RESEARCH COMMUNICATION

Antioxidants Intake And Status, And Oxidative Stress In Relation To Breast Cancer Risks: A Case-Control Study

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Abstract

A case control study was carried out to investigate associations between breast cancer risk, antioxidant status and oxidative stress among women in Klang Valley and Selangor. A total of 57 newly diagnosed cases aged 30 to 66 years old participated and were matched for age and ethnicity with 139 controls with no diagnosis of cancer or other chronic diseases. An interview based questionnaire designed to collect information on demographic and socioeconomic status, as well as reproductive, medical and dietary history was used. Anthropometric measurements including weight, height, waist and hip circumference were made and a 10 ml fasting venous blood sample was taken for glucose testing and analysis of plasma vitamin antioxidants and malondialdehyde. Hair and toenail samples were taken for selenium analysis. Results showed that the mean intake of vitamin A, vitamin E and selenium among cases (606.8±334.8μg/d, 6.1± 2.4 g/d, 56.9 ± 16.2 μg/d) was lower than controls (724.7 ± 414 μg/day, 6.9 ± 3.0 g/d, 60.8 ± 17.5 μg/d, respectively) (p<0.05 for all parameters). A similar trend was noted for plasma vitamin A and E and also selenium in hair and toenails. Poor antioxidant status as indicated by low plasma vitamin A (<284.3 μg/l or <366.3 μg/l) increased risk of breast cancer by approximately two fold, whilst low plasma vitamin E (<2.5 mg/dl, <2.8 mg/dl and <3.1 mg/dl) increased the risk by two to three fold [Adjusted OR 2.97 (95% CI 1.38-3.48), 2.32 (95% CI 1.07-2.41) and 2.12 (95% CI 1.00-4.21)]. Cases had a greater level of malondialdehyde 4.4 ± 1.1 mmol/g protein), an indicator of oxidative stress, as compared to controls (3.2 ± 1.7 mmol/g protein)(p<0.05). A high level of MDA (≥4.8 mmol/g protein) was associated with breast cancer [Adjusted OR 6.82 (95% CI 1.95-23.9)]. It is concluded that a poor antioxidant status and high oxidative stress are associated with breast cancer risk. Thus, it is essential for Malaysian women to obtain a good antioxidant status by consuming a diet rich in vitamins A and E as well as selenium and adopt healthy behaviour to reduce oxidative stress in order to prevent breast cancer.

Key Words: Breast cancer risk - antioxidant status - oxidative stress - selenium - malondialdehyde

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Introduction

Breast cancer is the commonest cancer among women in Malaysia (MAKNA, 2003) and developing countries (WHO, 1997). A total of 4,337 breast cancer cases were reported in the year 2002 by the National Cancer Registry Report (2002). Almost 30% of the cancer disease suffered by Malaysian women is breast cancer. Every woman in Malaysia has a 1 in 19 chance of getting breast cancer in their lifetime. Chinese women have the highest risk of getting breast cancer with the ratio of 1:14, followed by Indian women 1:15 and Malays 1:24 (National Cancer Registry, 2002).

Among the risk factors contributing to breast cancer are menarche at early age, having first pregnancy at late age, low number of children and late menopause, related to endogenous hormones (Hulka & Stark, 1995; World Cancer Research Fund, 1997) and the use of hormone replacement therapy (Rodriguez et al., 2001). Higher levels of hormone, especially estrogen, could play the role in enhancing the first phase of carcinogenesis in breast (Del Giudice et al., 1998; Hankinson et al., 1998). The most prominent modifiable risk factor of breast cancer is diet. High intake of animal fat and meat more than once a week may increase the risk (Lee et al., 2004) as it increases insulin resistance and IGF-1 (Stoll, 1996). In contrast, the intake of vegetables rich in antioxidant may prevent up to 33% incidence of breast cancer (World Cancer Research Fund, 1997). Thus, a reduction in antioxidant level due to the presence of free radicals may increase risk of breast cancer, particularly in alcohol drinkers (Terry et al., 2001) and smokers (Egan et al.,

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2002). Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) and antioxidants reaction capacity which stimulate the development of a disease such as breast cancer (Aghvami et al., 2006).

Several case control studies have reported a relationship with antioxidant status (Ching et al., 2000; Do et al., 2003). However, only a selected antioxidant micronutrient is being studied and oxidative stress has rarely being investigated in relation to diet, antioxidant and breast cancer, especially among Asian women. Similar case control studies in Malaysia only investigate the relationship between health behaviour and body size (Kamarudin et al., 2006) and fiber (Suzana et al., 2004). Therefore, this study aimed to investigate the relationship between the diet, antioxidant status and oxidative stress with risk of breast cancer among women in Klang Valley and Selangor of Malaysia. The results of this study are important for policy makers and health professionals involved in planning public health programme for cancer prevention in Malaysia. The study has been approved by the Research and Ethical Committee of Faculty of Medicine, Universiti Kebangsaan Malaysia (NN-026-2004). This study is part of a larger study investigating the relationship between diet, lifestyle, body composition, physical activity, hormone level and breast cancer as reported elsewhere (Rabeta et al., 2007, Suzana et al., 2008).

Materials and Methods

Study Design and Subjects

This case control study was carried out in Klang Valley and Selangor, Malaysia. Cases were newly diagnosed breast cancer women (stage 1 to III), not on any therapy for cancer (except for surgery and painkiller) and had not been diagnosed with other chronic diseases. They were patients at various health care centers including National Cancer Society (NCS) Health Centre, Kuala Lumpur General Hospital, Selayang Hospital, Hospital Universiti Kebangsaan Malaysia, Klang Hospital and Kajang Hospital. Whilst, controls were healthy women with no medical history of breast cancer, had no chronic diseases and were not pregnant and lactating at the time of the study. The controls were recruited at working institutions including China Press, Universiti Kebangsaan Malaysia, Universiti Putra Malaysia and residential areas at Taman Bukit Ampang and Bandar Baru Bangi. Both cases and controls were recruited through convenience sampling and matched for age (± 5 years) and ethnics. The study has been approved by the Ministry of Health Ethical Committee. Sample size was calculated using a computer software PS:Power and Sample Size Calculation (2002) version 2.1.31. The desirable sample size at study power 80% and odds ratio of 0.45 (Do et al., 2003), with the ratio of case to control at 1:2 were 87: 174.

Data collection

During recruitment, subjects were screened for stress using Hospital Anxiety and Depression Score (HADS) (Montazeri et al., 2003). This questionnaire consisted of 14 items, with seven questions for depression and another seven for anxiety and was developed to assist doctors in understand patients emotional well being. For the purpose of this study, the HADS questionnaire was translated to Malay language using a back to back translation. Those with high score for either depression or anxiety and also high blood sugar (>6.1 mmol/l) (screened using Accu-Check Advantage Glucometer, Roche, USA) or high blood pressure (>130/ 85 mmHg) (measured using a BP set (Omron model T 1 4, Omron, USA) were excluded from this study.

Eligible subjects were interviewed by trained interviewers using a set of questionnaire contains information on socio-demographics, medical and reproductive history modified from Breast Cancer Core Questionnaire by National Action Plan Breast Cancer (NAPBS)(1998). Habitual food intake was assessed using a validated structured diet history questionnaire (Suzana et al., 2000). In particular, cases were asked to provide estimation of habitual food intake prior they were diagnosed with cancer. Food models and household measures were used to facilitate diet history interview. Each estimated amount consumed was then converted into grams, using a list of commonly consumed foods with known weight for each portion size. Nutritionist Pro TM 2003 software was used to calculate nutrient intakes. Malaysian Food Composition Table (FCT) (Tee et al., 1997) was used as the nutrient database. However, the Malaysian FCT did not contain values for vitamin E and selenium. Thus, the USDA National Nutrient Database for Standard Reference (2007) was used as a database to estimate intake of these nutrients.

Subjects were also measured for anthropometric including height, body weight and waist circumference using standard protocol by Fidanza (1991). Body weight was measured using a digital weighing scale (SECA, Germany) to the nearest 0.1 kg. Height was measured using a SECA Bodymeter (SECA, Germany) to the nearest 0.1 cm. Waist circumference was measured using a flexible measuring tape to the nearest 0.1cm. The average of at least two measurements was calculated. The body mass index (BMI) was computed by dividing the weight (kg) over the square of height (m). Toenails and fingernails were collected from subjects. Hair was also cut randomly at different location on the head. All samples were stored in sealed plastic bags at room temperature. A total of 10 ml fasting venous blood taken by a nurse or medical laboratory assistance and centrifuged at 3000 rpm for 10 minutes. Plasma was aliquoted into Eppendorf tubes and stored at -80˚C for 6 months prior to analysis.

Biochemical analyses

A total of 0.1 ml of plasma was used to determine the lipid peroxidation level using thiobarbituric acid reactive substances (TBARS) following the method described by Ledwozyw et al. (1986). All procedures were done in dark to prevent further degradation of samples as a result of lipid peroxidation. TBARS is a pink chromogen product produced from the reaction between TBA and MDA. MDA is a secondary product of lipid peroxidation resulted from the presence of free radicals. Hundred microlitres of plasma were pipetted and mix with 400 ul

of distilled water in a test tube. A total fo 2.5 mls of TCA 1.22M/HCL 0.06M were added to the tube for the formation of protein precipitate following 15 minutes incubation at room temperature. Approximately 1.5 mls of 0.67% of TBA solution were added to the mixture before further incubation for 30 minutes in boiling water with each of the tube covered to prevent condensation. Tubes were then cooled at room temperature before added with 4 mls of 1-buthanol. The mixture was vortex for 3 minutes before centrifuged at 3000 rpm for 10 minutes. Three layers were formed but only the top 1-buthanol layer was taken for absorbance reading at 532 nm.

A total of 200 ul of plasma was used to analyse vitamin A and E using high-performance liquid chromatography (HPLC) method (Lee et al., 1992). The HPLC system, performed with a Hewlett Packard (HPLC series 1100, USA) equipped with degasser, quaternary pump, autosampler and diode array detector was used. Vitamins A and E were separated on an Ultrasphere octadecylsilyl (ODS) Hypersil reverse-phase column (5 um particle size, 250 mm x 4.6 mm I.D stainless steel column (Hewlett Parkard) with a mobile phase of methanol-butanol-water (89.5:5.5.5, v/v) at a flow rate of 1.2 ml/min. The detection wavelengths were at 340 nm for vitamin A and 290 nm for vitamin E.

Nails and hair selenium was measured using Inductively Coupled Plasma Mass Spectrometer (ICP-MS) method (Maxfield and Minar, 1983). Hair and toenails samples were prepared by appropriate nitric-perchloric acid digestion procedure (Ming and Big, 1990). The basis of the method was the measurement of atomic emission by an optical spectroscopic technique. Samples were nebulized and the aerosol that was produced was transported to the plasma torch where excitation occurred. Characteristic atomic-line emission spectra were produced by a radio-frequency inductively coupled plasma (ICP). The spectra was dispersed by a grating spectrometer and the intensities of the lines were monitored by photomultiplier tubes. The photocurrents from the photomultiplier tubes were processed and controlled by a computer system. A selenium standard was also run and a background correction technique was also required to compensate for variable background contribution to the determination of the trace elements.

Data analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 14.0. All the variables were tested for normality by the Kolmogorov-Smirnov test and test of homogeneity of variance before any statistical comparisons were made. Data transformation was carried out for variables whose distributions did not appear to be normally distributed. The qualitative data such as social and health factor, risks factor and FFQ data were analyzed by descriptive statistics and other statistical analysis. Continuously quantitative variables such as anthropometric measurements, biochemical data, dietary intake via DHQ and age were determined using t-test and Pearson correlation with p value less than 0.05. Crude OR calculation for binomial data were obtained from cross-tabulation-Cochran's and Mantel-Haenszel descriptive test. Then adjusted OR was calculated using a binary logistic regression analysis with the inclusion of established confounder factors such as educational level, type of occupation, relation, lactation, and waist circumference.

Results

Demographics data

A total of 250 subjects initially agreed to participate in this study, however, 10 subjects were dropped as they refused bloodwithdrawal and 18 subjects did not complete the interview process and four subjects were excluded because they had severe stress status as indicated by HADS score. Thus, a total of 57 cases and 139 controls aged 30 to 66 years old were recruited in the final analysis of this study. Most of the cases aged 40 years and above (82.9%), mean age of cases (47.2±7.4 y) and comparable with controls (46.8 ± 6.3 y) (Table 1). Most of the subjects

Table 1. Demographic and Socioeconomic Profile of Cases and Controls

Parameter	Cases (N=57)		Controls (N=139)	
	n	%	n	%
Ethnics				
Malay	34	59.6	77	55.4
Chinese	14	24.6	42	30.2
Indian	9	15.8	20	14.4
Religion				
Muslim	34	59.6	79	56.8
Buddhism	10	17.5	37	26.6
Christian	6	10.5	4	2.9
Hinduism	7	12.3	19	13.7
Age(year)				
30-35	2	3.5	8	5.8
36-40	2	3.5	20	14.4
41-45	13	22.8	30	21.6
46-50	24	42.1	38	27.3
>50	16	28.1	43	30.9
Educational level				
Non-educated	1	1.8	1	0.7
Primary school	26	45.6	18	12.9
Secondary school	22	38.6	83	59.7
University/college	8	14.1	37	26.6
Marital status				
Unmarried	3	5.3	8	5.8
Married	51	89.5	119	85.6
Divorced	1	1.8	5	3.6
Widower	2	3.5	7	5.0
Total household income per month				
<RM 500	1	1.8	3	2.2
RM 500- 1000	4	7.0	15	10.8
RM 1001-2000	22	38.6	37	26.6
RM 2001-3000	17	29.8	36	25.9
RM 3001-4000	4	7.0	18	12.9
RM 4001-5000	1	1.8	10	7.2
>RM 5000	8	14.0	20	14.4
Type of occupation				
Working	26	45.6	99	71.2
Not working/ Housewife	31	54.4	40	28.8
Living with				
Alone	0	0.0	5	3.6
Family/relative	57	100.0	134	96.4

Table 2. Sociodemographic, Reproductive History and Health Profile of Cases and Controls

Parameter		Cases (N=57)		Controls (N=139)		P value	OR (95% CI)
		n	%	n	%		
Age (year)	<40	5	8.8	23	16.5	0.165	0.5 (0.18-1.35)
	≥40	52	91.2	116	83.5		
Age of menarche (year)	<12	20	35.1	38	27.3	0.282	0.7 (0.36-1.35)
	≥12	37	64.9	101	72.7		
Household income/ month	<RM 1000	5	8.8	18	12.9	0.412	0.6 (0.23-1.83)
	≥RM 1000	52	91.2	121	87.1		
Educational level	Lower	27	47.4	19	13.7	0.000*	3.0 (1.56-5.58)
	Higher	30	52.6	120	86.3		
Working status	Working	26	45.6	99	71.2	0.001*	0.2 (0.09-0.36)
	Not working/ housewife	31	54.4	40	28.8		
Marital status	Not married	3	5.3	8	5.8	0.892	1.1 (0.28-4.30)
	Married	54	94.7	131	94.2		
Degree of relative	First	14	73.7	7	35.0	0.019*	6.3 (1.63-24.5)
	Second	5	26.3	13	65.0		
Pregnancy history	Yes	50	87.7	120	86.3	0.795	1.1(0.44-2.86)
	No	7	12.3	19	13.7		
Parity (times)	Less than 3	16	28.1	54	38.8	0.155	1.6 (0.83-3.18)
	More than 3	41	71.9	85	61.2		
Lactation	No	10	17.5	50	36.0	0.013*	2.6 (1.23-5.68)
	Yes	47	82.5	89	64.0		
Duration of lactation	<3 month	39	68.4	108	77.7	0.175	1.6 (0.81-3.19)
	≥3 month	18	31.6	31	22.3		
Menopausal status	Post-menopausal	21	36.8	50	36.0	0.908	1.0 (0.51-1.83)
	Pre-menopausal	36	63.2	89	64.0		
Hormone Replacement Therapy	Yes	2	3.5	6	4.3	0.796	1.2 (0.24-6.34)
	No	55	96.5	133	95.7		

* Significant difference at Chi-square test (p<0.05)

were Malays, followed by Chinese and Indians. Both cases and controls were mostly married and had total household income ranged between RM1001 to RM3000. Lower education was found to increase risk of breast cancer by 3 times [OR 3.0 (95%CI 1.56-5.58]. A higher percentage of cases were not working (54.4%) compared to controls (25.8%) (p<0.05). Thus, working reduced breast cancer risk by 80% [OR 0.2 (95%CI 0.09-0.36].

Reproductive history and health profile

Having a relative with breast cancer was found to be a strong risk factor was found to be strong risk factor, i.e. six fold. Interestingly, in this study lactating was found to increase risk of breast cancer by two fold (Table 2). Mean BMI of cases (26.1 ± 4.7 kg/m2) was not differed from controls (25.3 ± 4.5 kg/m2)(p>0.05). However, cases had a higher waist circumference (86.1 ± 10.8 cm) compared to controls (79.0 ± 10.1 cm). Thus, more cases (68.8%) had abdominal obesity according to WHO/ IOTF/ IASO (2000) classicification of waist circumference ≥ 80cm as compared to controls (42.6%) (p<0.05).

Nutrient intake and MDA value

The mean nutrient intakes of energy, vitamin A and E and plasma vitamin A and selenium in toenail were significantly lower in cases as compared to controls, whilst the mean protein intake and MDA value were higher in cases than controls (p<0.05 for all parameters) (Table 3). There was a trend that the higher intake of protein, vitamin A, E and selenium the lower breast cancer risk was noted (Table 4). At 75th percentile, more than 86.3g per day protein intake showed a reduction in breast cancer risk by 73% . A reduction of OR value was noted with the increased percentile of vitamin A intake (25th percentiles: 2.81, 50th percentile: 1.51, 75th percentile: 1.34). A decreasing OR value was also noted with the increased percentile of vitamin E intake (25th percentile, OR 2.32; 50th percentile, OR 2.18; 75th percentile 2.14). Selenium intake showed similar trend (Table 4). However, after adjustment for confounding factors including education, working status, family history, lactation and waist circumference, the risk of breast cancer only remain significant for protein, with protein intake of more or equal to 86.3g/d associated with 75% reduction in breast cancer risk (Table 5).

Table 3. Mean (±SD) Nutrient Intake, Plasma Lipid Peroxidation and Antioxidant Profile

Parameters (unit)	Cases (n=57)	Controls (n=139)
Energy (kcal/d)	1253 ± 286	1360 ± 367*
Protein (g/d)	62.1 ± 19.2	73.3 ± 33.6*
Carbohydrates (g/d)	172.1 ± 46.5	179.6 ± 49.8
Fat (g/d)	38.8 ± 13.1	41.9 ± 16.8
Vitamin A (µg/d)	606.2 ± 335	724.7 ± 414.4*
Vitamin E (mg/d)	6.1 ± 2.4	6.9 ± 3.0*
Selenium (µg/d)	56.9 ± 16.2	60.8 ± 17.5
Vitamin A (ug/l)	377.6 ± 417	431.5 ± 264.2*
Vitamin E (mg/dl)	3.3 ± 3.9	4.0 ± 2.7
Toenail selenium (µg/g)[#]	0.06 ± 0.09	0.11 ± 0.08*
Hair selenium (µg/g)[##]	0.06 ± 0.15	0.08 ± 0.18
MDA(mmol/g protein)	4.4 ± 1.1	3.2 ± 0.7*

*p <0.05, by independent sample t-test, [#][n=12], [##][n=36]

Table 4. Macronutrients, Antioxidant Intake and Status in Percentiles and Crude ORs

Parameter	Percentiles					
	25th	OR (95% CI)	50th	OR (95% CI)	75th	OR (95% CI)
Macronutrient						
Energy (kcal/d)	1068	0.57 (0.26-1.24)	1308	0.69 (0.34-1.42)	1521	0.93 (0.50-1.76)
Protein (g/day)	47.5	0.72 (0.35-1.47)	63.0	0.90 (0.47-1.70)	86.3*	0.27 (0.11-0.68)
Carbohydrate (g/d)	139.3	0.84 (0.45-1.59)	179.2	1.05 (0.50-2.21)	208.8	1.61 (0.75-3.46)
Fat (g/d)	28.8	0.66 (0.31-1.43)	38.9	0.69 (0.36-1.31)	49.2	1.25 (0.59-2.65)
Antioxidant intake						
Vitamin A (ug/d)	510.7*	2.81 (1.44-5.47)	829.2*	1.51 (0.78-2.94)	1202	1.34 (0.66-2.75)
Vitamin E (mg/d)	5.8	2.32 (1.20-4.48)	8.6*	2.18 (1.07-4.44)	10.3	2.14 (0.95-4.83)
Selenium (µg/d)	57.2	2.95 (1.22-7.12)	70.8*	2.17 (1.13-4.19)	92.5*	1.71 (0.84-3.52)
Antioxidant status						
Plasma vitamin A (µg/l)	284.3*	3.94 (2.07-7.57)	366.3*	2.65 (1.04-6.72)	564*	2.32 (1.18-4.57)
Plasma vitamin E (mg/dl) 2.74 (1.00-7.49)	2.5*	3.67 (1.61-8.33)	2.8*	2.76 (1.43-5.33)	3.18*	
Toenail selenium (µg/g)	0.04*	16.0 (3.27-78.3)	0.11	3.75 (0.87-16.2)	0.13	1.92 (0.36-10.4)
Hair selenium (µg/g)	0.03	2.96 (0.72-12.2)	0.11	1.00 (0.27-3.69)	0.19*	0.20 (0.05-0.84)
MDA	3.1	0.73 (0.28-1.96)	4.1	1.64 (0.86-3.15)	4.8*	6.41 (1.75-11.7)

*p<0.05, significant difference between cases and controls using independant Chi squared test

Biochemical indicators

With respect to biochemical indicators, the mean of plasma MDA, an indication of oxidative stress was higher among cases (3.9±1.7 mmol/g protein) as compared to controls (3.2±1.7 mmol/g protein) (p<0.05) (Table 3). Whilst, vitamin A status and selenium status as measured using toenail were better in controls than controls (p<0.05 for both parameters). The risk of breast cancer reduced with the increase in percentiles of plasma vitamin A, vitamin E, toenail and hair selenium. Inverse relationship was seen with MDA, of which the increased in percentiles of MDA, the higher the risk (Table 4). The breast cancer risk associated with these biochemical indicators remained significant after adjustment for confounding variable (Table 5). Women with low plasma vitamin A (ie. At 25th percentile, <284.3 µg/l or 50th percentile, 366.3 µg/l) were twice more likely to be at risk of getting breast cancer. Those with plasma vitamin E < 2.5 mg/dl, <2.8 mg/dl or <3.1 mg/dl had a higher risk of breast cancer, with adjusted OR at 2.9, 2.3 and 2.1, respectively. There was an inverse relationship between antioxidant status namely vitamin A (r = -0.237, p<0.05) and vitamin E (r = -0.453, p<0.05) with MDA. Thus, those with plasma MDA ≥ 4.8mmol/g protein had an increased risk of breast cancer by six times.

Table 5. Binary Logistic Regression Model for Dietary Intake and Antioxidant Status

Parameter	Crude OR	95% CI	Adj# OR	95% CI
Dietary Intake				
Protein (g/d)				
≥86.3/<86.3	0.27*	0.11-0.68	0.25*	0.12-0.54
Vitamin A (µg/d)				
<829.2/≥829.2	1.51*	0.78-2.94	1.31	0.59-2.87
Vitamin A (µg/d)				
<510.7/≥510.7	2.81*	1.44-5.47	1.38	0.67-2.84
Vitamin E (mg/d)				
<8.6/≥8.6	2.18*	1.07-4.44	1.57	0.68-12.4
Selenium (µg/d)				
<70.8/≥70.8	2.17*	1.13-4.19	1.56	0.72-3.39
Biochemical Indicators				
MDA (mmol/g protein)				
<4.8/≥4.8	4.41*	1.75-11.7	6.82*	1.95-23.9
Plasma vitamin A (µg/l) 25th percentile				
<284.3/≥284.3	3.94*	2.07-7.57	2.03*	0.62-6.65
Plasma vitamin A (µg/l) 50th percentile				
<366.3/≥366.3	2.65*	1.04-6.72	2.05*	0.19-4.10
Plasma vitamin E (mg/dl) 25th percentile				
<2.5/≥2.5	3.67*	1.61-8.33	2.97*	1.38-3.48
Plasma vitamin E (mg/dl) 50th percentile				
<2.8/≥2.8	2.76*	1.43-5.33	2.32*	1.07-2.41
Plasma vitamin E (mg/dl) 75th percentile				
<3.1/≥3.1	2.74*	1.00-7.49	2.12*	1.00-4.21

#Adjusted OR for type of education, type of occupation, relatives, lactation and waist circumference. *p<0.05, significant difference between cases and controls using Chi-squared test

Discussion

As indicated in this and other studies (Dite et al., 2003; Negri et al., 1997), family history, especially having a first degree relative with breast cancer has been consistently reported as an important risk factor by breast cancer. In particular, a study by Collaborative Group of Hormonal Factors in Breast Cancer (2001) found a significant association between breast cancer risk factor and enlarged number of first degree relative with breast cancer. Speiser (1996) reported that syndrome familial cancer was inherited in the dominant gene autosome. The findings of the study that socioeconomic factors (lower education) could increase the risk of breast cancer by 3 times were consistent with other study (Pan et al., 2004). In contrast, lifestyle behavior such as smoking and alcohol were not found to be associated with breast cancer as reported in other study (Pan et al., 2004), probably due to the small proportion of subjects who were smoking or consuming alcohol. Interestingly, this study found that women who were lactating had a higher risk of breast cancer by two folds. However, detailed questionnaire on duration of lactating was not asked in this study. Other studies found that lactating was a protective factor against breast cancer, with the longer women breastfeed the more they are protected against breast cancer (Collaborative Group on Hormonal Factors in Breast Cancer 2002,

Furberg et al., 1999).

This study found that gross measurement of obesity using BMI was not related to breast cancer, however, abdominal obesity as determined using waist circumference could raise the breast cancer risk to three folds after adjusting for confounding factors. Other study in Malaysia by Kamarudin et al. (2006) also showed similar finding. Thus, trimming the waist line is probably one of the prevention strategies to reduce breast cancer risk among Malaysian. A pilot intervention study by Fitzgibbon et al. (2004) found that the combination of weight reduction and breast health scheduled examination had decreased in breast cancer risk factor among women.

It seems that the higher the protein intake the lower the breast cancer risk. However, this study had not analysed the different effect from animal and protein intake. A recent study by Cho et al (2003) has found that intake of red meat could increased the cancer risk as compared to protein from plant product sources which was more protective. With respect to antioxidant intake, this study found that a higher intake of vitamin A, vitamin E and selenium, the lower the breast cancer risk. In particular, vitamin A intake of less than 510.7 μg/d or vitamin E intake less than 5.8 mg/d could increased breast cancer risk by 2.81 and 2.32, respectively. Whilst, selenium intake less than 70.8 μg/d could increased the risk of breast cancer by two fold. However, the contribution was not significant using binary logistic regression after controlling for cofounding factors (ie. education, working status, family history, lactation and waist circumference). These findings indicated that food intake at some extent is influenced by these external variables. Nevertheless, the study showed that poor antioxidant status as determined using plasma vitamin A and plasma vitamin E were associated with high risk of breast cancer, even after adjustment for confounding variables. In particular, low level of plasma vitamin A (ie. < 284.3 μg/l or < 366.3 μg/l) could increased breast cancer risk by two fold. Whilst, low level of plasma vitamin E (ie.< 2.5 to <3.1 mg/dl) could increased breast cancer risk by two to almost three folds. Similar trend was noted for toenail and hair selenium at univariate analysis, however, multivariate analysis could not be conducted for these parameters due to small sample size. Quite a number of subjects, especially Chinese refused to give their toenail and hair samples due to traditional belief that this practice will give bad luck.

A high level of oxidative stress as measured using MDA is associated with reduced level of plasma vitamin A and E. It should be borne in mind, in this study subjects who had emotional stress or chronic diseases that might resulted in high oxidative stress thus deprived antioxidant status were excluded. After adjusting for confounding factors, it was found that plasma vitamin A less than 284.3 μg/l or 366.3 μg/l increased risk of breast cancer by two fold. Whilst, the risk ranged between two to almost three folds with low plasma vitamin E (<2.5 mg/dl to < 3.1 mg/dl). Subsequently, high level of oxidative stress as measured using MDA was associated with almost six folds increased in breast cancer risk. Other studies had shown similar relationship between the antioxidants status with breast cancer risk (Ray and Husain, 2001, Ray and Husain, 2002, Kumaraguruparan et al., 2002; Kumaraguruparan et al., 2005, Torun et al., 1995, Aghvami et al., 2006).

In conclusion, the present study demonstrated that low level of antioxidant nutrients namely vitamin A, E and selenium and poor antioxidant status and also high oxidative stress could increased the risk of developing breast cancer. Thus, it is essential for Malaysian women to adopt a healthy lifestyle with consumption of diet rich in antioxidant nutrient and adopt a healthy behavior to reduce oxidative stress, in order to prevent breast cancer.

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