RESEARCH ARTICLE

Serum Adiponectin but not Leptin at Diagnosis as a Predictor of Breast Cancer Survival

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Abstract

Limited numbers of epidemiological studies have examined the relationship between adipokines and breast cancer survival. Preoperative serum levels of obesity-related adipokines (leptin and adiponectin) were here measured in 370 breast cancer patients, recruited from two hospitals in Korea. We examined the association between those adipokines and disease-free survival (DFS). The TNM stage, ER status and histological grade were aslo assessed in relation to breast cancer survival. Elevated adiponectin levels were associated with reduced DFS of breast cancer (P_{trend}=0.03) among patients with normal body weight, predominantly in postmenopausal women. There was no association of leptin with breast cancer survival. In conclusion, our study suggests that high levels of adiponectin at diagnosis are associated with breast cancer survival among women with normal body weight.

Keywords: Breast cancer - leptin and adiponectin level at diagnosis - prognosis - serum biomarker

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Introduction

While the incidence of breast cancer has increased over the past 30-40 years, the mortality rate has remained stable or has even decreased in the last 10-15 years, because of the result of earlier detection and improved treatment (Stuckey, 2011; Gadgil et al., 2012; Haghighat et al., 2012). Considering the substantial increase in breast cancer survival, the discovery of prognostic factors related to the breast cancer recurrence and survival is more important to the surveillance after primary treatment. Although commonly available prognostic factors include pathology criteria such as lymph-node status, tumor size, histologic grade and estrogen receptor (ER) status, these factors do not predict accurately exact clinical outcome probably due to heterogeneity of breast cancers.

A number of studies indicate that obesity is associated with increased risk of more aggressive breast cancer and cancer survival (Cleary and Maihle, 1997; Sweeney et al., 2004; Loi et al., 2005; Porter et al., 2006). Obesity plays a complex role in breast cancer through the increased inflammation, angiogenesis, and alterations in serum levels of potential growth regulators (McTiernan, 2005; Fontana et al., 2007). Several hypotheses have been proposed to explain the association between obesity and breast cancer including circulating estrogen level, the level of insulin and IGF, adipocyte action, etc (Maccio and Madeddu, 2011). Adipose cells were initially considered as a fat-storing tissue but are now known to have much more complex and dynamic functions, notably acting as an endocrine organ secreting a range of adipokines, including adiponectin and leptin (Miyoshi et al., 2003). In particular, adipocytes produce several "adipokines" and inflammatory cytokines which can influence aromatase activity and estrogen-dependent cell proliferation (Maccio and Madeddu, 2011). Leptin is a well-known factor which is involved in the regulation of body weight and body composition and is an important mediator of obesity.

Two obese-related adipokines (leptin and adiponectin) have been recently studied for their influence on the breast cancer risk and tumor biology (Vona-Davis and Rose, 2007). Leptin is secreted by normal or malignant breast tissue, and has been associated with increased aromatase activity leading to a functional cross-talk relationship with estrogen (Oh et al., 2011). On the other hand, the biological functions of adiponectin and the individual isoforms are currently the subject of intense investigation, with emerging roles in both protection from the consequences of insulin resistance, and the modulation of endothelial function. It was reported that lower serum adiponectin levels were found in women diagnosed with postmenopausal breast cancer compared to those without this disease (Tworoger et al., 2007; Gulcelik et al., 2012). Adiponectin is negatively correlated with body weight, BMI, and body fat (Miyoshi et al., 2003), but leptin correlates positively. Although their biological activities

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as their effects on breast neoplastic cells are largely in opposition to each other, the exact interplay between these different adipokines is yet nor well clarified. Recently, a great deal of attention has been given to the study of the epidemiologic association of adiponectin levels in blood with breast cancer incidence, as well as with the potent protective effects against inflammation, adverse lipid profiles, and atherosclerosis. Conversely, leptin has been implicated as a growth-promoting factor for cancer including *in vitro* proliferation of several human breast cancer cell lines (Dieudonne et al., 2002; Hu et al., 2002; Frankenberry et al., 2006; Ray et al., 2007).

We hypothesized that the serum levels of adiponectin and leptin at diagnosis might represent the aggressiveness of breast cancer and might be an independent predictor of breast cancer survival. In the present study, we examined the association between preoperative serum adiponectin or leptin levels and disease-free survival (DFS) and determined the potential of both markers as noninvasive biomarkers to predict the recurrence of breast cancer.

Materials and Methods

Study population

A total of 925 histologically confirmed incident breast cancer cases with peripheral blood were recruited in the Seoul National University Hospital and Asan Medical Center between 2004 and 2007 (Miyoshi et al., 2003). At the time of interview, before any adjuvant chemotherapy and/or surgery, peripheral blood were collected and processed for the DNA extraction and serum separation. Peripheral blood was collected into 10-ml serum storage tubes and was centrifuged at 3000g for 10 minutes at room temperature. Serum were stocked in 0.3-ml aliquots in cryovials and stored at -80°C until the time of measurement. After excluding the subjects with inadequate follow-up information, about 40 % (N=370) of subjects were randomly selected (Figure 1). As we mentioned our previous report (Sung et al., 2012), for the follow-up of patients, a retrospective chart review was used to collect clinical information and pathologic features including cancer stage based on 6th AJCC classification, tumor size, lymph-node invasion, distant organ metastasis, histologic grade, nuclear grade, estrogen receptor (ER) and progesterone receptor (PR) status, surgical treatment and medical adjuvant therapy (adjuvant chemotherapy, radiation therapy, and hormone receptor therapy). In addition, the death database from the Ministry of Security and Public Administration was used to collect the death information for recruited patients. The study design was approved by the Committee on Human Research of Seoul National University Hospital (IRB No. H-0503-144-004).

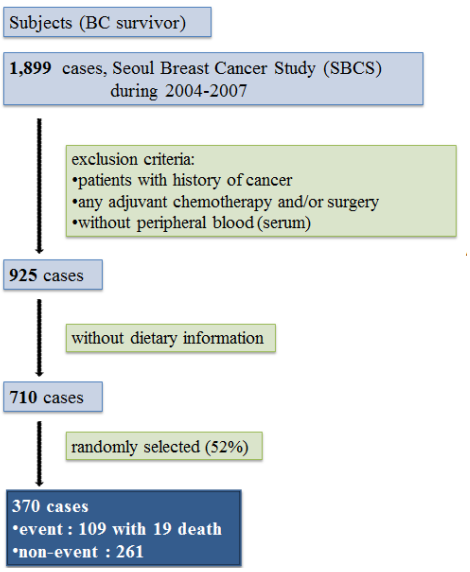

Figure 1. Flow Diagram of the Study Population Selection

Assays for serum adiponectin and leptin

The serum levels of proteins were determined using the Adiponectin Human ELISA kit (Mesdia (Adipomark), KOREA) and Leptin (human) EIA Kit (assay designs, USA) according to the manufacturer's instruction. Reproducibility within the assay was evaluated with an intra-assay coefficient of variation of 5.0% for adiponectin and 7.4% for leptin, respectively. The minimum detectable dose (MDD) was defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations (SD)). The mean MDD of adiponectin and leptin was 1.39ng/ml and 23.4pg/ml, respectively (mean of independent assays).

Statistical analyses

The distributions of adiponectin and leptin according to demographic factors and clinicopathological variables were compared by the use of the Mantel-Haenszel chi-square test (categorical variables) and the Pearson correlation coefficients test (continuous variables). Since the levels of adiponectin was normally distributed (the Kolmogorov–Smirnov test, $p<0.10$), but not leptin, which log square transformed values were used in correlation test and survival analysis.

DFS was defined as the time from date of surgery to the date of the first locoregional recurrence, first distant metastasis, second primary cancer or death from any cause. Patients known to be alive with no evidence of disease were censored at the last follow-up date (October 30, 2009).

Cox's proportional hazard regression models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI). The proportional hazard assumption of the Cox model was examined by graphic evaluation of Schoenfeld's residual plot. The multivariate model included age, TNM stage, ER/PR status, histologic grade (I-II and III), and nuclear grade (I-II and III). Additionally, stratified analyses were performed according to BMI group ($<25kg/m^2$ $p<\geq 25kg/m^2$) and menopausal status. All statistical procedures were conducted using SAS version 9.2 (SAS Institute, Cary, NC). All P values reported were two-sided.

Results

After a median follow-up of 4.2 years, there were 109 events including 19 deaths from any cause among all 370

patients. (Table 1) summarized the patients' characteristics and univariate and multivariate–adjusted HRs for DFS by patients' characteristics. Participants of this study had an average age of 46.6 years and the subject of DFS event had an average age of 46.2 years. Most patients were premenopausal (64.0%) and had a BMI less than 25 kg/m^2 (77.8%). In the multivariate analysis, the TNM stage remained as independent and significant prognostic factors for DFS (p<0.001), and ER status was shown in marginal association with DFS (HR=1.73, p=0.06). PR status, histological and nuclear grade, adjuvant chemotherapy, and hormone therapy was associated with DFS, but the association was disappeared the significance after adjustment for covariates.

In all patients, the median (25 and 75 percentile) of adiponectin and leptin were measured at 9.7ug/ml (6.9-13.2ug/ml) and 8.0ng/ml (6.6-9.3ng/ml), respectively. There was an inverse correlation between serum adiponectin and leptin level in the participants (r=-0.133; p=0.01). (Table 2) shows the distributions of patients' characteristics according to the tertile (low, middle, and high) of adiponectin and leptin. Women with higher adiponectin levels (P_{trend}=0.02) or with lower leptin levels (P_{trend}<0.01) were more likely to be heavier than women

Table 1. Hazard Ratios for Disease-Free Survival of 370 Breast Cancer Cases

	No. (%)	Event	HR	HR adj[1]	HR adj[2]
Age(mean±SD)	46.6±10.6	46.2±11.4			
≤ 39 yr old	100 (27.0)	34 (31.2)	Ref	Ref	Ref
40-49 yr old	135 (36.5)	32 (29.4)	0.67 (0.41-1.09)	0.89(0.52-1.50)	1.04(0.59-1.83)
≥ 50 yr old	135 (36.5)	43 (39.5)	0.86 (0.55-1.36)	1.01(0.61-1.69)	1.07(0.62-1.85)
Menopause status					
pre	235 (64.0)	66 (60.6)	Ref	Ref	Ref
post	132 (36.0)	43 (39.5)	1.19 (0.81-1.75)	1.08 (0.56-2.05)	1.22 (0.62-2.39
BMI(kg/m^2)					
< 25	288 (77.8)	80 (73.4)	Ref	Ref	Ref
≥ 25	82 (22.2)	29 (26.6)	1.14 (0.75-1.75)	1.07 (0.67-1.71)	1.03 (0.63-1.70)
TNM					
0 - I	160 (44.7)	28 (27.2)	Ref	Ref	Ref
II	130 (36.3)	38 (36.9)	1.43 (0.89 - 2.28)	1.30 (0.79-2.15)	1.24 (0.69-2.22)
III-IV	68 (19.0)	37 (35.9)	3.35 (2.09 - 5.38)	3.12 (1.89-5.13)	2.96 (1.67-5.26)
			P_{trend} <0.0001	P_{trend} <0.0001	P_{trend} <0.0001
ER					
Positive	221 (60.8)	47 (45.2)	Ref	Ref	Ref
Negative	142 (39.1)	57 (54.8)	2.33 (1.58-3.44)	1.58 (0.93-2.68)	1.73 (0.98-3.07)
PR					
Positive	205 (56.6)	44 (42.3)	Ref	Ref	Ref
Negative	157 (43.4)	60 (57.7)	2.10 (1.42-3.11)	1.39 (0.82-2.35)	1.43 (0.79-2.58)
Histologic grade					
I-II	178 (56.7)	37 (40.7)	Ref	Ref	Ref
III	136 (43.3)	54 (59.3)	2.07 (1.35-3.15)	1.06 (0.64-1.76)	1.25 (0.55-2.80)
Nuclear grade					
I-II	190 (57.1)	42 (43.3)	Ref	Ref	Ref
III	143 (42.9)	55 (6.7)	1.89 (1.26-2.83)	0.95 (0.58-1.55)	0.82 (0.36-1.83)
Adjuvant chemotherapy					
Yes	241 (67.9)	86 (83.5)	Ref	Ref	Ref
No	114 (32.1)	17 (16.5)	2.62 (1.54-4.47)	2.27 (1.24-4.17)	1.84 (0.94-3.59)
Radiotherapy					
Yes	215 (59.9)	57 (54.3)	Ref	Ref	Ref
No	144 (40.1)	48 (45.7)	0.73 (0.50-0.50)	0.78 (0.52-1.16)	0.81 (0.53-1.24)
Hormone chemotherapy					
Yes	236 (66.1)	49 (46.7)	Ref	Ref	Ref
No	121 (33.9)	56 (53.3)	0.36 (0.25-0.53)	0.43 (0.20-0.94)	0.57 (0.22-1.47)

*Event means the number of recurrence and death; Adj[1]: adjusted for age, bmi, TNM, ER and PR status; Adj[2]: adjusted for Adj[1] and histological and nuclear grade

Table 2. Patient Details in Relation to Adiponectin and Leptin Levels

	Adiponectin (μg/ml)				Leptin			
	Low (<8.0)	Middle (8.0-11.8)	High (≥11.8)	P	Low (<7.1)	Middle (7.1-8.6)	High (≥8.6)	P
Age, mean(SD)	46.8 (9.6)	46.4 (11.8)	46.1 (10.4)	0.50	43.6 (10.8)	48.4 (9.8)	47.2 (10.7)	0.22
BMI < 25 (kg/m^2), %	70.1	78.1	85.4	0.02	89.2	83.1	61.9	<0.01
TNM stage, %				0.14				0.17
0-I	41.9	47.9	44.2		50.9	46.3	37.4	
II	34.2	39.7	35.0		32.5	33.9	42.3	
III-IV	23.9	12.4	20.8		16.6	19.8	20.3	
Tumor size <2 cm, %	38.7	51.2	51.2	0.07	53.3	49.2	38.9	0.06
Lymph-node negative, %	55.8	67.5	55.9	0.11	59.3	59.5	60.7	0.83
Histologic gradeI-II, %	52.6	57.7	59.3	0.60	58.7	52.4	58.9	0.97
Nuclear gradeI-II, %	53.7	56.4	60.9	0.55	55.6	55.5	60.0	0.50
ER positive, %	58.0	65.0	59.5	0.49	64.7	68.3	50.0	0.02
PR positive, %	58.8	55.7	55.4	0.84	57.8	61.5	50.8	0.27

with low adiponectin and high leptin, respectively. On the other hand, women with higher leptin level had a larger tumor size (P_{trend}=0.06) and ER-negative (P_{trend}=0.02) compared to women with low leptin level.

Although there was no clear association overall between adiponectin or leptin concentration at diagnosis and breast cancer survival using all the patients with events and censoring at the 5- year-follow-up point (Table 3), elevated adiponectin levels were associated with DFS of breast cancer survivor with normal body

Table 3. Multivariate HR (95% CIs) of Breast Cancer Survival by tertile of Plasma Adiponectin and leptin Concentration Among Women with Normal Weight

	Total			Women with normal weight(BMI<25kg/m²)		
	All	Event	HR* (95%CI)	All	Event	HR (95%CI)
No. of subject	370	109		288	80	
all patients with events and censoring after 5 year f/u time						
Adiponectin*						
Low	124 (33.5)	43 (39.5)	Ref	87 (30.2)	30(37.5)	Ref
Middle	123 (33.2)	28 (25.7)	0.84 (0.48-1.46)	96 (33.3)	21(26.3)	0.81 (0.42-1.56)
High	123 (33.2)	38 (34.9)	0.99(0.61-1.63)	105 (36.5)	29(36.3)	0.84 (0.47-1.49)
P trend			0.99			0.57
Leptin						
Low	120 (32.4)	38 (34.9)	Ref	107 (37.2)	30 (37.5)	Ref
Middle	124 (33.5)	31 (28.4)	0.73 (0.43-1.24)	103 (35.8)	26 (32.5)	0.74 (0.40-1.36)
High	126 (34.1)	40 (36.7)	0.77 (0.46-1.28)	78 (27.1)	24 (30.0)	0.89 (0.48-1.63)
P trend			0.3			0.67
Ratio						
Low	128 (34.6)	45 (41.3)	Ref	81 (28.1)	31 (38.8)	Ref
Middle	121 (32.7)	26 (23.9)	0.72 (0.41-1.25)	95 (33.0)	18 (22.5)	0.67 (0.33-1.34)
High	121 (32.7)	38 (34.9)	1.01 (0.61-1.67)	112 (38.9)	31 (38.8)	0.77 (0.44-1.34)
P trend			0.98			0.38
Among women with less than 5 year f/u time						
No. of subject	136	69		109	54	
Adiponectin						
Low	40 (29.4)	25 (36.2)	Ref	30 (27.5)	19 (35.2)	Ref
Middle	45 (33.1)	22 (31.9)	0.81 (0.41-1.60)	34 (31.2)	17 (31.5)	0.75 (0.34-1.63)
High	51 (37.5)	22 (31.9)	0.60 (0.31-1.18)	45 (41.3)	18 (33.3)	0.45 (0.21-0.94)
P trend			0.13			0.03
Leptin						
Low	47 (34.6)	24 (34.8)	Ref	43 (39.5)	21 (38.9)	Ref
Middle	49 (36.0)	21 (30.4)	0.77 (0.40-1.50)	41 (37.6)	18 (33.3)	0.87 (0.41-1.84)
High	40 (29.4)	24 (34.8)	0.86 (0.44-1.69)	25 (22.9)	15 (27.8)	0.81 (0.39-1.69)
P trend			0.61			0.55
Ratio						
Low	47 (34.6)	30 (43.5)	Ref	32 (29.4)	22 (40.7)	Ref
Middle	34 (25.0)	14 (20.3)	0.68 (0.32-1.47)	28 (25.7)	11 (20.4)	0.50 (0.21-1.17)
High	55 (40.4)	25 (36.2)	0.80 (0.43-1.51)	49 (45.0)	21 (38.9)	0.61 (0.30-1.22)
P trend			0.55			0.21
Among women with more than 5 year f/u time						
No. of subject	234	40		179	26	
Adiponectin						
Low	84 (35.9)	18 (45.0)	Ref	57 (31.8)	11 (42.3)	Ref
Middle	78 (33.3)	6 (15.0)	0.37 (0.10-1.37)	62 (34.6)	4 (15.3)	0.47 (0.09-2.52)
High	72 (30.8)	16 (40.0)	1.56 (0.71-3.43)	60 (33.5)	11 (42.3)	1.62 (0.56-4.67)
P trend			0.27			0.32
Leptin						
Low	73 (31.2)	14 (35.0)	Ref	64 (35.8)	9 (34.6)	Ref
Middle	75 (32.1)	10 (25.0)	0.71 (0.67-1.91)	62 (34.6)	8 (30.8)	0.95 (0.27-3.34)
High	86 (36.8)	16 (40.0)	0.58 (0.24-1.40)	53(29.6)	9 (34.6)	0.91 (0.26-3.17)
P trend			0.22			0.88
Ratio						
Low	81 (34.6)	15 (37.5)	Ref	49 (27.4)	9 (34.6)	Ref
Middle	87 (37.2)	12 (30.0)	0.95 (0.36-2.53)	67 (37.4)	7 (26.9)	0.54 (0.11-2.57)
High	66 (28.2)	13 (32.5)	1.57 (0.65-3.79)	63 (35.2)	10 (38.5)	1.30 (0.44-3.86)
P trend			0.35			0.6200

*Adjusted for age, BMI, ER & PR status, TNM, histological and nuclear grade; Event means the number of recurrence or death

Table 4. Multivariate HR (95%CIs) of Breast Cancer Survival by Plasma Adiponectin Level Among Women with Time and Normal Weight (BMI<25m/kg²)

Adiponectin	Premenopusal women (n, %)			Postmenopausal women		
level	All	Event	HR* (95%CI)	All	Event	HR (95%CI)
Low	18 (25.7)	11 (31.4)	1.00	12 (33.3)	8 (42.1)	1.00
Middle	21 (30.0)	12 (34.3)	1.11 (0.41-3.03)	11 (30.6)	5 (26.3)	0.25 (0.06-1.06)
High	31 (44.3)	12 (34.3)	0.57 (0.21-1.55)	13 (36.0)	6 (31.6)	0.15 (0.03-0.71)
P trend			0.22			0.02

*Adjusted for age, BMI, ER & PR status, TNM, histological and nuclear grade; Event means the number of recurrence or death

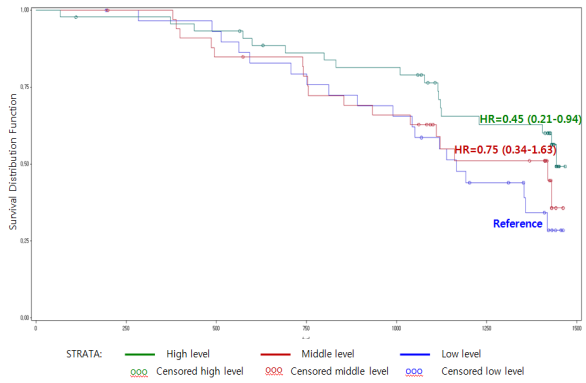

Figure 2. Kaplan-Meier Survival Curves. Stratified by Tertiles of Adiponectin Level Among Women with Less 5 Year Follow-Up Time and low BMI (<25 kg/m2) after Adjusted for age, BMI, ER & PR Status, TNM, Histological and Nuclear Grade: Days from Diagnosis to first Event (Recurrence, Second Occurrence, Breast Cancer-Specific Mortality, Total Mortality) Against Overall Survival Probability

with low adiponectin and high leptin, respectively. On the other hand, women with higher leptin level had a larger tumor size (P_{trend}=0.06) and ER-negative (P_{trend}=0.02) compared to women with low leptin level.

Although there was no clear association overall between adiponectin or leptin concentration at diagnosis and breast cancer survival using all the patients with events and censoring at the 5- year-follow-up point (Table 3), elevated adiponectin levels were associated with DFS of breast cancer survivor with normal body weight (BMI≤25 kg/m^2 (P_{trend}=0.03) with adjustment for age, ER/PR status, histological grade, nuclear grade and TNM stage during less than five years survival period (P_{trend}=0.03, Table 3 and Figure 2). On the other hand, the association between the high level of adiponectin and DFS was not observed in patent with more than 5 years survival time. In particular, the protective effect of adiponectin was observed predominantly in postmenopausal women (HR=0.25 and 0.15 for low vs. middle and low vs. high, respectively, P_{trend}=0.0191, in Table 4), but the effect was not find in premenopusal women. However, there was no association of leptin and the ratio of adiponectin and leptin level with the breast cancer survival for any subgroup analysis (Table 3).

Discussion

In the present study, women with higher leptin levels had a larger tumor size and ER-negative compared to women with low leptin levels. Despite their association, the level of leptin at diagnosis did not observe any effect on the recurrence or death of breast cancer survival regardless of the menopausal status. On the other hand, breast cancer patients with low preoperative adiponectin levels had poorer DFS than patients with elevated adiponectin levels among women with normal weight at diagnosis within 5 year survival time. In particular, the inverse association was observed predominantly in postmenopausal women.

Because of the inverse correlation of adiponectin levels with obesity, it has been suggested that the decreased levels of adiponectin may explain the increased risk of breast cancer in obesity (Chen et al., 2006; Nkhata et al., 2009; Karimi and Roshan, 2013) predominantly for postmenopausal women (Jarde et al., 2009; Nkhata et al., 2009). According to data from the experimental model, adiponectin appears to reduce the proliferation of breast cancer cells (Dieudonne et al., 2006), and to inhibit vascular endothelial growth factor-induced cell migration (Brakenhielm et al., 2004). In three retrospective case-control studies in which adiponectin levels were measured after diagnosis, adiponectin levels were inversely associated with breast cancer risk (Miyoshi et al., 2003; Mantzoros et al., 2004; Chen et al., 2006); furthermore, the association appeared stronger for postmenopausal women (Mantzoros et al., 2004). Since then, the first prospective case-control study nested within Nurses' Health Study (NHS) and NHS II cohort suggested the protective effect of adiponectin on the risk of postmenopausal breast cancer, particularly in a low-estrogen environment (Tworoger et al., 2007). On the other hand, two case-control studies nested in a prospective cohort in northern Sweden (Cust et al., 2009) and nested in the cohort of US women (Gaudet et al., 2010) did not confirm a significant difference the level of adiponectin in blood between cases and controls.

Contrast to the study related to the breast cancer risk, only few studies have examined the effect of adiponectin on breast cancer survival and mortality. The first demonstration of the association between adiponectin level and the breast cancer mortality among survivor was reported in the Health, Eating, Activity, and Lifestyle (HEAL) study; higher levels of adiponectin were associated with longer breast cancer survival (HR high p<low=0.39). We also supported the similar association between preoperative adiponectin levels and breast cancer survivor with normal body weight (BMI≤25kg/m^2) during less than 5 year survival time; however, the association was not observed during more than 5 year survivor. For the Korean women, the hazard ratio of recurrence following diagnosis of primary breast cancer was increase less than 5 years survival period (Lee and Park, 2009). In postmenopausal women, BMI is significantly correlated with serum levels of estrone and estradiol (r=0.38, p<0.001 and r=0.41, p < 0.001 respectively) (Cauley et al., 1989); this is important as blood levels of estradiol, bioavailable estradiol and free estradiol have been associated with increased risk of breast cancer recurrence (Rock et al., 2008). This has raised concerns that aromatase inhibitors (AIs), which target the aromatase enzyme to lower blood estrogen levels, may be have reduced effectiveness in postmenopausal women who are overweight or obese. Another report was to examine the modified effect of the association between adiponectin level and breast cancer recurrence. They suggested that adiponectin have prognostic significance in breast cancer recurrence and interventions related to the factor may protective against recurrence in ER/PR-negative patients (Oh et al., 2011). Tworoger et al. suggested the hypothesis that adiponectin may play a role in breast cancer etiology, particularly in a low-estrogen environment (Tworoger et al., 2007). They found that the association between adiponectin and postmenopausal breast cancer was strongest among women who had never used postmenopausal hormone

and those with low circulating estradiol levels, suggested that adiponectin may only influence breast cancer etiology in a low estrogen environment. For the prognosis of postmenopausal breast cancer survivor, the protective effect of preoperative adiponectin level was also observed on breast cancer survival in our present study.

Although earlier prospective study (Stattin et al., 2004) failed to an association between leptin and breast cancer risk, recent several nested case-control studies (Cust et al., 2009; Harris et al., 2011; Gross et al., 2013; Ollberding et al., 2013) suggested that high level of leptin was associated with increased breast cancer risk. A case-control study nested within the Multiethnic cohort (MEC) found an increased risk of postmenopausal breast cancer among women with the highest prediagnostic levels of leptin among normal weight women (Ollberding et al., 2013). A recent study examining the relationship of leptin in the circulation and in the breast tissue of healthy women (Llanos et al., 2012), reported correlation for leptin r=0.62(p=0.009) for normal women, r=0.36 (p=0.08) for overweight women, and r=0.03 (p=0.86) for obese women. In an analysis conducted in the Northern Sweden Health and Disease Cohort (Cust et al., 2009), the association between prediagnostic leptin and the risk of breast cancer differed by the stage at diagnosis with an inverse association reported for early stage and a suggestive positive association reported late stage disease when examined among pre- and postmenopausal combined. For the retrospective studies, no association was found even after stratification by menopausal status (Mantzoros et al., 1999; Petridou et al., 2000), the association similar for early- and late-stage disease reporting positive findings (Chen et al., 2006; Wu et al., 2009).

On the other hand, the recurrence/mortality of breast cancer was not associated with the level of leptin. Leptin is also significantly associated with the progression of breast cancer and with poor survival (Chen et al., 2006). In contrast, one study showed, for tamoxifen-treated postmenopausal obese patients, disease free survival (DFS) of the leptin-positive group was higher than that of the leptin-negative group (Cust et al., 2009). Intratumoral leptin mRNA levels in ER-positive tumors are significantly higher than in ER-negative tumors and intratumoral leptin mRNA levels significantly correlated with serum leptin levels (Hou et al., 2007). These two findings show that the direction of the association between leptin level and prognosis is still debatable, and that serum leptin reliably reflects leptin status. Recently, A prospective study was examined the association between the level of leptin and breast cancer recurrence but not found any association among 747 Korean breast cancer patients (Oh et al., 2011). Another earlier prospective study was also not observed the independent association of leptin level with prognosis in early-stage breast cancer, although leptin is strongly correlated with obesity and insulin (Goodwin et al., 2005). In present study, we also not observed any difference and association of leptin level between survivor and recurrence/mortality patients.

This study has several limitations. First, we didn't have the information of detail subtype adiponectin by molecular weight. High- and low-molecular-weight forms of adiponectin circulate in human plasma, which may have different biological activities (Waki et al., 2003; Pajvani et al., 2004; Fisher et al., 2005). Second, although this study is clinical follow-up study, adiponectin levels at diagnosis may be influenced by cancer process and potentially could bias the results. We collected only one fasting blood sample; therefore, we cannot assess the effect of change in leptin or adiponectin, which would require testing interventions to change these analytes such as weight loss, or physical activity. However, any misclassification based on these measures would most likely be non-differential and therefore would leads to an underestimation of the true association. Third, we were unable to assess risk in specific subgroups, including ER-negative disease, because of small numbers. Although this study has several limitations, there are several strengths to the current study, including the prospective design allowing for prediagnostic biomarker and covariate assessment. Thus, a confirmatory study should also take account of the effect of adiponectin at diagnosis as prognostic biomarker for breast cancer survival. Fourth, at the end of the follow up time, we found 109 events. Unfortunately, we should select the comparable subject from 925 patients based on the age distribution of event group, because of the laboratory analysis cost. That is the reason why the average of age for event and non-event was similar. Therefore, it should consider the potential bias due to this selection. Further investigation is necessary to assess the role of adiponectin as a breast cancer risk biomarker and its potential direct effect on breast carcinogenesis pathway.

In summary, this study suggested that the adiponectin level at diagnosis is associated with breast cancer recurrence and could use the predictor of prognosis for breast cancer survival with normal weight during less than 5 years survival times.

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