SERUM LEVEL OF DIPEPTIDYL PEPTIDASE-4 AS A POTENTIAL BIOMARKER FOR MEDULLARY THYROID CANCER

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Aim: Adipokines are the proteins secreted from adipose tissue and play an important role in the control of metabolism. Dipeptidyl peptidase-4 (DPP4) is a novel adipokine with different biological role. As indicated by various studies, serum levels of DPP4 had been associated with body mass index (BMI), insulin resistance, metabolic syndrome and malignancy. The aim of this study was to assess the serum levels of DPP4 in patients with medullary thyroid cancer (MTC) in comparison with these in the control group. Materials and Methods: This study was performed on 45 MTC patients (24 females and 21 males) and 45 healthy controls (21 females and 24 males). DPP4 and insulin serum levels were measured by ELISA, fasting glucose serum levels by enzym calorimetric method and insulin resistance index (HOMA-IR) by calculation using relevant equation. BMI (kg/m²) was also calculated. Results: Our data did not demonstrate a significant difference between serum DPP4 levels in MTC and healthy group (41.06 ± 22.08 ng/ml vs 39.94 ± 20.77 ng/ml, p > 0.05). Additionally, no significant difference was found in serum insulin and HOMA-IR concentrations between MTC patients and the controls (p > 0.05). Conclusions: This study suggests that the fluctuation in the levels of DPP4 does not play an important role in prognosis, early detection and diagnosis of MTC. Furthermore, higher levels of DPP4 cannot be considered as a risk factor for MTC.

Key Words: dipeptidyl peptidase 4, insulin resistance, medullary thyroid cancer, body mass index, metabolic syndrome.

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Abbreviations used: BMI — body mass index; DPP4 — dipeptidyl peptidase-4; FNA — fine needle aspiration; HOMA-IR — homeostatic model assessment of insulin resistance; MTC — medullary thyroid cancer.

Medullary thyroid cancer (MTC) is a malignant type of thyroid disorder, which arises from thyroid parafollicular cells (C cells), producing calcitonin [1] and accounts for 5–10% of thyroid cancers [2]. MTC occurs both in sporadic and hereditary forms. Hereditary form is divided into three types: multiple endocrine neoplasia type 2A, multiple endocrine neoplasia type 2B and familial medullary thyroid carcinoma [3]. Therefore, diagnosis and treating of MTC are challenging and distinction between hereditary and sporadic diseases is required [4]. Early diagnosis of MTC and its treatment in the early stages of the disease is important and can result in increased lifespan [5]. Currently, physicians diagnose MTC by physical examination, palpable nodules discovery and high levels of calcitonin. The studies have proven that fine needle aspiration (FNA) biopsy is the best way to identify MTC cells [6], however, there is a high risk and this invasive procedure is associated with infection, bleeding, inflammation and cyst fluid formation [7]. This diagnostic method is also costly, time-consuming and stressful process for patients [6]. Considering these disadvantages of FNA, biochemical blood markers are a good potential replacement for biopsy in cancer patients. Despite various studies, an appropriate tumor biomarker has not yet been identified for MTC. Similarly, owing to some ambiguous problems around assay methodology, sensitivity, specificity, and cost effectiveness, the routine assessment of serum calcitonin remains yet controversial [8].

Dipeptidyl peptidase-4 (DPP4/DPPIV) is a new identified adipokine [9], found in two forms: homodimer and tetramers with molecular weights of 220–290 kDa and 900 kDa, respectively [10]. DPP4 is a glycoprotein that exists on the surface of epithelial cells, especially in kidney, intestine, liver, as well as in the endothelial cells, fibroblasts and lymphocytes [9, 11]. DPP4 shows paracrine and autocrine effects and disrupts insulin signaling in the brain [11], thus DPP4 has a variety of roles in metabolism, immunity, endocrinology and cancer biology [12]. Based on literature review, DPP4 has three major functions including serine exopeptidase activity, binding to the extra-cellular matrix and the third major function is stimulation and activation of T cells [13]. Considering these functions, DPP4 can control the activity of many molecules such as cytokines, chemokines, incretin and neuropeptides [14]. Many recent studies point at the significant role of DPP4 in the early stages of malignant transformation and tumor progression [15]. These studies have attempted to determine whether this adipokine acts as a tumor suppressor or tumor activator [16]. DPP4 with exopeptidase activity can destruct the numerous chemokines which have different effects on tumor growth in a variety of neoplasms [14]. Also, binding of DPP4 to extracellular matrix proteins can promote tumor growth by adjusting the tumor cell adhesion, migration and invasion [16]. The expression of DPP4 has been reduced in several cancers, including melanoma [17] and lung cancer [18]. On the
other hand, the increase of this adipokine is shown in other types of cancer such as primary tumors of the lung [19], ovarian cancer [20], prostate cancer [21] and thyroid cancer [22].

The serum level of DPP4 in patients with MTC has yet to be determined. The purpose of this study was to assess DPP4 serum levels in patients with MTC and control group and to find a correlation between the serum level of DPP4 as a potential biomarker and prognosis of the MTC. Therefore, the assessment of alteration in serum level of DPP4 in MTC may help in prevention, early detection and diagnosis.

MATERIALS AND METHODS

Study population. This research is a case — control study carried out on patients with MTC who were referred to the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences and Endocrine Research Center at Taleghani Hospital. The case population consisted of 45 MTC patients, including 24 women and 21 men (mean age 29.46 ± 13.97 years). MTC diagnosis was confirmed by histopathology. Additionally, 45 people with no thyroid dysfunction (21 women and 24 men, with mean age of 27.53 ± 13.66 years) were selected as control group with normal thyroid function tests (TSH: 0.3–3.5 mIU/l, T4: 4.5–12.5 μg/dl, T3: 25–35% and T4: 75–210 ng/ml). Additional written informed consent was obtained from all individual participants. The study was approved by the Institutional Review Board and Ethics Committee of Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Clinical profile and blood collection. Anthropometric characteristics including height and weight were taken by the height measuring scaled balance (Seca, German company), with weight and height sensitivities of 250 g and 0.5 cm, respectively. The measurements were made with light clothing and no shoes. Body mass index (BMI) was calculated from the measured height and weight (kg/m²). 3 ml of blood were collected from antecubital vein of all subjects in the fasting state. After 5 min, the time of coagulation, samples were centrifuged at 3000 rpm for 10 min, and serum samples were separated and stored at −80 °C until further analysis. Serum samples were used to measure concentrations of DPP4, glucose and insulin levels.

Biochemical assay. In both groups, the serum DPP4 level was measured by using Human kit ELISA (Enzyme Linked Immunosorbert Assay) with sensitivity of 0.39 ng/ml (Cusabio Biotech Co., Ltd Manufacturer & Supplier, China). Serum insulin levels were quantified by an ELISA (Mercodia AB Company, Uppsala, Sweden), and serum glucose levels was measured using enzymatic colorimetric methods (Glucose Oxidase, Pars Azmoon Company) with a coefficient of variation (CV) of 2.5%. The insulin resistance was calculated using the following equation:

\[ \text{HOMA-IR} = \frac{\text{Fasting Serum Insulin} (\muIU/ml) \cdot \text{Fasting Plasma Glucose} (\text{mmol/l})}{22.5}. \]

Statistical analysis. Data analysis was performed using SPSS 19. Kolmogorov — Smirnov test was used to check the normality of samples. BMI, age, glucose and DPP4 data were in the normal distribution range except for insulin and homeostatic model assessment of insulin resistance (HOMA-IR), thus the logarithms of insulin and HOMA-IR were used for analyzing the data. All results were expressed as mean ± SD. Independent t-test were used to examine differences in mean variables and control groups. Correlations between variables were established using of Pearson’s correlation coefficient. A p-value < 0.05 was considered statistically significant.

RESULTS

As shown in Table 1, there was a slight increase in the levels of the DPP4 in the patient group (41.06 ± 22.08 ng/ml) compared with healthy individuals (39.94 ± 20.77 ng/ml), however, it was not statistically significant (Fig. 1). There were no significant differences between the two groups in terms of age and BMI, insulin and HOMA-IR (p > 0.05). In all MTC subjects serum glucose levels were higher compared to the control group (94.46 ± 12.38 mg/dl vs 85.26 ± 15.90 mg/dl, p = 0.003). Fig. 2 shows the mean of DPP4 for all the subjects as well as in each sex group separately. The changes of DPP4 levels in males in both groups were higher than in females, but these changes were not statistically significant. An attempt was made in order to find a correlation between changes in serum DPP4 concentration in patients with MTC vs baseline and changes in the values of each variable. There is a weak correlation between serum level of DPP4 and other variables, and this correlation was not statistically significant (Table 2).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 45)</th>
<th>Case (n = 45)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>27.53 ± 13.66</td>
<td>29.46 ± 15.97</td>
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<tr>
<td>BMI, kg/m²</td>
<td>25.84 ± 1.10</td>
<td>26.21 ± 1.30</td>
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<td>DPP4, ng/ml</td>
<td>39.94 ± 20.77</td>
<td>41.06 ± 22.08</td>
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<td>Insulin, µIU/ml</td>
<td>2.27 ± 0.53</td>
<td>2.35 ± 0.58</td>
<td>0.50</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>85.26 ± 15.90</td>
<td>94.46 ± 12.38</td>
<td>0.003</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.70 ± 0.55</td>
<td>0.89 ± 0.60</td>
<td>0.124</td>
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</tbody>
</table>

Note: Data are means ± SD.

Table 2. The Pearson correlation coefficient among DPP4 levels and other variables in group of patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DPP4</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
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<tr>
<td>Age, years</td>
<td>−0.046</td>
<td>−0.764</td>
<td></td>
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<tr>
<td>BMI, kg/m²</td>
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<tr>
<td>Insulin, µIU/ml</td>
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<tr>
<td>Glucose, mg/dl</td>
<td>−0.04</td>
<td>0.980</td>
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<tr>
<td>HOMA-IR</td>
<td>−0.119</td>
<td>0.437</td>
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</tr>
</tbody>
</table>

Note: r – Pearson’s correlation coefficient between variables.

DISCUSSION

The aim of this study was to determine the serum level of DPP4 in MTC patients compared with the healthy individuals. Thyroid cancer is the most common endocrine malignancy in the past four decades with increasing trends in incidence [4]. MTC is rare type
of thyroid cancer, originated from the thyroid parafollicular cells (C cells) secreting calcitonin (Ct) [23]. Despite using different strategies for screening and diagnosis of MTC, its early diagnosis is not yet fully possible. In the meantime, adipokine is one of the intervening factors used as a biomarker by researchers in order to diagnose some type of cancers at the early stage of the disease process [24]. DPP4 is a recently discovered adipokine with 766 amino acids, released by fat tissue [25]. In 2014, Arrebola et al. [15] demonstrated that the current DPP4 attracted international scientific community’s attention due to the particular complexity of its three-dimensional structure. This feature determines molecular and functional properties of DPP4 [11]. This adipokine is a ubiquitously expressed transmembrane glycoprotein with aminopeptidase activity that cleaves polypeptides from N-terminus dipeptides with proline or alanine in their second positions [26, 27] like neuropeptide Y, incretins and chemokines [28]. It also stimulates some molecules within cells such as p56, phospholipase C-γ and mitogen-activated protein kinase [29]. Therefore, as a consequence of these functions, DPP4 plays significant role in several diseases, especially cancer and immune disorders [33]. A better understanding of the role of DPP4 in the processes associated with disease will make it as a very attractive target for the development of more effective treatment strategies [31].

In 1995, Tanaka et al. [32] demonstrated that DPP4 was a new molecular marker for thyroid cancer. Northern blot analysis of 22 different thyroid tissues showed that DPP4 was more specific marker for various thyroid cancers. They also showed that DPP4 was strongly expressed in papillary and follicular thyroid cancers [32]. In 2004, Maruta et al. [33] demonstrated that the positive activity of DPP4 was the best marker in order to distinguish between follicular thyroid cancer and follicular thyroid adenoma in comparison with all laboratory tests and clinical findings. In 2013, Larrinaga et al. assessed the activity of 10 peptidases in a series of 30 papillary thyroid cancer samples, 10 follicular thyroid cancer samples, and 14 thyroid nodular hyperplasia samples, and they showed that the activity of DPP4/CD26 was significantly higher in papillary thyroid cancer than in follicular thyroid cancer, thyroid nodular hyperplasia, and non-tumor tissues [34]. Based on the results reported in this article, a slight increase of DPP4 was observed in the serum of MTC patients compared to the control group; however the difference was not statistically significant. Also, we found no differences in the serum insulin and insulin resistance index in two groups. There was also no correlation between DPP4 and other variables (BMI, age, insulin, glucose, and HOMA-IR) in the patient group.

In summary, the results of our study were different from above-mentioned studies. A mild increase in DPP4 of MTC patients in comparison with healthy controls showed that DPP4 cannot be considered as a marker for MTC. The main limitation of this study was the effect of genetics or ethnicity which was not controlled. Also, MTC had low prevalence, so the variety of samples was restricted. Therefore, further studies are needed in order to examine the content of DPP4 in thyroid tissue samples. In addition, given that the DPP4 was measured in MTC, it is suggested that the level of this adipokine should be studied in other types of thyroid cancer for more accurate interpretation of data in regards to its relationship with different type of thyroid cancers.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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REFERENCES


