RESEARCH ARTICLE

Lack of Association between Serum Adiponectin/Leptin Levels and Medullary Thyroid Cancer

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Abstract

Background: Adipokines are bioactive proteins that mediate metabolism, inflammation and angiogenesis. Changes in the secretion of key serum adipokines - adiponectin and letpin - may be associated with obesity, cancer and metabolic disorders. Thyroid cancer is one of the most important types of endocrine cancer. Therefore, investigating the association between serum levels of adiponectin and leptin and thyroid cancer might be important. The purpose of this study was to assess adiponectin and leptin levels in medullary thyroid carcinoma (MTC) cases in order to identify novel tumor markers. Materials and Methods: This research was based on a case-control study, including 45 patients with medullary thyroid cancer (21 men and 24 women) and 45 healthy controls (24 males and 21 females). Adiponectin and leptin levels were measured by ELISA in both groups. Height and weight were measured and body mass index (kg/m2) was calculated. Results: Adiponectin and leptin levels were not significantly different between medullary thyroid carcinomas and the control group. Also, there was no correlation among age and body mass index and the disease. Conclusions: These results suggest that changes in serum adiponectin and leptin levels do not play an important role in the diagnosis or could act as as biomarkers for medullary thyroid cancer.

Keywords: Adiponectin - leptin - medullary thyroid carcinoma - body mass index

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Introduction

Thyroid cancer is the most common endocrine cancer, comprised of four subtypes: papillary thyroid carcinoma, follicular, medullary, and anaplastic (Segev et al., 2003; Griebeler et al., 2013). In comparison with papillary and follicular cancer, medullary carcinoma has fewer outbreaks, but is the most aggressive (Hedayati et al., 2011, 2015). Unlike other cancers, a novel biomarker has not been identified for medullary carcinomas and the physician suspects MTC through physical examination, palpable nodules discovery and high levels of Calcitonin and genetic markers in accordance with the American Thyroid guideline, however owing to the some ambiguous problems around assay methodology, sensitivity, specificity, and cost effectiveness, the routine assessment of serum calcitonin remains controversial (Hedayati etal., 2006, 2015). Therefore, it is important to investigate novel biomarkers for the disease, with adipokines being prime candidates.

Adipose tissue is an active endocrine organ that release a number of biologically active molecules called adipokines and adipocytokines that impressed the whole body homeostasis (Ahima and Flier, 2000; Pontikides and krassas, 2007). The most abundant adipokines are leptin and adiponectin. They mediate hemostasis, cell proliferation, angiogenesis and inflammation. There is some possibility that these adipokines have a role in modulating the risk of cancer or increasing the risk of cancer progressing (Paz, 2011). Leptin levels are positively correlated with adipose tissue mass, and therefore increases in obesity (Uddin et al., 2010). Leptin bind to its cognate receptor (OBR) and activates several signaling pathways that are important pathways, including JAK / STAT, MAPK, PI3K/AKT, AMPK (Peso, 1997; Liang et al., 2002). These and other signaling pathways may use leptin acts as a growth factor to stimulate growth, migration and invasion of tumor cells, and therefore could play a role in the pathogenesis of thyroid cancer (Bank, 2000; Howard et al., 2010). Besides leptin, adiponectin levels are decreased in obesity and may also have a role in cancer (Barb et al., 2007).

Adiponectin is a type of adipokine derived from adipose tissue, and has two receptors called AdipoR1 and AdipoR2, which are found in abundance in skeletal muscle and liver but widely are expressed in a variety of normal and cancer cells as well (Kadowaki, 2005). Adiponectin binding to receptors and leads to increased uptake of glucose and fatty acid oxidation in muscle (AdipoR1) and decreased glucose production by the liver (AdipoR2)

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(Berg et al., 2002). This hormone also plays a role in the secretion of estrogen and insulin-like growth factor (IGF) that are important factors in increasing the risk of cancer (Kaklamani et al., 2008). Obesity, inflammation, insulin resistance, metabolic syndrome, cardiovascular disease and cancer have been shown to be significantly associated with adiponectin levels (Otvos et al., 2011). Several basic etiology studies have demonstrated that adiponectin has anti-tumor effects (Barb et al., 2007; Otvos et al., 2011). In one study, adiponectin levels are inversely associated with risk of breast cancer (Tworoger et al., 2007). Furthermore, adiponectin inhibits cell growth in colon and prostate cancer, and also can block cell cycle progression in colon cancer cells (Bub et al., 2006; Kim et al., 2010). Adiponectin and leptin both have critical effects on cell proliferation, apoptosis, cell invasion, angiogenesis and also regulated tumor formation (Paz, 2011). Fewer studies have been shown the relationship of adiponectin and leptin levels in patient with papillary thyroid cancer, and high levels of leptin and adiponectin reduction in the serum of these patients demonstrated (Mitsiades et al., 2011; Di Cristofano, 2013).

The association between leptin and adiponectin levels among the various types of thyroid cancer are still not well documented.

The aim of this study to achieve a more accurate understanding of the relationship between serum leptin and adiponectin changes in patients with medullary thyroid cancer in comparison with the control group. In this case-control study, for the first time, serum concentrations of leptin and adiponectin were evaluated in patients with MTC and compared to healthy subjects.

Materials and Methods

Patients who were referred to the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Science and Endocrine Research Center at Taleghani Hospital with written consent were used in this study. The case population consisted of 45 patients, including (24 females and 21 males, mean age 29.46±13.97 years) with medullary thyroid cancer (MTC). Diagnosis of MTC was confirmed by pathologists, also, 45 (21 females and 24 males, mean age 27.53±13.66 years) healthy people were selected as a control group from first-degree relatives of patients without thyroid disorders and with normal thyroid function tests (TSH: 0.3-3.5mIU/L, T4: 4.5-12.5µg/dl, T=Up: 25-35% and T3: 75-210ng/mL) with age, sex and BMI matched with each case group. Those individuals, who were using drugs affecting thyroid function and also obesity drugs, were excluded. This study was approved by an Institutional Review Board and Ethics Committee of Cellular and Molecular research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences.

Anthropometric profiles

Height was measured without shoes with Stadiometer (Seca, German company) with a sensitivity of 0.5 cm, and weight with light clothing and without shoes was measured by scale balance (Seca, German company) with a sensitivity of 250g for computing body mass index (kg/m^2). Body mass index is a measure of weight adjusted for height that was calculated by as weight (kg) divided by height2 (m) according to the World Health Organization. Demographic information such as age and sex were also recorded.

Blood collection and hormone assays

Blood samples were collected from both groups. For serum preparation, 3 ml of blood from a vein of the left arm in a fasting position was collected and incubated for 5 min at room temperature to clot, then centrifuged at 3000rpm for 10 minutes. The isolated serum samples were stored at -80^{O}C (Japan's Sanyo C Company) until assayed.

In groups, serum leptin and adiponectin levels were measured using ELISA according to the manufacturers' instructions. Human leptin and adiponectin hormones were determined by the sandwich ELISA. The kits used were prepared by the Sweden Company (Mercodia AB Company, Uppsala, Sweden). The sensitivity of adiponectin and leptin kits was 1.25ng/mL and 0.05ng/mL respectively. The ELISA reader was from Tecan Austrian Company and Sunrise Model.

Statistical analysis

Kolmogorov-Smirnov test were used to check the normality of samples. BMI and age data were normally distributed, but leptin and adiponectin were not, thus the logarithm of leptin and adiponectin was used for analyzing the data. Mean ± SD was used for data description. Independent t-test was used to examine differences in mean variables in patients and control groups. Also for checking differences of mean data between women and men, Independent t-test was used for both groups. Pearson correlations were calculated between each adipokine and variable (BMI and Age). All data was analyzed using statistical software (SPSS 19). A P-value <0.05 was considered statistically significant.

Results

Table 1 summarizes the demographic and anthropometric characteristics of all the subjects, as well as in each sex group separately. Means for age and body mass index, do not show significant differences. The results of adiponectin and leptin are given in Table 2. Although average levels of leptin in patients were higher than the control group, this difference was not statistically significant (Fig1). The levels of leptin between control (0.65±0.5 ng/ml) and patient (0.8±0.46 ng/ml) and adiponectin between control (0.8±0.14 μg/ml)

Table 1. Demography and Anthropometric Characteristics of Participants

Group	Sex	BMI(kg/m^2)	Age (year)	
Control	Female (21)	25.67±1	31.52±14.37	NS
	Male (24)	25.93±1.18	24.04±12.25	
Case	Female (24)	26.5±1.36	29.16±14.2	NS
	Male (21)	26.4±1.21	29.8±14.05	
P value (cases/controls)		0.141	0.509	

*NS indicates not significant, *Values are expressed as mean ± SD

Table 2. Serum Levels of Leptin and Adiponectin in Participants

Group	Sex	Adiponectin (μg/ml)	Leptin (ng/ml)	
Control	Female (21)	0.82±0.16	1.03±0.3	NS
	Male (24)	0.78±0.12	0.32±0.4	
Case	Female (24)	0.84±0.18	0.94±0.46	NS
	Male (21)	0.78±0.13	0.63±0.4	
P value(cases/controls)		0.849	0.162	

*NS indicated not significant, * Values are expressed as mean± SD

Table 3. Pearson Correlation Coefficient between Adiponectin and Leptin and Independent Variables (BMI and Age)

	Leptin		Adiponectin	
	(r)	(p)	(r)	(p)
Age (year)	0.278	0.046	` -0.397**	0.007
BMI (kg/m^2)	`-0.070	0.647	0.175	0.250
Leptin (ng/ml)			-0.087	0.568

**Correlation is significant at the 0.01 level

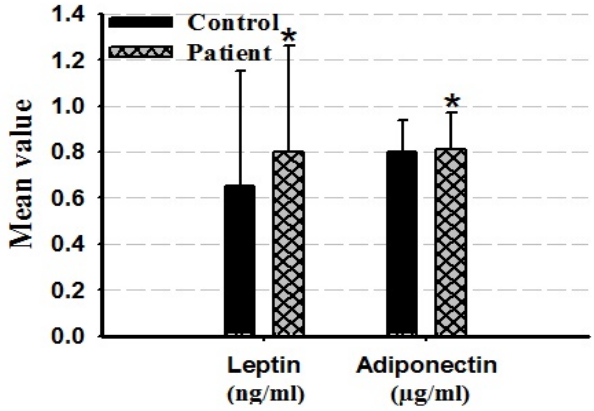

Figure 1. Comparison of Mean Serum Leptin and Adiponectin Concentrations between Control And Patients (Medullary Thyroid Cancer). Asterisk indicates no significant differences between patients and controls

and patient (0.81±0.16 μg/ml) did not show significant differences (P>0.05). But a significant difference (P<0.05) was observed in Leptin hormone levels in males between healthy and cancer groups (0.32±0.4ng/ml versus 0.63±0.4ng/ml, P=0.013). As shown in Table 3, adiponectin in the patient group was inversely significant correlated with age (r = -0.397, P<0.01). This means that in the patients group (medullary thyroid cancer) with increasing age, the levels of adiponectin in serum significantly decreased.

Discussion

Leptin and adiponectin are adipokines that are substantially produced by white adipose tissue, and play important roles in energy hemostasis, immune regulation and weight adjustment (Ahima, 2006), and also may have a role in the pathophysiology of cancer progression (Paz-Filho, 2011). A number of etiology studies have tested the relationship between adipocytokines and cancer, however conflicting results were obtained (Paz, 2011, Booth et al., 2015). Although these data are not conclusive, overall they suggest that some of adipocytokines, inhibit carcinogenesis and whilst others contribute to cancer progression (Booth et al., 2015). The propose of this study was to investigate the relationship between changes in serum leptin and adiponectin levels with increased risk of medullary thyroid carcinoma. Leptin is 16kD adipokine with a 167-amino acid that is produced by adipose tissue, which can also be released from other sites such as the intestine, stomach, liver and skeletal muscle (Baratha, 2002). Leptin acts through its receptor (ob, R) and mediates signaling related to oncogenesis via major pathways like PI3K/AKT (phosphatidylinositol 3-kinase/protein kinase). AMPK (5' AMP-activated protein kinase) and IRS (insulin receptor substrate). Leptin stimulates growth, migration and invasion in tumor cells (Fruhbeck, 2006). Several studies demonstrate relationships between leptin and thyroid cancer: in one study, leptin and its receptor are expressed in papillary thyroid cancer (Cheng et al., 2010). In another study, leptin stimulated cell proliferation and inhibited apoptosis of papillary carcinoma cells, via activation of PI3K/Akt (Uddin et al., 2010).

In a study from the Obesity Research Center and the Institute of Endocrinology and Metabolism Shahid Beheshti University of Medical Sciences, Tehran, the correlation between papillary thyroid cancers and leptin were examined. The findings shown a significant increase in serum leptin concentrations in these patients compared with controls (Hedayati et al., 2011). In a study at the Ankara Hospital Research Center, 43 patients with papillary thyroid cancer were reported to have serum leptin concentrations that were significantly higher than the control (Akinciet al., 2009). This was also found in the current study, where no significant differences were observed between serum leptin concentrations and medullary thyroid cancer. Although serum leptin concentrations of women in both groups were higher than that of men, this was not statistically significant and is likely related to larger proportion of adipose tissue in women relative to men. Adiponectin is a 30-kD adipokine that is expressed by adipocytes and modulates a number of metabolic processes including glucose uptake and fatty acid oxidation (Berg et al., 2002; Kadowaki, 2005). Additionally, adiponectin is inversely associated with insulin resistance and obesity, and may have a role in inflammation, metabolic disorder and cancer (Park and Scherer, 2011). Adiponectin can influences cells through pathways regulated by JNK (jun N-terminal kinase), STAT3 (signal transducer and activator of transcription 3), PPARs (peroxisome proliferator-activated receptors) and can act on tumor cells directly (Mandal et al., 2011).

The association between adiponectin and thyroid cancer is still unknown, however, one study reported that patients with papillary thyroid cancer have reduced levels of circulating adiponectin (Mistsiades et al., 2011). In the current study, 90 persons were matched for age, sex, and BMI. Serum adiponectin of patients with medullary thyroid cancer and healthy groups were measured - the obtained data showed that adiponectin level were not significantly different between the groups, which is in agreement with studies on prostate cancer (Steven et al., 2014), pancreatic (Pezzilli et al., 2010) and colorectal cancer (Danese et al., 2013).

These results have a different and a new key link between serum levels of leptin and adiponectin with thyroid cancer. Obtained data indicate that changes in the levels of adiponectin and leptin do not play an important role in diagnosis or risk factor in medullary thyroid cancer.

The main limitation of this study was that the impact of genetics and ethnicity that was not specified or controlled. Secondly, medullary thyroid cancer incidence has low prevalence that due to the low number of samples to be restricted. Therefore, prospective studies with larger sample sizes would allow for a better understanding of the relationship between adiponectin and leptin with thyroid cancer progression.

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