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Bone gamma-carboxy glutamic acid-containing protein and Retinol Binding Protein-4, are they different in medullary thyroid carcinoma patients?

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Abstract:

Background: Medullary thyroid carcinoma (MTC) is the third most common of all thyroid cancers (5-8%). Osteocalcin (OC), is a bone protein which is synthesized by osteoblasts. Retinol Binding Protein-4 (RBP-4) is an adipokine in the circulation. Adipokines could regulate inflammation, immunity and carcinogenesis. The aims of this study were analysis the correlation between MTC and plasma levels of OC and RBP-4.

Material & methods: Forty six MTC patients and 44 individuals as control group were studied. The mean age of cases was 34±11.3 years old (Mean ±SD) and in control group was 38±9.3. After informed consent, 10 ml of blood from the antecubital vein obtained and plasma was isolated. The plasma OC and RBP-4 concentration were measured by sandwich ELISA method. Obtained results were analyzed by SPSS version 16 with independent t-test method.

Results: The plasma level of OC concentration were 33.1±3.5 and 12.5±1.2 ng/ml (Mean ±SD) and Odds Ratio (OR) value was 1.0 among patients and control group respectively 1.04. In patients, mean plasma level of RBP-4 was 82.5±2.7 and in control group was 22.8±1.6 μg/ml and OR value was 2.1. The confidence interval was 95%. These differences of plasma levels were statistically significant (P= 0.001).

Conclusion: This study has shown differences between plasma level of OC and RBP-4 in two mentioned groups. These increased level were observed in both gender and different ages, it may be consider that plasma concentration of OC and RBP-4 had potency for helping in diagnosis or confirmation of medullary thyroid carcinoma across the other markers.

Key words: Medullary thyroid carcinoma, OC, RBP-4.

Introduction:

Thyroid carcinoma accounts for 1% of all Human cancers [1]. Thyroid carcinoma is the most frequent malignant tumor of the endocrine system [2]. Medullary thyroid carcinoma (MTC) derives from Para follicular C cells and may developed in either sporadic (75%) or autosomal dominant hereditary form (25%)[3, 5, 11]. Osteocalcin (OC), also known as a bone gamma-carboxy glutamic acid-containing protein (BGLAP), is a non-collagenous protein found in bone and dentin [6-8]. This protein is a 49-residue polypeptide (5.8kDa), highly conserves in different species during the evolution [9]. In human, OC gene is located on 1q25-q31, and regulated at transcriptional level by 1, 25 dihydroxy vitamin D_3 [10]. This molecule consists of three parts, a 23-residue signal peptide that is cleaved during translation, a 26- residue propertied that targets the protein for carboxylation and a 49-residue mature protein [9, 11]. Retinol Binding Protein-4 (RBP-4) is a 21 k.Da protein that was first reported to be an adipokine in 2005 [7]. The RBP-4gene is located on chromosome 10 (10q23–q24)[4], and encodes a protein of 201 amino acids with molecular mass of 21 k.Da. Liver has the highest expression level of RBP-4, however, adipose tissue has the second highest rate of expression [3, 6, 22]. Some studies have shown that plasma level of OC is higher than in patients with thyroid diseases and patients with some types of thyroid carcinoma with distant metastases to bone tissue [17, 18].Calcitonin-secreting cells are involved in bone metabolism and calcium homeostasis, so OC concentrations can be affected so on. Researchers suggest that between plasma levels of OC with adiopkine such as Adiponectin and Leptin are related significantly, so adipokines concentration are associated with bone dynamics. In this study, to investigate the correlation between thyroid malignancies with bone and adipose tissues metabolism, we evaluated the plasma level of OC and RBP-4 in patients with MTC.

Material & methods:

Patients: The case population consisted of 46 individuals, (22 males and 24 females) with MTC. They were referred to the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Science, Tehran, Iran. Also, 44 persons were selected as control group (20 male and 24 females) from referred to the laboratory with normal thyroid function tests (TSH: 0.3 =3.5 mIU/L, T4: 4.5=12.5µg/dL, T=Up: 25=35% and T3: 75=210 ng/mL) with age, sex, BMI matched with case group. Both groups were also matched for age and sex. The clinical examination was performed by endocrinologist. The diagnosis of MTC was confirmed by pathological documents. Patient inclusion criteria were: the presence of MTC based on pathology specimens confirmed. Exclusion criteria existed other diseases of MTC, according to a specialist or use of drugs other than thyroid medication or have abnormal body mass index (BMI). Inclusion criteria for the control group as well as normal thyroid tests and not taking certain medications several days before the sampling. Exclusion criteria included healthy individuals being affected by a particular disease; abnormal tests, he or having an abnormal BMI.

Sampling &Methods: For preparation of plasma, 10mL of whole blood was collected in EDTA treated tubes, from antecubital vein in sitting position. Then, plasma separated by 10min centrifugation at 3000rpm and the obtained plasma were aliquted in three 0.5mL micro tubes. The isolated plasma samples from each individual were stored in 1mL eppendorf micro tubes at deep freeze. In both groups, OC and RBO-4 hormone was measured by sandwich ELISA method (E90471Hu with 0/078 ng/ml sensitivity for OC) (CSB-E 09423h with0.1µg/ml sensitivity for RBP-4). ELISA method results were recorded by ELISA reader (Sun Rise Model, Tecan Co. Austrian).

Statistical Analysis: All the data were in normal distribution. The independent t-test was used to compare means of variables between two groups. Further, data was analyzed using statistical software (SPSS 16), and significant level was considered at0.05.

Results:

Altogether, 90 individuals, including 46 females and 44 males, participated in this study. Among these, 46 individuals were diagnosed with MTC and 44 individuals were control groups. The mean age of cases was 34±11.3 (Mean±SD) years and in controls was 38±9.3. As can be seen in Table 1, analysis of OC and RBP-4 plasma levels in MTC patients and controls has shown that levels of these hormones in patients are higher than comparison control group. The statistical analysis demonstrated that the differences between two groups were significant ($p \leq 0.001$) and Odds ratio 1.04 for OC and 2/1 for RBP-4 were obtained.

Discussion:

In this study we showed that plasma levels of OC and RBP-4 in patients with medullary thyroid carcinoma are higher than in control group subjects. These increased levels were also seen in males and females affected with medullary thyroid carcinoma. As these increased levels were observed in both gender and different ages, so they could be related to medullary thyroid carcinoma and they are independent of sex and age.

Additional studies were performed to indicate the role of bone as an endocrine tissue [19, 20]. For example it was shown that hyperthyroid patients have high bone turnover of formation [21]. Another research demonstrated that increasing in OC level caused increasing in Leptin [22]. Toivonen J et al, had shown identified patients with differentiated thyroid carcinoma of whom have high bone turnover when use LT4 as a suppressive drug [23].

The plasma level of OC increases in hyperparathyroidism and decreases in hypoparathyroidism [21-24], This increase in plasma level of OC is probably related to possible effect of thyroid hormones (T3, T4 and Calcitonin) on osteoblasts, since the thyroid hormones are important hormones in body metabolism [19]. On the other hand, OC is the most important hormone derived from bone tissue and has a lot of metabolic effects [7]. So because of thyroid hormone importance on metabolism and its effect on bone formation, changes in thyroid condition and thyroid hormones may result in changes in the circulating OC level [21].

Plasma levels of RBP-4 positively correlate with retinol levels[7, 24],Therefore,

subject's retinol status can influence circulating RBP-4 levels. RBP-4 does not interact only with retinol, produced a complex with transthyretin to prevents glomerular filtration of RBP-4 through the kidney [3]. Serum RBP-4 levels are increased with body mass index (BMI) in obese subjects[7, 24]. Besides increased BMI, increased circulating RBP-4 levels have a relationship with increasing visceral adipose tissue content [5, 8, 11]. RBP-4 was recently reported to be expressed by adipose tissue and associated with insulin resistance and components of the metabolic syndrome in patients with T2DM, IGT or obesity [1]. Takashima N et al showed that plasma RBP-4 and retinol levels were lower, whereas RBP-4/retinol ratio was elevated in patients with T2DM compared to controls [13]. LIM et al on 2008 shown that in patients with hypothyroidism their plasma level of RBP-4is increased [6]. In another research by Wu H et al,observed the level of RBP-4 in patient with degree of obesity are higher than normal subjects and in both gender male and female is appropriated with fatty mass [2]. In Alexander Tschoner et al study observed that in patients who remove their body fat with surgery, the RBP-4 level were decreased [11]. The increase in plasma level of RBP-4 is probably related to effect of thyroid hormones (T3, T4 and Calcitonin)on adipocytes, because the thyroid hormones are important hormones on fatty metabolism [2]. In adipose tissue, changes in thyroid status result in alterations of lipolysis capacity. Regulation of fat mass is controlled by multiple neuroendocrine signals. Thyroid hormones influence adipose tissue development and metabolism. Hyperthyroidism induces a transient hyperplasia participate to cell size reduction, whereas an opposite pattern is observed during hypothyroidism. T3 modulates both proliferation and differentiation of adipocytes [1, 5]. In humans, hyperthyroidism enhances and hypothyroidism decreases lipolysis through different mechanisms.

Strongly high OC and RBP-4 levels in medullary thyroid cancer patients in comparison with control group subject potentially suggest OC and RBP-4 as a protein marker of medullary thyroid carcinoma. It means that osteoblast and adipose tissues secreted hormones, proteins, and peptides potentially may have the application in diagnosis, confirmation, and/or treatment follow up.

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Table 1. Comparison results of OC & RBP-4 plasma levels in MTC patients with controls group

Subjects	***Case***		***Control***		***P-Value***
	Male	***Female***	***Male***	***Female***	
Frequency	22	24	20	24	-
	Total: 46		*Total*: 44		
OC levels(ng/ml)	32.2	34	12.04	13.1	0.0001
	Mean: 33.1		*Mean*: 12.5		
RBP-4 level (µg/ml)	85.7	79.4	24.2	21.5	0.0001
	Mean: 82.5		*Mean*: 22.8		