measure of sampling adequacy showed a score of 0·684. They were labelled as Healthy and Western dietary patterns based on their food groups. The healthy pattern includes eleven food groups (whole bread, fruits, vegetables, vegetable oils, nuts and seeds, legumes, spices, seafood, low-fat meat, skinless poultry and low-fat dairy products). The other pattern in terms of Western pattern was characterised by higher consumption of food rich in starch, high-fat meat, and poultry, high-fat dairy products, hydrogenated fat, fast food, salt, sweets and desserts.

The associations between the two estimated dietary patterns and BBD risk are presented in Table 3. In the multivariable-adjusted model, women in the higher score of the Healthy pattern score had OR for BBD of 0·26 (95 % CI 0·08, 0·81) compared with individuals with low consumption ($P<0·05$). Strong associations were observed between the Western pattern and BBD risk before (OR 5·37; 95 % CI 2·75, 10·46) and after (OR_{adj} 5·59; 95 % CI 2·06, 15·10; $P<0·01$) adjustments for energy and folate intakes, oral contraceptive usage and abortion status (Table 3).

The correlations between dietary patterns and relative expression levels of the studied genes in BBD participants are shown in Fig. 2. Since BBD is a multi-factorial disease which could partly be attributed to lifestyle and dietary factors as major contributors, we found significant correlations only in the adjusted models. The Western pattern was associated with the over-expression of *HIF-1α* after making adjustments for carbohydrate (g/d), crude fibre (g/d), height (cm) and age (years) (r_{adj} 0·309, $P<0·05$), whereas the Healthy pattern was inversely correlated with the expression of *HIF-1α* (r_{adj} −0·340, $P<0·05$), *VEGF-A* (r_{adj} −0·286, $P<0·05$) and *PPAR-γ* (r_{adj} −0·338, $P<0·05$) when adjustments made for potential covariates (*HIF-1α*: energy (kJ/d), protein (g/d), caffeine (mg/d), waist circumference (cm), and BMI (kg/m^2); *VEGF-A*: dietary fibre (g/d); *PPAR-γ*: protein (g/d), soluble fibre (g/d), caffeine (mg/d) and plasma levels of insulin growth factor binding protein-3 (mg/l)).

Table 4 presents the OR and corresponding 95 % CI found out to show associations between the fold change in expressions of *PPAR-γ*, *VEGF-A* and *HIF-1α* and low (<median) and high (≥median) scores of the identified dietary patterns in BBD patients. Unconditional logistic regression analysis showed that higher score of the Healthy pattern was correlated with less fold change in the expression of *PPAR-γ* (OR 0·26; 95 % CI 0·08, 0·86) and *HIF-1α* (OR 0·24; 95 % CI 0·07, 0·85).

Table 5 shows the OR and 95 % CI to indicate associations between fold change in expressions of *PPAR-γ*, *VEGF-A* and *HIF-1α* and the median scores of the estimated dietary patterns in the study population. After making adjustment for dietary covariates (intake levels of vitamin C and carbohydrate), the over-expression of *PPAR-γ* among those cases with higher scores of the Healthy dietary pattern was 65 % lower than the controls (OR_{adj} 0·35; 95 % CI 0·13, 0·94). Greater adhesion to the Healthy pattern decreased the risk of high expression levels of *VEGF-A* in the adjusted model rather than the BBD patients with less adhesion (OR_{adj} 0·38; 95 % CI 0·13, 1·08; $P=0·071$). Higher scores of attaining a Healthy diet decreased the expression of *HIF-1α* in cases rather than controls after adjustment for confounding variables (intake levels of vitamin C, carbohydrate, folate and caffeine) (OR_{adj} 0·30; 95 % CI 0·10, 0·90). Therefore, high adherence to a Healthy dietary patterns may promote down-regulation of *PPAR-γ* and *HIF-1α* in BBD. Greater adhesion to the Western dietary pattern *v.* controls significantly increased the up-regulation of *PPAR-γ* (OR_{adj} 8·08; 95 % CI 2·36, 27·62), *VEGF-A* (OR_{adj} 5·22; 95 % CI 1·93, 14·09) and *HIF-1α* (OR_{adj} 7·37; 95 % CI 2·11, 25·66), rather than BBD patients with less adhesion.

Discussion

To the best of our knowledge, this is the first study investigating the relationship between dietary patterns and the expression levels of *PPAR-γ*, *VEGF-A* and *HIF-*1α in BBD patients. Conducting PCA over the dietary data of the present study provided two major dietary patterns more specified in terms of Healthy and Western.

Our results showed that the identified Healthy pattern (high consumption of whole bread, fruits, vegetables, vegetable oils, legumes, spices, nuts and seeds, seafood, low-fat meat, skinless poultry and low-fat dairy products) was inversely associated with the BBD risk after making adjustment for potential covariates. Similarly, Tiznobeyk *et al.*[5] showed that a Healthy pattern (whole grains, vegetable oils, olives, fruits, vegetables, legumes,

Table 1. General characteristics of benign breast patients according to median values of relative expression levels of studied genes (Mean values and standard deviations)

Characteristics	*PPAR-γ* expression level (*n* 54)					*VEGF-A* expression level (*n* 65)					*HIF-1α* expression level (*n* 53)				
	<7·10†		≥ 7·10			<5·65†		≥ 5·65			<0·37†		≥ 0·37		
Continuous variables	Mean	SD	Mean	SD	*P*‡	Mean	SD	Mean	SD	*P*‡	Mean	SD	Mean	SD	*P*‡
PPAR-γ expression	N.D.	N.D.	N.D.	N.D.	N.D.	8·3	35·6	165	172	0·001*§	96·5	149	39·5	88·3	0·099§
VEGF-A expression	6·3	16·4	79·1	79·3	0·001*§	N.D.	N.D.	N.D.	N.D.	N.D.	26·8	41·9	37·0	76·5	0·578
HIF-1α expression	1·6	2·5	11·0	34·0	0·304§	2·6	4·7	10·2	30·9	0·310§	N.D.	N.D.	N.D.	N.D.	N.D.
Age at diagnosis (years)	38·5	7·3	36·3	7·4	0·273	37·0	6·9	36·9	7·5	0·918	35·5	8·5	37·4	6·5	0·369
Age at menses (years)	13·1	1·4	13·6	1·5	0·249	13·0	1·3	13·7	1·5	0·069	13·1	1·2	13·1	1·5	0·976
Waist circumference (cm)	84·5	7·8	83·2	8·3	0·553	85·8	8·3	83·9	8·9	0·380	84·2	8·2	84·1	7·9	0·950
Lean body mass	44·3	5·5	40·7	9·9	0·106	45·4	5·2	43·3	4·5	0·097	43·1	11·0	43·9	4·7	0·733
Total energy (kJ/d)	11 497	3372	11 146	3539	0·715	11 372	3451	11 422	3414	0·954	12 547	3129	11 079	3329	0·115
Dietary fibre (g/d)	26·7	9·3	30·9	21·9	0·359§	26·6	8·8	34·9	21·6	0·056§	30·2	11·4	28·3	14·4	0·587
Crude fibre (g/d)	10·1	4·3	10·0	4·3	0·947	10·2	4·1	11·3	5·2	0·373	11·3	4·4	10·1	4·5	0·343
Dietary carbohydrate (g/d)	353	122	602	1388	0·356	348	120	596	1293	0·276	396	118·9	347	139	0·177
Dietary protein (g/d)	112	54·7	118	77·2	0·762	106	51·6	117	73·5	0·504	120	48·5	104	52·1	0·269
Dietary fat (g/d)	105	48·5	113	56·1	0·613	107	49·4	107	53·4	0·994	132	62·2	100	49·1	0·046*
Categorical variables	*n*	%	*n*	%	*P*‖	*n*	%	*n*	%	*P*‖	*n*	%	*n*	%	*P*‖
BMI (kg/m^2)															
<25	6†	23	6	22·2	0·967	9	27·3	8	25·8	0·758	8	30·7	4	16	0·398
25–29·9	13	50	13	48·2		11	33·3	13	41·9		11	42·3	11	44	
≥30	7	27	8	29·6		13	39·4	10	32·3		7	27	10	40	
Number of pregnancy															
≤2	19	79·2	16	80	0·946	20	69	16	72·7	0·770	15	78·9	12	57·1	0·141
>2	5	20·8	4	20		9	31	6	27·3		4	21·1	9	42·9	
Number of breast-fed children															
≤2	22	81·5	23	85·2	0·715	26	76·5	25	80·6	0·683	24	88·9	16	64	0·033*
>2	5	18·5	4	14·8		8	23·5	6	19·4		3	11·9	9	36	
Family history of breast cancer															
Yes	4	14·8	4	14·8	1·000	6	17·6	5	16·1	0·870	4	14·8	6	24	0·401
No	23	85·2	23	85·2		28	82·4	26	83·9		23	85·5	19	76	
Supplements usage															
Yes	18	66·7	16	59·3	0·573	22	64·7	17	54·8	0·417	14	51·9	18	69·2	0·196
No	9	33·3	11	40·7		12	35·3	14	45·2		13	48·1	8	30·8	

VEGF-A, vascular endothelial growth factor-A; *HIF-1α*, hypoxia-inducible factor-1α; *n*, number.
*$P < 0·05$.
† Median-based stratifications were formed for fold change expression of studied genes.
‡ The *P* value was obtained by independent-sample *t* test.
§ Non-parametric distribution.
‖ The *P* value was obtained by χ^2 test. Some missing data existed in general variables (missing data included: *n* 1 for age at diagnosis, age at menses, waist circumference, lean body mass, BMI, family history of breast cancer and supplement usage; *n* 8 for number of pregnancy and number of breast-fed children).

Table 2. Factor loading matrix for the identified dietary patterns in benign breast patients (*n* 77) and controls (*n* 231)

		Factor loading†			
		Crude intake KMO = 0·685		Residual intake KMO = 0·536	
Food groups	Food items	Healthy	Western	Component 1	Component 2
Seafood	Fish, shrimp and other seafood	0·510‡	0·199	0·429‡	0·325
Low-fat meat	Low-fat lamb, low-fat beef, low-fat veal	0·461	0·291	0·687‡	0·143
High-fat meat	High-fat lamb, high-fat beef, high-fat veal, liver, others	0·224	0·589‡	0·648‡	–
Low-fat poultry	Skinless chicken and other poultry	0·367‡	–	0·452‡	–
High-fat poultry	Eggs, chicken and other poultry with skin	–	0·324‡		
Low-fat dairy products	Low-fat milk, low-fat yogurt, yogurt drink (Dough)	0·411‡	−0·230	–	0·330‡
High-fat dairy products	High-fat milk, high-fat yogurt, ice cream, cheese, Kashk	–	0·399‡		
Nuts and seeds	Seeds, almonds, peanuts, pistachios, walnuts, others	0·382‡	0·259	0·241	0·276‡
Spices	Pepper, cinnamon, turmeric, saffron, caraway, others	0·503‡	–	–	0·507‡
Vegetables	All kinds	0·693‡	–	0·231	0·636‡
Fruits	All kinds and fruit juices	0·595‡	–	−0·184	0·635‡
Legumes	Lentil, split pea, chickpea, green peas, beans, green broad bean, soya, others	0·405‡	–	–	0·255‡
Vegetable oils	Olive oil, rapeseed oil, soya oil, sunflower oil, maize oil, others	0·208‡	–	−0·397‡	0·127
Hydrogenated fat	Hydrogenated vegetable oils, solid fats from animal origin, animal butter	–	0·392‡	0·114	−0·150‡
Sweets and desserts	Biscuits, cookies, confectioneries, cube sugar, sugar, honey, jam, candy, chocolate, fruit syrup, soda, others	0·316	0·420‡	0·243‡	−0·127
Salt	Salt	0·170	0·645‡	0·334‡	–
Food rich in starch	Rice, white bread, refined cereals, spaghetti, noodle, maize, popcorn, potato	–	0·544‡	0·255	−0·483‡
Whole bread	Sangak bread and barley bread	0·146‡	–	–	–
Fast food	Pizza, hamburger, cheeseburger, sausage, lunch meat, French fries, potato chips, puffy, mayonnaise, others	−0·162	0·677‡	–	–
Variance explained (%)		15·56‡	9·82‡	12·24‡	11·57‡

KMO, Kaiser–Meyer–Olkin.
† Exploratory factor analysis using the factor procedure. Loading factor <0·1 in absolute values was suppressed.
‡ Greater values of each factor loadings were considered to correspond food group to that factor[32].

Table 3. Risk of benign breast diseases according to the median of scores estimated for certain dietary patterns in the study population† (Number values and percentages; odds ratios and 95 % confidence intervals)

		Low scores§		High scores			High scores					High scores			
Dietary pattern‡	*n*	*n*	%	*n*	%	*P*‖	Low scores	OR_{Crude}	95 % CI	*P*	Low scores	OR_{adj}	95 % CI¶	*P*
Crude intake														
Healthy pattern														
Case	77	29	37·7	48	62·3	0·043*	1·00	1·64	0·96, 2·78	0·066	1·00	0·26*	0·08, 0·81	0·021*
Control	231	115	49·8	116	50·2									
Western pattern														
Case	77	12	15·6	65	84·4	<0·001*	1·00	5·37*	2·75, 10·46	<0·001*	1·00	5·59*	2·06, 15·10	0·001*
Control	231	115	49·8	116	50·2									
Residual intake														
Component 1														
Case	77	20	26·0	57	74·0	<0·001*	1·00	2·82*	1·59, 5·00	<0·001*	1·00	2·35	0·94, 5·86	0·065
Control	231	115	49·8	116	50·2									
Component 2														
Case	77	37	48·1	40	51·9	0·422	1·00	1·09	0·65, 1·82	0·742	1·00	0·47	0·18, 1·22	0·121
Control	231	115	49·8	116	50·2									

* $P < 0·05$.
† Logistic regression analysis in crude and multivariate models was used to explore the associations between the study participants (dependent variable) and factor score of the identified dietary patterns (independent variable).
‡ A detailed list of food items comprising the 'Healthy' or 'Western' dietary pattern is shown in Table 2.
§ Median-based stratifications were formed for the score rate of each variable.
‖ Comparison of proportions was performed with χ^2 test.
¶ Adjusted for energy intake (<10 000 kJ/d/≥10 000 kJ/d), folate (<400 μg/d/ ≥400 μg/d), oral contraceptive usage (yes/no) and abortion (yes/no).

Table 4. Associations between fold change in expressions of *PPAR-γ*, vascular endothelial growth factor-A (*VEGF-A*) and hypoxia-inducible factor-1α (*HIF-1α*) according to median scores of identified dietary patterns in benign breast patients† (Odds ratios and 95 % confidence intervals)

Dietary pattern‡		Fold change of *PPAR-γ* (*n* 54)					Fold change of *VEGF-A* (*n* 64)					Fold change of *HIF-1α* (*n* 52)				
		<7·10§	≥7·10	*P*‖	OR	95 % CI	<5·65§	≥5·65	*P*‖	OR	95 % CI	<0·37§	≥0·37	*P*‖	OR	95 % CI
Healthy pattern	Low scores§	6¶	30·0	70·0	0·047*	1·00	10	45·5	0·600	1·00		5	29·4	70·6	0·038	1·00	
	High scores	21	61·8	38·2		0·26*	23	54·8		0·68	0·24, 1·94	22	62·9	37·1		0·24*	0·07, 0·85
Western pattern	Low scores§	5	62·5	37·5	0·704	1·00	5	50·0	0·100	1·00		2	40·0	60·0	0·662	1·00	
	High scores	22	47·8	52·2		1·81	28	51·9		0·92	0·24, 3·58	25	53·2	46·8		0·58	0·09, 3·84

* $P < 0·05$.
† Logistic regression analysis was used to explore the associations between fold change expressions of the studied genes (dependent variable) and factor score of the identified dietary patterns (independent variable).
‡ A detailed list of food items comprising the 'Healthy' or 'Western' dietary pattern is shown in Table 2.
§ Median-based stratifications were formed for the score rate of each variable.
‖ Comparison of proportions was performed with χ^2 test.
¶ Data are presented as number (percent).

nuts, fish, poultry, eggs and low-fat dairy products) might decrease the risk of BBD among Iranian women. In a prospective cohort study (6593 adolescent girls and 122 incident BBD cases), Boeke *et al.*[6] suggested that dietary carotenoid intake in adolescents might decrease the risk of BBD. In another cohort study Fung *et al.*[7] showed that DASH pattern scores were correlated with a lower risk of incidence of oestrogen-receptor-negative breast cancer. In a prospective Black Women's Health Study from 1268 breast cancer cases, Boggs *et al.*[41] reported that vegetable intake might decrease the risk of oestrogen- and progesterone-receptor-negative breast cancer. We observed significant inverse associations between the Healthy pattern and expression levels of *PPAR-γ*, *VEGF-A* and *HIF-1α*. This could be partly explained by the effect of nutritional active components on the expression of angiogenic factors. Fruits, vegetables and spices contain bioactive compounds that have shown anti-angiogenic properties in experimental studies[42,43]. *In vitro* experiments revealed that *HIF-1α* expression might be inhibited by silibinin, isoflavones and resveratrol present in fruits and vegetables[42]. Genistein, quercetin, curcumin, allicin, capsaicin, gingerol and perillyl alcohol can induce down-regulation of *VEGF*, thereby decreasing angiogenesis[42,43]. Kaempferol, a flavonol found in a variety of vegetables and fruits, impedes tumour growth, angiogenesis and *VEGF* expression via *HIF*-dependent pathway *in vitro*[44]. Fang *et al.*[45] revealed that apigenin not only may induce the down-regulation of *HIF-1α* and *VEGF* (in breast, ovarian, prostate and colon cancer cell lines) but also repressed angiogenic factors under *in vivo* conditions[45]. Vegetable oils were shown to be inversely associated with BBD in the prospective cohort of Nurses' Health Study II[3]. The present study showed that the Healthy dietary pattern contains vegetable oils which are rich in tocopherol. γ-Tocopherol enhanced the mRNA expression of *PPAR-γ* in SW480 colon cancer cell lines *in vitro*[46]. *n*-3 PUFA binds to the transcription factor *PPAR-γ*[47]. Short-time exposure to linoleic and CLA, dietary *PPAR-γ* ligands, could induce apoptosis, thereby inhibiting colon cancer metastasis[26]. DHA and EPA impressed the signalling pathways and caused cell cycle arrest[27]. In a large observational study (1971 controls and 1577 colon cancer cases), Murtaugh *et al.*[48] showed that *PPAR-γ* genotypes modified the correlation between prudent diet scores, vegetables and fruits and the risk of colon cancer. In that project, cases with the PPARγ2 PP and XA (i.e. PA/AA) genotypes, who had lower intake of refined grains or higher scores of prudent diet or higher consumption of lutein, exhibited a lower risk of colon cancer[48]. However, it is unknown whether this alteration in colorectal risk is associated with either direct influence of dietary pattern constitutes on tumorigenesis or acting as *PPAR-γ* ligands.

The present pattern labelled as Western (high intake of food rich in starch, high-fat meat, and poultry, high-fat milk, and dairy products, hydrogenated fat, fast food, salt, sweets and desserts) was correlated with higher risk of BBD. This pattern was linked to 5·59-fold increased risk for BBD. However, the previously reported unhealthy dietary pattern (refined grains, sweets, red meat, high-fat dairy products and animal fats) was not correlated with BBD risk in Iranian women[5]. Nevertheless, dietary patterns previously labelled as Western or Unhealthy were different

Table 5. Fold change expressions of *PPAR-γ*, vascular endothelial growth factor-A (*VEGF-A*) and hypoxia-inducible factor-1α (*HIF-1α*) in cases compared with controls according to median scores of identified dietary patterns in participants*
(Odds ratios and 95 % confidence intervals)

Fold change of *PPAR-γ*		Cases/controls			Cases with *PPAR-γ* < 7·10†		Cases/controls			Cases with *PPAR-γ* ≥ 7·10	
Healthy pattern‡			OR_{Crude}	95 % CI	OR_{adj}	95 % CI		OR_{Crude}	95 % CI	OR_{adj}	95 % CI
	Low scores†	6/115	1·00		1·00		14/115	1·00		1·00	
	High scores	21/116	3·47	1·35, 8·91	1·46§	0·48, 4·43	13/116	0·921	0·41, 2·04	0·35§	0·13, 0·94
Western pattern‡											
	Low scores†	5/115	1·00		1·00		3/115	1·00		1·00	
	High scores	22/116	4·36	1·59, 11·91	4·40‖	1·61, 12·04	24/116	7·93	2·32, 27·07	8·08‖	2·36, 27·62
Fold change of *VEGF-A*		Cases/controls			Cases with *VEGF-A* < 5·65†		Cases/controls			Cases with *VEGF-A* ≥ 5·65	
Healthy pattern‡			OR_{Crude}	95 % CI	OR_{adj}	95 % CI		OR_{Crude}	95 % CI	OR_{adj}	95 % CI
	Low scores†	10/115	1·00		1·00		12 /115	1·00		1·00	
	High scores	23/116	2·28	1·03, 5·00	1·00¶	0·37, 2·65	19/116	1·57	0·72, 3·38	0·38¶	0·13, 1·08
Western pattern‡											
	Low scores†	5/115	1·00		1·00		5/115	1·00		1·00	
	High scores	28/116	5·55	2·07, 14·88	5·44**	2·02, 14·64	26/116	5·15	1·91, 13·89	5·22**	1·93, 14·09
Fold change of *HIF-1α*		Cases/controls			Cases with *HIF-1α* < 0·37†		Cases/controls			Cases with *HIF-1α* ≥ 0·37	
Healthy pattern‡			OR_{Crude}	95 % CI	OR_{adj}	95 % CI		OR_{Crude}	95 % CI	OR_{adj}	95 % CI
	Low scores†	5/115	1·00		1·00		12/115	1·00		1·00	
	High scores	22/116	4·36	1·59, 11·91	1·35¶	0·40, 4·49	13/116	1·07	0·47, 2·45	0·30¶	0·10, 0·90
Western pattern‡											
	Low scores†	2/115	1·00		1·00		3/115	1·00		1·00	
	High scores	25/116	12·39	2·86, 53·53	12·79††	2·94, 55·49	22/116	7·27	2·11, 24·96	7·37††	2·11, 25·66

* Logistic regression analysis in crude and multivariate models was used to explore the associations between fold change expressions of the studied genes (dependent variable) and factor score of the identified dietary patterns (independent variable).
† Median-based stratifications were formed for the score rate of each variable.
‡ A detailed list of food items comprising the 'Healthy' or 'Western' dietary pattern is shown in Table 2.
§ Adjusted for vitamin C (<75 mg/d/≥75 mg/d) and carbohydrate (<130 g/d/≥130 g/d).
‖ Adjusted for BMI (≤24·99 kg/m^2/ 25–29·99 kg/m^2/ 30≤ kg/m^2).
¶ Adjusted for vitamin C (<75 mg/d/≥75 mg/d), carbohydrate (<130 g/d/≥130 g/d), folate (<400 μg/d/≥400 μg/d) and caffeine (<200 mg/d/≥200 mg/d).
** Adjusted for age (<40 years/ ≥40 years).
†† Adjusted for weight (<72 kg/ ≥72 kg) and height (<162 cm/≥162 cm).

https://doi.org/10.1017/S0007114520001737 Published online by Cambridge University Press

in the composition of food items, they are correlated with an elevated risk of breast cancer[8,11,49–51]. In a hospital-based case–control study, Heidari *et al.*[51] revealed that the Unhealthy dietary pattern (sweets, soft drinks, solid oils, processed meat, potato and salt) had increased the risk of breast cancer among Iranian women. In postmenopausal breast cancer patients from the E3N-EPIC cohort, Cottet *et al.*[8] proposed that alcohol-contained Western pattern with high positive loading for appetisers, potatoes, rice/pasta, cakes, French fries, pulses, canned fish, meat products, pizza/pies, eggs, alcoholic beverages, butter and mayonnaise) might increase the risk of breast cancer. The presence of genotoxic by-products (polycyclic aromatic hydrocarbons and heterocyclic amines) which are produced especially by eating red meat could increase the risk of breast cancer[52]. Pyrolysis of fat over a direct flame and high temperature renders the red meat a carcinogenic food[52]. Moreover, higher dietary intake of food rich in starch and sweets might increase the risk of breast cancer by enhancing blood glucose and insulin, thereby promoting cellular proliferation and tumour growth[53]. We observed significant correlations between Western dietary pattern and *HIF-1α* expression. Park *et al.*[54] injected colon cancer cells into male mice (age 4 weeks) and then divided them into two groups of diet: control (10 % energy from fat) and high fat (60 % energy from fat). They showed that consistent consumption of high-fat diet (mostly from animal sources) contributed to the enhancement of angiogenesis, phosphorylation of Akt, and extracellular signal-regulated kinase (ERK) 1/2 and expression of *HIF-1α*[54]. In this sample of BBD patients, high total energy intake was significantly consumed by individuals with low expression levels of *HIF-1α*. It seems that ATP magnitudes produced in postprandial status increase substantially and can attenuate and switch off the AMP-activated protein kinase activity in the meantime, which might result in elevated *HIF-1α* protein levels[55]. Increased *HIF-1α*-dependent metabolism could enhance the glycolysis under aerobic conditions and oxidative phosphorylation by altering the expression of cytochrome C oxidase subunit 4 and up-regulation of GLUT (GLUT1 and GLUT3)[56]. Cancer cells under normoxic state can show remarkable *HIF-1α* overexpression[57]. This process is cell specific and multiple signalling pathways interfere with the modulation of *HIF-1α* transcription[57]. Insulin receptor consists of an intracellular signalling pathway mediated by phosphatidylinositol-3 kinase (PI3K)/Akt[58]. Active PI3K has a role in the regulation of *HIF-1α* expression. In addition, the insulin receptor entails the intracellular mitogen-activated protein kinase-1 (MEK1)/ERK pathway, leading to cancer cell growth[57]. MEK1/ERK is involved in increasing the *trans*-activation of *HIF-1α* and related phosphorylation[57]. Miele *et al.*[59] indicated that insulin and insulin-like growth factor-I (IGF-I) could induce *VEGF* overexpression through the activation of PI3K/protein kinase B and MAPK-related mechanisms. Dietary factors could intervene in the IGF-I function and contribute to the reduction of angiogenesis, metastases and tumour growth in prostate cancer[60].

Although we used a FFQ validated for nutritional biomarkers[35–38], information bias is inevitable for FFQ-based studies. There were some limitations. First, the sample size for BBD patients was small. Next, the participants in the control group were determined eligible based on the subjective records and this is a potent limitation. Then, the response rate of patients is usually different from controls, which could lead to bias in the accuracy of recalling. Finally, there is limited opportunity in configuring a multivariate regression model by a large number of potential dietary- and non-dietary covariates, and therefore, the results are prone to variation across different studies because of the excluded potentially lifestyle-related confounders. Despite these limitations, the present study has some strengths. Our research is the first study designed specifically to investigate the association between the expression of relevant genes and dietary patterns in the context of BBD. Since BBD is a multifactorial disease and lifestyle and dietary factors are major risk contributors, we found significant associations between the expression of relevant genes and estimated dietary patterns after adjustment for potential confounders. Therefore, conducting studies with large-scale designs to confirm the associations between dietary patterns and the expression of angiogenesis-related genes in BBD patients is recommended.

Conclusion

These findings provide evidence that Healthy dietary patterns (high loads of whole bread, fruits, vegetables, legumes, nuts, seeds, spices, vegetable oils, seafood, low-fat meat, skinless poultry and low-fat dairy products) might be associated with the prevention of BBD risk. A Healthy diet was shown to have an inverse contribution to the expression of genes prone to tumorigenesis in BBD patients.

Acknowledgements

We are grateful to all participated women and valued colleagues of Nour-Nejat private hospital. We are thankful for Mrs. Neda Rezvani for drafting the initial proposal.

This research was founded by Drug Applied Research Center, grant number: 5-153640 and Nutrition Research Center, grant number: 5-71-1257, Tabriz University of Medical Sciences, Tabriz, Iran. This article was outlined using a data set obtained from M.Sc. thesis registered at Tabriz University of Medical Sciences (registration no. 5-97-1295).

Conceptualisation, S. P.; methodology, S. P., B. B., D. S., A. B., N. A. and S. A.; validation, S. P.; formal analysis, S. P., S. J. and M. F.-G.; investigation, S. P., V. M., S.-S. P, N. A. and S. A.; writing – original draft preparation, S. P., M. F.-G., S. J., S.-S. P., A. B., N. A. and S. A.; writing – review and editing, S. P; project administration, S. P. and V. M.; supervision, S. P.; funding acquisition, S. P.

There are no conflicts of interest.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114520001737

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