Expression of inflammatory cytokines by adipose tissue from patients with endometrial cancer

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Summary

Obesity results in increased mortality from many forms of cancer. We looked at the levels of gene expression for TNFα, IL-6, IκB kinase (inhibitor of NF-κB), CD 68 (glycoprotein expressed on macrophages) and leptin in samples of adipose tissue from individuals with endometrial cancer versus patients with benign conditions. This is a prospective study which included patients of a gynecologic oncology group. A piece of omental tissue was harvested from them during surgery. RNA was purified from all samples. Relative amounts of RNA for IκB, TNFα, IL-6, CD68 and leptin were calculated. Pearson's correlation method was used to correlate RNA levels with BMI. Logistic regression method was used to compare gene expression for cancer and control groups. The total sample size was 56 (24 endometrial cancer and 32 controls). IκB, TNFα and IL-6 levels increased linearly with increasing BMI in the control group. There was no correlation of IκB, TNFα, IL-6 or CD-68 levels with cancer status of the patients. Leptin had a weak protective effect against endometrial cancer (odds ratio = 0.92). Obesity is associated with increased expression of certain inflammatory cytokines in the adipose tissue. However, increased levels of these inflammatory markers in the adipose tissue of the omentum are not associated with presence of endometrial cancer.

Key words: Endometrial cancer; Inflammation; Cytokines; Obesity.

Introduction

Obesity is an epidemic that seriously threatens public health. According to the Centers for Disease Control and Prevention, an estimated 64% of US adults are either overweight or obese (body mass index, BMI, greater than 25, based on the National Health and Nutrition Examination Survey 1999-2000) [1]. Obesity is associated with metabolic dysfunction including resistance to insulin leading to diabetes, and hyperlipidemia associated with an increased risk of cardiovascular disease. Obesity also results in increased mortality from many forms of cancer [2].

In attempting to understand the increased mortality from cancer among obese individuals, a role for inflammation has been suggested. There is increasing evidence that excess adipose tissue induces a chronic inflammatory state. Adipose tissue secretes cytokines such as tumor necrosis factor α (TNFα) and interleukin 6 (IL-6) [3, 4], normally associated with activation of the immune system. Recently published reports go further in suggesting that inflammation associated with obesity is related to infiltration of immune cells, specifically macrophages, into the adipose tissue of obese animals [5, 6]. Also, although cultured adipocytes and pre-adipocytes do not normally express macrophage-specific genes, pre-adipocytes can undergo conversion to macrophages [7]. The relation between cancer and inflammation is complex, but there is evidence that TNF and IL-6 may be linked to cancer development through their downstream target, nuclear factor kappa-B (NF-κB), which promotes cell proliferation, inhibits cell death, or apoptosis and is involved in tumor promotion, angiogenesis and metastasis [8-10]. Excess adipose tissue also secretes increased amounts of leptin, a hormone responsible for appetite regulation [11]. Leptin has also been shown to promote angiogenesis which may contribute to cancer progression [12, 13]. In this study, we measured the expression of inflammatory cytokines and leptin in the omental adipose tissue of patients with endometrial cancer, which has been particularly prevalent in the obese population [14]. We also examined the relationship of these markers to BMI in subjects with benign conditions. Although there are a number of other potential mechanisms for the association of obesity with endometrial cancer, e.g., higher levels of endogenous sex steroids [14, 15], current knowledge suggests that the proinflammatory state associated with obesity may be a potential mechanism for cancer risk.

Methods

This is a prospective study which included 56 patients all of whom were patients of the gynecology-oncology group at Stony Brook University Medical Center between 2006 and 2008 (Table 1). Twenty-four patients in this sample had pathologically confirmed diagnosis of endometrial cancer. For all cases this was a newly diagnosed malignancy and none had had chemotherapy or radiation treatment prior to the surgery. Patients from the control group underwent abdominal operations for benign conditions, such as ovarian cysts, fibroids, etc. All diagnoses were confirmed by pathology reports. Patients with concurrent non-gynecologic malignancy were excluded from the study.

Upon obtaining informed consent from patients, a section of omental adipose tissue was collected during the surgery and stored frozen. All adipose tissue samples were ground under

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liquid nitrogen and about 100 mg of each sample was emulsified in 1 ml Qiazol Lysis Reagent. RNA was extracted from all emulsified adipose tissue samples using Qiagen RNeasy Lipid Tissue Mini Kit. cDNA was synthesized from all RNA samples and then real-time PCR was performed on all cDNA samples. Relative amounts of RNA in the samples were calculated based on the real-time PCR for I B (protein responsible for inactivation of NF-κB), TNFα, IL-6, CD68 (macrophage cell surface marker) and leptin genes and normalized to the amount of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) RNA in each sample. Relative amounts of RNA were used as estimates of levels of gene expression in the tissue samples.

Age and BMI of the cancer and control patients were compared with independent samples t-tests. Pearson's linear correlation coefficients were calculated for the levels of IκB, TNFα, IL-6, CD68 and leptin expression versus BMI and age of the patients in the control group (patients with benign conditions) and linear regression curves were plotted. All five markers as well as age and BMI of the patients were included as independent variables in the multivariable logistic regression models in order to find correlations of their expression in omental tissue with cancer status. Presence versus absence of endometrial cancer was set as a dependent variable for the logistic regression. All calculations were performed with the SPSS 13 software package.

Results

The total sample size was 56. There were 24 patients in the cancer group and 32 patients in the control group. Baseline demographics are summarized in Table 1. Patients in the endometrial cancer group were on average ten years older than controls and 83.3% were obese (defined as BMI > 30) versus 50% in the control group (Table 1). The majority of cancer patients (75%) had Stage I disease.

Using Pearson's correlation method we found that expression of IκB, TNFα, and IL-6 increases linearly with increasing BMI ($p = 0.019$, 0.009 and 0.028, respectively) independently of patient age. Linear regression curves for these cytokines and BMI (controlled for age) are shown in Figure 1. No statistically significant correlation was found between the expression of CD68 and leptin and BMI.

Using multivariable logistic regression model we found that none of the inflammatory markers that we tested (IκB, TNFα, IL-6 and CD 68) were predictive of the patients' cancer status when controlling for age and BMI (Table 2). This means that the levels of inflammatory markers were not significantly different in the omental tissue of endometrial cancer patients and controls of comparable age and BMI. Leptin, however, was found to have a very small, but statistically significant protective effect against endometrial cancer (odds ratio, OR = 0.92, Table 3).

Table 1. — *Basic demographics of the cancer and control groups*.

	Cancer patients n = 24	Controls n = 32	*p* value
Mean age (range)	61 (44-80)	51 (33-71)	0.001
Mean BMI (range)	36.6 (21.8-51.4)	31.4 (19.1-50.6)	0.013
% obese (BMI > 30)	83.3	50	
% smokers	25	22	
Cancer stage	Stage 1 n = 18 Stage 2 n = 1 Stage 3 n = 4 Stage 4 n = 1	Stage 0 n = 32	

Table 2. — *Logistic regression models for IκB, TNFα, IL-6 and CD68 (controlled for age and BMI)*.

Variable	Odds ratio [Exp(B)]	95% Confidence interval	*p* value
IκB	1.21	0.725-2.02	0.465
TNFα	0.921	0.671-1.26	0.609
IL-6	0.793	0.596-1.06	0.112
CD68	0.945	0.677-1.32	0.742

Table 3. — *Multivariable logistic regression model for endometrial cancer and leptin*.

Variable	Odds ratio [Exp(B)]	95% Confidence interval	*p* value
Leptin	0.92	0.84-0.97	0.041
Age	1.12	1.03-1.2	0.005
BMI	1.08	0.98-1.18	0.109

Discussion

The purpose of this study was to examine how the levels of inflammatory markers expressed by adipose tissue vary with increasing BMI and how they relate to the presence of endometrial cancer. A search for the link between obesity and cancer is warranted because of the impact that it has on cancer-related mortality. A prospective study of 900,000 US adults begun in 1982 with a 16-year follow-up found that deaths from cancer were 52% higher for males and 62% higher for females with a BMI > 40 compared to non-obese subjects [2]. Although increased risk was reported for multiple cancers, the relative risk of death from uterine cancer for non-smoking women was especially high at 6.25 in the study of Calle *et al*. [2].

In this study, we examined whether inflammation associated with excess adipose tissue is associated with endometrial cancer. We used TNFα, IL-6, IκB and CD68 as the markers of inflammatory response in the omental fat harvested from our patients. TNFα and IL-6 are inflammatory cytokines and I B is a protein responsible for inactivation of NF-κB. CD68 is a glycoprotein expressed on the surface of macrophages. The expression of this gene implies either infiltration of adipose tissue with macrophages of bone marrow origin or differentiation of pre-adipocytes into inflammatory cells. Both phenomena have been hypothesized in the literature [5, 7, 16].

We first looked at whether there was a correlation of the levels of gene expression for IκB, TNFα, IL-6 and CD68 in the omental tissue with BMI of the patients in the control group that underwent surgery for benign conditions. We did observe a statistically significant increase

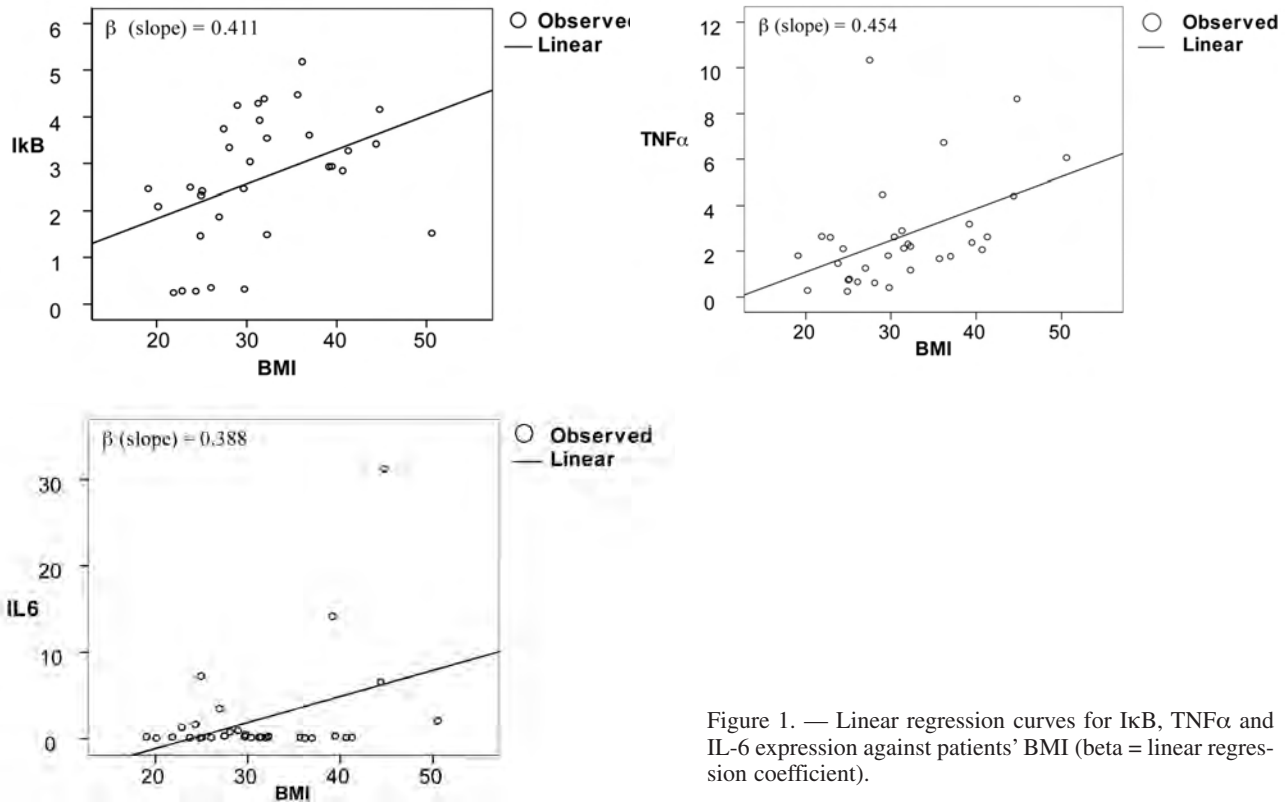

Figure 1. — Linear regression curves for IκB, TNFα and IL-6 expression against patients' BMI (beta = linear regression coefficient).

in the levels of IκB, TNFα and IL-6 associated with increase in BMI (Figure 1). Given the larger mass of adipocytes in the individuals with higher BMI, this upregulation of gene expression is expected to translate into even higher overall levels of these inflammatory cytokines.

TNFα and IL-6 have been shown by numerous studies to be potent activators of NF-κB family of proteins, some of which besides activating normal immune response have been shown to lead to cell growth and proliferation and thus act as oncogenes [8, 9]. There is also evidence that NF-κB can mediate metastasis and angiogenesis [8, 10] and thus play an important role at the later stages of cancer progression. NF-κB is constitutively active in a lot of human tumors including multiple myeloma, leukemias, breast and prostate cancers [8]. NF-κB is a downstream target of not only inflammatory agents, but also some common carcinogens, such as cigarette smoke [8]. Since the levels of inflammatory cytokines increase with increasing BMI, we suspected that endometrial cancer, which is highly associated with obesity, may show association with higher expression of TNFα, IL-6 and I B (a marker for the amount of NF-κB).

When the data on the expression of inflammatory genes were examined for the relationship to endometrial cancer, we did not see a statistically significant association of IκB, TNFα, IL-6 or CD68 expression by omental adipose tissue with cancer status of the patients (Table 2). Therefore we conclude that expression of inflammatory markers by adipose tissue within the peritoneal cavity is not elevated at least in the early stages of endometrial cancer since the majority of our patients had Stage I disease. However, with the data currently available, it is not possible to determine the significance of inflammatory cytokines for the long-term prognosis of these patients and whether or not there are subpopulations with higher expression of inflammatory genes and poorer clinical outcomes. Also, we only examined the gene expression profile in the omentum of our patients. The benefit of studying the omentum is that it is in a close physical proximity to the sites of gynecological malignancies. However, it is not known whether systemic inflammatory response associated with secretion of cytokines by peripheral fat deposits plays a role in cancer progression.

We also looked at the level of leptin expression in adipose tissue. Some studies have shown that leptin has angiogenic properties and as such may have important implications in cancer progression [13, 17], although these results have not been reproduced in all studies, particularly in vivo models [18]. Other molecular mechanisms by which leptin may promote cell proliferation have also been described. For instance, there is a study from China that looked specifically at proliferation of endometrial cells promoted by leptin-induced activation of COX-2 [19]. There also have been some clinical studies suggesting an association of leptin levels with cancer stage [20]. Ashizawa *et al*. in 2010 found that elevated serum leptin/adiponectin ratios were associated

with increased risk of endometrial cancer [21]. Interestingly, there is some evidence that leptin may be synergistic with estrogens as shown in a breast cancer model [22]. Contrary to the data from breast cancer, we found that leptin expression in omental fat appeared to have a mild protective effect against endometrial cancer in our patients (OR = 0.92, $p = 0.041$).

We conclude that obesity is associated with increased expression of certain inflammatory cytokines in adipose tissue. However, our study did not demonstrate any convincing evidence that increased levels of these inflammatory markers in adipose tissue of the omentum are associated with endometrial cancer.

Our study has important limitations. A relatively small sample size and bias towards early stages of cancer precluded us from stratified analysis according to cancer stage. Also, to really establish the role of cytokines secreted by excess adipose tissue, a study comparing adipose tissue from different depots would be helpful. The relation between obesity, cancer and inflammation may be more complex than could be uncovered in the context of a clinical study and further laboratory studies are needed.

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