RESEARCH ARTICLE

Medullary Thyroid Carcinoma - Circulating Status of Vaspin and Retinol Binding Protein-4 in Iranian Patients

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Abstract

Background: Vaspin and Retinol binding protein-4 (RBP4) are new adipokines mainly produced by adipose tissue. Considering that medullary thyroid carcinoma (MTC) is a malignant neuroendocrine tumor, and to date the relationship between serum levels of vaspin and RBP4 with MTC has not been studied, in this matched case-control study we evaluated their possible significance to this tumor type. Materials and Methods: A total of 45 patients with MTC (21 males and 24 females) and 45 healthy persons as a control group (24 males and 21 females) were selected. The two groups were matched for age, sex and body mass index. Serum Vaspin and RBP4 levels were measured by enzyme-linked immunosorbent assay (ELISA) methods in both groups. Also, weight and height were measured and body mass index was calculated too. Results: In total, patients with MTC had significantly higher serum vaspin levels compared to the controls (0.52ng/ml vs. 0.45ng/ml, P=0.0241). However, no significant difference was found in serum RBP4 concentrations between the patients with MTC and the controls (15.2±2.55 μg/ml versus 15.1±3.34 μg/ml, p>0.05). Conclusions: The results of this study demonstrated that serum RBP4 levels in MTC patients are not significantly different from those found in healthy individuals and did not correlate with MTC. On the other hand, higher levels of serum vaspin are associated with an increased risk of MTC. Thus Vaspin may be a novel and promising biomarker for diagnosis or confirmation of MTC in conjunction other specific tumor markers.

Keywords: Vaspin - retinol binding protein 4 - adipokine - medullary - thyroid carcinoma

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Introduction

During the past decade, Studies suggest that adipose tissue is not only a fat depot, storing energy and regulating energy balance, but also as an active endocrine organ has a major role in the secretion of a large number of bioactive peptides, collectively called Adipokines or Adipocytokines (Ouchi et al., 2011; Bluher, 2014). Adipokines play important roles in the modulation of a number of signaling cascades in target tissues. In addition, they have a role in obesity, blood pressure, glucose and lipid metabolism, insulin resistance, type 2 diabetes, inflammation and atherosclerosis in a paracrine and/or endocrine manner (Sahin-Efe et al., 2012; Jung and Choi, 2014). Also, the relationship between Adipokines and several types of cancer has been documented (Ghaemmaghami et al., 2013). In recent years, the number of identifying Adipokines has been increasing, such as Leptin, Apelin, Visfatin, Vaspin, Chemerin, Omentin, Adiponectin, Retinol binding protein-4, Angiotensinogen, Serum amyloid A, Zinc-alpha2-glycoprotein (Sahin-Efe et al., 2012; Bluher, 2014).

According to the importance of cancer on the one hand, and the influence of Adipokines on many diseases such as cancer, on the other hand, relationship between Adipokines and cancer has recently become a hot topic. S'pome Adipokines such as Leptin (Hedayati et al., 2011), Resistin (Lee et al., 2012; Ghaemmaghami et al., 2013) and Visfatin (Ghaemmaghami et al., 2013) have been documented to be influential in cancer biology.

Vaspin (visceral adipose tissue-derived serine protease inhibitor), is a 50kDa novel insulin-sensitizing Adipokine that was originally isolated from the visceral white adipose tissues of obese, diabetic Otsauka Long-Evans Tokushima Fatty (OLETF) rats (Hida et al., 2005). Vaspin mRNA is expressed in human visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) (Kloting et al., 2006). Vaspin mRNA expression in adipose tissue and serum Vaspin levels is positively associated with BMI (Lee et al., 2011; Saboori et al., 2013) and insulin sensitivity (Wada, 2008; Jian et al., 2014). In the other word, elevated levels of Vaspin were reported in obese subjects and patients with type 2 diabetes mellitus compared to healthy subjects, suggesting its compensatory role in human obesity related

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diseases and insulin resistance (Kloting et al., 2006). Also, studies have shown changes in serum Vaspin levels correlated with metabolic syndrome (Mirzaei, 2013), nonalcoholic fatty liver disease (NAFLD) (Aktas et al, 2011), coronary artery disease (CAD) (Kadoglou et al., 2011). A limited number of studies have investigated the relationship of Vaspin and cancer. In one study, Fazeli et al, investigated the association of Vaspin with colorectal cancer (Fazeli et al., 2013) and in another study; Erdogan et al, have examined the relationship between Vaspin and endometrial cancer (Erdogan et al., 2013).

Retinol-binding protein 4 (RBP), is an another novel Adipokine that belong to the lipocalin family of proteins transporting small hydrophobic molecules (Flower, 1996). RBP4 mainly produced by the liver and mature, lipid-laden adipocytes (Tsutsumi et al., 1992). RBP4 encoding gene is located on chromosome 10 (10q23-q24), a region that has been linked to elevated fasting blood glucose and increased risk for type 2 diabetes in different populations (Duggirala et al., 1999; Meigs et al., 2002). This gene encodes a protein with a molecular mass of 21 kDa and 201 amino acids (Colantuoni et al., 1983). RBP4 is a specific carrier of retinol (vitamin A) in circulation and transports retinol from the liver to peripheral tissues (Newcomer and Ong, 2000). It has been reported that elevated RBP4 levels were positively correlated with body mass index (BMI), impaired glucose tolerance, insulin resistance, Type 2 diabetes mellitus, metabolic syndrome cardiovascular diseases (Kotnik et al., 2011; Cheng et al., 2014). Studies also showed that RBP4 has a role in several types of cancers such as pancreatic, ovarian, squamous, colorectal and prostate cancer (Fabris et al., 1984; Putzki et al., 1990; Patz et al., 2007; Tsunoda et al., 2009; Abulaizi et al., 2011; Lorkova et al., 2012; El-Mesallamy et al., 2013; Uehara et al., 2013; Cheng et al., 2014).

Thyroid carcinoma is the most common neoplasm of the endocrine system which account for approximately 1-5% of all human cancers. Medullary thyroid carcinoma (MTC) is a rare malignant neuroendocrine tumor that originates from calcitonin (CT) producing parafollicular C of the thyroid gland (Alvandi et al., 2011). MTC represents 5% to 10% of all thyroid neoplasms and responsible for approximately 13% of all thyroid cancer-related deaths (Alvandi et al., 2011; Hedayati et al., 2011). MTC occurs in both sporadic (SMTC) and hereditary (HMTC) forms. Approximately 75% of MTC cases occur in the more common sporadic form, whereas hereditary form accounts for 25% of all MTC cases (Alvandi et al., 2011; Hedayati et al., 2011). Hereditary form of MTC can be classified into three clinical distinct forms: multiple endocrine neoplasia type 2A (MEN2A), type 2B (MEN2B) and familial medullary thyroid carcinoma (FMTC). Germ-line mutations of REarranged during Transfection (RET) proto-oncogene is responsible for HMTC form. RET gene, which is located on chromosome 10q11.2, consists of 21 exons and encodes transmembrane receptor tyrosine kinase protein that is expressed in tissues and with neural crest origin (Hedayati et al., 2006; Alvandi et al., 2011; Hedayati et al., 2011; Majidi et al., 2011).

The aim of this study was to investigate the relationship between changes in serum Vaspin and RBP4 levels with increased risk of medullary thyroid carcinoma. In this case-control study, for the first time, we evaluated the serum concentrations of Vaspin and RBP4 in patients with MTC and compare them with healthy subjects.

Materials and Methods

Patients and controls:

The case population consisted of 45 individuals, (24 females and 21 males, mean age 33.4±10.9 years) who had been histopathologically diagnosed with MTC. They had undergone total thyroidectomy and were referred to the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Science. Also, we selected a healthy age, sex and BMI matched control group (21females and 24 males, mean age 32.2±10.0 years) from first-degree relatives of patients without thyroid disorders and 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).

The clinical tests were performed by endocrinologists and the diagnosis of MTC were confirmed by pathologists. Written informed consent was obtained from all subjects, prior to the collection and analysis of blood samples. This study has been approved by the Institutional Review Board and Ethics Committee of Cellular and Molecular research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences.

Physical measurements

Anthropometric parameters, including weight and height of patient and control groups, were obtained from weight and height measurement by calibrated height measuring scaled balance (Seca, German company). Participants' height and weight were measured in light indoor clothing with shoes removed. Weight with 250g and height with 0.5cm sensitivity was reported. These data were used to calculate body mass index (BMI). 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 characteristics, including sex and age were also recorded. Those individuals, who were using drugs that can affect the thyroid functions, were excluded.

Biochemical measurements

Blood samples were collected from all of the participants. Three ml of venous blood was obtained from an antecubital vein after the groups rested in sitting position. For preparation of sera, blood samples were collected in a sterile test tube, allowed to clot on the bench

Table 1. Demographic and Anthropometric Characteristics of the Patients with Medullary Thyroid Carcinoma and Controls

Variable	Cases (n=45)	Controls (n=45)	P value
Female/male ratio	24/21	21/24	0.532
Age (years)	33.4±10.9	32.2±10.0	0.708
BMI (Kg/m^2)	26.2±1.3	25.8±1.1	0.141

Table 2. Serum Vaspin and RBP4 Levels in the Patients with Medullary Thyroid Carcinoma and Controls

Variable	Cases	Controls	P value
Vaspin (ng/ml) (median(min-max)	0.52 (0.29-1.69)	0.45 (0.22-1.31)	0.0241
RBP4 (μg/ml) (Mean ±SD)	15.24±2.55	15.05±3.34	p>0.05

at room temperature, and then centrifuged at 3,000 rpm for 10 min. The sera were aliquoted into 1 ml Eppendorf microtubes and kept frozen at -800c until tested.

Serum Vaspin levels were measured by enzyme-linked immunosorbent assay (ELISA) method in both patient and control groups. Research Human Vaspin ELISA Kits were provided from CUSABIO Biotech Co., Wuhan, China. Serum Vaspin concentrations were determined on the basis of the sandwich type ELISA method according to the manufacturer's instructions by an ELISA microplate reader (Tecan Sunrise, Tecan Austria). The sensitivity of the human Vaspin kit was 0.78pg/ml. Intra assay Coefficient of Variation for the Vaspin assay was 7.9%.

To detect RBP4 in the serum samples, quantitative ELISA method was used by Research Human RBP4 ELISA kit (CUSABIO Biotech Co., Wuhan, China), with the sensitivity of 0.1μg/ml according to the manufacturer's instructions. Intra assay Coefficient of Variation for the RBP4 assay was 5.9%.

Statistical analysis

Normal distribution of data was evaluated by the Kolmogorov-Smirnov (KS) test. All data were in normal distribution except Vaspin. Comparisons of variables with a normal distribution were made using the Independent t-test between two groups, and values were provided as mean ±SD. For parameters with an abnormal distribution, the Mann-Whitney U test was used for comparisons, and values were given as median (minimum-maximum). Statistical data analyses were performed using MedCalc

Results

Table 1 shows the general characteristics, including anthropometric measurement and demographic profiles of patients with MTC (45 subjects) compared to the control group (45 subjects). There were no significant differences in sex (P=0.532) and age (P=0.708) between the patients and controls. Also, there was no significant difference in BMI between both groups (P=0.141).

Serum Vaspin levels in patient and control groups as median (minimum-maximum) are shown in Table 2. As shown in the table, patients with MTC had significantly higher serum Vaspin levels compared to the controls (0.52 *vs*. 0.45ng/ml, P=0.0241).

In addition, serum concentrations of RBP4 in patients with MTC and controls as Mean ±SD. are shown in Table 2. As the table shows, serum RBP4 concentrations in MTC patients were not significantly different from control subject (subjects) (15.24±2.55μg/ml *vs*. 15.05± 3.34μg/ml, p>0.05).

Discussion

Medullary thyroid carcinoma (MTC) is a rare malignant tumor, but clinically significant tumor yet (Alvandi et al., 2011; Ghazi et al., 2014; Sheikholeslami et al., 2014; Yeganeh et al., 2015; Zarif-Yeganeh et al., 2015). In this study, we evaluated the relationship of serum Vaspin and RBP4 levels with medullary thyroid carcinoma.

VAT-derived serine protease inhibitor (Vaspin) is a novel Adipokine expressed in visceral WAT containing 392-395- amino acids that display 40% homology with α1-antitrypsin. Vaspin is expressed not only in human visceral and subcutaneous white adipose tissue, but also in liver, pancreas, stomach and hypothalamus is expressed (Bluher, 2012). Hida et al, for the first time showed that tissue expression and serum levels of Vaspin were increased at the peak of obesity and insulin resistance and decreased with the aggravation of diabetes and weight loss in OLETF rats (Hida et al., 2005). Also, they reported that the administration of Vaspin to OLETF rats improves glucose tolerance and insulin sensitivity (Hida et al., 2005). In obese human, Vaspin mRNA expression was detectable in both visceral and subcutaneous white adipose tissue, whereas it was undetectable in visceral and subcutaneous white adipose tissue of lean human (Kloting et al., 2006; Lee et al., 2011). A number of studies have reported that serum Vaspin levels are higher in obese human than group with normal weight (Saboori et al., 2013; Feng et al., 2014). A series of studies have recently shown that serum Vaspin levels are increased in patients with T2DM in comparison to individuals with normal glucose tolerance. Also, expression of Vaspin mRNA has been detected in patients with type 2 diabetes mellitus whereas was undetectable in normal glucose tolerant subjects (Feng et al., 2014; Jian et al., 2014). It has been postulated that expression of Vaspin mRNA in human white adipose tissue and its increased serum levels in obese individuals might be a compensatory mechanism to obesity, insulin resistance and T2DM (Kloting et al., 2006).

There are limited studies about the relationship of Vaspin with cancer. Recently Erdogan et al, reported that low serum Vaspin levels were associated with an increased risk of endometrial cancer in postmenopausal women (Erdogan et al., 2013). Fazeli et al, reported that colorectal cancer patients had higher serum levels of Vaspin in comparison to the control group (Fazeli et al., 2013). In the present study, for the first time, we showed that serum Vaspin levels were higher in patients with MTC compared to the control group. Therefore, Vaspin may have a role in carcinogenicity and cancer development. However, to date, there are no publications related to this matter and detailed mechanism how Vaspin can be involved in carcinogenicity remains to be determined at molecular levels. Thus, further studies are needed to investigate the

key molecular mechanisms involved in carcinogenesis and cancer development.

Recently, a number of studies documented that Vaspin has anti-apoptotic effects. Jung et al. (2011) demonstrated that Vaspin protects vascular endothelial cells from apoptosis induced by free fatty acids via activation of the PI3-kinase/Akt signaling pathway. Zhu et al. (2013) showed that Vaspin decreased expression of Bax and increased expression of Bcl-2 in human osteoblasts (hOBs). Also, they showed Vaspin protects hOBs from apoptosis by activating the MAPK/ERK signaling pathway. Another study has reported that Vaspin can inhibit endothelial cell apoptosis induced by Methylglyoxal by inhibition of caspase-3 via suppression of NADPH oxidase derived ROS generation. Therefore, it was concluded that Vaspin can activate PI-3 kinase/Akt and MAPK/ERK signaling pathways and prevent apoptosis, so it can have a role in cell proliferation and cancer development (Phalitakul et al., 2013).

Retinol binding protein 4 (RBP4) is a recently identified adipokine. RBP4 circulates in blood in complex with transthyretin (TTR) (Kotnik et al., 2011). STRA6, stimulated by retinoic acid gene homolog 6, is high-affinity cell surface receptor for RBP4 that acts as a retinol transporter in the membrane and mediates retinol uptake. STRA6 is highly expressed in brain, kidney, eye, testis, adipose tissue, spleen, and blood-organ barriers. RBP4 binds to STRA6 after dissociation from TTR (Nair et al., 2010). Kahn et al. for the first time discovered the role of RBP4 in obesity and insulin resistance (Abel et al., 2001). Previous investigation showed that individuals with a BMI of 40.0 and above had a higher rate from all cancers than those with normal weight (Cheng et al., 2014). In addition to obesity, metabolic syndrome and insulin resistance associated with increased risk of occurrence and mortality of many cancers (Wang et al., 2011). It has been demonstrated that RBP4 play an important role in obesity, insulin resistance, Type 2 diabetes mellitus and metabolic syndrome (Kotnik et al., 2011; Cheng et al., 2014) indicating that RBP4 also may play a role in inflammation and cancer.

In our study, we found no differences in the serum RBP4 levels between two groups. Alternations in serum levels of RBP4 have been reported in patients with cancer of different origins (Fabris et al., 1984; Putzki et al., 1990; Patz et al., 2007; Tsunoda et al., 2009; Abulaizi et al., 2011; Lorkova et al., 2012; El-Mesallamy et al., 2013; Uehara et al., 2013; Cheng et al., 2014). Studies showed altered serum levels of RBP4 in pancreatic cancer (Fabris et al., 1984; Abulaizi et al., 2011; El-Mesallamy et al., 2013). Also, the results of investigations reported that serum RBP4 is a potential new biomarker of ovarian cancer (Lorkova et al., 2012; Cheng et al., 2014). Tsunoda et al, reported RBP4 methylation in esophageal squamous cell carcinoma (Tsunoda et al., 2009). The result of Putzki et al, study showed significant decrease in serum RBP4 levels in patients with colorectal cancer (Putzki et al., 1990). Indeed, proteome analysis shows that serum RBP4 is a potential new biomarker of lung (Patz et al., 2007) and prostate cancer (Uehara et al., 2013). We concluded that serum RBP4 levels in MTC patient are not significantly different from those found in healthy individuals. Thus, the serum RBP4 levels in these patients do not appear useful biomarker for diagnosis or confirmation of MTC beside in other specific tumor markers. According to the results of this study, increased serum Vaspin levels in Iranian patients with medullary thyroid carcinoma can be used as a valuable marker for diagnosis or confirmation of medullary thyroid carcinoma. Several studies have suggested that Adipokines such Vaspin can be considered as a candidate for the treatment of obesity, but its side effects are carefully examined (Kloting et al., 2011; Bluher, 2014). As mentioned earlier, because Vaspin can up-regulate PI-3 kinase/Akt and MAPK/ERK pathways, and also, aberrant up-regulation of these pathways can be involved in the pathogenesis of diseases such as cancer, so the use of this Adipokine for the treatment of obesity and its related diseases are needed comprehensive prospective studies.

However, our study had some limitations. First, because of the relatively small sample size, it is probably not be representative of the general population. Second, in this study, the insulin resistance was not measured in both groups. Finally, waist-to hip ratio (WHR) in the studied population was not measured. Also, the measurement of further Adipokines should be investigated in order to clarify the role of Adipokines in MTC and use them as a tumor marker.

In conclusion, there was no significant difference in circulating RBP4 levels between MTC patients and controls. Therefore, serum RBP4 may not be a biomarker for MTC. In contrast, we observed that higher serum Vaspin levels were associated with an increased risk of medullary thyroid carcinoma. Also, increased serum Vaspin levels in patients with MTC in comparison to healthy subjects potentially suggest that Vaspin can be a novel and promising biomarker of MTC. But, the exact molecular mechanism by which Vaspin involved in MTC carcinogenesis should be investigated in future comprehensive studies.

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