Jurnal Sains Kesihatan Malaysia **6** (1) 2008: 61-77

Association between Oxidative DNA Damage, Fruits and Vegetables Intake with Breast Cancer: A Preliminary Study in Klang Valley

(Hubungan di antara Kerosakan DNA Oksidatif, Pengambilan Buah-Buahan dan Sayur-Sayuran dengan Kanser Payudara: Suatu Kajian Awal di Lembah Klang)

SUZANA SHAHAR, KIM TIU TENG, NOR FADILAH RAJAB
& FATIMAH ARSHAD

ABSTRACT

A preliminary study was conducted to determine the level of oxidative DNA damage, fruits and vegetables intake among 50 breast cancer patients (cases) as compared to 50 healthy women (controls) with no known medical history of breast cancer in Klang Valley. Both groups were matched for age and ethnicity. Data on socio-demographic, health status and medical history, fruits and vegetables intake, and supplements intake were obtained through an interview-based questionnaire. Anthropometry measurements included weight, height, and waist and hip circumference were also carried out on subjects. A total of 3mL fasting venous blood was drawn to assess lymphocytes oxidative DNA damage using Alkaline Comet Assay. Results indicated that the mean intake of fruits and vegetables was lower in cases (4.09 ± 1.17 servings/d) than controls (4.77 ± 0.90 servings/d)($p < 0.05$) The intake of fruits and vegetables from family groups of solanaceae, myrtaceae, caricaceae, apiaceae, brinjal, rutaceae, broccoli, orange, carrot, watermelon were 0.5 - 1 servings/week significantly higher among controls as compared to cases ($p < 0.05$ for all parameters). However, the intake of fruits from rosaceae family and apple was higher among controls than cases ($p < 0.05$). The estimated intake of β-carotene, carotenoids, vitamin A, vitamin C ($p < 0.001$), α-carotene and lycopene ($p < 0.05$) from fruits and vegetables were higher among controls than cases. Mean DNA damage level of cases (4.55 ± 1.78 % DNA in tail, %TD; 0.35 ± 0.21 tail moment, TM) were 3.5 and 3.9 times higher than the value of controls (1.3 ± 0.70% TD; 0.09 ± 0.09 TM) ($p < 0.001$) and the damage increased with higher values of waist hip ratio (% TD, $r = 0.396$, $p < 0.05$; TM, $r = 0.349$, $p < 0.05$) and waist circumference (% TD, $r = 0.334$, $p < 0.05$; TM, $r = 0.360$, $p < 0.05$). There was an inverse relationship between oxidative DNA damage with intake of total fruits and vegetables, cauliflowers and water convolvulus and also consumption from rutaceae and solanaceae families. Similar trend was noted for estimated *intake of vitamin A, carotenoids, vitamin C, β-carotene and lycopene. In conclusion, the intake of fruits and vegetables of five servings/d and the consumption of specific families and types of fruits and vegetables might protect against oxidative DNA damage and further reduce breast cancer risk.*

Key words: *DNA oxidative damage, Breast cancer, Alkaline Comet Assay, Fruits and vegetables*

ABSTRAK

Satu kajian awal telah dijalankan untuk menentukan tahap kerosakan oksidatif DNA, pengambilan buah-buahan dan sayur-sayuran di kalangan 50 pesakit kanser payudara (kes) berbanding dengan 50 wanita sihat yang tidak mengalami kanser payu dara (kawalan) di sekitar Lembah Klang. Kedua-dua kumpulan dipadankan dari segi umur dan bangsa. Data sosio-demografi, status kesihatan dan sejarah perubatan, pengambilan buah-buahan dan sayur-sayuran, serta pengambilan supplemen telah diperolehi melalui temu duga menggunakan soal selidik. Pengukuran antropometri yang merangkumi berat, tinggi, ukurlilit pinggang pinggul juga dilakukan ke atas subjek. Sejumlah 3mL darah venus berpuasa telah diambil untuk menilai kerosakan oksidatif DNA limfosit dengan kaedah Asai Komet Beralkali. Hasil menunjukkan bahawa pengambilan buah-buahan dan sayur-sayuran adalah lebih rendah di kalangan kes (4.09 ± 1.17 hidangan/h) berbanding kawalan (4.77 ± 0.90/h) ($p < 0.95$). Pengambilan buah-buahan dan sayur-sayuran daripada kumpulan keluarga solanaceae, myrtaceae, caricaceae, apiaceae, brinjal, rutaceae, brokoli, oren, lobak merah dan tembikai di kalangan subjek kawalan adalah 0.5 - 1 hidangan/minggu lebih tinggi secara signifikan berbanding dengan kes ($p < 0.05$ untuk semua parameter). Walau bagaimanapun, pengambilan buah-buahan daripada keluarga rosaceae dan epal adalah lebih tinggi di kalangan subjek kes berbanding dengan kawalan ($p < 0.05$ untuk kedua-dua parameter). Pengambilan nutrien β-karoten, karotenoid, vitamin A, vitamin C ($p < 0.001$), α-karoten dan likopen ($p < 0.05$) adalah lebih tinggi di kalangan subjek kawalan berbanding dengan kes. Min aras kerosakan DNA subjek kes (4.55 ± 1.78% DNA in tail, % TD; 0.35 ± 0.21 tail moment, TM) adalah 3.5 dan 3.9 kali lebih tinggi berbanding kawalan (1.3 ± 0.70 % TD; 0.09 ± 0.09 TM) ($p < 0.001$), dan kerosakan ini meningkat dengan peningkatan nisbah pinggang pinggul (% TD, $r = 0.396$, $p < 0.05$; TM, $r = 0.349$, $p < 0.05$) dan ukurlilit pinggang (% TD, $r = 0.334$, $p < 0.05$; TM, $r = 0.360$, $p < 0.05$). Terdapat hubungan songsang di antara kerosakan DNA dengan pengambilan buah-buahan dan sayur-sayuran total, bunga kobis dan water convolvulus dan pengambilannya dari keluarga rutaceae dan solanaceae. Tren yang sama diperolehi bagi anggaran pengambilan vitamin A, karotenoids, vitamin C, β-carotene dan likopen. Kesimpulannya, pengambilan buah-buahan dan sayur-sayuran sebanyak lima hidangan/h dan pengambilannya dari kumpulan *keluarga dan jenis tertentu mungkin melindungi kerosakan oksidatif DNA dan dijangka mengurangkan risiko kanser payudara.*

Kata kunci: Kerosakan oksidatif DNA, Kanser payudara, Asai Komet Beralkali, Buah-buahan dan sayur-sayuran

INTRODUCTION

Breast cancer is the leading cause of cancer mortality in women. In the year 2005, breast cancer contributed to 502,000 deaths globally (WHO 2006). In Malaysia, 3738 incidence of breast cancer was reported in the year 2003 (National Cancer Registry 2004). According to the Second Report of National Cancer Registry (2004), age-standardized incidence rate of breast cancer are 52.8 in 100,000 populations. Several factors, such as family history, null parity, and later age at giving birth are well established breast cancer risk factors (Collaborative Group on Hormonal Factors in Breast Cancer 2002). Many studies suggested that environmental factors (Hejar et al. 2004) and genetic susceptibility (Parsons 2005) also contributed to breast cancer risk.

Oxidative DNA damage might play an important role in the process of carcinogenesis (Valko et al. 2006). At high concentration, reactive oxygen species were important mediators to the damage of cell structures, nucleic acids, lipids and proteins (Valko et al. 2006). Due to this, consumption of fruits and vegetables which containing a mixture of natural antioxidant and phytochemical compounds are linked to the protection of biological system from the oxidative DNA damage and further reduced breast cancer risk. In Malaysia, studies on breast cancer are scarce, and limited only to health risk factors as compared to diet and oxidative DNA damage. For example, a study (Suzana et al. 2004) reported that fiber intake less than 10 gram per day contributed to 2.2 times breast cancer risk. Other studies showed that obesity, family history, use of oral contraceptive (Norsa'adah et al. 2005) and breast feeding (Hejar et al. 2004) reduced breast cancer risk. Therefore, the aim of this study was to evaluate the relationship of fruits and vegetables consumption and oxidative DNA damage in breast cancer patients and healthy women.

EXPERIMENTAL METHODS

STUDY DESIGN

This is a preliminary comparative study on 50 breast cancer patients (cases) and 50 healthy women (controls) with respect to oxidative DNA damage and fruits and vegetables intake. Cases were newly diagnosed breast cancer patients (as diagnosed using biopsy), not at the 4th stage of breast cancer, not undergoing chemotherapy or radiation, not lactating and not suffering from any other chronic diseases. They were recruited from outpatients department and surgical ward of Kuala Lumpur Hospital (HKL), Selayang Hospital and Hospital Universiti Kebangsaan Malaysia (HUKM). Controls were healthy women without past medical history of breast cancer and had undergone self-examination or mammogram, not suffering from any chronic diseases and not lactating. They were staffs at HKL, Universiti Kebangsaan Malaysia (Kuala Lumpur's campus) or residents of housing area at Sri Gombak and Titiwangsa. Cases and controls were matched according to age (± 5 years) and ethnicity. Sample size was calculated with 95% confidence interval and 80% study power following the method of Sample Size Determination in Health Studies (Lwanga & Lemeshow 1991). Socio-demographic data, health status and medical history, fruits and vegetables intake and supplements intake were obtained through interview based questionnaire. In addition, anthropometric measurements including weight, height, waist and hip circumferences were also carried out on subjects. In a sub-sample of 30 subjects, a total of 3 mL fasting venous blood sample was collected using heparinized tube for genotoxicity analysis using the Alkaline Comet Assay. The study has been approved by the Research and Medical Research Ethical Committee of the Universiti Kebangsaan Malaysia (NN-026-2004). This study is part of a larger study investigating the relationship between diet, lifestyle, body composition, physical activity and hormone level and breast cancer as reported earlier (Rabeta et al. 2007).

FOOD FREQUENCY QUESTIONNAIRE (FFQ)

A pre-tested quantitative FFQ which consists of 58 vegetables and 26 fruits commonly consumed by Malaysian population was used in this study. Participants were asked to describe the frequency of consuming a particular food per day, per week, per month or per year over the past one year. The FFQ was also used to estimate the intake of selected antioxidant nutrients namely α-carotene, β-carotene, total carotenoids, cryptoxanthin, lutein, lycopene, vitamin A and vitamin C. The nutrient was analyzed using the Nutritionist Pro software based on Malaysian Food Composition Table (Tee et al. 1997) for vitamin A, and C β-carotene, and USDA food database (USDA 2007) for other nutrients investigated. Fruits and vegetables consumed were quantified according to the Atlas of Food Exchanges & Portion Sizes (Suzana et al. 2002). One serving of vegetables is equal to half cup of cooked dark green vegetables with edible stem, root vegetables or sweet potatoes. While one serving of fruits equal to half cup or half medium size guava; one slice papaya, watermelon or pineapple; and one medium banana, orange, pear or apple.

ALKALINE COMET ASSAY

The Alkaline Comet Assay conducted in this study was modified according to the standard method (Singh et al. 1988). All of the steps were performed in the dark. A total of 100 μl 5% normal melting point (NMA) agarose was spread on top of each frosted slides. The slides were immediately covered with cover slips and kept at room temperature for 5 minutes to allow the agarose gel to solidify. Fresh whole blood of 10 μl was mixed with 80 μl of low melting point (LMA) agarose to form a suspension. The cover slips were gently removed and the suspension was gently pippeted onto the first layer of agarose, then covered with cover slips again and kept on ice for 5 minutes to solidify. After removal of the cover slips, the slides were immersed in cold lysing solution (2.5M NaCl, 10 μM Na_2EDTA, 10mM Tris, pH 10) with Triton X-100 and 10% DMSO prepared one hour before used. After one hour, the slides were removed from the lysing solution, drained and placed in electrophoresis tank. Electrophoresis tank was then filled with freshly prepared electrophoresis solution (1mM Na_2EDTA and 10N NaOH, pH 13) to cover all slides. Before electrophoresis, the slides were left in the solution for 20 minutes to allow unwinding of DNA double helix. Electrophoresis was conducted at 25 volt, 300 mA, and 20 minutes. After electrophoresis, the slides were taken out of the tank and neutralized using neutralizing buffer (0.4M Tris, pH 7.5) for 5 minutes and repeated three times. Onto each slide, 35 μl ethidium bromide (EtBr 10 μg/ml) was added and covered with a cover slip. All slides were placed in a humidified airtight container to prevent drying of the gel and analyzed the very next day. A total of 100 cells were captured by Leica Leitz Laborlux S, Germany and analyzed by Comet Score™.

STATISTICAL METHODS

Statistical tests were conducted using Statistical Packages for Social Sciences (SPSS) Version 12.0. Descriptive data were presented as mean and percentage between cases and controls. In order to investigate the difference of lymphocytes oxidative DNA damage, fruits and vegetables intake among cases and controls, Independent Sample *t*-test were used. Chi squared test was used to analyze categorical data. The relationship between oxidative DNA damage and consumption of fruits and vegetables were analyzed using Pearson Correlation Coefficient.

RESULTS

One hundred women aged 26 - 65 years, consisted of fifty breast cancer patients and fifty controls participated in this study. The ethnic composition of the cases was 70% Malays, 18% Chinese, 12% Indians, and controls was 70% Malays, 18% Chinese, and 12% Indians. The mean age of cases (49.62 ± 7.56 years) was comparable with controls (47.04 ± 8.99 years). The mean weight of cases and controls were 60.39 ± 9.46 kg and 58.83 ± 10.93 kg, respectively; height 156.65 ± 5.35 cm and 158.02 ± 7.82 cm, body mass index (BMI) 24.65 ± 3.91 kg/m^2 and 23.58 ± 4.18 kg/m^2.

As shown in Table 1, a higher percentage of cases had larger waist circumference and waist hip ratio (WHR) as compared to controls ($p < 0.05$ for both parameters). Cases were also more likely to have family history of breast cancer (32%) and use birth control (36%) as compared to controls (14% for family history and 18% for use of birth control) ($p < 0.05$) and use of oral contraceptive ($p < 0.05$) were significantly associated with breast cancer risk in this study. The association for alcohol consumption and smoking could not be computed due to small percentage of subjects who had these attributes.

Table 2 shows that the mean intake as expressed by serving size per day of fruits (1.49 ± 0.60 servings/day), vegetables (3.29 ± 0.73 servings/day) ($p < 0.05$) and total fruits and vegetables (4.77 ± 0.90 servings/day) ($p < 0.01$) of controls were higher than cases (1.23 ± 0.51, 2.86 ± 0.98, 4.09 ± 1.17 servings/day, respectively). When consumption of fruits and vegetables were classified according to family group, the intakes from the family of apiaceae caricaceae, myrtaceae, rutaceae and solanaceae were significantly higher among controls than cases ($p < 0.05$ for all parameters). However, cases showed a higher consumption of fruits in rosaceae family and apple than controls ($p < 0.05$). Controls consumed a significantly higher amount (approximately half to one serving/ week) of specific types of fruits and vegetables namely brinjal, broccoli, carrot, orange and watermelon than cases (less than half to one serving/week) ($p < 0.05$ for all parameters). Figure 1 shows that the intake of α-carotene, lycopene ($p < 0.05$), β-carotene, carotenoids, lutein, vitamin A and vitamin C ($p < 0.001$ for all parameters) estimated from fruits and vegetables among controls were significantly higher than cases.

Table 3 shows that cases (5.35 ± 1.79 % TD; 0.435 ± 0.257 TM) displayed a significantly higher oxidative DNA damage as assessed using alkaline Comet Assay compared to controls (1.41 ± 0.81 % TD; 0.086 ± 0.086 TM)($p < 0.001$). Oxidative DNA damage (% TD and TM) among cases in the 31 - 40 years old ($p < 0.05$), 41-50 years old ($p < 0.001$) and above 50 years old ($p < 0.001$) were significantly higher than controls. Among cases, it appeared that DNA damage was the highest among those aged > 50 years old. Furthermore, a higher oxidative DNA damage was showed in subjects with WHR (% TD $r = 0.396$, $p < 0.05$; TM $r = 0.349$, $p < 0.05$) and waist circumference (% TD $r = 0.334$, $p < 0.05$; TM $r = 0.360$, $p < 0.05$).

There was an inverse relationship between oxidative damage with total fruits and vegetables intakes (% TD $r = -0.279$, $p < 0.05$; TM $r = -0.298$, $p < 0.05$) (Table 4). Similar trend was noted for fruits and vegetables intake from rutaceae family (% TD $r = -0.284$, $p < 0.05$), solanaceae family (TM $r = -0.298$, $p < 0.05$), cauliflowers (TM $r = -0.571$, $p < 0.05$) and water convolvulus (TM $r = -0.674$, $p < 0.05$). As shown in Table 5, there was an inverse relationship between the estimated intake of selected antioxidants from fruits and vegetables with oxidative DNA damage, particularly vitamin A, carotenoids and vitamin C that showed significant association in both % TD and TM. Whilst, β-carotene and lycopene only showed significant association with either % TD or TM only.

TABLE 1. Physical and health characteristics of cases and controls

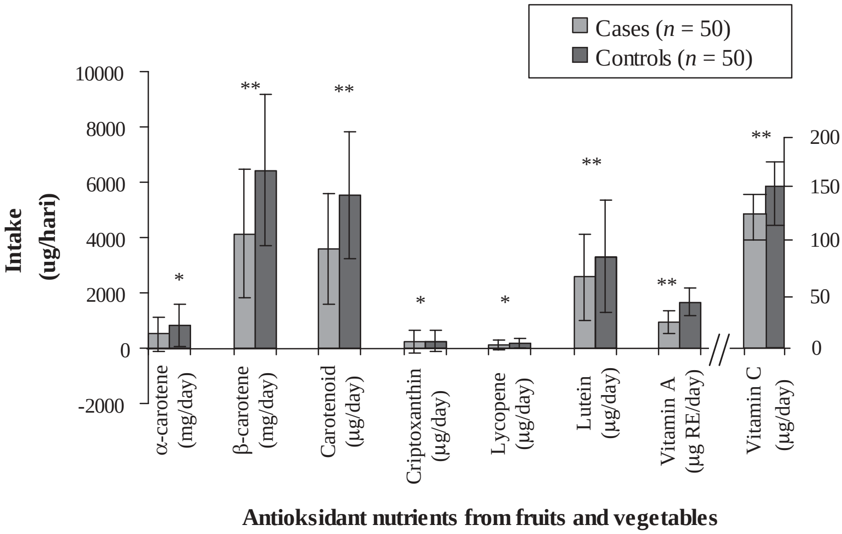
Characteristic	Cases (n = 50)	Controls (n = 50)	p
	n (%)	n (%)	
BMI			
≥ 25 kg/m^2	19 (38)	15 (30)	0.398
< 25 kg/m^2	31 (62)	35 (70)	
Waist (cm)			
≥ 80 cm	36 (72)	22 (44)	0.005**
< 80 cm	4 (28)	28 (56)	
WHR			
≥ 0.85	30 (60)	18 (36)	0.016*
< 0.85	20 (40)	32 (64)	
Alcohol intake			
Yes	10 (20)	2 (4)	-
No	40 (80)	47 (94)	
Smoking			
Yes	6 (12)	0 (0)	-
No	46 (92)	50 (100)	
Family history			
Yes	16 (32)	7 (14)	0.031*
No	34 (78)	43 (86)	
Menopausal status			
Yes	27 (54)	23 (46)	0.424
No	23 (46)	27 (54)	
Age of menarche (year)			
< 11	5 (10)	2 (4)	0.240
≥ 11	45 (90)	48 (96)	
First pregnancy (year)			
≥ 30	42 (84)	39 (78)	0.444
< 30	8 (16)	11 (22)	
Lactation			
Yes	35 (70)	35 (70)	1.000
No	15 (30)	15 (30)	
Use of birth control			
Yes	18 (36)	9 (18)	0.043*
No	32 (64)	41 (82)	
Use of Hormone Replacement Therapy			
Yes	2 (4)	0 (0)	
No	48 (96)	50 (100)	0.15

* p < 0.05, **p < 0.01, Chi square

TABLE 2. Mean (± SD) intake of fruits and vegetables, fruits and vegetables according to family classification and types of fruits and vegetables frequently consumed among cases and controls

Food groups	Cases (n = 50)	Controls (n = 50)	p
	Mean ± SD	Mean ± SD	
Fruits (servings/day)	1.23 ± 0.51	1.49 ± 0.60	0.022*
Vegetables (servings/day)	2.86 ± 0.98	3.29 ± 0.73	0.015*
Total fruits and vegetables (servings/day)	4.09 ± 1.17	4.77 ± 0.90	0.001*
Fruits & vegetables according to family classification (servings/week)			
Alliaceae	1.62 ± 2.06	1.76 ± 1.76	0.732
Amaranthaceae	0.80 ± 0.80	1.37 ± 0.95	0.053
Anacardiaceae	0.52 ± 0.76	0.56 ± 1.15	0.856
Apiaceae	1.10 ± 1.28	2.22 ± 1.34	0.013*
Asteraceae	0.38 ± 0.70	0.48 ± 0.76	0.507
Brassicaceae	5.06 ± 2.00	6.03 ± 3.20	0.128
Caricaceae	0.56 ± 1.20	1.09 ± 0.88	0.013*
Convolvulaceae	0.58 ± 1.4	0.94 ± 1.15	0.165
Cucurbitaceae	3.40 ± 2.03	2.75 ± 3.15	0.223
Leguminosae	2.26 ± 2.23	2.27 ± 2.22	0.459
Malvaceae	0.53 ± 0.50	1.30 ± 0.52	0.216
Musaceae	1.08 ± 1.98	1.70 ± 1.47	0.079
Myrtaceae	0.24 ± 0.83	0.60 ± 0.48	0.029*
Rosaceae	2.55 ± 1.19	1.29 ± 1.59	0.002*
Rutaceae	0.94 ± 1.28	1.75 ± 0.99	0.001*
Solanaceae	3.63 ± 2.27	4.77 ± 2.68	0.037*
Vitaceae	0.49 ± 0.45	0.79 ± 0.92	0.224
Fruits and vegetables frequently consumed (≥1 servings/week)			
Apple	2.12 ± 0.63	1.76 ± 0.66	0.006*
Banana	1.84 ± 0.62	1.98 ± 0.65	0.274
Brinjals	1.54 ± 0.58	1.84 ± 0.74	0.026*
Broccoli	1.52 ± 0.71	2.00 ± 0.83	0.002*
Cabbage	1.72 ± 0.73	2.00 ± 0.86	0.661
Carrot	1.70 ± 0.70	2.16 ± 0.71	0.002*
Cauliflowers	1.90 ± 0.81	1.98 ± 0.65	0.589
Chinese cabbage	1.72 ± 0.76	1.96 ± 0.76	0.112
Chinese mustard leaves	1.72 ± 0.73	2.00 ± 0.86	0.062
Four angled beans	1.72 ± 0.70	1.80 ± 0.64	0.552
Garlic	1.92 ± 0.83	1.96 ± 0.63	0.798
Grapes	1.84 ± 0.71	2.00 ± 0.78	0.287
Kale	1.78 ± 0.74	1.90 ± 0.81	0.448
Mung beans	1.74 ± 0.80	1.78 ± 0.76	0.799
Orange	1.68 ± 0.47	1.96 ± 0.53	0.006*
Papaya	1.82 ± 0.69	2.12 ± 0.82	0.051
Potatoes	2.04 ± 0.67	1.94 ± 0.77	0.489
Spinach	1.98 ± 0.75	1.92 ± 0.74	0.683
String beans	1.82 ± 0.75	2.00 ± 0.85	0.266
Tomatoes	1.88 ± 0.77	2.18 ± 0.75	0.051
Water convulvolus	1.68 ± 0.71	1.96 ± 0.75	0.059
Watermelons	1.64 ± 0.69	2.12 ± 0.75	0.001*

*p < 0.05, Independent sample t-test



*p < 0.05, **p < 0.01, Independent Sample t-test

FIGURE 1. Mean antioxidants intake from fruits and vegetables among cases and controls

TABLE 3. Oxidative DNA damage according to age group among cases and controls

	% TD[a]		p	TM[b]		p
	Cases (n = 30)	Control (n = 30)		Cases (n = 30)	Control (n = 30)	
Mean ± SD	5.35 ± 1.79	1.41 ± 0.81	0.000**	0.435 ± 0.257	0.086 ± 0.086	0.000**
Age Group (year)						
≥ 30	3.73 ± 0.00	1.12 ± 0.64		0.157 ± 0.000	0.110 ± 0.060	
31- 40	4.45 ± 1.55	2.13 ± 0.72	0.006*	0.279 ± 0.060	0.130 ± 0.116	0.006*
41 – 50	3.72 ± 1.42	1.03 ± 0.57	0.000**	0.286 ± 0.140	0.046 ± 0.053	0.000**
> 50	5.35 ± 1.79	1.41 ± 0.81	0.000**	0.440 ± 0.257	0.086 ± 0.086	0.000**

[a] % DNA damage in tail
[b] Tail moment
*p < 0.05, ** p < 0.001, Independent sample t-test

DISCUSSION

The findings that family history of breast cancer and usage of oral contraceptive were associated with breast cancer were consistent with other studies reported previously (Antoniou et al. 2003; Gammon et al. 2004; Nagata et al. 2006). In this study, it was found that abdominal obesity as measured using WHR and waist circumference would increase risk of breast cancer by approximately three times. This finding is consistent with other study in Malaysia (Kamarudin et al. 2006).

TABLE 4. Correlation between oxidative DNA damage, fruits and vegetables intake, and fruits and vegetables intake according to family classification, fruits and vegetables frequently consumed among subjects

Food groups	% TD[a]		TM[b]	
	r	p	r	p
Fruits	-0.207	0.113	-0.301	0.019*
Vegetables	-0.227	0.082	-0.181	0.166
Total fruits and vegetables	-0.279	0.031*	-0.298	0.021*
Fruits & vegetables according to family classification				
Alliaceae	-0.169	0.196	-0.201	0.123
Amaranthaceae	-0.128	0.329	-0.065	0.619
Anacardiaceae	-0.067	0.610	-0.117	0.375
Apiaceae	-0.063	0.632	-0.034	0.799
Asteraceae	0.046	0.729	0.123	0.351
Brassicaceae	-0.007	0.961	0.067	0.610
Caricaceae	-0.162	0.217	-0.198	0.129
Convolvulaceae	-0.204	0.117	-0.211	0.105
Cucurbitaceae	0.155	0.237	0.098	0.456
Leguminosae	0.080	0.542	0.116	0.376
Malvaceae	0.022	0.866	0.071	0.590
Musaceae	-0.165	0.207	-0.201	0.123
Myrtaceae	-0.236	0.070	-0.196	-0.134
Rosaceae	0.178	0.173	-0.029	0.825
Rutaceae	-0.284	0.028*	-0.195	0.136
Solanaceae	-0.165	0.207	-0.298	0.021*
Vitaceae	0.024	0.854	0.078	0.554
Fruits and vegetables frequently consumed (≥ 1 serving/week)				
Apple	0.177	0.176	0.012	0.930
Banana	-0.409	0.130	-0.319	0.247
Brinjals	-0.233	0.403	-0.240	0.389
Broccoli	-0.214	0.444	0.065	0.819
Cabbage	0.056	0.843	0.249	0.370
Carrot	-0.194	0.489	-0.063	0.822
Cauliflowers	0.408	0.131	-0.571	0.026*
Chinese cabbage	-0.065	0.817	-0.018	0.949
Chinese mustard leaves	-0.112	0.624	0.033	0.906
Four angled beans	-0.172	0.541	-0.041	0.884
Garlic	-0.238	0.394	-0.328	0.232
Grapes	-0.330	0.230	-0.284	0.304
Kale	-0.165	0.556	0.285	0.303
Mung beans	-0.544	0.036*	0.331	0.227
Orange	-0.318	0.249	-0.193	0.491
Papaya	-0.207	0.460	-0.284	0.305
Potatoes	-0.165	0.556	-0.237	0.394
Spinach	-0.185	0.510	-0.221	0.429
String beans	-0.272	0.326	0.092	0.743
Tomatoes	-0.091	0.747	-0.172	0.539
Water convulvolus	-0.487	0.065	-0.674	0.006*
Watermelons	-0.203	0.469	-0.285	0.303

[a] % DNA damage in tail
[b] Tail moment
*$p < 0.05$, Pearson Correlation Test

TABLE 5. Correlation (*r*) between oxidative DNA damage, antioxidant nutrients from fruits and vegetables among cases and controls

Micronutrients	% TD[a]		TM[b]	
	r	p	r	p
α-carotene	-0.031	0.821	0.067	0.611
β-carotene	-0.367	0.004*	-0.217	0.096
Carotenoid	-0.396	0.002*	-0.316	0.014*
Criptoxanthin	0.120	0.363	0.079	0.550
Lycopene	-0.223	0.087	-0.260	0.045*
Lutein	-0.224	0.086	-0.140	0.280
Vitamin A	-0.477	0.000**	-0.316	0.004*
Vitamin C	-0.323	0.012*	-0.278	0.032*

[a] % DNA damage in tail
[b] Tail moment
*$p < 0.05$, ** $p < 0.001$, Pearson Correlation Test

Studies on breast cancer and fruits and vegetables intake are usually controversial. Case controls study (Adzersen et al. 2003), cohort study (Fung et al. 2005) and meta-analysis (Gandini et al. 2004) showed protective effects of fruits and vegetables consumption to breast cancer risk. However, some other studies (Smith-Warner et al. 2001 & Malin et al. 2003) did not found positive association between fruits and vegetables consumption with breast cancer risk. The controversial results might be due to variation of study methodology and subjects characteristics. A randomized controlled trial might give a clearer picture of specific anti-carcinogenic effects of bioactive compounds but it takes longer time and the process is complicated. However, case control study or cohort study might provide or identify a potential compounds in fruits and vegetables beneficial to prevent breast cancer risk.

In this study, the classification of fruits and vegetables according to family group which contain specific phytochemical compounds identical to the specific families might deepened the horizon of study on breast cancer. It was found that the consumption of fruits and vegetables from the family of apiaceae, caricaceae, myrtaceae, rutaceae and solanaceae were significantly higher among controls than cases. Fruits belonged to myrtaceae family; such as guava is rich in vitamin C, flavonoids and phenolic compounds that exert free radical scavenging ability especially at hypoclorous acid (Neergheena et al. 2006). Rutaceae family which is more commonly known as citrus family is rich source of vitamin C and secondary limonoids compounds. The seeds and fruits of citrus contain triterpenoids complex such as aglycon and glucoside that are potent antibacterial and antitoxicity agents (Rodrigues et al. 2000). In addition, carrots and coriander leaves that belonged to apiaceae family are high in β-carotene and α-carotene contents (Tee et al. 1997). Coriander leaves are high in polyphenol compounds that are potential hydrogen peroxide scavenger (Hashim et al. 2005). Papaya is the representative of caricaceae family that is high in lycopene, criptoxanthin and carotenoids (Tee et al. 1997). A study showed that consumption of 400 g/day papaya increased antioxidant level in healthy male subjects (Rahmat et al. 2004). Vegetables such as tomatoes, brinjals, pepper and potatoes those are bright in colors belong to solanaceae family. For example, tomatoes are high in lycopene that exert two times higher ability than β-carotene and one time higher than α-tocopherol to quench singlet oxygen (Agarwal & Rao 2000). In this study, controls reported a higher intake of specific fruits and vegetable (ie. almost half to one serving per week more than cases) namely brinjal, broccoli, carrot, orange and watermelon.

The finding of this study is similar to the results of previous study (Smith-Warner et al. 2001) that showed 0.5 serving/week consumption of broccoli reduced (RR 0.86; 95% CI 0.72-1.02) breast cancer risk by 14%. Broccoli is the source of sulfur compounds such as isothiosianate and dithiolthion that manage to activate phase I and phase II detoxification enzyme (Finley 2003). Interestingly, this study and also other study (Simon et al. 2000) showed that rosaceae family, such as apple and plum exerts inverse association with breast cancer risk. This may require further investigation.

The findings that selected antioxidant nutrient intake from fruits and vegetables namely α-carotene, lycopene, β-carotene, carotenoids, lutein, vitamin A and vitamin C were higher among controls than cases, thus might contribute to protective effect against breast cancer were similar to other case-control studies among Germany (Adzersen et al. 2003), Korean (Do et al. 2003) and African American populations (Simon et al. 2000). A meta-analysis (Gandini et al. 2004) also showed that β-carotene reduced the risk of breast cancer. Study on antioxidant showed that subjects with a lower level of antioxidant displayed a higher breast cancer risk (Ching et al. 2002).

In this study, the lymphocyte levels of oxidative DNA damage seen among cases and controls were similar or slightly lower to those reported from previous studies (Andreoli et al. 1997; Zhu et al. 1999; Colleu-Durel et al. 2004). In the present study, the breast cancer patients participated as cases were newly diagnosed patients and did not suffer from other chronic diseases. While cases were healthy subjects without any chronic diseases and history of breast cancer that might contribute to the lower lymphocytes oxidative DNA damage levels. In this study, oxidative DNA damage increased proportionally with advanced age among cases. Comet assay studies of the effect of age on DNA damage in human results have reported conflicting results (Zhu et al. 1999 & Smith et al. 2003). The higher DNA damage was also noted among subjects with higher WHR and waist circumference due to the larger accumulation of adipose tissue in the abdominal which are indicator for diseases related to obesity and oxidative stress.

The higher the intake of fruits and also from specific family namely rutaceae, solanaceae, cauliflowers and water convolculus the lower oxidative damage were observed. This study is the first study of its kind to show the association between oxidative DNA damage with fruits and vegetables intake according to family group classification. Other studies of oxidative DNA damage and fruits and vegetables intake showed controversial results (Zhu & Loft 2001; Giovannelli et al. 2002). However, different study population might gives variation in the results. Fruits and vegetables that contain abundant phytochemicals and antioxidant showed the ability to lower oxidative DNA damage in vivo (Cao et al. 1998; Pool-Zobel et al. 1998; O'Neill et al. 2002). The ability of fruits and vegetables to reduce oxidative DNA damage might work in synergy or there are magic bullets that manage to quench specific carcinogenic compounds.

Fruits in rutaceae family contain flavonol, flavones and flavonon that are powerful in free radical quenching (Rodrigues et al. 2000). Vegetables in solanaceae family display colorful appearance due to specific flavonoids. For example, brinjals contain nasunin as specific bioactive compounds that are powerful in hydroxyl radicals (Noda et al. 2000). Red and green bell peppers contain capsaicins that manage to reduce oxidative stress (Toskulkao & Tekittipong 1996). These antioxidant compounds might be the contributing factors for the selected fruits and vegetables to reduce oxidative DNA damage and further reduced breast cancer risk. Studies have shown that cruciferous vegetables have the ability to reduce oxidative DNA damage (Giovannelli et al. 2002). So far, no study examined the ability of water convolvulus to reduce oxidative DNA damage. However, this vegeTable 1s high in carotenoids that might explain the protective effects towards oxidative DNA damage.

In this study the intake of selected antioxidants nutrient, particularly vitamin A, C, carotenoid, β-carotene and lycopene reduced oxidative damage. Study have shown that high consumption of vitamin C and β-carotene reduced oxidative damage (Giovannelli et al. 2002) but other study showed inverse correlation (Bianchini et al. 2000). Study showed that lymphocytes treated with ascorbic acid and α-tocopherol has lower DNA damage when the cells are pretreated with hydrogen peroxide (Sierens et al. 2001). Vitamin C is water soluble vitamin that manages to quench radicals present in water soluble phase and it is able to recycle vitamin E to its original form. Carotenoids that are pro-vitamin A can scavenge free radicals and prevent lipid peroxidation and repair the DNA damage.

In conclusion, this study suggested that consumption of fruits and vegetables of specific family groups and types and a total serving of more than four per day might protect against oxidative DNA damage and further reduced the risk of breast cancer. Further larger population study need to be conducted to investigate the effects of fruits and vegetables consumption particularly the tropical kind and also 'ulam' on oxidative DNA damage.

ACKNOWLEDGEMENTS

This study was funded by project of MAKNA & UKM. Thanks to the staffs of Department of Nutrition and Dietetics, Department of Biomedical Sciences and the subjects who volunteered in this study, and special thanks to Ms Rabeta, for helping in data collection.

REFERENCES

Adzersen, K. H., Jess, P., Freivogel, K. W., Gerhard, I. & Bastert, G. 2003. Raw & cooked vegetables, fruits, selected micronutrients and breast cancer risk: a case-control study in Germany. *Nutr. Cancer.* 46 (2): 131-137.

Agarwal, S. & Rao, V. A. 2000. Tomato lycopene and its role in human health and chronic diseases. *Canad. Med. Assoc. J.* 163: 739-744.

Andreoli, C., Leopardi, P. & Crebelli, R. 1997. Detection of DNA damage in human lymphocytes by alkaline single cell gel electrophoresis after exposure to benzene or benzene metabolites. *Mutat. Res.* 377: 95-104.

Antoniou, A., Pharoah, P. D. P. & Narod, S. 2003. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: A combined analysis of 22 studies. *Am. J. Human Gen.* 71: 1117-1130.

Bianchini, F., Elmstahl, S., Martinez-Garcia, C., van Kappel, A. L., Douki, T., Cadet, J., Ohshima, H., Riboli, E. & Kaaks, R. 2000. Oxidative DNA damage in human lymphocytes: correlations with plasma levels of alpha-tocopherol and carotenoids. *Carcinogenesis* 21: 885-890.

Cao, G., Booth, S. L., Sadaeski, J. A. & Prior, R. L. 1998. Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruits and vegetables. *Am. J. Clin. Nutr*. 68: 1081-1087.

Ching, S., Ingram, D., Hahnel, R., Beiby, J. & Ross, E. 2002. Serum levels of macronutrients, antioxidants and total antioxidants status predict risk of breast cancer in a case control study. *J. Nutr.* 132: 303-306.

Collaborative Group on Hormonal Factors in Breast Cancer. 2002. Alcohol, tobacco and breast cancer. *Br. J. Cancer* 87: 1234-1245.

Colleu-Durel, S., Guitton, N., Nourgalieva, K., Legue, F., Léveque, J., Danic, B. & Chenal, C. 2004. Alkaline single-cell gel electrophoresis (comet assay) : A simple technique to show genomic instability in sporadic breast cancer. *Eur. J. Cancer* 40: 445-451.

Do, M. H. Lee, S. S., Jung, P. J. & Lee, M. H. 2003. Intake of dietary fat and vitamin in relation to breast cancer risk in Korean women : A case-control study. *J. Korean Med. Sci.* 18: 534-540.

Finley, J. W. 2003. The antioxidant responsive element (ARE) may explain the protective effects of cruciferous vegetables on cancer. *Nutr. Rev.* 61: 250-254.

Fung, T. T., Hu, F. B., Holmes, M. D., Rosner, B. A., Hunter, D. J., Colditz, G. A. & Willett, W. C. 2005. Dietary patterns and the risk of postmenopausal breast cancer. *Int. J. Cancer* 116(1): 116-121.

Gammon, M. D., Eng, S. M., Teitelbaum, S. L., Britton, J. A., Kabat, G. C., Hatch, M., Paykin, A. B., Neugut, A. I. & Santellaf, R. N. 2004. Environmental tobacco smoke and breast cancer incidence. *Environ. Res.* 96: 176-185.

Gandini, S., Merzenich, H., Robertson, C. & Boyle, P. 2004. Meta-analysis of studies on breast cancer risk and diet : The role of fruit and vegetable consumption and the intake of associated micronutrients. *Eur. J. Cancer* 36: 636-646.

Giovannelli, L., Saieva, C., Masala, G., Testa, G., Salvini, S., Pitozzi, V., Riboli, E., Dolara, P. & Palli, D. 2002. Nutritional and lifestyle determinants of DNA oxidative damage : A study in Mediterranean population. *Carcinogenesis.* 23(9): 1483-1489.

Hashim, M. S., Lincy, S., Remya, V. Teena, M. & Anila, L. 2005. Effect of polyphenolic compounds from Coriandrum sativum on H_2O_2-induced oxidative stress in human lymphocytes. *Food Chem.* 92: 653-660.

Hejar, A. R., Chong, F. B., Rosnan, H. & Zailina, H. 2004. Breast cancer and lifestyle risks among Chinese women in the Klang Valley in 2001. *Med. J. Mal.* 59(2): 226-232.

Kamarudin, R., Shah, S.A., Hidayah, N. 2006. Lifestyle factors and breast cancer: A case control study in Kuala Lumpur, Malaysia. *Asian Pac. J. Cancer Prevention.* 7(1): 51-54.

Lwanga, S.,K. & Lemeshow, S. 1991. *Sample Size Determination in Health Studies. A Practical Manual*. Geneva: World Health Organization.

Malin, A. S. Qi, D., Shu, X. O., Gao, Y. T., Friedmann, J. M., Jin, F. & Zheng, W. 2003. Intake of fruits, vegetables and selected micronutrients in relation to the risk of breast cancer. *Int. J. Cancer* 105(3): 413-418.

Nagata, C., Mizoue, T., Tanaka, K., Tsuji, I., Wakai, K., Inoue, M. & Tsugane, S. 2006. Tobacco smoking and breast cancer risk: An evaluation based on a systematic review of epidemiological evidence among the Japanese population. *Jap. J. Clin. Oncol*. 36 (6): 387-394.

National Cancer Registry. 2004. *The Second Report of National Cancer Registry-Cancer Incidence in Malaysia 2003.* http://crc.gov.my/ncr/Report_22.htm. Online on 22nd of August 2006.

Neergheena, V. S., Soobratteea, M. A., Bahoruna, T. & Aruomab, O. I. 2006. Characterization of the phenolic constituents in Mauritian endemic plants as determinants of their antioxidant activities in vitro. *J. Plant Physiol*. 163: 787-799.

Noda, Y., Kaneyuki, T., Igarashi, K., Mori, A. & Packer, L. 2000. Antioxidant activity of nasunin, an anthocyanin in eggplant peels. *Toxicol*. 148: 119-123.

Norsa'adah, B., Rusli, B. N., Imran, A. K., Naing, I. & Winn, T. 2005. Risk factors of breast cancer in women in Kelantan, Malaysia. *Singapore Med. J*. 46(12): 698-704.

O'Neill, K. L., Standage, S. W., Hughes, B. G. & Murray, B. K. 2002. Fruits and vegetables and the prevention of oxidative DNA damage. In R. R. Watson (Ed.). *Vegetables, fruits, and herbs in health promotion,* New York: CRC Press, 135-146.

Parsons, R. 2005. The oncogenetic basis of breast cancer. Dlm. D. F. Roses (pynt). *Breast cancer*, New York: Churchill Livingstone, 15-26.

Pool-Zobel, B. L., Bub, A., Leigibel, U. M., Treptow-van Lishaut, S. & Rechkemmer, G. 1998. Mechanisms by which vegetable consumption reduces genetic damage in humans. *Can. Epidemiol. Biomarkers Prev.* 7(10): 891-899.

Rabeta, M. S., Suzana, S., Fatimah, A., Ahmad Rohi, G., Normah, H. and Nor Fadilah, R. 2007. Abdominal obesity increased breast cancer risk among women in Klang Valley. *Mal. J. Health Sci.* 5(2): 17-28.

Rahmat, A., Abu Bakar, M. F., Faezah, N. & Hambali, Z. 2004. The effects of consumption of guava (*Psidium guajava*) or papaya (*Carica papaya*) on total antioxidant and lipid profile in normal male youth. *Asia Pac. J. Clin. Nutr.* 13(Suppl.): S106.

Rodrigues, A., Sandstr¨om, A., C´a, T. Steinsland, H. Jensen, H. & Aaby, P. 2000. Protection from cholera by adding lime juice to food. Results from community and laboratory studies in Guinea-Bissau, West Africa. *Tropic. Med. Inter Health* 5: 418-422.

Sierens, J., Hartley, J. A., Campbell, M. J., Leathem, A. J. C. & Woodside, J. V. 2001. Effect of phytoestrogen and antioxidant supplementation on oxidative DNA damage assessed using the comet assay. *Mut. Res.* 485: 169-176.

Simon, M. S., Djuric, Z., Dunn, B., Stephens, D., Lababidi, S. & Heilbrun, L. K. 2000. An evaluation of plasma antioxidant levels and the risk of breast cancer: a plot case control study. *The Breast J.* 6(6): 388-395.

Singh, N. P., McCoy, M. T., Tice, R. R. & Schneider, E. L. 1988. A simple technique for quantification of low levels of DNA damage in individual cells. *Exp. Cell. Res.* 17: 184-191.

Smith, T. R., Miller, M. S., Lohman, K. K., Case, L. D. & Hu, J. J. 2003. DNA damage and breast cancer risk. *Carcinogenesis* 24(5): 883-889.

Smith-Warner, S. A., Spiegelman, D., Yaun, S. S., Goldbohm, R. A., Graham, S., Miller, A. B., Potter, J. D., Rohan, T. E., Speizer, F. E., Toniolo, P., Willett, W. C., Wolk, A., Zeleniuch-Jacquotte, A. & Hunter, D. J. 2001. Intake of fruits and vegetables and risk of breast cancer : A pooled analysis of cohort studies. *JAMA* 285(6): 769-776.

Suzana, S., Rafidah, G., Noor Aini, M. Y., Nik Shanita, S., Zahara, A. M. & Shahrul Azman, M.N. 2002. Atlas makanan: Saiz pertukaran & porsi. Kuala Lumpur: MDC Publisher.

Suzana, S., Azhar, Y. & Fatimah, A. 2004. Association between dietary fibre and cancer: a case-control study in Malaysia. *Mal. J. Nutr.* 10(2): 173-182.

Tee, E. S., Ismail, M. N., Nasir, M. A. .& Khadijah, I. 1997. *Nutrient Composition of Malaysian Foods.* Ed. ke-4. Kuala Lumpur : Malaysian Food Composition Database Programme, Institute for Medical Research,

Toskulkoa, C. & Tekittipong, S. 1996. Capsaicin inhibits exercise induced lipid peroxidation in cardiac and skeletal muscles of rat. *Phytotherapy Res.* 6: 34-37.

USDA. 2007. United State Department for Agriculture : Agricultural Research Service. http://www.ars.usda.gov , online on 8th of December 2007.

Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M. & Mazur, M. 2006. Free radicals, metals, and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact*. 160: 1-40.

WHO. 2006. *The World Health Report.* Geneva: World Health Organization.

Zhu, C. Q., Lam, T. H., Jiang, C. Q., Wei, B. X., Lou, X., Liu, W. W., Lao, X. Q.& Chen, Y. H. 1999. Lymphocyte DNA damage in cigarette factory workers measured by the Comet Assay. *Mutat. Res.* 444: 1-6.

Zhu, C. Y. & Loft, S. 2001. Effects of brussels sprouts extracts on hydrogen peroxide-induced DNA strand breaks in human lymphocytes. *Food Chem. Toxicol.* 39: 1191-1197.

Suzana Shahar
Kim Tiu Teng
Department of Nutrition and Dietetics
Faculty of Allied Health Sciences
Universiti Kebangsaan Malaysia
Jalan Raja Muda Abdul Aziz
50300 Kuala Lumpur.

Nor Fadilah Rajab
Department of Biomedical Sciences
Faculty of Allied Health Sciences
Universiti Kebangsaan Malaysia
Jalan Raja Muda Abdul Aziz
50300 Kuala Lumpur.

Fatimah Arshad
Department of Dietetics
International Medical University Malaysia
No. 126, Jln 19/155B, Bukit Jalil
57000 Kuala Lumpur.

Corresponding author: suzanas@medic.ukm.my
Received: September 2007
Accepted for publication: January 2008