Short Communication

Population-based, Case-control Study of Blood C-Peptide Level and Breast Cancer Risk[1]

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Abstract

Insulin resistance has been suggested to be associated with an increased risk of breast cancer. Insulin sensitivity can be measured using blood C-peptide, a marker of insulin secretion. It is thus conceivable that blood C-peptide levels may be associated with breast cancer risk. To evaluate this hypothesis, we analyzed data from a subset (143 case-control pairs matched by age and status of menopause) of women who participated in the Shanghai Breast Cancer Study, a population-based, case-control study conducted in Shanghai during 1996–1998. Fasting blood samples were collected from study subjects to measure C-peptide levels. For cancer patients, the samples were collected before any cancer therapy. Conditional logistic regression was used to estimate adjusted odds ratios and 95% confidence intervals related to C-peptide levels. Breast cancer risk was increased with increasing levels of C-peptide (trend test, $P = 0.01$), with an odds ratio of 2.7 (95% confidence interval = 1.2–5.9) observed for the highest compared with the lowest tertile of C-peptide concentration after adjusting for body mass index and age at the first live birth. The risk was not altered after fully adjusting for other traditional risk factors for breast cancer. This positive association was observed in both pre and postmenopausal women and regardless of the levels of waist-to-hip ratio or body mass index. The results from this study were consistent with the insulin-resistance hypothesis for breast cancer and suggest that increased levels of C-peptide may contribute to the development of breast cancer.

Introduction

The incidence of breast cancer among women in urban Shanghai has been increasing at an alarming rate recently. The incidence rates were 18.3/100,000 in 1972–1974 and 27.5 in 1993–1994, respectively, a 50.5% increase over the last 2 decades (1). Accompanying the rapid economic development in Shanghai during this period, there are substantial changes in dietary and other life-style exposures. The consumption of animal foods has been increased rapidly (2), and, in the mean time, physical activity levels declined (3), resulting in a significant increase in the prevalence of obesity (4) and type 2 diabetes mellitus (5). It has been suggested that such changes may have contributed to the recent increase in breast cancer incidence (1).

Although some studies have shown that factors related to insulin resistance and/or type 2 diabetes, such as obesity (6–8), overeating (9), and sedentary lifestyle (10, 11), are associated with breast cancer risk (12), only a limited number of epidemiological studies have directly investigated the association of blood insulin and C-peptide with breast cancer risk (13, 14), and results from previous studies have been inconsistent (15, 16). C-peptide is a marker of pancreatic insulin secretion, and this marker may reflect more accurately an individual's level of insulin secretion because of its longer half-life than insulin (17). To evaluate the relationship between C-peptide levels and breast cancer risk, we analyzed data from a subset of participants in the Shanghai Breast Cancer Study.

Materials and Methods

The Shanghai Breast Cancer Study is a population-based, case-control study conducted among Chinese women in Shanghai during 1996–1998. This study was designed to recruit all women newly diagnosed with breast cancer between the ages of 25 and 64 during the period from August 1996 to March 1998, as well as a representative random sample of controls from the general population. All cases and controls were permanent residents of urban Shanghai who had no prior history of cancer and were alive at the time of the interview. Through a rapid case-ascertainment system, supplemented by the population-based Shanghai Tumor Registry, 1602 eligible breast cancer cases were identified during the study period, and in-person interviews were completed among 1459 (91.1%) of them. The major reasons for nonparticipation were refusal (109 cases, 6.8%), death before the interview (17 cases, 1.1%), and inability to locate (17 cases, 1.1%). Cancer diagnoses for all patients were confirmed by two senior study pathologists through the review of tumor slides.

Controls were randomly selected from the female general population, and frequency was matched to cases by age (5-year intervals). The number of controls in each age-specific stratum was determined in advance according to the age distribution of the incident breast cancer cases reported to the Shanghai Cancer Registry from 1990 to 1993. The Shanghai Resident Registry, which keeps registry cards for all permanent residents in urban Shanghai, was used to randomly select controls. For each age-predetermined control, a registry card identifying a potential control of the same 5-year age group was randomly selected. Only the women who lived at the registered address

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Table 1 Comparison of case- and matched-control subjects by demographic factors, major risk factors for breast cancer, and Serum C-peptide levels, Shanghai Breast Cancer Study, 1996–1998

	Cases (n = 143)	Controls (n = 143)	OR (95% CI)
Demographic factors			
Age (25^{th}, 75^{th} percentile)	54 (46, 59)	55 (46, 60)	
Education of high school or more (%)	43.4	38.5	1.3 (0.8–2.0)
Household income ≥15,000 (Yuan, %)	69.2	71.3	0.9 (0.5–1.5)
Major risk factors			
Family history of breast cancer in mother/sister (%)	3.5	2.1	1.7 (0.4–7.0)
Ever diagnosed with breast fibroadenoma (%)	7.0	2.8	3.0 (0.8–1.1)
No regular leisure physical activity in the last 10 years (%)	69.2	61.5	1.4 (0.9–2.3)
Current WHR ≥0.80 (%)	60.1	51.1	1.6 (0.9–2.7)
Current BMI ≥23 (%)	60.8	48.3	1.7 (1.0–2.7)
Age at menarche ≤13 yr (%)	24.5	18.9	1.4 (0.8–2.6)
Age at menopause ≥50 yr (%)[a]	48.0	40.2	1.5 (0.8–2.8)
Age at first live birth ≥30 yr (%)[b]	19.6	10.5	2.8 (1.1–4.5)
C-peptide levels (ng/ml)			
Mean ± SD	1.40 ± 1.33	1.11 ± 0.88	P = 0.01[c]
Median (25^{th}, 75^{th} percentile)	1.06 (0.76, 1.49)	0.88 (0.59, 1.20)	P = 0.03[d]

[a] Among postmenopausal women.
[b] Among parous women.
[c] P from paired t test, using log-transformed data.
[d] P from Wilcoxon signed rank test.

during the study period were considered to be eligible for the study. In-person interviews were completed among 1556 (90.3%) of the 1724 eligible controls identified. Reasons for nonparticipation included refusal (166 controls, 9.6%) and death before interviews (two controls, 0.1%).

A structured questionnaire was used to elicit detailed information on demographic factors, dietary habit in the last 5 years, regularly leisure physical activity in the last 10 years, tobacco and alcohol use, menstrual and reproductive history, hormone use, prior disease history, weight at different decadal ages, and family history of cancer. All participants were also measured for current weights, circumferences of the waist and hips, and sitting and standing heights. Fasting blood samples, 10 ml from each woman, were collected in the morning using EDTA or heparin vacutainer tubes from 1193 (82%) cases and 1310 (84%) controls after finishing the interviews and anthropometrics. To minimize the potential influence of breast cancer and its sequelae on the levels of biomarkers in the blood samples, specimens from breast cancer cases were collected as soon as possible after the initial cancer diagnosis. As a result, blood samples were collected before any cancer therapy from ~50% of cases. Immediately after collection, the samples were placed in portable insulated cases with ice pads (0°C–4°C) and transported to the central laboratory for processing. All samples were aliquoted and stored at −70°C within 6 h after collection.

To increase the comparability between cases and controls for molecular epidemiological studies, an individually matched case-control study was built into the Shanghai Breast Cancer Study. This sub-study included cases whose blood samples were collected before any cancer treatment (1 day on average before surgery and subsequently pathological confirmation), and for each case, a control was selected from the pool of controls included in the main study and individually matched to the index cases by age (±3 years), menopausal status (yes, no), and the date of sample collection (±30 days). To study the relationship between blood C-peptide levels and breast cancer risk, 150 cases and their individually matched controls were selected sequentially from the subjects included in this sub-study according to the time of study participation, and their serum samples were assayed for C-peptide. To eliminate the effect of between-assay variability on the study results, samples for each case-control pair were assayed in the same batch.

Serum C-peptide was measured using an enzymatically amplified one-step sandwich-type ELISA assay kit from Diagnostic System Laboratory, Webster, TX, and the ELISA assay was performed in the Division of Genetics, University of South Carolina School of Medicine according to the manufacture's instruction. An automated robotic system (BRIO; Diagnostic System Laboratory) was used for pipetting samples and reagents, for plate washing between steps, and for orbital shaking. Sample aliquots of 20 μl were pipetted into microtiter wells coated with anti-C-peptide antibodies and incubated with 200 μl of a buffered solution of anti-C-peptide antibody conjugated to horseradish peroxidase. Plates were orbitally shaken at 500–700 rpm at ambient temperature for 60 min and then washed. The tetramethylbenzidine chromogen solution (100 μl) was added to each well and incubated for 10 min with orbital shaking at ambient temperature. Enzyme reactions were stopped by the addition of 100 μl of a 0.2 N sulfuric acid solution. Optical absorbance was measured at 450 nm. A set of six standards across the range from 0 to 15 ng/ml of the analyte and two controls were run on each assay plate for assay calibration and quality control. Assay runs were rejected if coefficients of variation exceeded 10% for any standards or controls or if control values fell outside the limits established by the reagent manufacturer. Individual samples with coefficients of variation >10% were repeated in a separate assay run. Data were analyzed using a spline curve derived from the optical densities of the kit standards for each individual run.

The data analysis was restricted to 143 pairs of cases and controls, for which data were available for both case and control. Because the data were skewed, log-transformed data were used in the paired Student's t tests to compare the mean differences between cases and controls. The Wilcoxon signed rank tests were also used for comparisons of the median differences between cases and controls. ORs,[3] approximation of

[3] The abbreviations used are: OR, odds ratio; CI, confidence interval; BMI, body mass index; WHR, waist-to-hip ratio; IGF, insulin-like growth factor.

Table 2 Association of blood C-peptide concentration and breast cancer risk from the Shanghai Breast Cancer Study, 1996–1998

	Cases (n = 143)	Controls (n = 143)	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
All subjects (ng/ml)				
≤0.70	29	46	1.0 (referent)	1.0 (referent)
0.71–1.07	45	49	1.9 (0.9–4.0)	1.8 (0.8–3.8)
>1.07	69	48	2.9 (1.4–5.9)	2.7 (1.2–5.9)
Trend test			P = 0.004	P = 0.01
Premenopausal (ng/ml)				
≤0.70	9	17	1.0 (referent)	1.0 (referent)
0.71–1.07	16	14	2.6 (0.8–9.0)	2.9 (0.7–12.5)
>1.07	20	14	3.5 (1.0–12.1)	3.1 (0.7–14.2)
Trend test			P = 0.06	P = 0.24
Postmenopausal (ng/ml)[b]				
≤0.70	20	29	1.0 (referent)	1.0 (referent)
0.71–1.07	29	35	1.5 (0.6–4.0)	1.6 (0.6–4.3)
>1.07	49	34	2.5 (1.0–6.0)	2.9 (1.1–8.0)
Trend test			P = 0.02	P = 0.02

[a] All ORs are adjusted for BMI and age at the first live birth.
[b] ORs are also adjusted for age at menopause.

relative risk, were used to measure the association of breast cancer risk with serum C-peptide levels (18). Conditional logistic regression models were used to obtain maximum likelihood estimates of the ORs and their 95% CIs after adjusting for potential confounders, including age at menarche, age at menopause, and BMI as continuous variables. To evaluate a possible dose-response relation between C-peptide levels and beast cancer risk, cases and controls were categorized into three groups according to the tertile distribution of serum C-peptide concentrations among controls. ORs and 95% CIs for the top two tertiles were derived as compared with the lowest tertile group. Tests for trends across the tertiles were performed in logistic regressions by assigning the median value of each tertile to each of the corresponding tertile groups. All statistical analyses were based on two-tailed probability.

Results

The distributions of selected demographic characteristics and major risk factors for breast cancer are shown in Table 1. Cases and controls were comparable in age, with the median ages of 54 and 55 for cases and controls, respectively. All major risk factors reported previously (19, 20) were associated positively with the risk of breast cancer, although the ORs were statistically significant only for a high BMI and a late age at first live birth. All subsequent analyses included these two variables in the conditional logistic models to control for their potential confounding effects. The lack of statistically significant associations with other risk factors may be attributable to the small sample size of this study.

The median concentration of serum C-peptide was 1.06 ng/ml in cases and significantly higher than that in controls (0.88 ng/ml; Table 1). Of 143 cases, the information on clinic stage of cancer was available in 125 patients, with 89.6% being at an early stage. The median of C-peptide for cases at early stage (1.06 ng/ml) was similar to that for cases at late stage (0.98 ng/ml, P = 0.81) but statistically higher than that observed among their individually matched controls (0.88 ng/ml, $P < 0.001$). Serum C-peptide levels were weakly or not correlated to current BMI, WHR, and height, with Pearson correlation coefficients 0.29 ($P < 0.01$), 0.08 (0.32), and −0.06 (0.50) for controls and 0.15 (0.07), 0.19 (0.02), and −0.15 (0.08) for cases, respectively (data not shown in Table 1).

Table 2 presents ORs of breast cancer associated with serum C-peptide levels. Breast cancer risk increased with increasing levels of C-peptide (trend test, P = 0.01). The adjusted OR was 2.7 (95% CI = 1.2–5.9) for the highest *versus* the lowest tertile of C-peptide levels. The risk was not substantially altered after additional adjustment for other confounding factors, including age at menarche, regular leisure physical activities, a family history of breast cancer in mother/sister, and a history of fibroadenoma, with an OR of 2.6 (95% CI = 1.1–5.9) for the highest compared with lowest tertile of C-peptide. Findings were similar when analysis was restricted to those cases that were diagnosed at an early stage and their controls. The fully adjusted ORs across tertiles of C-peptide levels were 1.0, 1.6, and 3.5 (trend test, $P < 0.01$). Stratified analyses by menopausal status showed that this positive association existed in both pre and postmenopausal women, with 3-fold elevated risk observed for women in the highest *versus* lowest tertile of C-peptide levels. The test for interaction between C-peptide concentration and status of menopause was insignificant (P = 0.43).

Table 3 evaluates potential joint effect of C-peptide levels and BMI or WHR on the risk of breast cancer. A positive association with C-peptide was observed regardless of the level of WHR or BMI. Women who had a high level of blood C-peptide and a high WHR or BMI were at a particularly elevated risk of breast cancer.

Discussion

This is, to our knowledge, the first population-based, case-control study on the association of blood C-peptide with breast cancer risk, using fasting blood samples collected before any cancer therapy. Our study has shown that women who were at high C-peptide levels had an increased risk of breast cancer, and this association was independent of body weight and fat distribution, as well as menopausal status. These findings are supported by observations from two case-control studies reported previously (13, 14). Bruning *et al.* (13) reported from a case-control study in the Netherlands a dose-response relationship between blood C-peptide and breast cancer, with a nearly 3-fold elevated risk observed among women with the highest C-peptide levels, which was also independent of BMI and was observed in both pre and postmenopausal women. Blood sam-

Table 3 Joint effect of blood C-peptide concentration and BMI or WHR on the risk of breast cancer from the Shanghai Breast Cancer Study, 1996–1998

C-peptide levels (ng/ml)	WHR < 0.8 (median)		WHR ≥ 0.8 (median)	
	Case/control	OR (95% CI)[a]	Case/control	OR (95% CI)[a]
≤0.70	19/31	1.0 (referent)	10/15	1.3 (0.4–3.9)
0.71–1.07	17/22	1.6 (0.6–4.0)	28/27	2.7 (1.0–7.3)
>1.07	21/17	3.0 (1.1–8.5)	48/31	3.9 (1.6–9.8)
	BMI < 22.8 (median)		BMI ≥ 22.8 (median)	
	Case/control	OR (95% CI)[a]	Case/control	OR (95% CI)[a]
≤0.70	19/38	1.0 (referent)	10/8	3.0 (0.8–11.4)
0.71–1.07	18/22	2.3 (0.9–6.2)	27/27	2.6 (1.0–6.3)
>1.07	17/12	3.7 (1.3–10.1)	52/36	4.1 (1.7–9.8)

[a] All ORs are adjusted for age at the first live birth.

ples from the study, however, were collected after cancer treatment and nonfasting blood samples were used. A similar positive association was reported from a hospital-based, case-control study (14), in which postsurgery blood samples collected from premenopausal women were used in insulin assays. That study also used women with nonproliferative breast diseases as controls. Thus, selection bias may be a concern. In contrast, no evidence for a positive relationship between C-peptide and breast cancer was reported in a recent study (15), including only 45 breast cancer cases. Most of the cases in that study were diagnosed >5 years before C-peptide assays were performed. Thus, survival and prevalent bias may be a major concern. Very recently, Toniolo *et al.* (16) reported an insignificant positive association between blood C-peptide levels and breast cancer risk in a prospective study. The overall association between IGF-I and breast cancer risk reported in that study, however, was also weak and not statistically significant.

Cumulative data have implicated that insulin resistance probably plays an important role in the pathogenesis of breast cancer (6, 12, 21). Insulin can increase the bioavailability of IGF-I by increasing the synthesis of IGF-I and inhibiting the synthesis of IGF-binding protein 1 and 2. Insulin and IGF-I can synergistically stimulate mammary cell proliferation *in vitro* and *in vivo* (22). By decreasing the levels of sex hormone-binding globulin (12, 22), insulin also can lead to increased availability of free estradiol, the hormone that plays a major role in breast cancer etiology (23). Furthermore, several studies have confirmed that insulin stimulates the synthesis of both androgen and estrogen in ovarian tissue (21). Therefore, the link between insulin and breast cancer risk may be through the role of this molecule in regulating the level, bioavailability, and effect of both IGF-I and estrogen.

IGFs have been shown to be associated positively with the risk of breast cancer primarily among premenopausal women (16, 24). In our study, however, the risk associated with C-peptide was seen in both pre and postmenopausal women. It appears the association between C-peptide and breast cancer risk cannot be explained completely by IGF-I level. A similar positive association between C-peptide and breast cancer risk among pre and postmenopausal women was also reported in a previous case-control study conducted in the Netherlands (13). On the other hand, obesity has been found to be related to an increased risk of breast cancer among post but not premenopausal women (25, 26). It is believed that the positive association between obesity and breast cancer risk among postmenopausal women is primarily attributable to an elevated level of estrogens among overweight women. We found in this study that the blood C-peptide level was positively associated with the risk of breast cancer independent of body weight. This was somewhat unexpected, because obesity is related to hyperinsulinemia (6), and, thus, high level of blood C-peptide may be in the causal pathway between obesity and breast cancer risk. Most of the subjects included in this study, however, were nonobese.

Fasting blood samples are needed for measuring blood insulin or C-peptide, because insulin secretion is heavily influenced by recent meals. A major strength of this study is that all blood samples were collected in the morning after >8 h of fasting. The primary concern of the study is that postdiagnostic blood samples were used in the assays of C-peptide. The blood samples used in this study, however, were all collected before any cancer therapy. Thus, the observed case-control difference in blood C-peptide levels cannot be attributed to the influence of treatment. Because blood insulin levels are reduced with energy restriction (27) and the stress (and thus loss of appetite), a recent cancer diagnosis may result in reduced insulin secretion in some cases. Therefore, the difference in blood C-peptide level observed between cases and controls in this study may be somewhat underestimated, which may lead to conservative estimates of the association of C-peptide with breast cancer risk in this study. To minimize potential influence of recent dietary changes after cancer diagnosis on C-peptide levels, blood samples were obtained within days after breast cancer initial diagnosis, and, thus, possible lifestyle changes, if any, were likely to be limited. Selection bias attributable to refusals would be minimal in this study, because only a very small proportion of subjects (6.8% cases, 9.6% controls) refused to participate. Furthermore, the associations of traditional risk factors for breast cancer identified in the entire study (19) were similar to those identified in this subset, although some of these associations were not statistically significant because of the small sample size. This suggests that selection bias may not be a major concern in this study. We also performed analyses among cases who were diagnosed at an early stage and their controls, and a positive association between C-peptide and breast cancer risk was similar to that observed in all of the subjects combined. Moreover, the distribution of clinic stage of cancer was comparable between this subset and entire data of the Shanghai Breast Cancer Study, with the early stage being 89.6 and 89.3%, respectively.

In conclusion, we found that a high blood C-peptide level was associated with an increased risk of breast cancer, supporting the hypothesis that insulin resistance may play a role in breast cancer risk. The sample size of the study, however, was relatively small, and postdiagnostic blood samples were sued. Prospective studies with a larger sample size will be needed to additionally evaluate the association of blood C-peptide with breast cancer risk.

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