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Risk Factors for Lung Cancer among Northern Thai Women: Epidemiological, Nutritional, Serological, and Bacteriological Surveys of Residents in High- and Low-incidence Areas

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Lung cancer incidence among Northern Thai women is one of the highest in Asia (an annual age-adjusted incidence rate of 37.4 per 100,000), and the incidence rate significantly differs by geographical districts. Therefore, we conducted a comparative study of women living in the Sarapee area, which showed the highest (crude incidence rate, 40.9), and the Chom Tong area, which had one of the lowest incidence rates (8.5) in Chiang Mai Province, despite the two areas' geographical and cultural closeness. The women in this study were either family members of lung cancer patients or their neighbors. To find clues to the etiology of lung cancer, this study used various epidemiological and biochemical approaches: interviewing on lifestyle factors, duplicate meals, chemical examination of drinking water, biochemical analysis of sera, mutagenicity test of urine, and monitoring of fungi and bacteria in the living environment. We found that tobacco smoking (*Khiyo*, local cigars) was less frequently observed in Sarapee (high incidence), compared with Chom Tong (low incidence), and that the history of chronic benign respiratory diseases was the most distinct event among women in Sarapee, resulting in a significantly increased percentage of those with a history of both benign respiratory diseases and tobacco smoking. This population revealed increased levels of serum tumor necrosis factor (TNF)-α, an endogenous tumor promoter. Furthermore, significantly increased urine mutagenicity was found to be closely associated with history of benign respiratory disease in Sarapee. The fungus which was most commonly found in the air inside houses in Sarapee was identified as *Microsporum canis*. Additionally, significantly increased serum concentrations of a constituent of the fungus were found in Sarapee women, compared with those in Chom Tong. Our results suggest that tobacco (*Khiyo*) smoking alone may not be able to explain the very high incidence of female lung cancer in Northern Thailand, and that chronic benign respiratory disease, possibly caused by the infection of fungi such as *M. canis*, is likely to be involved in the etiology of female lung cancer in North Thailand.

Key words: Lung cancer — Women — North Thailand

Women living in North Thailand, in particular Chiang Mai Province, are known to have a high risk of lung cancer. According to the Cancer Registry for Chiang Mai Province, the annual average age-adjusted incidence rates of lung cancer in Chiang Mai were 49.8 and 37.4 per 100,000 for men and women in 1988–1991, respectively,[1, 2)] and the incidence rate of female lung cancer increased to 40.1 in 1992.[3)] This high incidence is notable among women in Asian countries: other rates are 12.1 estimated for all of Thailand, 11.7 for Japanese in Osaka, 3.0 for Indians in Bombay, 16.3 for Filipinas in Manila, and 18.1 for Chinese in Shanghai, with higher incidence rates of 21.9, 32.6, and 33.2 observed among Chinese women in Singapore, Hong Kong, and Tianjin, respectively.[4)] Lung cancer has been the most common malignancy among both sexes in Chiang Mai since the population-based cancer registry started in 1983.[5)] Although a declining trend of lung cancer incidence among Chiang Mai women has recently been noted (29.7

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in 1994[6]), the rate is still very high, and this recent reduction might be influenced by a much more predominant cause of death, AIDS.

The most common cell type of lung cancer among Chiang Mai women was reported to be adenocarcinoma (51.9% of all lung cancer), followed by squamous cell carcinoma (19.2%) and small cell carcinoma (11.5%), with almost half (45.8%) of all cases clinically diagnosed.[4, 7] Lung cancer of Chiang Mai women has the following features: (1) a sharply rising age-specific incidence rate from the relatively young age of about 40, and no substantial difference in age-specific incidence rates between men and women after age 50; (2) a characteristic clustering in the geographical distribution of high-incidence areas, although no ethical or cultural differences were found between high- and low-incidence areas, except for areas where mountain tribes live (Fig. 1). A local cigar called "*Khiyo*," which is made from shredded tobacco leaves and other flavorings such as tamarind shell and *Koy*-tree bark, wrapped with dry young banana leaves, has been suspected to generate the high incidence of lung cancer, since *Khiyo* contains a very high content of tar (28.5–200.8 mg per *Khiyo*) and has a high mutagenicity.[8] Only one case-control study (60 male cases and 55 female cases) has so far been conducted to investigate the etiology of lung cancer in Chiang Mai, but *Khiyo* was not found to be a significant risk factor.[9] Other types of studies than case-control comparison may also be useful for studying the risk factors underlying the large inter-area differences of lung cancer incidence, since neither biological markers that are altered owing to cancer in the body nor biological monitoring of meals and environment around patients' houses are, in general, used in case-control studies.

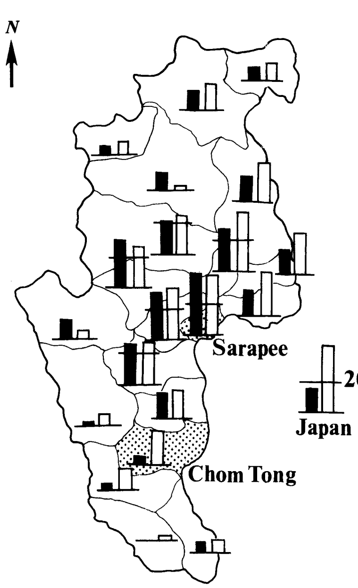

Fig. 1. Geographical distribution of lung cancer incidence in Chiang Mai Province by county: annual crude incidence rates in women (black bars) and men (white bars). Women in Sarapee showed the highest incidence among Chiang Mai counties, while Chom Tong had one of the lowest incidence rates, despite the two areas' geographical and demographical closeness. Incidence rates in Japan (1990) are shown for comparison.

In 1995, we started a Thai-Japan collaborative research project to clarify the etiology of lung cancer in Chiang Mai women under the auspices of the Monbusho (The Ministry of Education, Science, Sports and Culture of Japan) International Scientific Research Program; Special Cancer Research. In this project, five studies were conducted: (1) a comparative study of female residents living in high- and low-incidence areas; (2) a laboratory study to assess candidate causal factors of lung cancer, based on the results of the comparative study; (3) a large-sized case-control study in Chiang Mai University Hospital; (4) pathological study of lung cancer specimens; (5) molecular biological analysis using lung cancer DNA.

In this paper, we report the results of the comparative study, which was conducted by means of interviewing on lifestyle factors; duplicate meals; detection of heavy metals, arsenic and other chemicals in drinking water; biochemical analysis of sera; mutagenicity assay of urine; and monitoring of fungi and bacteria in the living environment. Our "multi-spectrum" approach used in this study has been shown to be a potent method for the assessment of novel etiological factors in human cancer, based on an understanding of the overall lifestyle and environment of the study subjects. Our results indicated that the very high incidence of female lung cancer in Chiang Mai cannot be attributed to tobacco (*Khiyo*) smoking alone, and that benign respiratory disease is very likely to be involved in the etiology of female lung cancer in North Thailand.

MATERIALS AND METHODS

Study subjects Study subjects were 57 and 59 female residents living in Sarapee and Chom Tong (Table I). Sarapee showed the highest incidence of female lung cancer in Chiang Mai Province (annual average crude incidence rate, 40.9), while Chom Tong had one of the lowest (8.5).[7] Their geographical locations are shown in Fig. 1. We selected Chom Tong because this area is geographically near, and demographically similar to Sarapee; the population (42,000 women) is comparable to that of Sarapee (33,000 women). Subjects were either family members or neighbors of lung cancer patients, aged between 50 and 74, the age group in which most female lung cancer occurred.

Interviewing and questionnaire Field surveys in Sarapee and Chom Tong were carried out in November of

Table I. Epidemiological Characteristics of Woman Subjects Living in Sarapee Area (High Incidence of Lung Cancer) and Chom Tong Area (Low Incidence)

Factors	Sarapee (*n*=57)	Chom Tong (*n*=59)	
Mean age (yrs, SE)	60.3±1.0	62.4±0.8	
Mean height (cm, SE)	149.9±0.8	149.4±0.9	
Mean weight (kg, SE)	48.0±1.4	44.6±1.4	
Marital status			
Married	32 (56.1%)	35 (59.3%)	
Widowed	18 (31.6)	22 (37.3)	
Divorced	2 (3.5)	1 (1.7)	
Separated	2 (3.5)	1 (1.7)	
Single	3 (5.3)	0 (0.0)	
Education			
None	11 (19.3%)	24 (40.7%)	
Primary school	46 (80.7)	35 (59.3)	
Occupation			
None	24 (42.1%)	32 (54.2%)	
Working	33 (57.9)	25 (42.4)	
Unknown	0 (0.0)	2 (3.4)	
Smoking status			
Current smokers	15 (26.3%)	36 (61.0%)	
Ex-smokers	23 (40.4)	17 (28.8)	
Never-smokers	19 (33.3)	6 (10.2)	$P<0.001$
History of benign respiratory disease			
Yes	25 (43.9%)	8 (13.6%)	
No	32 (56.1)	51 (86.4)	$P<0.001$

1995 and 1996, respectively. Study subjects were interviewed at the Sarapee Community Hospital or the Chom Tong Community Hospital by the same trained interviewers (nurses), using a standardized questionnaire. The questionnaire surveyed (1) race/tribe, religion, and birthplace; (2) past history of house removal; (3) marriage status, education, occupation history (of subjects, their husbands, and households), and social class (i.e., possession of real estate property, car, electric products, etc.); (4) types of residences and duration of living in the same area, sources of drinking water, conditions of toilet and kitchen (outside/inside houses, ventilation, types of fuel), and house surroundings (roadside, near factories, etc.); (5) the same information for previous dwelling places; (6) use of incense sticks; (7) house ventilation; (8) burning activity around houses (burning fallen leaves and wood); (9) current and past history of malnutrition, non-respiratory (e.g. malaria, fluke, hepatitis, etc.) and respiratory diseases (lung tuberculosis, pneumonia, bronchitis, etc.); (10) current and past history of medication; (11) family history of cancer; (12) age at menarche, menstrual status, and reproductive history; (13) food restriction; (14) types of meals (north/central Thai, Chinese, Western) by breakfast, lunch, and dinner; (15) current and past intake frequency of 80 selected food items; (16) intake frequency and amounts of beverages; (17) history of tobacco smoking and types of tobacco including various *Khiyo*; (18) passive smoking before and after marriage in houses (parents, husbands, other family members living together) and work places; (19) alcohol consumption; and (20) tobacco/betel nut/*Miang* (fermented tea leaf) chewing. The same questionnaire was used in the case-control study at Chiang Mai University Hospital.

Duplicate meals When the subjects visited the Sarapee and Chom Tong Community Hospital for interviewing and blood collection, they were asked to prepare duplicate meals (including foods taken between meals), starting from lunch of that day and ending just before lunch on the next day. Each food item was identified and weighed after collection. (Details were described elsewhere.[10]) Food samples were stocked at −80°C for further analyses in future, and it should be noted that this survey reflected food intake in the dry season.

Chemical analysis of drinking water Samples of drinking water at four houses and local cookshops in Sarapee were analyzed to detect heavy metals and arsenic by atomic absorption spectroscopy; other chemicals were analyzed by ion chromatography.

Biochemical examination of sera Peripheral blood samples of subjects were collected at the Community Hospital in Sarapee (in 1995) or Chom Tong (in 1996) between 9 and 10 a.m. under fasting conditions. Sera were immediately separated at the Hospitals, stocked at −80°C, and subjected to various biochemical assays in Japan to determine serum concentrations of total protein, albumin, α_1-, α_2-, β-, γ-globulin, total lipids, malondialdehyde, ferritin, vitamins A, B_2, B_{12}, E, folic acid, zinc, selenium, immunoglobulin E (IgE), and activity of cholinesterase, along with isolation of lymphocyte DNA. In addition, serum tumor necrosis factor (TNF)-α was measured at one time when all samples had been collected (Quantikine HS for human TNF-α, R&D Systems Ltd., Minneapolis, MN). Serum (1→3)-β-D-glucan, a constituent of the fungus found in Sarapee, was examined by the kinetic turbidimetric *Limulus* test.[11]

Urine mutagenicity Starting from the day of interviewing, 24 h urine samples of subjects were collected, and urine mutagens were absorbed on blue rayon (Funakoshi Co., Tokyo) within 9 h after collection, following the protocol of Morimoto *et al*.[12] Briefly, 0.4 g of blue rayon was added to 400 ml of each urine sample; the mixture was shaken for 30 min; the rayon was removed, washed with water, and dried with a paper towel. Then, 0.4 g of fresh blue rayon was again added to the same urine, and the procedure was repeated. The rayon was kept at −80°C in an air-tight bag. Measurement of urine mutagenicity in all rayon samples from Sarapee and Chom Tong was carried out in 1998. Mutagens in the rayon were eluted with methanol:ammonia (50:1); mutagens were again absorbed with 0.1 g of fresh blue rayon and eluted. The residue after evaporation was resolved with 0.1 ml of dimethylsulfoxide (DMSO) and subjected to the mutagenicity test (50 μl/plate), using *Salmonella typhimurium* TA98 and TA100 with and without S9 mix. Numbers of His+ revertants per plate were counted and compared with those in negative controls, and each measurement was duplicated. Mutagenicity was taken as the mean of colony numbers with duplicate measurements after subtraction of those in negative controls. Positive controls (50 μl/plate) were 2-aminofluorene without S9 mix (0.1 μg/plate for TA98 and 0.01 μg/plate for TA100) and 2-acetylaminofluorene with S9 mix (0.5 μg/plate). Blue rayon samples from two Japanese non-smoking women were added, and mutagenicity was measured with other samples for reference. All these assays were performed without awareness of sample identification at the Department of Biochemistry, Faculty of Medicine, Chiang Mai University.

Collection and identification of fungi and bacteria in the air inside houses In the afternoon of the day of interviewing, we visited houses of all subjects and collected fungi and bacteria in the air, using air-monitoring equipment (Bio Air Checker, Nikken Bio Medical Lab., Kyoto). We set the equipment at 1.0 m above the floor and 0.5 m inside the house entrance. The equipment blew the air to a culture plate for 1 min (50 liter air); two types of culture plates were used, Sabouraud glucose agar and standard SCD agar (Nikken Bio Medical Lab.). Inoculated plates were cultured for 1 week at 25°C, and the numbers of colonies per plate were counted. After the isolation of colonies, slide culture and giant culture for 2 weeks at 25°C were carried out to identify fungi.

RESULTS

Epidemiological questionnaire Characteristics of the woman subjects from Sarapee and Chom Tong are shown in Table I, together with those epidemiological factors which varied significantly between the two areas. All subjects were found to be native Thai, with most being Buddhist (96% and 100% in Sarapee and Chom Tong, respectively). Education levels for Sarapee women were higher than those of Chom Tong, although none had any secondary schooling. There was no significant difference in social class, which was estimated in terms of ownership of land (35% and 54% in Sarapee and Chom Tong, respectively), houses (93% and 92%), cars (16% and 19%), motorbikes (72% and 63%), TV's (91% and 88%), radios (79% and 58%), refrigerators (74% and 70%), and washing machines (23% and 15%). (Sarapee may be more urbanized than Chom Tong because of its nearer location to Chiang Mai City.)

To our surprise, there was a significantly lower percentage of current smokers among women from the high-incidence area, Sarapee, than among those from the low-incidence area, Chom Tong ($P<0.05$): current, ex-, and never-smokers were 26%, 40%, and 33% in Sarapee, respectively; 61%, 29%, and 10% in Chom Tong, respectively. Among smokers, 81% (13/15) of current smokers and all ex-smokers (23) in Sarapee used *Khiyo*; all current (36) and ex-smokers (17) in Chom Tong used *Khiyo*. We found no substantial difference between the two areas in amounts of tobacco (*Khiyo*) used. Daily consumption of *Khiyo* among current and ex-smokers in Sarapee was below one *Khiyo* for 11 current and 12 ex-smokers, 2 *Khiyo* for one current and 5 ex-smokers, 3 *Khiyo* for 2 ex-smokers, 4 *Khiyo* for 2 ex-smokers, and 7 *Khiyo* for one ex-smoker; it was below one *Khiyo* for 33 current and 16 ex-smokers, 2 *Khiyo* for 2 current and one ex-smoker in Chom Tong. Commercial cigarettes were used by 5 current smokers (2 used *Khiyo*; 1, 2, 3, 4 and 5 cigarettes per day) and 4 ex-smokers (3 used *Khiyo*; 1, 2, 4, and 10 cigarettes per day) in Sarapee. There was no significant difference between the areas in age at starting *Khiyo* smoking (mean±SE, 18.6±1.4 and 21.2±1.6 in Sarapee and Chom Tong, respectively). Most subjects in both Sarapee and Chom Tong consumed one *Khiyo* a day or less, a quantity which seems insufficient to explain the high incidence of lung cancer among Sarapee women. In addition, exposure to husband's smoking is more frequent in Chom Tong than in Sarapee ($P<0.05$): 24% (46%) and 42% (51%) of husbands were current (ex-) smokers in Sarapee and Chom Tong, respectively.

The most remarkable difference between the areas was the history of chronic benign respiratory disease. Those who had experienced chronic benign respiratory disease accounted for 44% (25/57) in Sarapee and 14% (8/59) in Chom Tong, with the most frequent disease being chronic bronchitis (20/25 in Sarapee, 2/8 in Chom Tong), followed by chronic obstructive pulmonary disease (COPD). In Sarapee, the ages at which they experienced these diseases were the teens (3), twenties/thirties (9), forties/fifties (22), sixties and over (9). Ten and three of 25 cases had suffered from the diseases over 20 and 30 years, respectively, and there was a total of eighteen respiratory disease cases at the time of the study. Those with a history of both *Khiyo* smoking (or commercial cigarettes) and chronic benign respiratory disease accounted for 32% (18/57) in Sarapee, in contrast with 14% (8/59) in Chom Tong (Fig. 2). We found no relation between smoking habits and history of benign respiratory diseases.

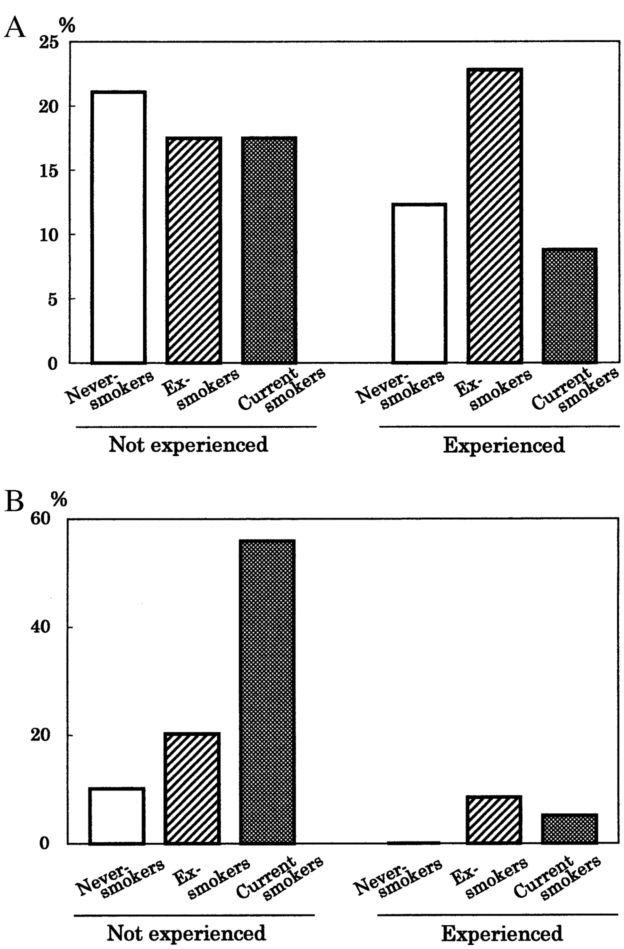

Fig. 2. Frequency distribution of women in (A) Sarapee area and (B) Chom Tong area by tobacco (*Khiyo*) smoking status("never," "ex-," and "current smokers") and history of benign respiratory disease ("not experienced" and "experienced").

Other significant differences between Sarapee and Chom Tong were found in sources of drinking water (5 and 35 of 57 were from tap and well in Sarapee; 29 and 18 of 59 from tap and well in Chom Tong, respectively), intake frequency of beef (28, 22, and 7 of 57 were never, ≤ once per week, and ≥ twice per week in Sarapee; 47 and 12 of 59 were never and ≤ once per week in Chom Tong, respectively), and intake frequency of fermented fish (8, 6, 6, and 37 of 57 were never, ≤ once per week, 2–3 times per week, and ≥ once per day in Sarapee; 1, 1, 1, and 56 of 59 were never, ≤ once per week, 2–3 times per week, and ≥ once per day in Chom Tong, respectively).

Duplicate meals The results were reported in our previous paper.[10] Briefly, intake amounts of potatoes, legumes, confectionery, and fruits revealed significant differences between Sarapee and Chom Tong ($P<0.001$). In particular, intake of fresh fruits in Sarapee (mean±SD, 18.2±43.7 g) is remarkably low compared with that in Chom Tong (107.8±131.7 g), or even with those estimated for urban (108 g) and rural areas (71.2 g) throughout Thailand. Although this significant difference in intake of fresh fruits observed in duplicate meals reflects the situation in the dry season, a lower intake frequency of fresh fruits in Sarapee even in the growing season was also reported in the questionnaire survey ($P=0.11$).

Drinking water Concentrations of cadmium, mercury, selenium, lead, arsenic, sexivalent chromium, nitrogen (as nitric and nitrous acids), fluorine, zinc, iron, copper, sodium, manganese, chlorine, calcium, magnesium, nitrogen as ammonia, and pH in drinking water in Sarapee were <0.001, <0.00005, <0.001, <0.001, 0.001–0.005, <0.005, <0.1–0.3, 0.16–0.37, <0.005–0.034, <0.03–0.10, <0.01–0.02, 8.1–43.5, <0.005–1.2, 4.7–51.1, 30.0–72.8, 1.8–19.2, <0.1 mg/liter, and 7.1–7.7, respectively. All measured values were within the ranges regarded as safe.

Serological analysis Concentrations of selected serum components were compared among women from Sarapee and Chom Tong (Table II). Although we found significant differences in several components between the two areas, none of them revealed abnormal deviations from the Japanese standard ranges, except for abnormally high levels of vitamin B_2, B_{12}, and folic acid in Chom Tong, and IgE in both areas. Specifically, serum levels of vitamin B_{12} and folic acid in Chom Tong were significantly higher than those in Sarapee: 54 of 57 and all women in measurement of vitamin B_{12} and folic acid exceeded the upper detection levels of 1600 pg/ml and 15 ng/ml in Chom Tong, respectively. However, the lower serum concentrations in Sarapee were still within the normal range, indicating that these women did not suffer from a deficiency of these

Table II. Mean Levels±SD of Serum Components among Women in Sarapee and Chom Tong

Serum components	Sarapee (n=57)	Chom Tong (n=59)	
Total protein	8.0±0.4 (g/dl)	7.6±0.5	P<0.01
Albumin	4.6±0.3 (g/dl)	4.5±0.3	P<0.05
α_1-globulin	0.21±0.03 (g/dl)	0.21±0.04	
α_2-globulin	0.72±0.08 (g/dl)	0.70±0.10	
β-globulin	0.80±0.09 (g/dl)	0.70±0.10	P<0.01
γ-globulin	1.7±0.4	1.5±0.3	P<0.01
Cholinesterase	388±91 (IU/liter)	382±120	
Total lipids	571±106 (mg/dl)	548±95	
Malondialdehyde (MDA)	3.4±0.4 (nM/ml)	3.5±0.6	
Ferritin	151±119 (ng/ml)	125±95	
Vitamin A	178±54 (IU/dl)	178±53	
Vitamin B_2	59±15 (ng/ml)	117±31	P<0.01
Vitamin B_{12}	801±349 (pg/ml)	1581±73[a)]	P<0.01
Vitamin E	1.1±0.3 (mg/dl)	1.0±0.2	
Folic acid	11±2 (ng/ml)	>15[b)]	P<0.01
Zn	88±12 (μg/dl)	85±10	
Selen	12±2 (μg/dl)	12±2	
Immunoglobulin E	1063±1579 (U/ml)	942±1911	

a) Fifty-four women showed concentrations over the upper limit of measurement (1600 pg/ml), and were calculated as 1600 pg/ml.
b) All women showed concentrations over the upper limit of measurement (15 ng/ml).

nutrients. Extremely high concentrations of IgE were observed in both areas, with a large fraction of the high serum IgE possibly being due to the infection of parasites. Interestingly, in Sarapee the significantly increased IgE was associated with a history of benign respiratory disease, and this increase was enhanced by tobacco smoking (mean±SE, 493±136 and 838±258 U/ml for 11 never-smokers and 21 ex- and current smokers without the history, respectively; 836±340 and 1762±545 in 7 never-smokers and 18 ex- and current smokers with the history; P<0.05 for the difference in mean between the first and last groups), suggesting that foreign substances entering the respiratory tract, along with immunological reactions, might be causing diseases. This association was not found in Chom Tong.

When we measured serum levels of the endogenous tumor promoter TNF-α, we found significantly increased levels among those with a history of both benign respiratory disease and tobacco smoking in Sarapee: mean±SE, 2.3±0.2 pg/ml for 11 never-smokers without the history and 3.2±0.3 pg/ml for 18 ex- and current smokers with the history (P<0.05, Fig. 3). In Sarapee, serum TNF-α significantly increased with a history of the disease among never-smokers, although an enhancement of TNF-α levels by tobacco smoking was not observed among those with a history of the disease. However, these associations were not observed in Chom Tong; there was no significant difference in mean TNF-α levels between Sarapee (mean±SE, 3.0±0.2) and Chom Tong (3.3±0.2, two non-informative cases). Serum TNF-α levels in both areas were about two-fold higher than those among the Japanese general population (data not shown).

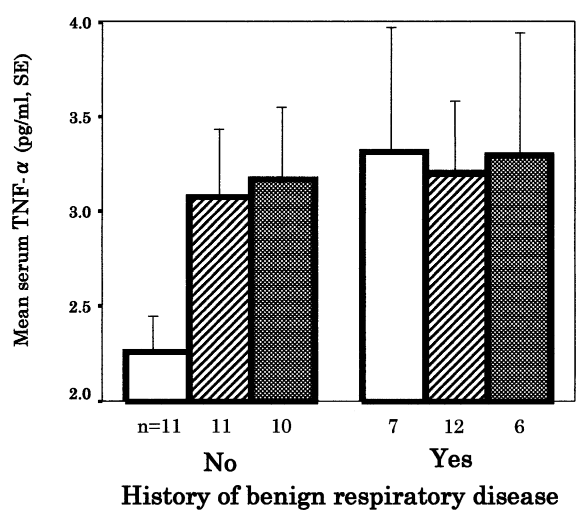

Fig. 3. Mean serum concentration of TNF-α (pg/ml) among the women from Sarapee by tobacco smoking (▭ never-smokers, ▨ ex-smokers and ▩ current smokers) and history of benign respiratory disease.

Urine mutagenicity Mean urine mutagenicity (14.3±3.0 and 33.7±7.4 by TA98 and TA100 with S9 mix, respectively) in Sarapee (*n*=55; two non-informative cases excluded) was significantly higher than that (5.2±1.7 and 17.8±3.6 by TA98 and TA100 with S9 mix, respectively; $P<0.01$ and $P<0.05$ for difference in mean values) in Chom Tong (*n*=59). Urine mutagenicity measured by TA98 and TA100 showed a good correlation ($r=0.47$, $P<0.01$). Compared with two samples from two Japanese female non-smokers (6.0 and 1.3 by TA98 with S9 mix; 12.3 and 27.3 by TA100 with S9 mix), urine mutagenicity in Sarapee was remarkably high. Increased urine mutagenicity was observed among those who had experienced benign respiratory disease in Sarapee, but not in Chom Tong; among those with no disease history, there was no significant difference in mutagenicity between Sarapee and Chom Tong (Fig. 4). Furthermore, mutagenicity among those who had experienced respiratory disease in Sarapee revealed a remarkable and synergistic increase with *Khiyo* smoking (Fig. 4). Urine mutagenicity did not show significant association with any other environmental or lifestyle factors surveyed in the questionnaire.

Collection and identification of fungi and bacteria in the air inside houses In addition to a lower frequency of tobacco smokers and a higher frequency of those who experienced benign respiratory diseases in Sarapee, association of the above biological markers with a history of disease and tobacco smoking was seen only in Sarapee, despite the similar consumption amounts and types of tobacco in both areas. We investigated fungi and bacteria as possible causes of those respiratory diseases, particularly in Sarapee. When we compared the growth of fungus colonies in culture plates (Sabouraud glucose agar) between Sarapee and Chom Tong, under the same culture conditions, the bulk of the plates from Sarapee showed much larger sizes of colonies than those from Chom Tong. Out of a total of 23 detected fungus species in Sarapee and Chom Tong, the most frequent in Sarapee was *Microsporum canis*, detected in 72% of the surveyed houses (*n*=53), followed by *Acremonium fulciforum*, which was found only in Sarapee (38%), and *Fusarium soranii* (36%); *M. canis* and *F. soranii* were found in 38% and 26% of the houses in Chom Tong. *M. canis* in Sarapee was detected in 62% of the houses of women with a history of benign respiratory disease.

Moreover, we found that sera of three woman subjects from Sarapee contained abnormally high concentrations of (1→3)-β-D-glucan (≥11 pg/ml), a constituent of cytoplasmic membrane of *Moniliales* including *Aspergillus*, *Candida*, and *Microsporum*, whereas none of the Chom Tong subjects showed this abnormality (Fig. 5). Sixteen of the 49 women examined in Sarapee showed detectable levels of serum (1→3)-β-D-glucan (≥3.17 pg/ml), while only 3 of 42 subjects in Chom Tong revealed detectable levels. Although air-monitoring data were available for 13 of the 16 women with detectable levels of (1→3)-β-D-glucan in Sarapee, and *M. canis* was found in a high percentage of their houses (10/13), we found no association between serum (1→3)-β-D-glucan levels and history of benign res-

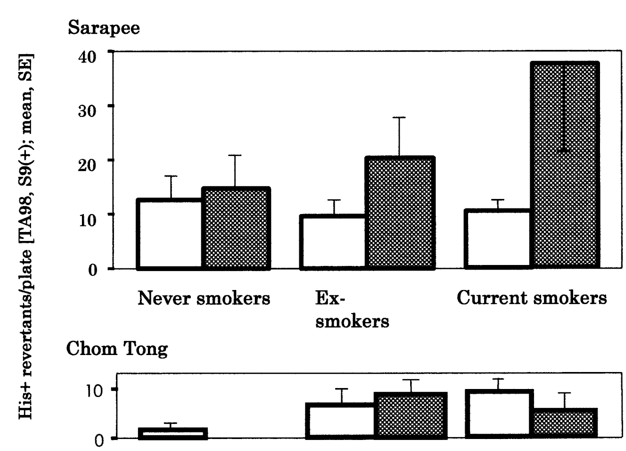

Fig. 4. Urine mutagenicity (mean numbers of His+ revertants/plate, TA98 with S9 mix) by tobacco smoking and history of benign respiratory disease (▭ no and ▩ yes) in Sarapee and Chom Tong. Increased mutagenicity was found among those with a history of both benign respiratory disease and tobacco smoking. Urine mutagenicity among women from Chom Tong was similar to that of two Japanese women measured for reference.

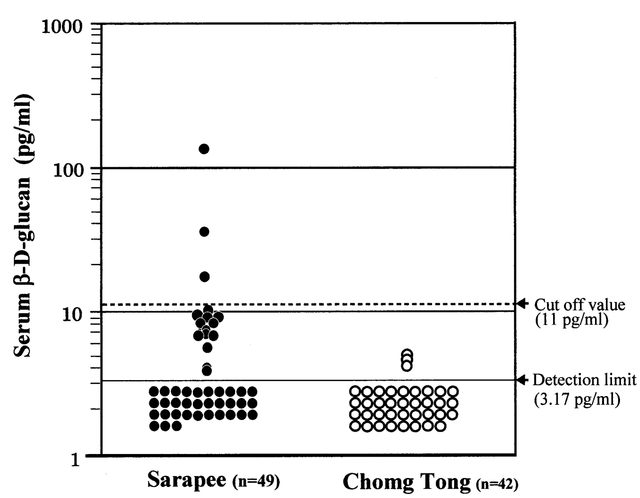

Fig. 5. Serum levels of (1→3)-β-D-glucan (pg/ml) among women in Sarapee and Chom Tong. High levels of β-D-glucan in Sarapee imply that women living there were heavily exposed to fungi such as *Microsporum canis*.

piratory disease, probably because this marker reflects only current exposure to the fungi.

DISCUSSION

The lifestyle and environment of residents of developing countries differ from those in developed countries, which may result in a different etiology for lung cancer, an etiology which we sought to understand using a wide-spectrum approach. We conducted a comparative study of female residents living in high-incidence (Sarapee) and low-incidence (Chom Tong) areas for female lung cancer, using various epidemiological and biochemical tools. Although this kind of study is, in general, limited in its ability to produce conclusive evidence, since it needs to be combined with or followed by other approaches, the present study did have a rationale: knowing that lung cancer incidence among women in Sarapee was about five times higher than in Chom Tong, despite the two areas' geographical and cultural closeness, biochemical approaches, which could not be used in our parallel case-control study, were used to elucidate the overall characteristics of lifestyle and environment among the women living in the high-incidence area.

In this study, we found the following: (1) tobacco (*Khiyo*) smoking was not related to the high incidence of female lung cancer, because of a lower percentage of smokers in the high-incidence area, Sarapee, and very low consumption of one *Khiyo* a day or less for them; (2) the history of chronic benign respiratory disease was the most outstanding feature among women in Sarapee, resulting in a significantly higher percentage of those with a history of both benign respiratory disease and *Khiyo* smoking in Sarapee; (3) a remarkably low intake of fresh fruits was observed in Sarapee women in the dry season; (4) extremely high levels of serum vitamin B_2, B_{12} and folic acid were observed among women in the low-incidence area, Chom Tong; (5) in Sarapee, high serum IgE levels were associated with a history of benign respiratory disease, suggesting that immuno-stimulating foreign substances might be causing disease; (6) increased serum levels of TNF-α were closely associated with a history of benign respiratory disease and tobacco smoking in Sarapee; (7) significantly increased urine mutagenicity was closely associated with a history of benign respiratory disease in Sarapee; (8) fungi detected inside houses showed distinct differences between the two areas, with the most frequent one in Sarapee found to be *M. canis*; significantly high concentrations of serum $(1\rightarrow3)$-β-D-glucan, probably derived from this fungus, were observed among some subjects from Sarapee. To summarize, tobacco smoking by itself is insufficient to explain the very high incidence of female lung cancer in Sarapee; benign respiratory disease, however, mainly chronic bronchitis possibly associated with the infection of fungi such as *M. canis*, is very likely to be involved in the etiology of female lung cancer in Northern Thailand. Given the potential implications of our findings, a follow-up study is warranted to confirm this involvement.

The association of high urine mutagenicity with a history of benign respiratory disease in Sarapee indicates the possibility that *M. canis*, together with *Khiyo* smoking, may act as an initiator in lung carcinogenesis. On the other hand, increased serum levels of the endogenous tumor promoter TNF-α among those with a history of both benign respiratory disease and *Khiyo* smoking indicate the possible involvement of *M. canis* as a tumor promoter.[13] Interestingly, the production of TNF-α by human monocytes has been reported to be enhanced by serum exposed to *Candida albicans*,[14] which has the same membrane components as *M. canis*.[11] Infection of *M. canis* may be involved in different stages of lung carcinogenesis, although serum TNF-α and urine mutagenicity measured in this study do not necessarily reflect the situation in the lung, and it should be noted that serum $(1\rightarrow3)$-β-D-glucan is a marker for exposure to *M. canis* only in the present, not the past. To overcome these limitations of this study and to confirm our findings, we are now conducting *in vitro* and *in vivo* studies to assess the role of *M. canis* in lung carcinogenesis.

Finally, the extremely high serum levels of vitamin B_{12} and folic acid among the women from Chom Tong may be noteworthy. The very low incidence of female lung cancer in Chom Tong, despite a pattern of prevalent *Khiyo* smoking, might in part be associated with those high serum levels. If such is the case, identification of the food(s) responsible for the high serum concentrations of these nutrients, would be useful for lung cancer prevention.

The findings in this study will also provide a solid base for our parallel case-control study in Chiang Mai University Hospital, as well as our on-going pathological and molecular biological studies aimed at characterizing lung cancer of women in Northern Thailand and elucidating its etiology.

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