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Serum Cotinine and Passive Smoking Status Associated with Non-Smoking Newly Diagnosed Women with Breast Cancer in Malaysia

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Abstract

Background: Evidence shows that exposure to passive smoking increases the risk of breast cancer. However, there is a lack of data on the role of serum cotinine level among passive smoker women with breast cancer. The purpose of this study was to investigate the association of serum cotinine level and passive smoking exposure with the risk of breast cancer.

Method: We conducted this case-control study on 78 women with newly diagnosed breast cancer and 83 healthy women, aged 21 to 59 years. Neither cases nor controls were ever smokers in their lifetime. The serum cotinine level, as a biological marker of secondhand smoking, was assessed among women exposed to passive smoking.

Results: The mean serum cotinine concentrations were higher among cases compared to controls although the difference was not statistically significant (4.6 ± 3.5 ng/mL vs. 2.8 ± 2.2 ng/mL, respectively, $P = 0.059$). However, serum cotinine significantly increased the risk of breast cancer (OR = 1.22; 95% CI = 1.02, 1.48, $P = 0.034$). Exposure to passive smoking at home and exposure from a smoker husband increased the risk of breast cancer compared with those with no exposure (OR = 2.17; 95% CI = 1.15, 4.08, $P = 0.016$; and OR = 2.67; 95% CI = 1.35, 5.29, $P = 0.005$, respectively).

Conclusion: Serum cotinine levels and passive smoking exposure appeared to be independent risk factors associated with the development of breast cancer.

Keywords: Breast cancer, Cotinine, Passive smoker, Newly-diagnosed, Women

Introduction

Cigarette smoke is known to contain at least 50 different carcinogens, of which the main types are polycyclic aromatic hydrocarbons (PAHs), tobacco-specific

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nitrosamines (TSNAs), and aromatic amines.[1] Epidemiologic studies conducted over the past few decades clearly showed the relationship of smoking with increased risk of mortality due to major cancers in both men and women.[2] A positive association between cigarette smoking and breast cancer is plausible because smoking-specific DNA adduct and *p53* gene mutations are more prevalent in the breast tissues of smokers compared with that in non-smokers.[3]

The effect of passive smoking on cancer is of great public health importance, but the existing data are inconclusive. Some studies have suggested that exposure to environmental tobacco smoke in the household can cause cancer, while others have found either no effects or dose-dependent ones. In a hospital-based case-control study on women with breast cancer in China, women who were ever exposed to second-hand smoking (SHS) had a higher breast cancer risk compared with women who were never exposed to SHS, with an adjusted odds ratio (OR) and 95% confidence interval (CI) of 1.35 (1.11 to 1.65).[4] The risk association of SHS with breast cancer was explored in California teachers study, a large prospective study of women.[5] Detailed lifetime information on SHS exposure by setting (home, work, or social) and by age of exposure were collected from 57,523 women who were lifetime non-smokers and had no history of breast cancer, among whom 1,754 were diagnosed with invasive breast cancer. For women exposed in adulthood (age ≥ 20), the risk increased at the highest level of cumulative exposure (Hazard Ratio (HR)=1.18; 95% CI, 1.00 to 1.40), primarily among postmenopausal women (HR=1.25; 95% CI, 1.01 to 1.56). A statistically significant dose response was detected when analysis was restricted to women with moderate to high levels of SHS exposure.[5]

In a prospective cohort study, Luo et al. (2011) showed the evidence of self-reported active and passive smoking of 79,990 women aged 50-79 and development of breast cancer.[6] In total, they identified 3520 incident cases of invasive breast cancer during an average 10.3 years of follow-up. The highest breast cancer risk existed women who had smoked for 50 years or more (HR=1.35; 95% CI, 1.03 to 1.77) among all lifetime non-smokers. In women who had never smoked, after adjustment for potential confounders, those with the most extensive exposure to passive smoking (≥ 10 years of exposure in childhood, ≥ 20 years of exposure as an adult at home, and ≥ 10 years of exposure as an adult at work) had a 32% excess risk of breast cancer compared with those who had never been exposed to passive smoking (HR=1.32, 95% CI, 1.04 to 1.67).[6]

Assessment of SHS using biological markers is becoming more and more popular.[7] Biomarkers specific to SHS are nicotine and its metabolites (cotinine) and metabolites of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Cotinine is the preferred blood, saliva, and urine biomarker for SHS. As the main metabolite of nicotine, cotinine has been utilized as a biomarker of cancer to validate the self-reported smoking status.[8] Morales et al. (2015) confirmed 100% accuracy of serum cotinine in the self-reporting of current and never smoking in 233 newly-diagnosed cancer patients.[8]

In a prospective Norwegian study of 1,741 individuals with lung cancer and a similar number of matched healthy controls, mean serum cotinine significantly predicted the risk of lung cancer.[9] In comparison to subjects with a cotinine level of ≤ 5 ng/mL, the OR of lung cancer increased linearly, reaching 55 (95% CI, 35.7 to 85.0) among individuals with a serum cotinine level of > 378 ng/mL. There is clear evidence that not only tobacco smoking, but also passive smoking exposure augments the serum levels of cotinine. In a lung cancer study in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, passive smokers presented significant differences in cotinine compared with non-smokers non-exposed to passive smoking. A one-hour per day increment in passive smoking gave rise to a significant 2.58 nmol/L (0.45 ng/mL) increase in mean serum cotinine ($P< 0.001$). In current smokers, a one-cigarette per day increment resulted in a significant 22.44 nmol/L (3.95 ng/mL) increase in mean cotinine ($P< 0.001$).[10]

In a follow-up study on 166 cohort of patients

Table 1. Distribution of the sociodemographic and reproductive characteristics of the participants

Characteristics	Cases (n = 78) n (%)	Controls (n = 83) n (%)	*P*-value	95% CI
Age (years, mean and SD)	47.7 (7.7)	43.2 (9.5)	0.001	-7.2, -1.8
Ethnicity			1.00[a]	
Malay	75 (96.2)	80 (96.4)		
Chinese	3 (3.8)	3 (3.6)		
Marital status			0.44[a]	
Ever married	76 (97.4)	78 (94.0)		
Never married	2 (2.6)	5 (6.0)		
Education level			0.028	
Tertiary	27 (34.6)	43 (51.8)		
Primary/Secondary	51 (65.4)	40 (48.2)		
Family history of breast cancer			0.017	
No	57 (73.1)	73 (88.0)		
Yes	21 (26.9)	10 (12.0)		
Age at menarche (years)			0.891	
≤ 13	45 (57.7)	47 (56.6)		
≥ 13	33 (42.3)	36 (43.4)		
Use of oral contraceptives			0.723	
No	42 (53.8)	47 (56.6)		
Yes	36 (46.2)	36 (43.4)		
Duration of oral contraceptives use (months, mean and SD)	37.9 (27.1)	29.3 (29.2)	0.093[b]	-24.7, 7.6
Age at first full-term pregnancy (years)			0.007	
≤ 30	58 (79.5)	69 (94.5)		
≥ 30	15 (20.5)	4 (5.5)		
Menopausal status			0.019	
Pre-menopausal	49 (62.8)	66 (79.5)		
Post-menopausal	29 (37.2)	17 (20.5)		
Age at menopause (years, mean and SD)	50.0 (3.6)	49.9 (3.3)	0.899	-2.6, 2.3
BMI (kg/m^2, mean and SD)	25.6 (5.3)	25.6 (4.3)	0.981	-1.5, 1.5

BMI = body mass index; CI: confidence interval; SD: standard deviation; [a]*P*-value based on Fisher's exact test; [b]Mann-Whitney U Test

with stage I and stage II breast cancer, smoking was one of the important prognostic indicators of breast cancer-specific mortality.[11] However, serum cotinine measurement was not associated with metastasis-free survival from breast cancer. Due to the inconclusive reports on the effects of passive smoking and serum cotinine on breast cancer, the present study aimed to compare the exposure of passive smoking and serum cotinine levels among newly-diagnosed women with breast cancer and healthy controls in Malaysia.

Materials and Methods

Study populations

This study was conducted from February 2014 to June 2015 in Kelantan, Malaysia. We obtained ethical approval from the Human Research and Ethics Committee of Universiti Sains Malaysia (USM) that complies with the Declaration of Helsinki. The participants were explained of the purpose of the study, detailed procedures to be undertaken, voluntariness, and assurance of confidentiality in the study. Written informed consent was obtained from all participants prior to entering the study. Eligible study population included women aged 21-59 years who attended the Oncology Clinic of Universiti Sains Malaysia Hospital (HUSM).

Study design

This case-control study recruited cases from HUSM, from women newly-diagnosed with histologically confirmed malignant breast cancer, ranging from stage I to stage IV, and those who were never smoked in their lifetime. Based on the culture in Malaysia, the majority of women are non-smokers and have a negative opinion and

image of smoking among women.[12] We used the outpatient register list of all women attending the Oncology Clinic for the cases. These cases had not yet received any therapy except for analgesics and/or surgery. We selected them based on convenience sampling on a first-come-first-serve basis. Controls were staff members of HUSM and USM campus comprising healthy volunteers, who were never smokers and had no known history of breast cancer, no medical illnesses, and no use of medications other than analgesics, and were not pregnant or lactating during the study period. We age-matched the controls to the nearest five years and recruited them by a convenience sampling method. If a woman refused to participate, the next eligible woman was approached for participation.

Questionnaire

We conducted a face-to-face interview using a questionnaire comprised of 22 questions. A team of trained research assistants led by the principal investigator (Zahali) conducted the interviews at the hospital appointment visits of the patients. The questionnaire included questions on sociodemographic status, reproductive history, family history of breast cancer, and smoking status. The contents of the questionnaire were based on an in-depth literature review. A three-member panel of experts reviewed the content validity. To minimize the measurement errors, we applied a number of methods: 1) the research assistants involved in the study were given a three-day hands-on training to have consistency in question wording, addressing the respondents, and reading the questions properly as written; 2) the questionnaire was pretested among 20 individuals and any inconsistencies in responses were verified and corrected; 3) the questions were made as simple as possible in the local Malay language understandable by the people; 4) the interviewer read the questionnaire out loud ; 5) we offered no leading questions during the interview; and 6) any missing data were reviewed on the spot, and we varied the data through asking the respondent at the same time. In terms of smoking status, the questions addressed lifetime, active, and passive smoking. Participants were considered as ever smokers, if they answered that they had smoked during their lifetime. However, as mentioned earlier, participants were eligible only if they were never smokers. In this study, we defined passive smokers as those who reported exposure to passive smoke at home and/or at work. We categorized passive smoking into exposure only at home, exposure only at work, and exposure both at home and at work. The participants were further asked about the number of smokers in their household, including the husband and/or other family members such as father and brother.

Anthropometric measurements

Anthropometric measurements included body height and weight. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). A portable stadiometer (SECA, Germany) was used to measure body height to the nearest 0.1 cm. We asked the participants to stand straight with their head positioned towards the Frankfurt plane horizontal on the robust of the stadiometer. Standing barefoot with heels almost together, the subjects were ensured to have their knees straight and their heels, buttocks, and shoulder blades touch the stadiometer in vertical position. We lowered the spacer of the stadiometer by the measurer until it touched the crown of the head and compressed the hair. A body composition analyzer (Tanita, Tokyo, Japan) determined the body weight. We placed the analyzer on a hard-flat level surface and checked for zero balance prior to measurements. Clothes weight was set to 0.5 kg. The participants had to be in light clothes, stand barefoot on the center of stainless-steel electrodes, and look straight ahead. To avoid inter-measurer bias, only one measurer performed all measurement procedures; one recorder assisted in positioning the participants during the measuring process. The standard measurement procedures were applied in all anthropometric measurements.[13] An average value of duplicate readings was used in data entry.

Blood sample collection and serum cotinine

Table 2. Distribution of passive smoking and serum cotinine among the participants

Characteristics	Cases (n = 78) n (%)	Controls (n = 83) n (%)	*P*-value
Passive smoking at home			0.016
No	35 (44.9)	53 (63.9)	
Yes	43 (55.1)	30 (36.1)	
Smokers at home			0.003[a]
None	35 (44.9)	53 (63.9)	
Husband only	37 (47.5)	21 (25.3)	
Husband and other family members	3 (3.8)	1 (1.2)	
Other family members only	3 (3.8)	8 (9.6)	
Passive smoking at workplace			0.904
No	70 (89.7)	74 (89.2)	
Yes	8 (10.3)	9 (10.8)	
Exposure to passive smoking (at home and workplace)			0.014
No	30 (38.5)	48 (57.8)	
Yes	48 (61.5)	35 (42.2)	
Serum cotinine (ng/mL, mean and SD)	4.6 (3.5)	2.8 (2.2)	0.059b

[a]*P*-value based on Fisher's Exact test; [b]Mann-Whitney U Test; SD: standard deviation

analysis

In the present study, only women exposed to passive smoking underwent serum cotinine analysis. A total of 5 mL of venous blood sample was collected from each participant in the morning. The blood samples were maintained in an icebox. The blood samples were processed within four hours after collection and centrifuged at 4000 rpm at 4˚C for 10 min. The serum sample was aliquoted and stored at -80˚C until subsequent analysis. The concentration of serum cotinine was assayed using an enzyme-linked immunosorbent assay (ELISA) kit (Abnova, Taiwan) according to the manufacturer's recommendations and instructions. The lower detection limit of serum cotinine was 1 ng/mL.

Sample size estimation

We estimated the sample size based on the data of a similar case-control study done among women with breast cancer in Poland. In the study by Kruk (2009),[14] sample size was estimated with a 95% CI and 80% study power; moreover, the prevalence of no exposure to passive smoking from husband was 44.2% in the case group and 67.4% in the control group. Based on the above calculation, our study required a total of 138 respondents in both groups.

Statistical methods

Data were analyzed using SPSS (Released 2017. IBM SPSS Statistics for Windows, version 25.0. Armonk, NY: IBM Corp.). The main objective of the present study was to determine the association between passive smoking and breast cancer. We used serum cotinine, the principal metabolite of nicotine, as a biomarker of passive smoking and the main outcome variable in this study. Continuous data such as age, duration of contraceptive use, BMI, and serum cotinine were summarized as mean and standard deviation or median and percentile values (25^{th} and 75^{th}). Categorical data were presented as frequencies and percentages. We tested the differences between cases and controls regarding mean values using independent t-test or Mann-Whitney U test depending on the data distribution. Chi-square test analyzed the categorical variables, while Fisher's exact test was used for variables with smaller expected frequencies. We performed multiple logistic regression analyses to specify the predictors of breast cancer after adjusting for confounders. Variables with a *P* -value of <0.25 in simple logistic regression analysis and clinically important variables (such as BMI) were included in the multivariate model. The results were presented as crude and adjusted OR. A *P* -value of ≤ 0.05 was considered as statistically significant.

Results

Table 1 compares the baseline characteristics in 78 cases and 83 controls. Overall, cases were slightly older compared with controls with respective mean ages of 47.7 (7.7) and 43.2 (9.5) years. The majority of the participants were Malay, married, and premenopausal women. Compared with controls, cases had lower education levels and were more than 30 years of age at the first full-term pregnancy. In addition, cases were exposed to oral contraceptives for a longer duration than controls, although there was no significant association between these two groups. Furthermore, only 26.9% of cases reported a family history of breast cancer. The groups were not significantly different in terms of BMI and age at menopause between.

Table 2 presents the self-reported exposure to passive smoking and serum cotinine concentration. Compared with controls, a significantly higher proportion of cases (55.1% vs. 36.1%, $P = 0.016$) reported exposure to tobacco smoke at home. More cases than controls (47.5% vs, 25.3%, $P = 0.003$) had their husband as the smoker at home. However, smokers in the household were not only limited to the husband, but also included extended family members such as the father and other siblings. In contrast, approximately 10% of the participants were exposed to passive smoking at their workplace; however, the difference was not significant between the groups. Significantly more cases were exposed to passive smoking when household and work places were combined. The mean serum cotinine concentrations were higher among cases compared with controls, but no statistically significant differences were detected (4.6 ± 3.5 ng/mL vs. 2.8 ± 2.2 ng/mL, respectively, $P = 0.064$).

Figure 1 shows the distribution of serum cotinine concentration among cases and controls. Approximately, 10.0% of cases and 17.9% of controls had serum cotinine concentrations below 1 ng/mL. The percentage of cases and controls with serum cotinine concentrations of 1 to 1.9 ng/mL were 42.5% and 53.6%, respectively. Moreover, 47.5% of cases and 28.6% of controls

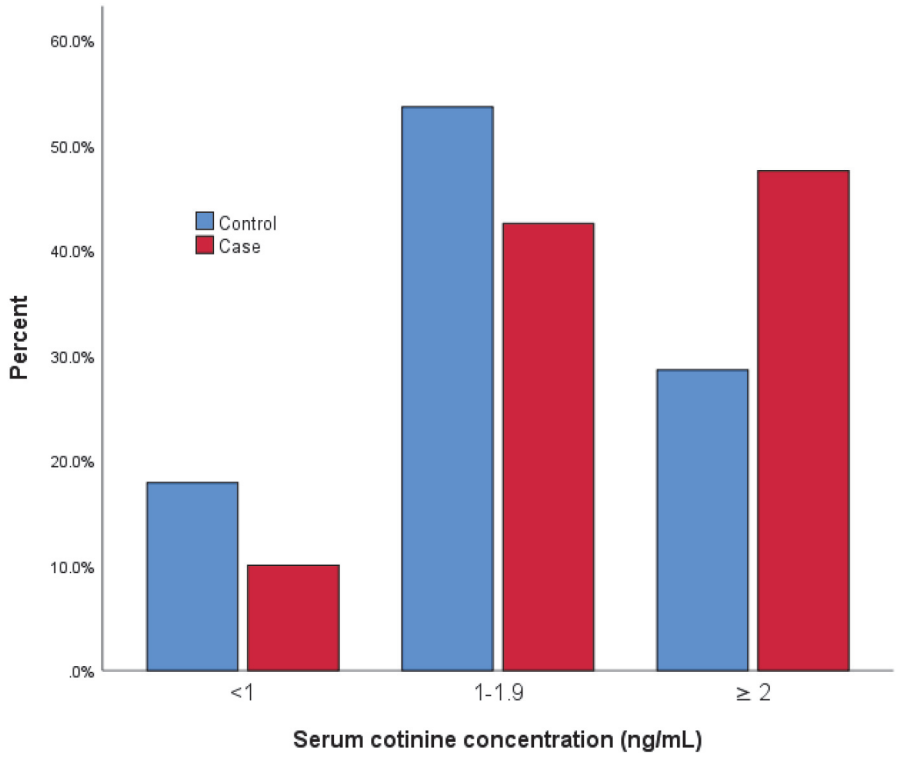

Figure 1. This figure shows the distribution of serum cotinine concentration among cases (n = 40) and controls (n = 28) exposed to passive smoking.

Table 3. Associated factors of breast cancer risk by multiple logistic regression model

Characteristics	Simple Logistic Regression		Multiple Logistic Regressiona	
	Crude OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Passive smoking at home				
No	Reference			
Yes	2.17 (1.15, 4.08)	0.016	-	
Number of smokers at home				
None	Reference			
Husband only	2.67 (1.35, 5.29)	0.005	-	
Husband and other family members	4.54 (0.45, 45.45)0.198		-	
Other family members only	0.57 (0.14, 2.29)	0.426	-	
Passive smoking at workplace				
No	Reference			
Yes	0.94 (0.34, 2.57)	0.904	-	
Exposure to passive smoking (at home and workplace)				
No	Reference			
Yes	2.19 (1.17, 4.12)	0.015	-	
Serum cotinine (ng/mL)	1.22 (1.02, 1.48)	0.034	1.22 (1.02, 1.48)	0.034

OR = odds ratio; CI = confidence interval; [a]Model was adjusted for age, BMI, and menopausal status

had serum cotinine concentrations above 2 ng/mL ($P = 0.26$).

Table 3 shows the results of multiple logistic regression. Among all factors included in the multiple logistic regression model, only serum cotinine was associated with breast cancer. The odds of a person (with an increase in 1 ng/mL of serum cotinine concentration) having breast cancer was 1.22 times (95% CI, 1.02 to 1.48, $P = 0.034$).

Discussion

In this study, after adjusting for confounders, serum cotinine was associated with an increased risk of breast cancer among non-smoking women. As the main metabolite of nicotine, cotinine has a half-life about 15 to 40 hours and is considered as a reliable biomarker of recent exposure to passive smoking. Different studies have commonly used cotinine levels as a biomarker to validate the self-reported smoking status.[8] In previous studies, a cut-off point of more than 15.0 ng/mL of serum cotinine levels was employed to classify people with active smoking.[15, 16] Using this definition, all participants in our study were non-smokers.

The increased cotinine levels among passive smokers in our study is consistent with the EPIC study conducted on a large European population.[10] In the foregoing study, a one-hour per day increment in passive smoking exposure increased the serum cotinine by 0.45 ng/mL.[10] Similarly, data from Health Survey for England showed that cotinine concentrations in non-smoking individuals who were married to smokers were strongly associated with the hours of exposure to passive smoking.[17] In Health Survey for England, the mean concentration of cotinine increased by 0.31 ng/mL when the partner smoked 30 or more cigarettes per day compared to non-smoker partners.[17]

There is limited data on the association between cotinine and breast cancer risk. Some studies, on the other hand, have investigated the association between cotinine and other types of cancer.[9, 18, 19] A recent large prospective study conducted among 11,856 non-smoking adults in the U.S. reported that with a two-fold increase in serum cotinine, the adjusted mortality rate ratios were 1.10 (95% CI, 1.03 to 1.17) for all-cancers and 1.13 (95% CI, 1.03 to 1.24) for smoking-related cancers, respectively.[20] A nested case-control study revealed that subjects with detectable levels of serum cotinine had 1.81-fold increased risk of colorectal cancer (95% CI, 0.98 to 3.33) compared to those who had undetectable levels of serum cotinine; however, the estimate rate was not

statistically significant.[18]

A nested case-control study was conducted among smokers and non-smokers to predict the association between cotinine level and lung cancer risk. In a subset of EPIC population, lung cancer risk increased monotonically with 12.4-fold increase in serum cotinine levels (95% CI, 7.1 to 21.9) among smokers in the highest decile (serum cotinine >1,800 nmol/L or >316.8 ng/mL) compared with non-smokers (serum cotinine <75 nmol/L or <13.2 ng/mL) after adjustment for the number of cigarettes per day.[19] A study on a Norwegian population reported a significantly increased risk of lung cancer among individuals with a serum cotinine level of >378 ng/mL with an OR of 55.1 (95% CI, 35.7 to 85.0) compared with individuals with a cotinine level of ≤5 ng/mL.[9] However, in another study, there was no significant association reported between passive smoking and the prognosis of breast cancer, particularly regarding the development of metastases based on a point estimate of serum cotinine concentration.[11]

The findings of the Global Adult Tobacco Survey (GATS) conducted among 14 low- middle-income countries showed the prevalence of passive smokers among women, ranging from 14.8% in Mexico to 68.8% in Vietnam.[21] According to the National Health and Morbidity Survey (2015), approximately 31% of non-smoking females in Malaysia were exposed to SHS.[22] An estimated 32.0% and 24.2% of non-smoker Malaysian women showed the evidence of passive smoking exposure at home and workplace, respectively.[22] In our study, 55.1% of cases and 36.1% of controls were exposed to passive smoking at home; however, only about 10% of the participants in both groups were exposed to passive smoking at workplace. Several studies have similarly reported high passive smoking exposure of non-smoking women at home and workplace.[23-25]

In agreement with other studies, we observed that exposure to passive smoking at home increased the risk of breast cancer among non-smoking women. In addition, this increase was most apparent among women living with the husband as the only smoker at home. Similarly, a hospital-based case-control study done among Chinese women showed an increased risk of breast cancer in women exposed to passive smoking at home.[4] A large prospective study conducted among European countries reported that women exposed to passive smoking at home ran the highest risk of breast cancer development with a HR of 1.30 (95% CI, 1.07 to 1.59).[26] Another study carried out on Chinese women reported a significant positive relationship between breast cancer and the degree of smoking in husbands.[27] In a study among Thai women, those exposed to passive smoking from a husband less than seven hours per week had 3.77 (95% CI, 1.11 to 12.82) times higher risk of breast cancer compared with women with no exposure to passive smoking.[28] A meta-analysis among Chinese women showed 1.41 OR (95% CI, 0.95 to 2.09) of breast cancer with exposure to heavy smoking (≥20 cigarettes per day) and 1.11 OR (95% CI, 0.98 to 1.25) of breast cancer with exposure to light smoking (<20 cigarettes per day) in their husbands.[29]

Studies have also investigated the association between exposure to passive smoking at workplace and breast cancer development.[5, 6, 29] Unfortunately, in our study, we observed that passive smoking at workplace alone was not significantly associated with the risk of breast cancer. This could be attributed to an under-reporting of passive smoking exposure in women at workplace. Similarly, California teachers study reported no significant association between exposure to passive smoking at workplace and breast cancer with HR=1.02 (95% CI, 0.93 to 1.13).[5] In addition, a prospective study on active and passive smoking among 79,990 women showed no significant association between exposure to passive smoking at workplace and breast cancer incidence (HR=1.01; 95% CI, 0.82 to 1.26). However, the results of a meta-analysis concluded that breast cancer risk was significantly associated with passive smoking exposure in the workplace by 1.66-fold (95% CI, 1.07 to 2.59) among Chinese females.[29]

To our knowledge, there is no previous data available on serum cotinine and breast cancer

risk in Malaysia. However, in the current study, only women exposed to passive smoking were included in serum cotinine analysis. Therefore, it is necessary that further studies be performed with larger samples to compare the serum cotinine concentration between the populations exposed and unexposed to passive smoking. Besides, the duration and intensity of lifetime passive smoking exposure from childhood to the adulthood should be prospectively investigated in detail.

Conclusion

In conclusion, increase in serum cotinine level among passive smoking women is associated with elevated breast cancer risks. Our study corroborates the evidence that exposure to passive smoking, especially from husbands' smoking at home, increases the risk of breast cancer development among non-smoking women. Clearly, active smoking should be avoided; furthermore, based on this study, we strongly support the proposed smoking prohibition particularly in public and private areas in order to protect non-smoking individuals from the effects of passive smoking.

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Conflicts of Interest

None declared.

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