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ARE ALLIUM VEGETABLES AND THEIR CONSTITUENTS
ASSOCIATED WITH REDUCED RISK OF BREAST CANCER?

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ARE ALLIUM VEGETABLES AND THEIR CONSTITUENTS ASSOCIATED WITH REDUCED RISK OF BREAST CANCER?

By

Roveena Noeline Goveas

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ABSTARCT

ARE ALLIUM VEGETABLES AND THEIR CONSTITUENTS ASSOCIATED WITH REDUCED RISK OF BREAST CANCER?

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Constituents of allium vegetables such as onions, garlic, leeks and chives have been shown to have anti-carcinogenic properties in *in-vitro* and *in-vivo* studies. However, the results from epidemiological studies have been inconsistent. This study examined the relation between consumption of allium vegetables in adulthood and breast cancer risk. We analyzed the data from 'Breast Cancer in Women of Polish Ancestry' study, a population based breast cancer case-control study designed to study the effects of dietary and lifestyle changes that occur with migration on breast cancer risk among Polish immigrants to the United States. Dietary intake was assessed by a food frequency questionnaire and a risk factor questionnaire was used to evaluate lifestyle factors. Analysis of 140 histologically confirmed breast cancer cases and 303 age and area of residence matched controls showed an inverse statistically significant association between increased garlic intake (>3 serving versus <1 servings/week, OR=0.39, 95% CI=0.18-0.83) and breast cancer risk. For total allium vegetable intake (garlic, onions, green onions, chives and leeks), the observed 40% reduction in risk (>10 serving versus <5 serving/week, OR=0.58, 95%CI=0.30-1.12) did not reach statistical significance. This study provides support for the protective role of increased garlic intake during adulthood on breast cancer risk.

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KEY TO ABBREVIATIONS

BMI	Body Mass Index
CI	Confidence Intervals
DADS	DiAllyl DiSulfide
DHQ	Diet History Questionnaire
DMBA	7, 12-DiMethylBenz (a) Anthracene
FFQ	Food Frequency Questionnaire
HCFA	Health Care Financing Administration
HRT	Hormonal Replacement Therapy
IRB	Institutional Review Board
ISCR	Illinois State Cancer Registry
KCI	Karmanos Cancer Institute
MET	Metabolic Equivalent
NCI	National Cancer Institute
NIH	National Institute of Health
OC	Oral Contraceptive
OR	Odds Ratio
PI	Principal Investigator
RDD	Random Digit Dialing
SAS	Statistical Analysis Software
SEER	Surveillance Epidemiology and End Results
US	United States

Introduction

Breast Cancer Incidence and Mortality

Breast cancer is the most common malignancy and leading cause of cancer related deaths among women world-wide (1). Annually, one million cases of breast cancer are reported world-wide with approximately 580,000 cases in developed countries. In 2000, breast cancer accounted for 1.6% of the overall female deaths in the world (2). The age adjusted incidence of breast cancer (adjusted to 2002 World Standard Population) varies world-wide, with the incidence per 100,000 population as high as 99.4 in North America, 88.7 in Denmark, 50.3 in Poland, to a low of 4.4 in Haiti and 3.9 in Mozambique, Africa. Within North America, United States (US) has the highest age adjusted incidence rate of 101/100,000 population (3). In the US, it was estimated (2007) that breast cancer is the most common cancer among women, excluding skin cancers, and the second most common cause of cancer related deaths after lung cancer (4). It is estimated that a women in the US has a 1 in 8 (12%) chance of developing invasive breast cancer at some point in her lifetime (5).

According to 2004 US statistics, age-adjusted incidence of invasive female breast cancer for all races was 117.7 per 100,000 women (adjusted to 2000 US Standard Population), with highest incidence in Whites, slightly lower in Blacks and lowest in American Indian /Alaska natives (6). The trend in breast cancer incidence has changed over the past 3 decades. There was a significant increase in incidence in the 1980's, followed by a slow rise of 0.4% (annual percent change) in the 1990's, however, there has been a 3.9%

(annual percent change) decrease from 2001-2004 (7). It has been hypothesized that the wide application of screening in the early 1980's led to a higher rate of detection of breast cancer than before and this led to the initial rise in the reported incidence of breast cancer. The results published from the Women's Health Initiative trial 2002, on use of Hormonal Replacement Therapy (HRT) and the increased risk in breast cancer, led to a marked decrease in the use of HRT and this has been postulated as the reason for the decrease in the incidence of breast cancer in the 2000's (5). In, addition there has been a significant decrease in the use of screening mammography, 76.4% (Confidence Intervals (CI) = 75.8-76.9) in 2000 to 74.6% (CI = 73.8-75.4) in 2005 (test for trend, $p<0.001$), which also could have contributed to the decline in detection of breast cancer (8).

According to 2004 US health statistics, it has been estimated that there are about 2.4 million prevalent cases of breast cancer (6). With improved screening rates and advancement in treatment, the mortality rate for breast cancer is decreasing (5). There was a annual percent decrease of 0.4% from 1975-1990, and the mortality rate further decreased from 1990-2004 by 2.2% per year (7). In 2000, mortality rate from breast cancer was 26.6/100,000 population and decreased to 24.4 / 100,000 people in 2004 (6) with the median age of death being 69 years (7). Mortality rate in 2004 was higher among Blacks than any other ethnic group (7). The overall five-year relative survival rate for 1996-2003 based on 17 Surveillance Epidemiology and End Results (SEER) geographic areas was 88%, among Whites it is 90% and 78% in Blacks (7).

Risk factors

The etiology of breast cancer is multi–factorial, with a small proportion being attributed to gene mutations and the remainder is suspected to be due to environmental factors. In addition, epidemiological studies on migrant populations have observed that migrants acquire the rates of breast cancer incidence of host countries within 1-2 generations. These studies have contributed to our understanding of the role of environmental factors as risk in cancers (9).

Non- modifiable factors like gender, advancing age, gene mutations and positive family history are considered some of the strongest non-modifiable risk factors. Approximately 7% of the breast cancer cases can be attributed to single gene mutations (10). Some of the well established genes associated with breast cancer are mutations in the tumor suppressor genes, BRCA 1 and BRCA 2. Fifty-two percent of the hereditary breast cancers can be attributed to BRCA 1, while 32% to BRCA2 gene mutations (11). It has been estimated that the risk of breast cancer in female carriers of BRCA2 mutation ranges from around 28% at 50 years to 84% at age 70 (11).

Family history of breast cancer in the first-degree relative is another strong, non-modifiable risk factor (12). However, eight of nine women with breast cancer usually have no positive family history of breast cancer (13). The age at which the relative developed breast cancer, the number of family members affected, and the blood relation to the affected relative are all important factors that determine the risk. In a collaborative reanalysis of 52 individual studies, it was observed that lifetime excess incidence of

breast cancer for a woman with one affected first-degree relative was 5.5% and 13% for a woman with two affected relatives (13).

Apart from gene mutations and family history, gender and age are important non-modifiable risk factors. Females are at a higher risk than males for developing breast cancer. Male breast cancer accounts for less than 1% of all reported breast cancers (6). In the US, it has been observed that the risk of developing breast cancer increases with advancing age. There is a gradual increase in risk from age 20 to 40, and much greater increase after age 40 which continues and then plateaus at around 80 years of age (6). On average, from 2000-2004, 95% of new breast cancers occurred in women older than 40 years (14). As per the 2004 National Cancer Institute (NCI) statistics, the median age at breast cancer diagnosis for all races was 61 years (7), and more than 80% of breast cancers are in post-menopausal women (6).

Exposure to hormones such as estrogens has been implicated as a risk factor for breast cancer. Earlier age at menarche and later age at menopause, thus longer duration of exposure to endogenous estrogens have been associated with increased risk of breast cancer (15-17). Similarly, exposure to exogenous estrogens and hormones like the use of Hormonal Replacement Therapy (HRT) has been implicated as potential risk factors for breast cancer. The US Preventive Services Task Force in their report in 2004, recommended against the routine use of HRT based on good evidence from studies conducted in representative populations, since the risks including that of breast cancer exceeded the benefits (18). The association of breast cancer with use of oral contraceptive

(OC) pills is not well established. In a concise review based on evidence on use of oral contraceptive pills and risk of breast cancer it was concluded that use of oral contraceptive pills did not led to a significant increase in breast cancer risk (19). Other studies, however, contradict these findings. In studies investigating "current use" as opposed to "ever used", increased risk have been found for current users (Odds ratio (OR) 4.0, CI= 1.8-9.0) (20).

The rising incidence of breast cancer in the developing world can be to some extent attributed to the changing behavioral patterns with the adoption of reproductive patterns similar to the developed nations such as increase in nulliparity, late age at first full term pregnancy and reduced duration of lactation. It has been estimated that risk of breast cancer increases on average by 3-5% per year delay in age at first full term pregnancy (16).The association between parity and breast cancer risk has been inconsistent. Some studies have shown a protective effect, while others do not support this observation (15, 16). It has also been observed that the protective role of multiparty is stronger for breast cancers arising at a later age (16). Lactation is another factor that has been shown to be protective against breast cancer risk. In a collaborative reanalysis of data from 47 epidemiological studies study it was observed that cases of breast cancer had a shorter average duration of breast feeding (9.8 months) in comparison to controls (15.6 months). It was estimated that the relative risk of breast cancer decreased by 4.3% for every 12 months of breast feeding. It was also observed that average duration of breast feeding differed among the developed and the developing nations, with an average of 8.7 and 29.2 months for developed and developing nations respectively (21).

Western lifestyle characterized by low physical activity, high caloric intake leading to energy imbalance resulting in increasing obesity is another factor implicated in increased risk of breast cancer (2). Obesity has a variable effect on breast cancer risk based on menopausal status. Several studies have demonstrated that obesity is associated with increased risk of breast cancer in post-menopausal women whereas such a relation has not been observed in pre-menopausal women (22, 23). The differential effect of adiposity on breast cancer has not been fully explained, although it has been proposed that the protective effect of obesity fro pre-menopausal women is due to the increase in anovulatory cycles that obese women experience, thus lower total exposure to estrogens (24-26). The increase in breast cancer risk due to obesity in postmenopausal women can be to some extent explained by the higher levels of circulating estrogens in postmenopausal women who are overweight (27, 28). Also, studies have observed that sex hormone binding globulins are lower in obese post-menopausal women contributing to a higher level of bioavailable estradiol (27-29).

Physical activity throughout lifetime by its effect on weight reduction and hormonal levels has been shown to be protective against breast cancer (30). In a systemic review of literature on physical activity and breast cancer (literature review through early 2006), it was concluded that there was an inverse association between physical activity measured during various lifetime periods, such as adolescence and adulthood, and breast cancer risk. The reduction in risk was observed to be stronger for postmenopausal women. However, it is not clear whether the magnitude of the protective effect is similar for

physical activity during adolescence versus adulthood and whether there is an additional protective effect for those who are active throughout their lifetime (31).

Various components of diet like dietary fat, fruit and vegetable intake have been studied extensively to evaluate their role in breast cancer. The role of dietary fat has received considerable attention. The possible role of dietary fat in breast cancer has been hypothesized from observations in ecological studies (32). However, results from case-control and cohort studies have been inconsistent, with case-control studies showing a positive association (OR ranging between 1.2-1.5) whereas no such association has been observed in cohort studies (33, 34). Several reasons underlie this inconsistency. Bingham et al., in 2003, observed that, the methods used to measure dietary intake play an important role in ascertaining the association between diet and diseases. They evaluated diet in 13070 women from the European Prospective Investigation of Cancer and Nutrition (EPIC) study with an FFQ and 7 day food diary. Analysis of the data from 168 incident cases of breast cancer and four age and date of entry matched controls (case:control:1:4) showed that saturated fat intake was associated with breast cancer risk with a hazard ratio of 1.22 (95% CI=1.06-1.40) when intake was measured by a 7 day food diary, however when intake was quantified using a Food Frequency Questionnaire (FFQ), no significant association was detected (35). The observed association for the 7 day food diary is of the same order of magnitude as the association observed from the meta-analysis of the case-control studies and dietary fat. Nevertheless, both cohort and case-control studies evaluating the association between breast cancer and specific types

of fat have reported protective effects with monounsaturated fatty acids and omega-3 fatty acids with considerable consistency (36).

Similar to dietary fat, the role of fruits and vegetable in breast cancer incidence has also been extensively studied. In a meta-analysis of case-control studies, a statistically significant protective effect of vegetables intake on breast cancer risk was observed (OR 0.86, CI=0.78-0.94). However, in the same paper, meta-analysis of the cohort studies did not find a significant reduction in risk (37). For cohort studies a pooled analysis by Smith-Warner et al., (2001), of 8 cohorts, also did not observe a significant reduction in risk either for total fruit and vegetable consumption (Relative Risk (RR)=0.93, 95% CI=0.86-1.00) or total fruits only or total vegetables only when comparing highest to lowest quartile of intake (38).

Alcohol consumption is another risk factor associated with breast cancer incidence. The modest positive association between alcohol intake and breast cancer of approximately 10% increase per one drink/day has been a consistent finding in several epidemiological studies over the past two decades (39). In a meta-analysis, it was summarized that relative risk of breast cancer for women was 1.10 for 12g/day of alcohol (1 drink per day) consumed in comparison to non-drinkers (40). Several biologically plausible mechanisms have been proposed for the association between breast cancer and alcohol consumption. Acetaldehyde, a metabolite of alcohol has shown to be carcinogenic and also, known to destruct folate and induce hyperregenaration (41). In addition, the increased risk for

breast cancer from alcohol consumption can also be partly explained by higher estrogen levels in heavy drinkers (42).

Assessment of diet in epidemiological studies

Using an appropriate and reliable instrument to measure dietary intake is crucial in epidemiological studies assessing the role of diet on disease. Twenty–four hour recall, food records and food frequency questionnaires are some of the tools used to measure dietary intake. Twenty-four hour recall and food records are helpful in assessing absolute intake over specific days. The food frequency questionnaire assesses relative intake over longer periods of time as well as remote diet from the past (43).

24 Hour recall: Intake over a period of 24 hours is assessed by trained interviewers. This method aims at assessing current diet over a specified amount of time, usually one year. Since the process is interviewer assisted, subjects can participate irrespective of their level of literacy. Details on foods consumed and quantification are subject to errors due to recall of short term memory, although the response is less influenced by the vigilant nature of the subject as details are recorded after intake. It is essential to consider an ideal balance of intake over the various days of the week, thus 24 hour recalls usually cover both weekdays and are uniformly administered over a year to incorporate changes in diet due to seasonality (43).

Food Record Method: This is also referred to as food diary. It is a detailed list of the food items consumed by the individual over approximately two to four days over the four seasons, or approximately 8-16 days of intake per individual. Subjects are trained to maintain a record of all food consumed over specific days including preparation

techniques. After completion, the food record is reviewed by a nutritionist to ensure accuracy and gather more information if required. Although this method overcomes the need for a trained interviewer, for a complete and accurate record, the individual need to be highly motivated and well trained. Like the 24 hour recall, the food diary is useful to capture the dietary habits of culturally diverse groups (43).

Food Frequency Questionnaire (FFQ):

In chronic disease and cancer epidemiology, it is often necessary to access diet from the remote past to capture an etiologically relevant window and a FFQ serves as a relatively useful tool in these situations. A FFQ has the advantage of being a relatively simple tool and can be interviewer or self administered. It consists of a list of questions on specific foods and may or may not include information for portion sizes (43). Some of the commonly used FFQ in epidemiological studies are the Willett FFQ, Block FFQ and Diet History Questionnaire (DHQ), which was recently developed by the National Institute of Cancer and is an improvement of the Block instrument. The Block FFQ and DHQ quantifies portion sizes by providing three standard ranges and evaluates portion sizes differently for men and women. Whereas, the Willet FFQ does not include separate questions on portion sizes but instead subjects are asked to report their frequency of intake in terms of a standard serving size. In a study conducted to compare the efficiency of these FFQ's it was observed that the DHQ and Block FFQ were superior tools in assessing absolute intake. Correlation coefficients of energy intake were better for DHQ and Block FFQ than the Willett FFQ. The same was true for 26 other nutrients before adjustment for energy intake. However, on adjusting for energy intake the correlation

coefficients of all three tools improved and fairly similar performance was observed. Also, it was noted that it is important to account for energy adjustment when using Willett FFQ as this reduces the error in portion sizes estimates (44). Frequency of consumption is usually assessed in terms of days, weeks, months or years. To design an FFQ, a food list must be constructed. The food list consists of foods that are consumed commonly by the study population and contain the nutrients of interest. However, if the study sample is culturally diverse, it may be cumbersome to design a FFQ to incorporate a diverse diet (43).

Information from a questionnaire is converted into nutrient values using standard nutrient databases, wherein, the nutrient values for each food intake is calculated taking into consideration the portion sizes and frequency of consumption (43).

Recall of Remote Diet: In studying diseases like cancer with long latent periods, it is often necessary to assess exposures from the remote past, and it becomes necessary for subjects to recall past exposures including remote food patterns. This may pose several limitations, as it is observed that diet recalled from the past is commonly influenced by the current diet. This is of significant concern, particularly if there has been a recent change in diet. Also, assessing diet from the past may contain elements of recall bias, because subjects may not be able to accurately recall their past diet from remote memory. However, it has been reported that diet over the past 10 years is recalled with considerable accuracy (43, 45, 46). Differential reporting between cases and controls is another potential problem when recalling dietary habits after the onset of the disease. If a particular exposure has been previously reported or is suspected to be associated with

disease, then there is a potential that cases may tend to over or under report the exposure due to knowledge of its association with the disease. The precision with which diet is reported may also vary based on case status (43).

Aim and hypothesis

Aim

To determine the role of allium vegetables on breast cancer risk by assessment of allium vegetable intake through a food frequency questionnaire in a breast cancer case-control study in Polish immigrant population.

Hypothesis

Reduced consumption of allium vegetables and their constituents such as organosulfurs are associated with increased risk of breast cancer in Polish immigrants to the United States.

Anti-carcinogenic properties of Allium Vegetables

Proposed mechanisms for protective role of allium vegetables in breast cancer

The genus Allium belongs to the family Alliaceae and includes onion, garlic, leek and chives. In addition to essential elements like water, carbohydrates and proteins, allium vegetables contain non-essential micronutrient components called phytochemicals. One of the bioactive compounds is an organosulfur, alliine (S-allylysteine sulfoxide). This is converted to allicin by the enzymatic action of allinase on disruption of cell membrane which occurs when being chopped, cut or crushed (47). Allicin is an unstable compound and spontaneously forms mono, di and tri sulfides. These allyl sulfides are biologically active and exhibit anti-carcinogenic properties. Some of the proposed mechanisms of their actions as shown in in-vitro studies are inhibition of cell proliferation, bioactivation of cytochrome enzymes and induction of apoptosis (48-51).

In Vitro

a) Inhibition of Cell proliferation: Allyl sulfur compounds in garlic have been shown to inhibit cell proliferation by alteration in cell cycle progression. It has been observed that DiAllyl DiSulfide (DADS) arrested cells in the G2/M phase of the cell cycle by suppressing the activity of p34cdc2 kinase complex, which regulates progression of cells from G2 into M phase of the cell cycle (50).

b) Bioactivation of cytochrome P450 enzymes: Detoxification of carcinogens is a defense mechanism of the body against carcinogens. Diallyl sulfide, a constituent in garlic has

been shown to suppress carcinogen activating cytochrome P450 2E1. Diallyl sulfide has also been shown to induce phase -2 enzymes and detoxify carcinogens in animal models (51).

c) Apoptosis: Along with detoxification, apoptosis is another natural process in the body wherein old and deranged cells are periodically lysed. This is an important mechanism against cancer prevention. Allyl sulfides have been shown to induce apoptosis in cancer cell lines. Cultures of human colon tumor cells exposed to DADS showed morphological changes of apoptosis such as DNA fragmentation and indistinct cell membranes in comparison to controls (48).

In Vivo: The anti-carcinogenic properties of garlic have also been demonstrated in in-vivo studies, wherein they reduced DMBA (7, 12-DiMethylBenz (a) Anthracene)-DNA binding in mammary cells and reduced DMBA induced mammary tumor formation (49) .

Literature review of epidemiologic studies assessing effect of allium vegetables on cancer

The allium vegetable group consisting of leeks, onions, garlic and chives has several medicinal attributes including anti-carcinogenic properties. A meta-analysis of studies evaluating the role of allium on gastro-intestinal system showed that a high intake of raw and cooked garlic was protective against stomach and colorectal caners (52). The likely protection of allium vegetables and their constituents in prostate cancers was hypothesized following results from laboratory experiments, which showed that constituents of garlic alter the expression of prostate biomarkers such as Prostate Specific Antigen (PSA) and Prostate Specific Membrane Antigen (PSMA) (53). Similar to the effect of constituents of allium vegetables on prostate tissue, constituents from garlic demonstrate anti-carcinogenic properties against mammary cell lines. However a thorough literature review revealed that few epidemiological studies have assessed the role of allium intake on breast cancer risk and the results that are published are inconsistent. Among the earliest studies that evaluated the association between allium intake, in addition to other dietary factors on breast cancer, was a case-control study in Greece (1986). This study interviewed 120 histologically confirmed cases and 120 hospital controls from orthopedic and trauma units. Interviews were conducted by a dietician and a physician and dietary history preceding the disease was collected using a FFQ. This study observed a non-significant inverse (protective) trend after adjusting for age, years of schooling and interviewer, when the highest quintile of consumption of

onion was compared to the lowest (square root of chi-square, z-value=-1.14, non-significant) (54).

Levi et al (1993) reported a case-control study conducted in Switzerland as the pilot phase of a larger study. They studied 107 histologically confirmed breast cancer cases and 318 hospital based controls, but excluded controls admitted for breast, gynecological, hormonal, metabolic or neoplastic conditions. After adjusting for factors such as age, education status and total energy intake, a significant inverse association was observed between onion intake and breast cancer with an odds ratio of 0.4, $p<0.01$, for highest versus lowest quartile of intake. However, the multivariate analysis did not adjust for other potential confounders such as menstrual and reproductive risk factors. Also, since this study enrolled hospital controls, generalization of these results is limited (55).

Challier et al (1998) evaluated the role of diet on breast cancer in a case-control study conducted in northeast France. In this study dietary information from the past—before diagnosis—was collected using a recalled 6 day diary and a FFQ. The study had 345 histologically confirmed cases and an equal number of controls from a preventive medicine centre. Cases and controls had similar age and socio-economic status, as reported by the authors. A statistically significant inverse association was observed between breast cancer risk and total garlic and onion consumption after adjusting for potential confounders like total calorie intake, parity, weight, and corporeal surface. The observed decrease in risk relative to those who consumed ≤ 6 servings/week, were:

(OR=0.52, 95%CI=0.34-0.78, OR=0.25, 95%CI=0.11-0.55, OR=0.4, 95%CI=0.25-0.64, and OR=0.30, 95%CI=0.17-0.52) for consumption of (7-10, 11-12, 13-16, and >16 servings/week, respectively) (56).

The foremost difficulty in evaluating the role of diet on chronic diseases like cancer in prospective studies is the long latent period and hence the considerably long follow-up. The only cohort study that evaluated the role of allium intake on breast cancer was the Netherlands cohort. The Netherlands cohort consisted of 120,852 people, with 62,573 women aged 55 to 69 years. At the end of 3.3 years of follow-up, there were 469 incident cases of female breast cancer. This study included newly developed cases of invasive breast carcinoma and excluded subjects with any prevalent cancers other than skin cancers. They also excluded cases of in-situ tumors and breast cancers other than carcinomas such as sarcomas, lymphomas, or unspecified types. Dietary history in the year preceding the study was assessed by a 150-item semi-quantitative questionnaire. Of the allium vegetables, only consumption of onions and leeks, but not fresh garlic, was included in the questionnaire. However, they did collect information on garlic supplements. Garlic supplement users were defined as those stating daily use of garlic supplement for at least one year in the five year period prior to the study. These researchers reported a follow-up of ninety-five percent. Analysis of the data from the 469 incident cases and a randomly selected sub-cohort of 1812 controls did not show a statistically significant association between onion and leek consumption and breast cancer incidence. Multivariate analysis was conducted after adjusting for potential confounders such as age, parity, menstrual and reproductive history, use of oral

contraceptive pills, benign breast disease, family history of breast cancer, body size as measured by Quetelet index, alcohol consumption, smoking, educational status and intake of vitamin C and b-carotene. The association of garlic supplements and breast cancer was analyzed in two subgroups. In the first, exclusive garlic supplement users were compared with non-users of supplements. The second sub-group compared those who used garlic supplements along with other supplements with those who used other supplements but not garlic. Consumption of garlic supplements did not show a statistically significant inverse association with breast cancer risk in either subgroup of garlic supplement users. Since this study assessed garlic supplement consumption during the past 5 years, and onion and leek consumption during the year preceding the study, it is likely that the etiologically relevant window for carcinogenesis was not captured since the average follow-up period was only 3.3 years. To account for the possibility that some of the observed cases could have had pre-clinical breast cancer at the time of enrollment in the cohort, the investigators re-analyzed the data after excluding cases diagnosed in the first year of the study but the findings did not change (57).

Methods

Study Design

This was a population based case-control study.

Dataset

We analyzed data from the 'Breast Cancer In Women Of Polish Ancestry' study. This is a population based breast cancer case-control study designed to evaluate the effects of diet and other the lifestyle factors on breast cancer risk in Polish immigrants to the United States and Polish natives. Dr. Dorothy R. Pathak was the Principal Investigator (PI) of the study and it was funded in 1997 by National Institute of Health/ National Cancer Institute (NIH/NCI). The study started in 2000 concurrently in Poland and the United States. Data collection sites were Gliwice, Katowice, Poznan and Bialystok in Poland and Detroit Metropolitan area in Michigan and Cook County in Illinois. This thesis analyzed data from the US component of this study i.e. Polish immigrants to the US.

Study Population

This study was conducted among Polish immigrants residing in Illinois and Detroit. Histologically confirmed cases of invasive breast cancer between January 1st 1994 and December 31st 2001 in the age group 20 -79 years were identified from the Illinois State Cancer Registry (ISCR), Illinois and the Karmanos Cancer Institute, Michigan. Once identified, permission was sought from the physicians in order to contact their patients. Cases with unknown place of birth were initially screened for race (White) and

subsequently for their place of birth through phone and only Polish-born subjects were eligible for the study. Population based controls were selected and matched on area of residence and frequency matched within a 5-year age group. Controls under the age of 65 years were selected using Random Digit Dialing (RDD). Census data (1990) for individual census tracts within Cook County, Illinois (Chicago) and Wayne, Oakland, and Macomb counties, Michigan (Detroit area) were used to calculate the percentage of the population born in Poland residing in each census tract and then the tracts were sorted in descending order based on these percentages. Tracts were stratified into low, medium and high density of Polish-born and controls were selected in proportion to the Polish-born population residing in the three density areas.

Controls in the age group between 65-79 years were selected from the Medicare records of the Health Care Financing Administration (HCFA). Similar to cases, controls were screened for their place of birth by telephone interview and were eligible to participate in the study only if Polish-born, and not previously diagnosed with breast cancer or any other cancer except squamous and basal cell cancer. An introductory letter describing the purpose of the study was sent to all eligible cases and controls. In situations where telephone numbers were not available, letters were mailed to screen for place of birth. Willingness to participate was also asked in the same introductory letter. By subsequent phone calls, home visits were arranged. For those identified by HCFA, telephone numbers were not available; therefore, residence addresses and zip code information were used to identify phone numbers. If telephone numbers where not obtainable then local addresses were traced and home visits were initiated.

Data collection

In order to capture the traditional Polish diet, information on diet was collected retrospectively from all subjects during 1985-1989, the last time period prior to introduction of market economy in Poland. Dietary intake was assessed by a food frequency questionnaire and a risk factor questionnaire was used to evaluate lifestyle factors.

Food Frequency Questionnaire

To evaluate the role of diet on caner it is desirable to assess relative intake over a considerably long period of time rather than an absolute intake on specific days. Also, given that incidence of breast cancer in Poland is one third that in US (58), we were interested in capturing the traditional Polish dietary and lifestyle patterns prior to the introduction of market economy in Poland in the 1990's. Hence we retrospectively assessed dietary intake during 1985-1989 for all subjects using a food frequency questionnaire. We used a carefully designed 142 item FFQ that incorporated foods common to both Polish and American diets. Frequency of consumption was assessed as an open ended question in times per days, week, month, or year. The FFQ was administered by trained interviewers at in-person home visits. On completion of the FFQ, the site nutritionist reviewed for completeness and for clarification of out of range responses. To calculate total caloric intake as well as specific nutrient intakes, nutrient data base that was developed for the 1986 Nurse's Health Study (Willett's data base) was used. Nutrient information for foods that were specific to the Polish diet and were not

included in the Willett's data base was obtained from the Polish nutrient database, DIET 1.

Risk factor questionnaire

Information on other established and potential risk factors such as age, age at first full term pregnancy, parity, age at menarche, age at menopause, use of oral contraceptive pills, hormonal replacement therapy, family history of breast cancer, height, weight, and physical activity were also collected for the same time period. Each participant provided information on date of migration to the US allowing for calculation of duration of stay in the US.

Description of variables

Dependent variable

The dependent variable, breast cancer was analyzed as a binary variable (case/control). Age at diagnosis for the cases was obtained from the Cancer Registries, and also confirmed during interview by questions regarding breast surgeries, like biopsy, lumpectomy or mastectomy. The distribution of age at diagnosis for the Polish-born cases was obtained from the Illinois State Cancer Registry for the 5 year period 1990-1995. We assumed a similar age distribution for cases from the Detroit Metropolitan Area. Controls were selected to be age matched to the distribution of age at diagnosis of the cases.

Independent variables

Main exposure of interest

1) Allium vegetable consumption:

The main exposure of interest was intake of allium vegetables, garlic intake and consumption of onions only. Information on dietary intake of allium vegetables during 1985 to 1989 was collected from subjects using the food frequency questionnaire. The questionnaire included questions on intake of yellow, white, red and green onions either raw or cooked. In addition questions on consumption of chives and leeks were also included. Consumption was assessed in terms of daily, weekly, monthly or yearly frequencies. All consumption frequencies were converted into weekly consumption by an appropriate conversion factor. For total allium vegetable intake, tertiles were created based on the distribution of this intake among the controls. The tertile cut points were: < 5 servings per week, 5 to less than 10 servings per week and equal to and greater than 10 servings per week. Similarly, the tertile cut points for garlic consumption were: less than 1 serving per week, 1 to 3 servings per week and > 3 servings per week. Intake of onions (raw yellow, white, red and green onions) was also categorized into 3 categories with the following cut-points: equal to or less than 2, > 2-5 and greater than 5 servings per week.

Covariates

Age at menarche: Age at menarche was assessed by the onset of natural menstruation. Median age at menarche was 14 years. Menarche was divided into 4 categories as follows: less than 12 years, 12 to less than 13, 13 to less than 15 and greater than 15

years. For the 4 women who reported their age at menarche as unknown, the median of 14 years was assigned.

Menopausal status and age at menopause:

Subjects who reported having menstrual cycles were considered as pre-menopausal. Women who had hysterectomy without removal of ovaries, were considered pre-menopausal if their age was less than 50, and postmenopausal if they were 50 years or older and their age at menopause was assigned to be 50. Post-menopausal women who reported that they stopped menstruating, were over 50 years of age, and did not report their age at menopause were assigned 50 years. If menopause was surgically induced with both ovaries removed, age at surgery was considered as age at menopause. In our study, 250 women were post-menopausal and 193 were pre-menopausal. Mean age at menopause of our study subjects with known age at menopause was 50.7 years. For analyses, age at menopause was categorized into 5 categories as follows: pre-menopausal were categorized in one category, post-menopausal were categorized into less than 50, equal to 50 to less than 52, 52 to less than and equal to 54 and greater than 54 years.

Age first full term pregnancy: Full term pregnancy was defined as any pregnancy with gestational age more than 24 weeks or 6 months, irrespective of the outcome. Age at first full term pregnancy was divided into 4 categories: nulliparous, less than 22, equal to 22 to less than 30 and equal to and greater than 30 years.

Parity: Parity was assessed by the total number of full term pregnancies. It was analyzed as a continuous variable including those who were nulliparous being assigned parity=0.

Lactation: Total duration of breast feeding was calculated as the sum of duration of breast feeding for each child. Total duration of lactation was divided into 4 categories: those

who were nulliparous and did not breast feed, parous but did not breast feed, breast fed for less than or equal to 12 months and more than 12 months.

Family history: History of breast cancer in the mother, sister or daughter was considered as positive first degree family history. This was analyzed as a binary variable.

Alcohol: Alcohol consumption was assessed by total intake of beer, wine and hard liquor during 1985 -1989. Among those who consumed alcohol, the median consumption was 0.5 drinks per week, which is equivalent approximately to 6 grams of alcohol per week (1 drink is approximately equivalent to 12 grams of alcohol) (39). This variable was divided into 3 categories: non drinkers, drinkers with less than 0.5 drinks per week and equal to and more than 0.5 drinks per week.

BMI (1985-1989): Body Mass Index (BMI) was calculated from height and weight in 1985 -1989 using the following formula - weight (kg)/ $[height]^2$ (m). It was divided into 4 categories under-weight (<18.5), normal (18.5-<25), overweight (≥25-≤30) and obese (>30). If height was available, but weight in 1985-1989 was missing, weight at age of 18, 30, 40, 50 or 60, based on subject's age range in 1985 was used for BMI calculation. Thus, if age in 1985-1989 was less then 25 years, then weight at 18 was used for calculations, similarly if age in 1895-89 was between 25-<35, 35-<45, 45-<55, 55-70 then weight at ages 30, 40, 50 and 60 were used respectively. If weight for the closest decade as described above was missing (13 participants), mean weight specific to case/control status, for a particular age range in 1985 was used for BMI calculation for these individuals. If height was missing, the BMI remained as missing. If the individual's age in 1985 was lower than the age at which maximum height was attained,

their 1985 BMI was also assigned to a missing category. Totally, information on BMI in 1985-1989 was missing for 13 participants.

Duration of stay in the US by 1985:

Each participant provided information on date of migration to the US allowing for calculation of duration of stay in the US. Duration of stay in the US was divided into three categories as follows: those who migrated after 1985, those who migrated before 1985 but the total duration since migration was less than 10 years, and those with 10 or more years since migration prior to 1985.

Hormone replacement therapy: All subjects were asked if they ever used hormone replacement therapy in form of pills or skin patches, creams, suppositories or injectables for relief of menopausal symptoms and/or bone loss. The response was recorded as yes/no and analyzed as a binary variable.

Oral Contraceptive Use (OC): Use of hormonal preparations for birth control was asked for all subjects and recorded as ever used / no. This was analyzed as a binary variable. We did not differentiate between past and current users.

Calorie intake (1985-89): Total calorie intake was calculated for all food items assessed by the FFQ. Quartile cuts were: (0 - ≤1882), (>1882– ≤2257), (>2257– ≤2735), (>2735).

Physical activity: Physical activity during 1985-1989 was assessed using the questionnaire which included daily activities like sitting, reclining and household activities such as sweeping, gardening, cooking, stair climbing and sleeping. It also included recreational activities such as recreational sports, walking, bicycling, aerobic exercise as well as job activity. Intensity of activity was expressed in terms of MET's (Metabolic Equivalent), which were extracted from the Compendium of Physical

Activities (59).Total MET's utilized were divided into quartiles as follows: (0 - ≤47.33), (>47.33 - ≤54.41), (>54.41- ≤63.23), (>63.23). Information on physical activity was missing for 2 participants, as they were unable to recall their activity in 1985-1989.

Statistical Analyses

We used logistic regression to analyze the data, as the dependent variable was binary (case/control). The independent variables were both categorical and continuous. Because controls were matched to cases on their age at diagnosis, and we included in the study all Polish-born cases that were diagnosed from January 1, 1994 through December 31, 2001, controls in 1985 were younger, 39 versus 41 for cases. Since cases were frequency matched to controls on age within 5 year interval, as well as on area of residence, we used conditional logistic regression. To account for the age difference between cases and controls in 1985, we also included age in 1985 as our adjustment variable in all data analyses. Firstly, we did a univariate analyses to evaluate the association of each of the variables with breast cancer. The univariate analyses was stratified by age at diagnosis and area of residence and adjusted for age of the participants in 1985-1989. BMI was initially analyzed separately for pre and post menopausal women as it is well established that the effect of body weight is different based on menopausal status. Since the effect estimates did not differ by menopausal status, results are reported for the whole group. Multivariate analysis was done with the main exposure of interest (total allium vegetable intake, garlic intake, onion intake) and other variables as covariates. For multivariate analysis we started with a saturated model with all the covariates in the model. Even though some of the covariates such as age at menarche, age at menopause, duration of

lactation, family history of breast cancer in first degree relative did not reach statistical significance in the univariate analysis, we included them in the multivariate analysis as adjusting variables, in order to obtain estimates that would be comparable to those in existing literature where these factors did confound the relationship between exposure of interest and breast cancer (54-57). Effect modification was assessed by incorporating cross product terms of the covariates with the main exposure of interest (total allium intake, garlic intake only, onion intake only) in the model and were retained in the model if statistically significant (p-value less than or equal to 0.05). Covariates were assessed for confounding effect by evaluating if the point estimate of the main exposure of interest changed by 15% or more when the covariate was included in the model relative to the unadjusted OR estimate. Similar to univariate analysis, our multivariate analysis was stratified by age at diagnosis and area of residence and adjusted for age in 1985-1989. Separate models were run for pre and post-menopausal women. Additional analyses were conducted for individual allium vegetables such as garlic and onion only. Measures of association are reported in terms of hazard / odds ratio with 95% confidence intervals. P-values less than or equal to 0.05 were considered statistically significant. Statistical Analysis Software (SAS) version 9.1 was used for all analyses.

Results

We analyzed the data on 140 breast cancer cases and 303 age and area of residence matched controls (Table 1). We had 109 cases from Chicago and 31 from Detroit. Among the controls 229 were from Chicago and 74 were from Detroit. The mean age of cases and controls in 1985 was 41 and 39 years respectively. The distribution of cases and controls according to area of residence is shown in Table 2.

A) Results Univariate analysis

Univariate analysis of each of the variables adjusted for age in 1985-1989 and age at diagnosis and area of residence stratified is discussed below and presented in Tables 3-6.

Age at menarche and age at menopause: Age at menarche and age at menopause were not statistically associated with breast cancer risk. The odds ratio for menarche later than 15 years was 0.65 (95% CI=0.31-1.36) in comparison to those with age at menarche below 12 years. Later age at menopause (>54 years) was around unity (OR 1.04, 95% CI=0.41-2.61) relative to those with menopause at <50 years.

Reproductive risk factors: Subjects with later age at first full term pregnancy (≥30 years) were twice more likely to have breast cancer than those younger than 22 years at first full term pregnancy (OR 2.78, 95% CI=1.27-6.09). We did not observe the protective effect of parity on breast cancer in our analysis.

Lactation: Subjects who breast fed for more than 12 months were not at a statistically significant lower risk of breast cancer in comparison to those were parous but did not breast feed (OR 0.84, 95% CI=0.38-1.85).

Hormonal use: We did not observe a statistically significant association between use of HRT and risk of breast cancer (OR 1.10, 95% CI 0.56-2.15). However, women who had ever used OC were a higher risk of breast cancer compared to those who never used OC, though the increase in risk was not statistically significant (OR 1.53, 95% CI 0.81-2.91). We did not differentiate between past and current OC users.

Family history: Family history of breast cancer in the first degree relative was reported by 14% of cases and 8% of the controls. Subjects with a positive family history of breast cancer were twice likely to have breast cancer, though the results did not reach statistical significance (OR 1.72, 95% CI: 0.86-3.45).

Alcohol consumption: Although alcohol consumption (more than 0.5 drinks per week) relative to non-drinkers, showed increase in risk, (OR=1.55, 95%CI= 0.70-3.43), it did not reach statistical significance. The magnitude of increased risk is of the same order at what has been reported in the literature (39).

Total caloric intake: The association between breast cancer and total caloric intake showed decreased risk in contrast to what has been previously reported in the literature. However, the observed inverse association was not statistically significant. (OR 0.56, 95% CI = 0.30-1.06).

BMI (1985-89): We did not observe a statistically significant association between BMI (1985-89) over 30 in comparison to those with BMI in the normal range of 18.5- <25 (OR 0.77, 95% CI=0.33-1.80).

Physical activity: Consistent with the previous studies there was an inverse association between adult physical activity and breast cancer risk. The odds ratio for highest versus lowest quartile was 0.53 (0.28-0.99).

Duration of stay: Migration to the US at an early age and hence longer duration of stay in the US was not statistically associated with increased risk of breast cancer. Odds ratio for those who migrated before 1985 and in the US for 10 or more years was 1.14 (95% CI: 0.60-2.16) in comparison to those migrated after 1985.

Allium vegetable intake: Total allium vegetable intake was inversely associated with breast cancer risk in the univariate analysis. Odds ratio for highest versus lowest tertile (>10 serv/week vs. <5 serv/week) of allium vegetable intake was 0.54 (95% CI = 0.31-0.94). Similarly, garlic intake was also significantly associated with breast cancer risk in the univariate analysis (OR 0.37, 95% CI= 0.19-0.73). However, onion intake, though showing inverse relationship with breast cancer risk did not reach statistical significance (OR 0.58, 95% CI =0.33-1.03).

In summary, in the univariate analysis we observed that physical activity, intake of allium vegetables and garlic intake alone were significantly associated with breast cancer risk in this population.

B) Results of Multivariate analysis

In the multivariate analysis, on assessing for interaction of each of the covariates with the main exposure of interest, we did not find any significant interactions. Similarly, we did not observe any confounding effect by any covariates, that is, none of the covariates changed the odds ratio of the main exposure of interest by 15% or more. In order to obtain OR's that could be compared to existing literature, we chose to keep the well known risk factors and potential confounders in our multivariate analyses. The adjustment covariates include: age at menarche, age at menopause, age at first full term

pregnancy, parity, duration of breast feeding, family history of breast cancer, BMI, physical activity, alcohol intake, use of HRT, use of OC and duration of stay in the US. Accordingly, our final multivariate model was adjusted for all the covariates mentioned above. Results of the multivariate analysis are shown in Table 7.

The association between allium vegetable intake and breast cancer did not differ between pre and post menopausal women. Combining both pre- and post-menopausal women, an inverse association (OR 0.58, 95% CI=0.30-1.12) though not statistically was observed between total intake of allium vegetables and breast cancer in the multivariate analysis after adjusting for the potential confounders mentioned above. The estimated reduction in risk was 40% for those consuming more than 10 servings per week compared to those consuming less than 5 servings per week. The average individual servings of allium vegetables are: 1 clove of garlic or 3 grams, 2 tablespoons of raw chopped yellow or white onion, 20 grams, fried onions or 38 grams, 2 tablespoons. of green onions or 12 grams, ¼ cup of boiled chopped leeks or 26 grams (60). For total allium intake the frequency of consumption was evaluated rather than absolute amount of allium vegetables consumed.

On analyzing allium vegetables separately, we observed that garlic intake of more than 3 servings per week was associated with a statistically significant inverse association with risk of breast cancer (1 serving of garlic is approximately equal to 1 clove or 3.3 grams). Odds ratio for consuming more than 3 servings per week in comparison to less than 1 serving per week was 0.39 (95% CI= 0.18- 0.83). Evaluation of the effect separately for

pre and post-menopausal women indicated that the magnitude of effect for garlic was similar in these two populations, though the results did not reach statistical significance in each group due to reduced sample size. Intake of onion was also associated with a decreased risk of breast cancer, though not statistically significant (OR 0.63, CI=0.32-1.24).

Discussion

To our knowledge, this is the first case-control study evaluating the role of allium vegetable intake on breast cancer in Polish immigrants to the US. This population based case-control study observed an inverse trend between total allium vegetable intake during adulthood and breast cancer risk. We observed a protective effect of increased consumption of allium vegetables (more than 10 servings per week); it was not statistically significant on adjusting for well established breast cancer risk factors such as family history of breast cancer in first degree relatives, age at menarche, age at menopause, age at first full term pregnancy, parity, duration of breast feeding, BMI (1985-1989), physical activity, alcohol intake, use of HRT, OC use and duration of stay in the US. The small sample size of our study could have limited the power to detect a significant difference. Higher tertile of intake was associated with greater protection as compared to lower tertile of intake after adjusting for potential breast cancer risk factors. The association between allium vegetable intake and breast cancer risk was not different based on menopausal status.

Our findings are consistent with previous studies that evaluated the risk of allium vegetable intake on breast cancer. Two case-control studies, in Switzerland and in France observed a significant protective effect of allium vegetable intake such as onion and garlic and reported a 40-60% reduction in breast cancer risk when comparing lowest versus highest levels of consumption (55, 56) . However, a case-control study in Greece and the Netherlands cohort studies did not find a statistically significant association

between allium vegetable intake and breast cancer risk (54, 57). The inconsistent findings from these studies could be due to lack of standardization in the quantification of allium vegetable intake.

We also observed a statistically significant protective effect of increased garlic consumption on breast cancer risk. Similar findings have been reported in a case-control study in Switzerland, although the findings were not statistically significant (age adjusted odds ratio for lowest versus highest quartile of garlic intake was 0.6) (55). In addition, it has been demonstrated that a serving of garlic (1 clove, 3grams) has higher proportion of active constituents such as thiosulfinates than an average serving of onions(61) , approximately 20 grams. Hence, it is biologically plausible to observe significant anti-carcinogenic effects with garlic than equivalent amounts of onion and other allium vegetables. Also, the protective role of garlic in cancers of the gastrointestinal system have been fairly consistent (52).

Previous case-control studies that evaluated the relation between allium vegetable intake and breast cancer selected hospital based controls (54, 55). However, it is well known that the use of hospital controls is likely to bias the results as the reason for hospitalization and the associated illness would modify dietary and other breast cancer risk factors. To minimize the influence of hospitalization on diet and lifestyle habits, we selected population-based controls. Also, population-based controls provide a greater scope for generalization of our findings.

Our study population was selected from two locations–Chicago and Detroit, with different population characteristics and lifestyles. However, as we matched our cases and controls on area of residence, the possibility of confounding by unmeasured socio-economic characteristics on the observed effect was minimal in our study. Also, we controlled for potential breast cancer risk factors that could likely confound the observed association between allium vegetable intake and breast cancer. Nevertheless, it is still possible that the observed findings are due to chance or confounding by some unknown variable.

Previous studies that assessed the role of allium intake on breast cancer have evaluated the intake of either onions and garlic or onions and leeks (54-57).This study provides more comprehensive assessment of allium vegetables intake since it takes into consideration intake of onions, garlic, leeks and chives. Nonetheless, it is essential that one views the results of this study with caution since by calculating total consumption of allium vegetables as a sum of consumption of each of the allium vegetables, we are assuming that a serving of any one of the allium vegetables has an equivalent effect on reducing the risk of breast cancer. It has been observed that an average serving of different allium vegetables does not contain the same amount of anti-carcinogenic constituents such as thiosulfinates. Block E, et al, showed that per micromol/gram wet weight the amount of total thiosulfinates was almost 100 times as high in garlic as it was in red and yellow onions, chives and leeks (61). This implies that to obtain the same amount of protective compounds from the other allium vegetables as from garlic the serving size of the other allium vegetables would have to be 100 fold greater in terms of

weight. However, the gram weight of an average serving for the other allium vegetables was only between 7-13 times as high (garlic 3grams, onions 20grams, leeks 38grams) (60). Additionally, the estimates of the absolute amounts of these constituents will differ depending on growing conditions and methods of preparation which has not been addressed in the Block E at al. paper (61).

A major limitation of our study, just as of any case-control study is a potential for recall bias. As we intended to capture the traditional Polish diet, we collected details on dietary and lifestyle factors during 1985 to 1989, the last time period before the introduction of market economy in Poland. Hence, subjects had to recall exposures 10-15 years in the past; this could have introduced an element of recall bias. Moreover, it is possible that cases may under or over report specific exposures such as consumption of allium vegetables and this could potentially bias our results towards the null.

Ethical considerations

The study was reviewed and approved by the Institutional Review Board (IRB) at Michigan State University. The approval was renewed in 2007.

Conclusions

This study provides support for the protective effect of garlic intake during adulthood on breast cancer risk. In these analyses we assessed intake during adulthood only, however, intake during specific time periods such as during adolescence needs to be considered. Moreover, effects of preparation techniques on the bioavailability of constituents of allium vegetables need to be addressed in future studies.

Tables

Table1: Frequency of cases and controls

Case status	Frequency
Cases	140
Controls	303
Total	**443**

Table2: Frequency distribution of cases and controls by area of residence

Area of residence	Controls	Cases	Total
Chicago	229	109	338
Detroit	74	31	105

Table 3: Univariate analysis of physical activity, caloric intake and duration of stay in the US

Physical activity, Caloric intake and duration of stay in US				
Variable	Categories	Controls N=(303)	Cases N=(140)	Odds ratio [a] (95% CI)
Physical activity	0- ≤47 mets	26	32	Reference
	>47- ≤54 mets	27	26	0.74 (0.40-1.35)
	>54- ≤63 mets	25	24	0.76 (0.41-1.42)
	>63 mets	23	19	0.53 (0.28-0.99)
Caloric intake	0- ≤1882 calories	25	35	Reference
	>1882- ≤2257calories	23	19	0.58 (0.31-1.06)
	>2257- ≤2735calories	24	27	0.82 (0.46-1.47)
	>2735 calories	28	19	0.56 (0.30-1.06)
Duration of stay in US	After 1985	47	44	Reference
	<10 years	24	24	0.88 (0.49-1.56)
	≥10 years	29	32	1.14 (0.60-2.16)

[a] Adjusted for age in 1985-1989 and stratified by age at diagnosis and area of residence.

Table 4: Univariate analysis of menstrual, reproductive factors and hormonal use

Menstrual and reproductive factors and hormonal use				
variable	Categories	Controls N=(303) %	Cases N=(140) %	Odds ratio [a] (95% CI)
Age at menarche	0- ≤12	20	24	Reference
	12- ≤13	22	22	0.89 (0.46-1.71)
	13- ≤15	39	40	0.92 (0.51-1.63)
	>15	18	14	0.65 (0.31-1.36)
Age at menopause	Pre-menopausal	42	46	2.21 (0.93- 5.25)
	0-<50	16	15	Reference
	50-<52	17	16	0.94 (0.43-2.05)
	52-≤54	15	14	0.82 (0.36-1.86)
	>54	10	9	1.04 (0.41-2.61)
Age at first full term pregnancy	Nulliparous	12	11	1.76 (0.78-3.96)
	0-<22	28	21	Reference
	22-<30	52	51	1.17 (0.68-2.02)
	≥30	8	17	2.78 (1.27-6.09)
Duration of breast feeding	Nulliparous	12	12	2.16 (0.87-5.35)
	0 months	15	12	Reference
	0-≤12 months	45	61	2.13 (1.09-4.15)
	> 12 months	28	15	0.84 (0.38-1.85)
Use of Hormonal therapy	Yes	11	14	1.10 (0.56-2.15)
Use of oral contraceptive pills	Yes	14	16	1.53 (0.81-2.91)
Family history in first degree relative	Positive	8	14	1.72 (0.86-3.45)

[a] Adjusted for age in 1985-1989 and stratified by age at diagnosis and area of residence.

Table 5: Univariate analysis of Body mass index

Body mass index, duration of stay in US and hormonal use				
Variable	Categories	Controls N=(303) %	Cases N=(140) %	Odds ratio [a] (95% CI)
Body Mass Index (1985-1989)	<18.5	6	4	0.65 (0.21-1.97)
	≥18.5-<25	57	65	Reference
	≥25-30	24	24	0.75 (0.43-1.30)
	≥30	8	7	0.77 (0.33-1.80)
	Missing	4	0	0

[a] Adjusted for age in 1985-1989 and stratified by age at diagnosis and area of residence.

Table 6: Univariate analysis of alcohol consumption, total intake of allium vegetables,, intake of garlic only and onions only.

Alcohol consumption, total allium vegetable, intake of onion, garlic intake				
Variable	Categories	Controls N=(303) %	Cases N=(140) %	Odds ratio [a] (95% CI)
Alcohol consumption	Non-drinkers	19	8	Reference
	<0.5 drink per week	42	48	1.70 (0.78-3.72)
	≥0.5 drink per week	39	43	1.55 (0.70-3.43)
Total allium vegetable intake	< 5 ser/week	32	39	Reference
	5-10 ser/ week	30	37	0.97 (0.57-1.63)
	≥10 ser/ week	38	24	0.54 (0.31-0.94)
Intake of garlic	<1 ser/week	42	48	Reference
	1- <3 ser/week	35	40	0.96 (0.59-1.55)
	>3 ser/week	23	12	0.37 (0.19-0.73)
Intake of onion	≤2 ser/week	38	42	Reference
	2-5 ser/week	30	37	0.95 (0.56-1.59)
	> 5 ser/ week	32	21	0.58 (0.33-1.03)

[a] Adjusted for age in 1985-1989 and stratified by age at diagnosis and area of residence.

Table 7: Multivariate analysis of total intake of allium vegetables, intake of garlic only and onions only

VARAIBLE	Categories	Controls N=(303) %	Cases N=(140) %	Adjusted odds ratio [b] (95% CI)
Total allium intake	< 5 ser/week	32	39	Reference
	5- <10 ser/ week	30	37	0.97 (0.54-1.73)
	≥10 ser/ week	38	24	0.58 (0.30-1.12)
Garlic	<1 ser/week	42	48	Reference
	1- 3 ser/week	35	40	0.91 (0.53-1.55)
	>3 ser/week	23	12	0.39 (0.18-0.83)
Onion intake	≤2 ser/week	38	42	Reference
	>2- ≤5 ser/week	30	37	0.96 (0.53-1.71)
	> 5 ser/ week	32	21	0.63 (0.32-1.24)

[b] Adjusted for age at menarche, age at menopause, age at first full term pregnancy, parity, number of full term pregnancies, duration of breast feeding, use of contraception, use of hormonal replacement therapy, alcohol consumption, total calorie intake, physical activity, BMI in 1985-1989, duration of stay in US.

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