DRAFT

Confounding factors in epidemiologic studies of spousal smoke exposure in Japanese Women

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Abstract

This paper reports the findings of a study of 400 Japanese women living in either Osaka or Okayama. The objective of the study was to determine whether certain lifestyle factors correlate with being a smoker or living with a smoker. Each of the subjects was asked a series of questions concerning diet and lifestyle, and each supplied a 50 ml sample of urine for cotinine analysis. The cotinine data were used both to confirm whether the subject was a smoker and to investigate correlations between reports of the husband's smoking and exposure to airborne nicotine.

The results reveals a very large percentage of misclassification of smoking status (around 20% of self-reported non-smokers being regular smokers). Moreover, it was found that living with a smoker correlated strongly with a variety of lifestyle factors thought to be independent risk factors for lung cancer or heart disease. These findings confirm that epidemiologic studies of spousal smoking that have not corrected for such confounding factors must be interpreted with caution.

The data also showed little correlation between ETS exposure, as determined by urinary cotinine, and typical questionnaire responses to questions on spouses smoking habits. In fact, cotinine levels in the non-smokers married to smokers were lower, though similar, to those in non-smokers married to non-smokers. To the extent that epidemiologic studies that have used spousal smoke exposure as a surrogate for ETS exposure have compared groups with similar ETS exposure, the studies are of little relevance to ETS but rather reflect differences in independent risk factors between smoking and non-smoking families.

Introduction

Epidemiologic studies have investigated, over the last decade, a possible association between exposure to environmental tobacco smoke (ETS) and lung cancer. The first study to raise the issue was published by Hirayama in 1981, and investigated, among other issues, the incidence of lung cancer in non-smoking women married to smoking husbands as compared to non-smoking women married to non-smokers. Since then 33 studies have been completed. While the majority of these (29 of 33) reported no statistically significant association, many have reported relative risks in excess of one (though generally below two). This has lead some organizations to claim a causal link between exposure to ETS and lung cancer. This has not been the case in Japan, where many scientists are still investigating the possible effects of ETS on various health end-points.

Any epidemiologic study that attempts to detect a possibly small risk from exposure to a substance that is encountered in many different environments must be wary of the possibility of confounding factors influencing the data. It is now accepted that studies of ETS exposure are susceptible to several different types of confounding.

One of the first to have been identified is that of misclassification of smoking status. With the decreasing social acceptability of smoking, there is a tendency for subjects to deny smoking when responding to an epidemiologic questionnaire. Because active smoking is a risk factor for lung cancer, assignment of actual smokers as non-smokers can result in misleading relative risks. Moreover, a considerable amount of concordance within marriage has been reported, with women married to smokers being more likely to be smokers that women married to non-smokers. These two factors combine to make it more likely that a reported non-smoking woman is actually a smoker if she is married to a smoker as compared to a non-smoker. This differential misclassification (with more misclassification present in the exposed group than in the non-exposed group) can falsely elevate the relative risks from epidemiologic studies.

More recently, diet has been as an important identified confounding factor in studies of ETS and lung cancer. Koo and others have reported that non-smokers living with smokers are more likely to consume more foods related to lung cancer risk, and consume fewer foods thought to be protective for lung cancer, than women married to non-smokers. This concordance of lifestyle factors also can falsely elevate the risk attributed to ETS.

Women in Japan always have been under strong social pressure not to smoke, although a not insignificant percentage of the female population (10 to 20%) is known to smoke despite that pressure. The social unacceptability of smoking by women in Japan increases the risk of misclassification of smoking status in Japanese epidemiologic studies that rely upon marriage to a smoker and self-reporting of smoking status as the primary or sole indices of exposure to ETS. Surprisingly, this issue has never been investigated in Japan even though a number of studies of ETS and lung cancer among non-smokers have been conducted among Japanese populations.

Until very recently, epidemiologic studies conducted in Asia of spousal smoke exposure and lung cancer have tended to report higher relative risks than similar studies in the United States. In attempting to explain this finding, Hirayama suggested that there may be a greater disparity in Japan between the level of ETS exposure by women married to smokers and women married to non-smokers than in the United States. This suggestion rests, in turn, on two hypotheses: first, because Japanese homes are smaller on average than homes in the United States, Japanese wives of smokers may be exposed to higher levels of ETS from spousal smoking than their American counterparts; and, second, because Japanese women tend to work less frequently outside the home than women in the United States, women married to non-smokers in Japan may have less background (i.e., non home-related) ETS exposure than do women married to non-smokers in the United States.

The suggestion of a greater disparity in Japan than in the United States in female exposure to ETS in the absence of a spouse who smokes has been questioned on several grounds. A number of commentators have pointed out that the suggestion is neither supported nor refuted by the available data, although a 10-country study conducted by the International Agency for Research on Cancer contains data that are inconsistent with the hypothesis. In addition, it has been noted that Japanese men tend to spend less time at home than their United States counterparts, thus reducing their wife's ETS exposure. The study reported in this paper was designed to shed further light on this issue.

Study Design

A total of 400 female subjects were included in the study, 200 from the city of Osaka and the other 200 from Okayama, which is more rural in nature than Osaka. The subjects were selected in a semi-random manner, with quotas being assigned by district within the target areas selected to provide a representative mix of socio-economic conditions and by age (range 20-55). The sample was limited to females who were married at the time the study was being conducted. Candidates were not asked at the screening stage about other lifestyle factors, such as smoking or whether they worked outside the home.

The majority of subjects were identified through door-to-door canvassing during the early evening hours when most candidates, both working and nonworking, could be expected to be at home. The subjects were told that the purpose of the study was to identify lifestyle factors common to women in the geographic area in which they lived. Those agreeing to participate in the study were asked to respond to an administered questionnaire, which took approximately 30 minutes to complete, focusing on a variety of lifestyle and dietary issues. The subjects then supplied approximately 50 ml of urine, which was immediately frozen and transported to Teikyo University, where all samples were stored at -30 degrees centigrade throughout the collection period. The subjects were given a token gift and offered information concerning the sugar and protein content of their urine as incentives to participate. A 10 percent response rate was achieved in the study.

Sample population

Table 1 defines the critical parameters of the sample population, segregated (1) by smoking status and (2) by whether the self-reported non-smokers were married to a smoker or to a non-smoker.

Table 1: Characteristics of the sample population

	number of subjects	mean age (years)	mean income (.0000 yen)	% working outside home
Smoker	78	34.5	615	68
NS/S	168	42.0	717	58
NS/NS	153	43.0	716	57
Total	400	41.0	695	59

NS/S: self-reported non-smoker married to a smoker
NS/NS: self-reported non-smoker married to a non-smoker

It is notable that the two non-smoking groups are highly comparable -- there are no significant differences in age, family income or the percentage of the subjects working outside the home. Moreover, the nonsmoking women do not differ materially in these respects from what was expected from Japanese national statistics for urban dwelling females of the age range studied. In contrast, the smoking group is significantly younger than the nonsmoking group and has a lower mean family income and with a higher percentage working. The percentage of female smokers selected for study is somewhat higher that the national average (approximately 15%) but is consistent with a population of less affluent urban workers.

All but one of the subjects selected were used in the analysis of results. This one subject was rejected because of incomplete questionnaire data.

Analysis of urine

Cotinine was measured in each urine sample by an enzyme linked-immunoassay (ELISA). In brief, this technique is based on the competitive binding of monoclonal antibody between cotinine immobilized in wells on a microtitre plate. This cotinine-antibody complex is then washed off the walls using a secondary anti-body (anti-mouse serum) labeled with an enzyme (horseradish peroxidase), which in turn reacts with a substrate (o-phenylenediamine). The resulting colored product is measured against samples with known concentrations of cotinine.

All samples were presented to the laboratory blind for an initial screen. After the magnitude of the cotinine concentrations had been estimated, the samples were re-run in duplicate against the appropriate calibration range. Ten percent of the samples were randomly selected and cross-checked against a gas chromatographic; and assay agreement was found to be at 10%. The lower limit of detection was determined to be 5.6 ng/ml.

Results

Misclassification of smoking status

Substantially higher smoking status misclassification rates were found in this study than in most comparable European and American studies. For purposes of clarification, those with cotinine levels below 200 ng/ml were defined as current non-smokers, those with levels between 200 and 600 ng/ml were defined as occasional smokers and those with levels in excess of 600 ng/ml were considered to be regular smokers. no way!

Table 2: Classification of smoking status by urinary cotinine.

Based on cotinine level	stated smoking status: Never	Ex	Current	Total
Non-smokers	274	23	11	308
Occasional smokers	3	2	7	12
Regular smokers	16	3	60	79
Total	293	28	78	399

24/321 24/(274+23+24)

321=

The proportion of self-reported non-smokers (never and ex-) who are true occasional or regular smokers was 24/321 (7.5%). This proportion was higher for self-reported ex-smokers (17.9%) than for self-reported non-smokers (6.5%).

never?

The proportion of self-reported non-smokers shown to be regular smokers was 19/321 (5.9%). (10.7% for self-reported ex-smokers and 5.5% for self-reported non-smokers).

never?

Of all those who reported current smoking or had levels of cotinine consistent with occasional or regular smoking, 24 denied current smoking, a rate of 23.5%. If this is restricted to those with levels consistent with regular smoking only (97 subjects), then 19 deny current smoking, a rate of 19.6% which is much higher than any reported Western study (where the largest such misclassification was 7.4%).

There was also a very strong concordance between husbands' and wives' current smoking. Based on self-reported data, the concordance ratio was 7.97 (95% C.I. 3.71-17.1). Similarly, a very strong concordance existed between husbands' and wives' ever smoking (ratio 6.48, 95% C.I. 2.73-15.3).

What is this?

Correlating husbands' reported current smoking and wives' current smoking as judged by cotinine levels yields a concordance ratio of 3.51 (95% C.I. 2.00-6.17) when based on regular/occasional smoking, and a ratio of 3.26 (95% C.I. 1.81-5.89) when based on just regular smoking. These data are somewhat more in line with Western data.

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A key issue with respect to the possibility of these high misclassification rates and high concordance ratios having an effect on the spousal smoke exposure/lung cancer epidemiology is the comparison of groups self reported non-smoking women married or not to smokers. The pertinent data are presented in Table 3.

Table 3: Data on concordance of smoking habit

True smoking group (based on cotinine)	Husbands' smoking status Never	Ever
Smoker	76	198
Occasional	2(2.4%)	1(0.5%)
Regular	4(4.9%)	12(5.7%)

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If one were to assign relative risks of 1, 2.5 and 10 to true non, occasional and regular smokers, then the average risk is 1.52 in the exposed group and 1.48 in the non-exposed group, a bias of 1.03. The confidence limits of this bias are wide -- 0.73-1.44 based on a Monte Carlo simulation of 5000 runs looking at the distribution.

Hence the data do indicate considerable misclassification of smoking status. This misclassification is much higher than has been reported in comparable Western studies. Unlike the Western studies, the misclassification is almost as likely to occur when the husband is not a smoker as it is if the husband is a smoker. This equates to a possible bias of around 1.03, though the confidence intervals of this encompass most of the published relative risks attributed to spousal smoke exposure and lung cancer.

Confounding lifestyle factors

The questionnaire sought information on a wide range of dietary and lifestyle factors. In particular, information was sought on the frequency of consumption of foods and drinks defined by narrow categories (such as smoked fish, miso soup, dark green vegetables, carrots). In addition, information was obtained on the type of cooking predominately used, type of heating in the home, size and ventilation of the home, type of workplace, and amount of exercise of both subject and husband.

These parameters were compared between subjects assigned from their questionnaire responses as smokers (S), non-smokers living with smokers (NS/S), or non-smokers living with non-smokers. (NS/NS). The data were evaluated using the Krusal-Wallis test for ordering values, and by a chi-squared test for qualitative variables.

The following variables were statistically significantly different at a 95% confidence level between NS/S and NS/NS.

goodness to present

Table 4: Comparisons of diet and lifestyle between non-smokers married or not to smokers.

Variables where frequency in NS/S is greater than in NS/NS	Variables where frequency in NS/NS is greater than in NS/S
Alcohol other than beer	Exercise
Butter	Husband and wife eat together
Smoked fish	Dark Green Vegetables
Orange juice	Carrots
Coffee	Fresh fruit
Pork	Vitamin supplements
Frying food	Milk
Use of air conditioner.	Boiling food
Daily contact with traffic fumes.	Hours with windows open at home.
Sharing workplace with smokers.	Number of hours husband spends at home.
Workplace seems smoky.	Number of hours husband spends with wife.

In all of these factors the trend is seen to go from NS/NS to NS/S to S; that is, non-smokers married to smokers lie somewhere between non-smokers married to non-smokers and smokers so far as these diet and lifestyle factors are concerned. This is illustrated in Table 5, which selects data for the most frequent consumption groups.

Table 5: Comparisons of NS/NS, NS/S and S for some dietary factors -- percentage of group consuming the food once a day or more.

Food	% of NS/NS	% of NS/S	% of S
Dark green vegetables	65	57	32
Carrots	33	28	15
Milk	53	44	39
Fresh Fruit	78	68	49
Vitamin supplements	21	12	9
Pork	3	6	4
Fried food	3	6	6
Alcohol (other than beer)	9	13	17
Beer	9	14	22
Green Tea (more than once per day)	82	76	71
Miso soup	54	58	61
Exercise (more than once a week)	10	8	0

In fact, the data show with remarkable consistency that non-smokers married to smokers have a poorer lifestyle in terms of exercise, a higher consumption of foods thought to be positively associated with lung cancer (such as pork, fried foods and alcohol), and a lower consumption of foods thought to be protective for lung cancer (such as fresh fruit, dark green vegetables, carrots, and vitamin supplements) than non-smokers married to non-smokers. This is likely to introduce a positive bias in any epidemiologic study that compares non-smoking women married or not to smokers, and could well account for the observation of relative risks in the order 1.3.

Stat sig but weak!

Comparisons of cotinine level and exposure to spousal smoking

While there was a strong correlation between self-reported numbers of cigarettes smoked per day (r=0.43, n=78, p<0.001), there was little correlation between the husbands' smoking habits and cotinine in true non-smokers (that is, eliminating subjects with cotinine values greater than 200 ng/ml), as is illustrated in Table 6.

Table 6: Cotinine levels in true non-smokers

	Husbands habit	
	Never	Ever
Number of subjects	78	219
Median cotinine (ng/ml)	14.4	12.6
Distribution (cotinine, ng/ml)		
<10	24(30.8%)	96(43.8%)
10-30	44(56.4%)	95(43.4%)
30-50	5(6.4%)	13(5.9%)
>50	5(6.4%)	15(6.8%)

It can be seen from Table 6 that not only are the median cotinine values slightly lower in the non-spousally exposed group, there is no indication at all from the distribution of a significant excess of high cotinine values in women whose husbands smoke.

Furthermore, there was no positive relationship between cotinine level with numbers of cigarettes smoked by the husband either per total weekday (r=-0.09), per total weekend day (r=-0.11), at home per weekday (r=-0.12) or at home per weekend day (r=-0.12). In fact, the last two correlations were marginally significantly (p<0.05) negative. There reportedly also was no positive relationship between the number of cigarettes smoked in the same room as the spouse either on a weekday (r=-0.09) or during a weekend day (r=-0.08).

Hence, a striking finding of this study is that there is no positive relationship between cotinine levels in true non-smokers and any index of smoking by the husband. It would therefore seem that in this population spousal smoking is not a relevant source of ETS exposure.

amount of

One factor that could explain this finding is that husbands spend a considerable time at work. The average hours worked per week by the husband was 53.2 (s.d. 12.5). Further, 70% of husbands spent fewer than 12 hours per day with their wives (if sleep is assumed to account for 8 of those hours, only 4 hours are left for possible ETS exposure). In fact, 50% of husbands were reported to have spent fewer than 8 hours in a typical day in the same room as their wives. On the other hand, 60% of the subjects worked, and 71% of these stated that they shared a workplace with a colleague who smoked.

These data suggest, then, that epidemiologic studies of Japanese women that have used questions relating to the numbers of cigarettes smoked per day by the husband have not used a good surrogate for exposure to ETS.

Conclusions

The findings of this study illustrate some of the complexities involved in epidemiologic studies of environmental tobacco smoke exposure in Japanese women and any health end-point.

The most significant finding is that spousal smoke exposure appears to be an entirely inadequate marker for exposure to ETS. In fact, non-smokers married to smokers, had lower cotinine values than non-smokers married to non-smokers. This is not entirely surprising if one considers the findings both that most husbands spend relatively little time with their spouses and that many women work and are likely to be exposed to ETS outside of the home. If this is not a phenomena of recent history, and we believe that it is not, then the epidemiologic studies carried out to date in Japan have measured cases and controls with equivalent exposures to ETS. And if this is so, then presumably the increased relative risks reported in these studies must be due to factors other than ETS exposure.

One possible explanation for this is misclassification of smoking status. A very high proportion of self-reported female non-smokers in this study turned out actually to be smokers. More importantly, these misclassified subjects are far more likely to be regular smokers than occasional smokers. The percentage of misclassification was similar whether the subject is married or not to a smoker. However, the number of misclassified smokers in this study is relatively small (19 regular and 5 occasional) and the estimate of bias from this source is subject to large variation, so large that misclassification bias alone might explain the elevated risks for lung cancer found in some Japanese studies.

Perhaps a more likely explanation, based on the findings of this study, is that non-smokers living with smokers are exposed more frequently and with a greater intensity to a variety of independent risk factors for lung cancer (and other diseases such as cardiovascular disease) than non-smokers living with non-smokers. The combination of risk from these factors could well explain the increased relative risks found in spousal smoke exposure studies.