

## RESEARCH ARTICLE

# Association of Obesity and Breast Cancer Risk: *The Role of Estrogen, Tumor Necrosis Factor-alpha, and Adiponectin as Risk factors (preliminary study)*

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**BACKGROUND:** Breast cancer is the most frequent cancer diagnosed among women. Many factors influence the carcinogenesis of breast cancer. The aim of this study to analyze the role of obesity (waist circumference and body mass index), serum Estradiol levels, TNF- $\alpha$ , and Adiponectin in the occurrence of breast cancer.

**METHODS:** This was observational study with case-control design. Eleven breast cancer patients as cases and twelve FAM (Fibroadenoma Mammæ) patients as controls were analyzed. The serum Estrogen, TNF- $\alpha$  and Adiponectin were examined in their association with breast cancer risk.

**RESULTS:** Women with breast tumor and waist circumference  $\geq 80$  cm have significantly higher breast cancer risk than women with breast tumor and waist circumference  $< 80$  cm (OR 8,75; 95% CI : 1,24 – 61,88;  $p = 0,029$ ). Women with breast tumor and higher serum TNF- $\alpha$  levels ( $\geq 2,30$  pg/ml) have higher breast cancer risk (19,25 times) than women with breast tumor and have lower serum TNF- $\alpha$  levels (95% CI : 1,77 - 209,55,  $p = 0,015$ ). Whereas, women with breast tumor and lower Adiponectin/TNF- $\alpha$  ratio ( $< 2,13$ ) have higher breast cancer risk (22,5 times) than women with breast tumor and higher Adiponectin/TNF- $\alpha$  (95% CI: 2,60 – 194,51;  $p = 0,005$ ).

**CONCLUSION:** These results suggest that high concentration of serum TNF- $\alpha$ , waist circumference  $\geq 80$  cm and low Adiponectin/TNF- $\alpha$  ratio in women with breast tumor are significantly associated with an increased risk for breast cancer.

**KEYWORDS:** Obesity, breast cancer, adiponectin/TNF- $\alpha$  ratio.

Breast cancer is the most common cancer affecting women worldwide. Multiple risk factors are associated with increasing risk of breast cancer. This include genetic, familial and hormonal factors

The association of obesity with breast cancer risk, especially in post menopause women are well established. Aromatization of adrenal androgen into estrogen at adipose tissue is proposed to play role in the development of breast cancer.

Obese people have more adipose tissue. Adipose tissue is not merely a fat-storing tissue but also an endocrine organ, producing various cytokines including TNF- $\alpha$  and Adiponectin. TNF- $\alpha$  regulates immune responses, inflammation and programmed cell death (apoptosis). The ultimate fate of a cell exposed to TNF- $\alpha$  is determined by signal integration between its different effectors (1). Adiponectin is an adipocytokines discovered through systemic analysis of adipose-expressed genes. Funashashi has found the lower level of Adiponectin in plasma of individual with central obesity (2). Miyoshi and Montzoros reported the association of low serum Adiponectin levels with an increased risk for breast cancer (3,4). Moreover, Miyoshi also found that tumors arising in women with low Adiponectin levels are more likely to show a biologically aggressive phenotype (3).

Today, there are still few researches on the correlation of serum Adiponectin levels with the risk of breast cancer and the molecular mechanism which is able to explain the role of Adiponectin in carcinogenesis

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## Materials and Methods

### STUDY DESIGN AND STUDY PARTICIPANTS

This was observational study with case-control design, breast cancer patients as cases and FAM patients as controls. During 6 months period from March to August 2006, 90 women with breast tumor (suspected of breast cancer or FAM) in the screening centers of Rumah Sakit Kanker Dharmais Jakarta were identified as potential subjects. Thirty patients refused and 60 patients agreed to participate and were included in this study. Written informed consents as to the study were obtained from all participants. Information about demography, medical history, menstrual and menopause status, and parity was assessed by interview questionnaires. Anthropometric information (weight, height and waist circumference) was obtained according to standard anthropometric measurement. Blood samples for Estrogen, TNF- $\alpha$  and Adiponectin assay were obtained before surgery. Among the 60 participants, 35 patients were treated with excision, mastectomy, breast-conserving surgery or biopsy. Histological diagnosis of breast cancer was confirmed in 11 patients, and histological diagnosis of FAM was confirmed in 12 patients. Final analytic sample included 23 participants.

### MESUREMENTS OF BIOCHEMICAL MARKERS

All venous blood samples drawn before surgery, and the serum was immediately separated by centrifugation and stored at -20°C until use. Serum Estradiol levels were measured by immunochemiluminescent using the kits from DPC (Coefficient of Variation 3.57%-9.7%). Serum TNF- $\alpha$  levels were measured

by Quantitative sandwich enzyme immunoassay using the kits from R&D System (Coefficient of Variation 0.4%-14.5%). Serum Adiponectin levels were measured by ELISA using the kits from Daiichi Pure Chemicals (Coefficient of Variation 2.5%). Serum Estradiol measurements was performed in Prodia Clinical Laboratory – Kramat Raya Jakarta Branch. Serum TNF- $\alpha$  and Adiponectin measurements was performed in Research & Development Laboratory of Prodia Clinical Laboratory.

### STATISTICAL ANALYSIS

Statistical analysis were performed with SPSS for windows ver. 11.5. Univariate analysis were performed to calculate mean, maximum and minimum value and SD. Comparison of the Body Mass Index, waist circumference, serum Estradiol, TNF- $\alpha$ , and Adiponectin levels between cases and control group were analysed using Mann-Whitney non-parametric test. Spearman correlation statistics were used to determine the correlation among the various variables. The relationship between obesity, serum Estradiol levels, serum TNF- $\alpha$  levels, serum Adiponectin Levels, Adiponectin/TNF- $\alpha$  ratio and breast cancer risk were determined using crosstabs test to obtain OR and 95% CI. Obesity was defined if BMI  $\geq 27$  kg/m<sup>2</sup> or waist circumference  $\geq 80$  cm. Serum Estradiol levels was categorized according to reference value adjusted with menopausal or menstrual status. The category of serum TNF- $\alpha$  levels was defined using cut off point that was calculated with ROC analysis. Serum Adiponectin levels and Adiponectin/TNF- $\alpha$  ratio were categorized using the median value.

## Results

Table 1 shows mean values and SDs of BMI (Body Mass Index), waist circumference and the measured biomarkers between breast cancer (cases) and FAM (control). They reveal significance differences of age ( $p < 0.001$ ), BMI ( $p < 0.001$ ), Waist Circumference ( $p = 0.001$ ), Adiponectin ( $p = 0.017$ ) and Adiponectin/TNF- $\alpha$  ratio ( $p = 0.005$ ) between women with breast cancer

(cases) and women with FAM (control). Adiponectin and the ratio Adiponectin/TNF- $\alpha$  are inversely associated with breast cancer risk. However, no significance differences of serum Estradiol and TNF- $\alpha$  levels are noted between women with breast cancer (cases) and women with FAM (control).

**Tabel. 1 : Basic Characteristics of Subjects (total 23 patients)**

Variables	FAM (Control)	Breast Cancer (Cases)	p
Total samples	12	11	
Age (years)	27,67 ± 5,91	42,27 ± 10,57	< 0,001*
Body Mass Index (kg/m <sup>2</sup> )	20,36 ± 2,62	25,97 ± 3,19	< 0,001*
Waist Circumference (cm)	70,48 ± 7,28	82,23 ± 8,11	0,001*
Estradiol (pg/ml)	91,16 ± 52,62	105,69 ± 75,46	0,595
TNF- $\alpha$ (pg/ml)	1,84 ± 0,87	2,93 ± 1,65	0,057
Adiponectin ( $\mu$ g/ml)	6,17 ± 2,79	3,85 ± 1,25	0,017*
Rasio Adiponectin/TNF- $\alpha$	4,00 ± 2,51	1,56 ± 0,74	0,005*

FAM = Fibroadenoma Mammariae, p = probability, \* = statistically significance (p < 0,05)

Correlation analysis using Spearman statistics reveal that there are no significant correlation between age and BMI, waist circumference, serum Estradiol levels, serum Adiponectin levels, serum TNF- $\alpha$  levels, Adiponectin/TNF- $\alpha$  ratio. Inversely, we found significant negative correlation between BMI and serum Adiponectin levels (r = -0.520, p = 0.011), BMI and Adiponectin/TNF- $\alpha$  ratio (r = -0.508, p = 0.013), waist circumference and serum Adiponectin levels (r = -0.531, p = 0.009), waist circumference and Adiponectin/TNF- $\alpha$  ratio (r = -0.636, p = 0.001). Significant positive correlation also noted between waist circumference and serum TNF- $\alpha$  levels (r = 0.526, p = 0.01).

**Table 2. Correlation among variables**

Variables	rp	
Body Mass Index – Serum Adiponectin levels	- 0,520	0,011*
Body Mass Index – Adiponectin/TNF- $\alpha$ levels ratio	- 0,508	0,013*
Waist Circumference – Serum TNF- $\alpha$ levels	0,526	0,010*
Waist circumference – Serum Adiponectin levels	- 0,531	0,009*
Waist circumference – Adiponectin/TNF- $\alpha$ ratio	- 0,636	0,001*

r = Coefisien of correlation, p = probability, \* = statistically significance

Table 3 show the results of association analysis using crosstabs method between BMI, Waist Circumference, Estradiol, TNF- $\alpha$  Adiponectin, Adiponectin/TNF- $\alpha$  ratio with breast cancer incident as cases and FAM as control. There was evidence for significant association between waist circumference and breast cancer risk (OR' 8.75; 95% CI, 1.24 – 61.68; p = 0.029). We also found association between serum TNF- $\alpha$  levels with breast cancer risk and statistically significant (OR, 19.25; 95% CI, 1.77 – 209.55; p = 0.015). An inverse association between Adiponectin doesn't achieve statistical significance. However, there is evidence strong inverse association between Adiponectin/TNF- $\alpha$  ratio with Breast Cancer Risk (OR, 22.5; 95% CI, 2.60 – 194.51; p = 0.005). For BMI and Estradiol, there is positive association with Breast Cancer risk but it did not achieve statistical significance.

**Table 3. Association of BMI, Waist Circumference, Estradiol, TNF- $\alpha$ , Adiponectin, Adiponectin/TNF- $\alpha$  ratio with Breast Cancer Risk**

Variables	Cut Off	OR	CI, 95%	p
BMI	27 kg/m <sup>2</sup>	9.17	0.86 – 97.69	0.860
Waist Circumference	80 cm	8.75	1.24 – 61.88	0.019*
Estradiol	Ref. Value	9.17	0.86 – 97.69	0.066
TNF- $\alpha$	2.30 pg/ml	19.25	1.77 – 209.55	0.015*
Adiponectin	6.62 $\mu$ g/ml	7.14	0.68 – 75.22	0.102
Adiponectin / TNF- $\alpha$	2.13	22.5	2.60 – 193.51	0.005*

## Discussions

The association between BMI with breast cancer risk has been established. There are several mechanisms to explain Obesity cause the increasing production of growth-promoting steroid hormones including estrogen that can bind to its receptor in the nucleus of breast tumor cells and promote breast cancer growth and metastasis.

In this study, we found that BMI in women with breast cancer subjects were higher than BMI in women with FAM. From cross-tab test to analyze the correlation between BMI and breast cancer risk, we obtained OR 9.17; 95% CI 0.86-97.69,  $p=0.860$ . It means, this study result wasn't similar to what has been previously reported. It was probably due to the 2 subjects with extremely high BMI in FAM patients and small amount of samples.

In this study, the mean of waist circumference in breast cancer patients ( $82.23 \pm 8.11$  cm) was higher than FAM patients ( $70.48 \pm 7.28$  cm) and the difference was statistically significance ( $p = 0.001$ ) (Table 1). If the status of waist circumference was changed to obese (central obesity) and non-obese (normal), according to Indonesian Society for the study of Obesity (ISSO) criteria (waist circumference limit is 80 cm) then we got significant correlation ( $p = 0.029$ ) between breast cancer occurrence and waist circumference with OR 8.75 (95% CI: 1.24 – 61.68) (Table 3).

Central obesity is often associated with the occurrence of insulin resistance and hyperinsulinemia.

Increased insulin level, lead to decreasing Steroid Hormone Binding Globulin (SHBG) synthesis by the liver, and also capable to reduce the synthesis of Insulin-like growth factor binding protein1 (IGFBP1), either by the liver or by other local tissues, and in result, the IGFBP1 level in blood decreases as well. Besides, insulin stimulates insulin-like growth factor 1 (IGF-1) synthesis. Combination of both situations can increase the IGF-1 bioavailability. While, recently more evidence show correlation between IGF-1 and the development of breast cancer. In MCF-7 cell culture, IGF-1 directly increased the ER transcriptional activity in the absence of Estradiol. The presence of Estradiol would increase the ER transcriptional activity even more (5,6). The laboratory experiment showed that IGF family was not merely having mitogenic effect in breast cancer cells, but the anti-apoptotic effect as well. Besides, in central obesity, hiperinsulinemia takes place and the expression of abnormal insulin receptor can result in signalling deviation through Insulin Receptor Substrate 1 Pathway (4,6). Synergy interaction between the elevated Estrogen level and insulin signaling deviation proved to be able to increase proliferation activity either in normal or cancer breast cells.

In this study, the mean of Estradiol level in breast cancer patients ( $105.69 \pm 75.46$  pg/ml) was not significantly different ( $p = 0.595$ ) from the mean of Estradiol level in FAM patients ( $91.16 \pm 52.62$  pg/ml). Standard deviation of the mean of Estradiol



level was too large as the tests were done in different menstruation periods, whereas Estradiol level shows variation in different periods. Related to that, normal or high Estradiol level was categorized based on the reference values according to the menstruation period on the sampling day. Mann-Whitney test was performed and we found that Estradiol value was significantly different ( $p = 0.048$ ) between breast cancer patients and FAM patients, and this result was similar with the references. However, categorizing Estradiol value like this has some disadvantages particularly towards the data accuracy because the data was obtained from interview. Difficulty was found during the interview because most of the patients were uncertain about the first day of their last menstruation and how many days their menstruation periods were. Therefore, categorizing based on the assumption that menstruation cycle (for patients who could not answer the question for sure) was 28 days. Then, cross-tab test was performed (Table 3) and from the analysis we found no association between Estradiol and breast cancer occurrence with the odds ratio of 9.17 (95% CI: 0.86 – 97.69), and it wasn't statistically significant ( $p = 0.066$ ). The high odds ratio but not statistically significant, was probably due to the limitation in collecting data as previously explained.

In this study, we obtained that the mean of TNF- $\alpha$  level in breast cancer patients ( $2.93 \pm 1.65$  pg/ml) was higher compared to the mean of TNF- $\alpha$  level in FAM patients ( $1.84 \pm 0.87$  pg/ml) but not statistically significant with  $p = 0.057$ . When we further observed, we found data with extremely high TNF- $\alpha$  level in both FAM and breast cancer patients. Extremely high TNF- $\alpha$  level (6.75 pg/ml) was found in the patient with breast cancer along with paget's disease of the nipple and clinical inflammation was found around the nipple. Besides, the interview revealed that the patient had analgesic/anti-inflammatory drug. (Patients having anti-inflammatory or infection should have been excluded, but because most of patients used anti-inflammatory, the exclusion was not carried out). So in this matter, extremely high TNF- $\alpha$  level was influenced by another clinical condition (acute inflammation). The patient with extremely high TNF- $\alpha$  level (4.32 pg/ml) in FAM group appeared to be clinically healthy (there was no acute inflammation/infection), but the patient was one of the 2 patients in FAM group that had waist circumference > 80 cm, although their BMI was normal. Besides, the histological examination result of the patient showed epitheliosis (another patient with mild epitheliosis had rather high TNF- $\alpha$  level,  $\geq 2.1$  pg/ml,

while most of patients in FAM group had TNF- $\alpha < 2$  pg/ml). From analysis of cross-tab test (Table 3) we found that TNF- $\alpha$  level correlated with breast cancer occurrence with odds ratio 19.25 (95% CI: 0.86 – 26,78), and it was statistically significant ( $p = 0.015$ ).

In individual with obesity, especially central obesity, TNF- $\alpha$  level increased. In this study, we found positive correlation between central obesity and TNF- $\alpha$  level with  $r = 0.519$  which was statistically significant ( $p = 0.008$ )

TNF- $\alpha$  is pro-inflammatory cytokine that plays role in the regulation of immune response, inflammation and apoptosis. TNF- $\alpha$  have pleiotropic effect and cells that exposed to TNF- $\alpha$  will affected by the signal and activated effectors, I $\kappa$ B kinase (IKK), c-jun-N-terminal protein kinase (JNK) or caspase. Activation of caspase trigger apoptosis, on the contrary activation of IKK inhibits apoptosis through the NF $\kappa$ B transcription factor.

TNF- $\alpha$  acts through its receptor (TNFR1) to activate the NF $\kappa$ B transcription factor gene. In acute inflammation condition (in normal epithelial cells), NF $\kappa$ B gene activation results in increased expression of genes encoding pro-inflammatory mediators (cytokines), and genes that regulate the balance between cell proliferation and apoptosis. In inflammatory immune cells (myeloid), NF $\kappa$ B activation can cause cell death (apoptosis), but what is more important is to regulate the short expression of pro-inflammatory mediators to repair tissue damage. In pre-cancer epithelial cells, NF $\kappa$ B activation can increase the cell-survival, and also the tendency to become malignant. It occurs because of the increase in inflammation & cell-survival gene expression and the inhibition in the "machine" that activates cell death (7). Apoptosis is a mechanism to eliminate damage or abnormal cells including cancer and pre-cancer cells. Related to the explanation and the significant positive correlation between TNF- $\alpha$  and occurrence in breast cancer, then women with breast tumor (FAM) having high TNF- $\alpha$  level may have higher risk to become malignant, and in breast cancer patients it may cause the cancer cells to survive more.

Besides through the inflammatory and apoptosis mechanisms, TNF- $\alpha$  is also able to increase the formation of Reactive Oxygen Species (ROS) that is mediated by neutrophil and other cells. This process can cause DNA damage and inhibit DNA-repair in tumor cells (8).

In this study, we found that the mean of Adiponectin level in breast cancer patients ( $3.85 \pm 1.25$   $\mu$ g/ml) was

lower compared to the mean of Adiponectin level in FAM patients ( $6.17 \pm 2.79 \mu\text{g/ml}$ ) and was statistically significant with  $p = 0.017$ . This study result was similar to the result by Miyoshi (2003), Mantzoros (2004), and Chen (2005) who have found significant correlation between Adiponectin level and breast cancer (3, 9, 10). The former researchers used patients that had negative mammography results as control, while in this study, the control group was patients with benign breast tumor which was confirmed to be FAM by histopathology.

Adiponectin is a protein exclusively secreted by adipocytes and acts as insulin-sensitizer (3). Regarding to this, adiponectin secretion in obese subjects and other insulin resistance circumstances will decrease. In this study, we proved these phenomena and found a negative correlation between BMI and waist circumference with adiponectin level ( $r = -0.520$  for BMI and  $r = -0.531$  for waist circumference), which was statistically significant ( $p = 0.011$  for BMI and  $p = 0.009$  for waist circumference).

Then we categorized the subjects based on cut off value of adiponectin and the median of adiponectin level between subjects, and analyzed them using the cross-tab, and we observed that adiponectin level didn't correlate with the risk of breast cancer with odd ratio 2.40 (95% CI : 0.44 – 12.98;  $p = 0.309$ ). Actually, categorizing the samples using median value didn't have much clinical value, so we categorized the samples again using the mean value of healthy women subjects,  $6.62 \mu\text{g/ml}$  (11). Based on this categorization, we got a higher odds ratio, i.e. 7.14 (95% CI : 0.68 – 75.22), but this value was not statistically significant ( $p = 0.102$ ). Ideally, we should have used a cut off value obtained from analysis of ROC curve, i.e.  $6.88 \mu\text{g/ml}$ , but when we used this value as cut off, we couldn't analyze the odds ratio due to no sample in one of the groups. We needed a larger number of samples to be able to analyze this. By further observation, in this study, we found a subject in the breast cancer group having an extremely high level of adiponectin. This subject also had an extremely high TNF- $\alpha$  level. Besides, BMI of this subject was normal ( $24.94 \text{ kg/m}^2$ ) but her waist circumference was 83.3 cm (central obesity). In obese subjects, we usually find high level of TNF- $\alpha$  but low level of adiponectin. Thus, this subject possibly had acute inflammation that cause increased level of TNF- $\alpha$  as well as adiponectin. This finding raised a question whether acute inflammation could induce an increase in adiponectin level as a response to that inflammation. This could be answered

by an experimental study. Besides, subjects with the second highest adiponectin level ( $4.86 \mu\text{g/ml}$ ) had a histological examination finding that differed from other patients, intracystic papillary carcinoma (other patient's pathology anatomy findings were ductal carcinoma). This aroused a suspect that this high concentration had correlation with histopathology.

Until now, the mechanism that explains the relationship of adiponectin and the risk of breast cancer hasn't been much known. Presently, there is no study towards the effect of adiponectin to breast tissues and breast cancer, but it is presumed that adiponectin can influence their growth and differentiation. In this study, we didn't find a correlation between adiponectin level and the estradiol level, but there were a correlation between adiponectin level and BMI and also between adiponectin level and waist circumference. These findings confirmed the study done before by Miyoshi, and meant that adiponectin influence the risk of breast cancer, but not through the mechanism of estrogen. A mechanism that might be able to explain the relationship of adiponectin and breast cancer is through the insulin resistance. Insulin is suggested to stimulate the proliferation of breast cancer cells by forming a binding and stimulates the signaling of insulin and IGF-1 (3).

Ouchi (2000) found that Adiponectin could attenuate the TNF- $\alpha$  induced phosphorylation of I $\kappa$ B-B and then activated NF $\kappa$ B without through other signal transduction pathways (JNK, P38 kinase, Akt kinase) mediated by TNF- $\alpha$ . NF $\kappa$ B is an important protein in inflammation and can activate the signal transduction pathway in cancer cells and pre-cancer inflammation cells and can stimulate the malignancy process (12). In a study using rats as a model for inflammation related cancer, it was showed that NF $\kappa$ B and TNF- $\alpha$  played roles in cancer development (13). There might be an association between TNF- $\alpha$  and Adiponectin in carcinogenesis.

Other research supporting the role of Adiponectin in apoptosis process was done by Yokota, et al (2000). In that research, Yokota et al evaluated the expression of apoptosis related genes in the presence of Adiponectin in M1 cells. After treated with Adiponectin for 48 hours, there was a down-regulation of Bcl-2 (anti-apoptotic gene) expression, and also of Bcl-xL gene expression. On the contrary, adiponectin treatment didn't have effect towards the regulation of pro-apoptotic gene expression (Bax, Bak, and p53). Thus, in obesity where the concentration of adiponectin is low, there is no down-regulation of anti-apoptotic gene, and the apoptosis process is blocked (14).

In this study, there was no direct correlation between TNF- $\alpha$  and adiponectin ( $r = -0.228$ ,  $p = 0.272$ ). Based on the theory explained above, there is an association between TNF- $\alpha$  and Adiponectin towards the process of apoptosis and impact in apoptosis process plays a role in carcinogenesis. Therefore, in this study, we tried to analyze the correlation between the ratio of Adiponectin/TNF- $\alpha$  and breast cancer. We performed a cross-tab test, but before that we categorized the value of Adiponectin/TNF- $\alpha$  ratio based on the median in subject population, i.e 2.13. We didn't use the cut off from ROC curve analysis due to the small number of samples and there was no sample in one of the category, and in result the cross-tab test to calculate the odds-ratio couldn't be performed. According with the above theory, cross-tab analysis showed a correlation between Adiponectin/TNF- $\alpha$  ratio with the occurrence of breast cancer with odds-ratio 22.5 (95 CI:2.60 – 194.51) which was statistically significant ( $p = 0.005$ ). This correlation was stronger and more significant than the correlation of TNF- $\alpha$  itself with the occurrence of breast cancer, and much stronger and significant than the correlation of adiponectin itself which was weak and insignificant. This could be understood because Adiponectin was a protection while TNF- $\alpha$  was a risk factor and the ratio between both variables gave a corroborating effect.

Adiponectin and TNF- $\alpha$  have been known to play role in carcinogenesis through the mechanism of inflammation that eventually affects the apoptosis process. Besides that mechanism, recent studies showed the possibility of other mechanism, through the angiogenesis process.

It is known that angiogenesis plays role in cancer development and from recent observations, it was shown that angiogenesis was also important in obesity. Treatment with anti-angiogenic agent will reduce the adipose tissue, and this shows that angiogenesis process is an important component in obesity development. Besides, it is also known that obese cancer patients have worse prognosis, decrease in disease-free interval and increase in metastasis risk. This also strengthen the suggestion that angiogenesis is increased in obesity. A study by Silha, *et al* (15) proved the increase of vascular growth factors (angiogenic and anti-angiogenic factors).

Angiogenesis is stimulated by hypoxia and erythropoiesis (EPO). Vascular Endothelial Growth Factor (VEGF) is the most specific angiogenesis inducer, produced by both normal and cancer cells

that experience hypoxia. TNF- $\alpha$  blocks the synthesis of EPO but not the production of VEGF (16). Burgel, and Ouchi performed an experimental study using human umbilical vein endothelium cells (HUVECs) and found that Adiponectin could stimulate the growth of new blood vessels (angiogenesis) by stimulating the crosstalk between Adenosine Monophosphate-activated protein kinase signal and Akt in endothelial cells (17).

Different from study by Ouchi (2004), the study by Brakenhielm *et al* showed that Adiponectin was a negative regulator for angiogenesis process. In vitro, Adiponectin attenuated the proliferation and migration of endothelial cells. Furthermore, the study by Brakenhielm *et al* showed that in vivo, Adiponectin stimulated the apoptosis of endothelial cells by activating the cascade pathway of caspase-8, caspase-9, and caspase-3. These co-supporting in vitro and in vivo results showed that Adiponectin was a potential angiogenesis inhibitor. The difference between the study result by Brakenhielm and Ouchi was understood as Ouchi used endothelial cells derived from large blood vessel, while Brakenhielm used endothelial cells derived from capillary. Brakenhielm claimed that their choice of endothelial cells was more relevant to the in vivo condition, as angiogenesis process commonly developed from small blood vessel (capillary), and not the large blood vessel (17,18).

Thus, it could be concluded that Adiponectin played a role in carcinogenesis through inflammation process which eventually associated with apoptosis, and through angiogenesis.

In conclusion, we have shown a significant association between high serum TNF- $\alpha$  levels, waist circumference  $\geq 80$  cm, low Adiponectin/TNF- $\alpha$  ratio and increasing risk for breast cancer. However, we have not found a significant association between high serum Estradiol levels and/or low Adiponectin levels with breast cancer risk. The results suggest that waist circumference, serum TNF- $\alpha$  levels and Adiponectin/TNF- $\alpha$  ratio might be used as a breast cancer risk predictor in women with breast tumor. In addition, Adiponectin/TNF- $\alpha$  ratio was the strongest predictor for breast cancer risk (OR 22.5). The limitation of this study because of the design of this study is observational case-control study that cannot indicate causal relationship, selection bias and samples heterogeneity. A large number of subjects is needed to confirmed our preliminary results.

**Acknowledgement:**

The Authors thank the Prodia Foundation for Research and Training for the invaluable support in this research. And thank to Dharmais Cancer Hospital for technical assistance in collecting samples in this research, especially to Samuel Haryono, MD., Sutjipto, MD., and Ramadhan, MD.

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