İstanbul Med J 2019; 20(6): 535-40
DOI: 10.4274/imj.galenos.2019.26817

Can VAP-1 Protein be used as a Biomarker in Thyroid Cancer?

VAP-1 Proteini Tiroid Kanserinde Biyobelirteç Olarak Kullanılabilir mi?

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ABSTRACT

Introduction: The aim of this study is to determine whether vascular adhesion protein-1 (VAP-1) glycoprotein can be used as a biomarker in thyroid cancer.

Methods: Retrospective analysis of the pathology results of patients who were operated for thyroid malignancy or multinodular goiter in our hospital was performed. A total of 46 patients, including 16 papillary carcinomas, ten follicular carcinomas, ten benign nodules, and ten healthy tissues, were included in the study. Patients with cancer other than thyroid cancer, who were pregnant, who had a chronic systemic disease, and impaired liver function tests were excluded from the study. The level of tissue VAP-1 was assayed by immunohistochemistry.

Results: In our study, 13 papillary carcinoma tissues, five follicular carcinoma tissues, six benign nodules, and one healthy tissue were stained positively. Although there was a statistically significant difference between papillary carcinoma and healthy tissue, no statistically significant difference was found between the other groups.

Conclusion: VAP-1 glycoprotein can be used as a biomarker in the diagnosis of papillary thyroid carcinoma.

Keywords: Thyroid cancer, VAP-1 protein, goiter

ÖZ

Amaç: Bu çalışmanın amacı, vasküler adhezyon proteini-1 (VAP-1) glikoproteinin tiroid kanserinde biyobelirteç olarak kullanılıp kullanılamayacağını belirlemektir.

Yöntemler: Hastanemizde tiroid malignitesi veya multinodüler guatr nedeniyle opere edilen hastaların patoloji sonuçlarının retrospektif analizi yapıldı. Çalışmaya 16 papiller karsinom, on foliküler karsinom, on iyi huylu nodül ve on sağlıklı doku olmak üzere toplam 46 hasta alındı. Tiroid kanseri dışında kanseri olan, gebe olan, kronik sistemik hastalığı olan ve karaciğer fonksiyon bozukluğu olan hastalar çalışma dışı bırakıldı. Doku VAP-1 seviyesi immünohistokimya ile test edildi.

Bulgular: Çalışmamızda 16 papiller karsinom olgusunun 13'ü, 10 foliküler karsinom olgusunun 5'i, 10 benign dokunun 6'sı ve 10 normal dokunun 1'i pozitif olarak boyanırken diğer olgular negatif olarak boyandı. Papiller karsinom ve normal doku arasında istatistiksel olarak anlamlı bir fark olmasına rağmen, diğer gruplar arasında istatistiksel olarak anlamlı bir fark bulunmadı.

Sonuç: VAP-1 glikoprotein papiller tiroid karsinomu tanısında biyobelirteç olarak kullanılabilir.

Anahtar Kelimeler: Tiroid kanseri, VAP-1 proteini, guatr

Introduction

Thyroid cancer is a common disease among head and neck cancers, and the incidence of it has been increasing all around the world (1). The five-year survival is only 59% in late-stage compared to nearly 100% for an earlier, localized stage (2).

Most of the patients present with a thyroid nodule, but only 5-15% of nodules are malignant (3). One of the most effective methods of decreasing mortality is the early diagnosis, and fine-needle aspiration cytology (FNAC) is most commonly used for this purpose (4). The use of FNAC can reduce unnecessary thyroid operations by 25% (5). Although malignant nodules can be detected by FNAC, 10% to 25% of thyroid nodules are diagnosed as indeterminate nodules (6). FNAC results may vary from non-specific cytology to malignancy, but as a result, the biomarker may be needed to support this outcome preoperatively. Given the limitations of diagnosis by FNAC, investigators have examined molecular markers as cytopathologic adjuncts to improve the accuracy of diagnostic testing of thyroid nodules. A major aim of the research is to

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Cite this article as/Atıf: Baki A, Tosun İ, Yıldız M. Can VAP-1 Protein be used as a Biomarker in Thyroid Cancer?. İstanbul Med J 2019; 20(6): 535-40.

Received/Geliş Tarihi: 02.04.2019
Accepted/Kabul Tarihi: 22.10.2019

improve upon the preoperative diagnosis of indeterminate nodules in order to avoid surgery for benign nodules. To reduce repetitive diagnostic tests and operations, there has been an extensive investigation into molecular markers that can be detected on FNAC specimens to stratify a patient's risk of malignancy more accurately.

Many biomarkers are used to differentiate between benign and malignant thyroid nodules, including cytokeratin-19, fibronectin-1, intracellular sodium/iodide, high molecular weight cytokeratin, cyclin D1, and galectin-3 (7,8). They can be used alone or in combination. A single biological marker is not yet able to distinguish between benign or malignant thyroid nodules. The ideal biomolecule that can make this distinction has long been the subject of research.

Vascular adhesion protein-1 (VAP-1), which is another promising glycoprotein marker, weighs 170 kDa. This protein is an endothelial adhesion molecule generally involved in the interaction between leukocytes and endothelial cells, including leukocyte rolling, adhesion, and transmigration into sites of inflammation (9). Recently, many studies have investigated the role of VAP-1 in cancers. In the head and neck, liver, and melanoma tumors, VAP-1 expression is found in intra-tumoral vessels (10-12). VAP-1 has been shown to enhance tumor growth in mice (13). In humans, serum VAP-1 is correlated with angiogenic factors in lung cancers and is more concentrated in metastatic prostatic cancers (14,15).

The primary aim of this study was to investigate tissue VAP-1 levels in benign and malignant thyroid lesions and to compare them with healthy tissue levels. We also aimed to determine whether VAP-1 could be used as a biomarker in thyroid cancer.

Methods

The pathology specimens of patients who underwent total thyroidectomy for thyroid malignancy or multinodular goiter were examined. The study was carried out in four groups, including papillary carcinoma, follicular carcinoma, benign tissue, and healthy tissue. The pathologic tissues of 53 patients were retrospectively reviewed. Patients with other cancers other than thyroid cancer, who were pregnant, who had a chronic systemic disease, and impaired liver function tests were excluded from the study. Of the 46 patients included in the study, 16 were papillary thyroid carcinoma (PTC), ten were follicular thyroid carcinoma (FTC), ten were benign nodules, and ten were healthy patients. Thyroid cancer patients underwent total thyroidectomy and neck dissection. The American Common Cancer Committee TNM classification system was used for staging. Our study was approved by the Ethics Committee of Ümraniye Training and Research Hospital (decision no: 165, date: 24.11.2017). For the study, approval was obtained from the patients.

Laboratory Study

VAP-1 (A-8: sc-166713; Santa Cruz Biotechnology) clone was used as the primary antibody. Paraffin blocks suitable for immunohistochemical analysis were selected, and 3-micron thick sections were taken on the poly-L-lysine coated slide. The immunohistochemical study was completed automatically on the VENTANA BENCHMARK XT device by the device instructions. The staining patterns in the tissue were evaluated as negative and positive.

Statistical Analysis

In this study, IBM SPSS Statistics Version 22 (IBM Turkish Limited Company, İstanbul, Turkey) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviations, and median value) were calculated. When the groups were evaluated together, nonparametric data were assessed by the chi-square test. We performed a receiver operating characteristic (ROC) curve analysis to evaluate the predictive value of VAP-1 for papillary thyroid cancer. Significance was assessed at $p<0.05$ levels.

Results

There were 46 patients in our study. Sixteen of the patients had PTC, ten of them had the FTC, ten with benign tissue, and ten with healthy tissue. The mean age of the patients included in the study was 43.6±5.2, 42.7±5.7, 43.7±4.7, and 43.4±5.2 years, respectively. Thyroglobulin (ng/mL) levels of the patients were higher in cancer patients than in benign and healthy tissues. In PTC and FTC patients, it was found to be 89.4±56.7 and 88.7±55.2, whereas it was 15.2±13.6 and 11.3±10.4 in benign tissue and healthy tissue, respectively. FT4 (pmol/L), thyroid-stimulating hormone (μIU/mL) values, and liver function tests were normal in all groups. Of the PTC patients, 14 were stage 1-2, two were stage 3-4, while eight of the FTC patients were stage 1-2, and two were stage 3-4 (Table 1).

In tissue staining, 13 of the PTC, five of the FTC, seven of the benign nodules, and one of the healthy tissues were stained positively while the others were negatively stained (Figure 1, 2) (Table 2).

Table 1. Demographic and laboratory values of patients

Characteristics	Papillary carcinoma	Follicular carcinoma	Benign tissue	Healthy tissue
Age	43.6±5.2 (n=16)	42.6±5.7 (n=10)	43.7±4.7 (n=10)	43.4±5.2 (n=10)
Tg (ng/mL)	89.4±56.7	88.7±55.2	15.2±13.6	11.3±10.4
FT4 (pmol/L)	12.5±4.7	13.4±3.8	14.1±2.6	11.50-23.12
TSH (μIU/mL)	3.5±3.1	3.6±3.4	3.2±2.3	3.1±1.6
Hepatic disease	-	-	-	-
Systemic disease	-	-	-	-
Thyroid cancer stage	-	-	-	-
I/II (n, %)	14 (87.5%)	8 (80%)	-	-
III/IV (n, %)	2 (12.5%)	2 (20%)	-	-

Tg:thyroglobulin, TSH: thyroid-stimulating hormone, FT4: Free T4, ng/mL: nanogram/milliliter, pmol/L: picomole/liter, μIU/mL: micro unit/milliliter, n: number of patients

When all groups were compared, a statistically no significant difference was found (p=0.48) (Table 3). Although there was a statistically significant difference in comparisons between PTC and healthy tissue groups (p<0.01), respectively, no significant difference was found between the other groups (p=1), (p=0,52), (p=1), (p=1), (p=0.52) (Table 4).

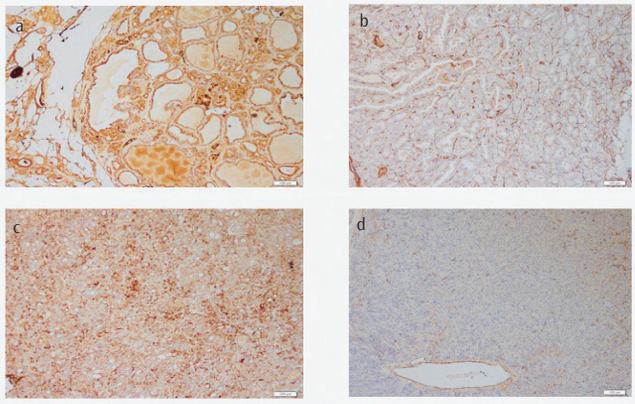

Figure 1. a) Papillary carcinoma positive staining. b) Papillary carcinoma negative staining. c) Follicular carcinoma positive staining. d) Follicular carcinoma negative staining

The area under the ROC curve was 0.83 (95% confidence interval, 0.65-1; p=0.007). The decision on optimal cut off value for tissue VAP-1 was based on maximizing the sum of sensitivity and specificity. The cut-off value of VAP-1 was 0.5 µg/mL, with a 78% specificity and 100% sensitivity (Figure 3).

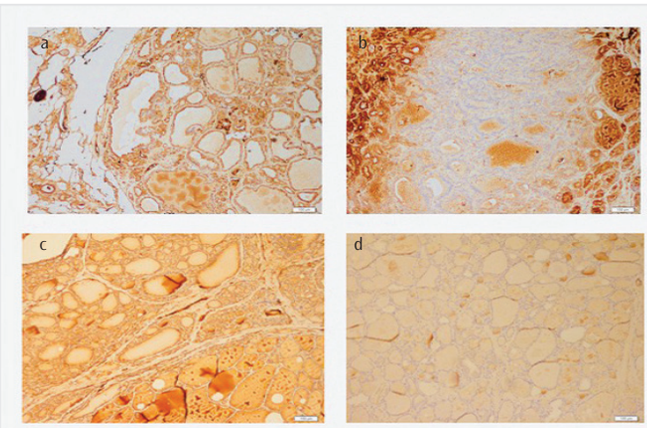

Figure 2. a) Benign tissue positive staining. b) Benign tissue negative staining. c) Healthy tissue positive staining. d) Healthy tissue negative staining

Table 2. Staining characteristics of the patients

	Positive stained	Negative stained
	n	n
Normal tissue	1	9
Benign nodule	7	3
Papillary cancer	13	3
Follicular cancer	5	5

n: number of patients

Table 3. Comparison of all groups together

	n	x^2	p
Papillary cancer	16	6.66	0.48
Follicular cancer	10		
Benign nodule	10		
Normal tissue	10		

Chi-square test, N: number of patients, p value ≤0.05

Table 4. Comparison of binary groups

Comparison of papillary cancer and normal tissue	**n**	**x^2**	**p**
Papillary cancer	16	6.25	0.01*
Normal tissue	10		
Comparison of papillary cancer and follicular cancer groups	**n**	**x^2**	**p**
Papillary cancer	16	6.25	1
Follicular cancer	10		
Comparison of papillary and benign nodules	**n**	**x^2**	**p**
Papillary cancer	16	6.25	0.52
Benign nodule	10		
Comparison of follicular cancer and benign nodules	**n**	**x^2**	**p**
Follicular cancer	10	0.00	1
Benign nodule	10		
Comparison of follicular cancer and normal tissue	**n**	**x^2**	**p**
Follicular cancer	10	0.00	1
Normal tissue	10		
Benign nodule and normal tissue comparison	**n**	**x^2**	**p**
Benign nodule	10	0.40	0.52
Normal tissue	10		
Chi-square test, N: number of patients, p value ≤0.05*			

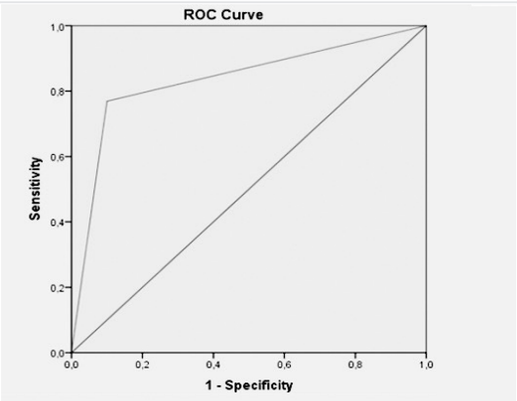

Figure 3. Receiver operating characteristic (ROC) curve. Tissue VAP-1 staining in papillary thyroid cancer and healthy tissue were used to draw the ROC curve, and the specificity, sensitivity, area under the ROC curve (AUC), and cut-off value were determined

VAP-1: vascular adhesion protein-1, AUC: area under the curve

Discussion

Biomarkers have been used for many years to detect thyroid cancer and to determine its prognosis (16). A marker that can identify benign and malignant thyroid nodules and predict their behavior is continuously sought. Difficulties in identifying thyroid nodules and determining their prognosis persist (17). For thyroid nodules, an accurate biomarker is necessary to measure the likelihood of preoperative malignancy.

The process of tumor growth and metastasis involves a variety of cell-cell and cell-extracellular matrix interactions mediated by cell adhesion molecules such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1, platelet endothelial cell adhesion molecule-1, and selectins. During tumor growth and metastasis, each process requires cell adhesive interactions involving specific adhesion molecules and receptors (18,19). VAP-1 is one of the endothelial adhesion molecules, which is upregulated at sites of inflammation and mediates binding of lymphocytes to vessels of inflamed tissue (20). In many studies, serum VAP-1 levels are a significant predictor of the prognosis of cancer diseases.

The tissue and serum VAP-1 protein levels were significantly lower in colorectal cancer compared with healthy colon tissue (21). The low serum VAP-1 was associated with poor prognosis in patients with colorectal cancer (22). The low tissue VAP-1 may be part of a mechanism used by the tumor to prevent the recruitment of antitumor defense cells (21). The serum concentration of VAP-1 was significantly elevated in patients with gastric cancer, and clinicopathological analysis revealed that low serum VAP-1 levels in tumors were associated with tumor size increase, serosal invasion, lymph node metastasis, peritoneal dissemination and poor prognosis (23). Serum VAP-1 levels were also found higher in patients with hepatocellular cancer (24). In human breast cancer, tumor VAP-1 mRNA expression is associated with improved prognosis (25,26). In contrast, increased VAP-1 protein expression was found to be associated with poor prognosis in astrocytomas (27).

Hu et al. (28) find that serum VAP-1 levels are significantly lower in thyroid cancer group than in healthy control and benign thyroid nodule groups. Another important finding of their study is that serum VAP-1 has relatively high sensitivity and specificity in predicting thyroid cancer. In this study, we evaluated VAP-1 protein expression in different thyroid pathologies and healthy thyroid tissue at tissue level for the first time in the literature. In our study, 13 of 16 cases of PTC, five of ten cases of FTC, six of ten benign tissues, and one of ten healthy tissues were stained positively. Although there was a statistically significant difference between PTC and healthy tissue, no statistically significant difference was found between the other groups. The cut-off value of VAP-1 was 0.5 µg/mL, with a 78% specificity and 100% sensitivity for papillary carcinoma. Therefore, the combined application of ultrasonographic features and VAP-1 examination in FNA material could be a potential way to improve the accuracy of diagnosing PTC.

It was shown that the genes encoding chemokines CCL20, CXCL8, and the adhesion molecule L-selectin were overexpressed in PTC in comparison to healthy thyroid tissue, and these chemokines could be associated with tumor-related inflammation and lymphocyte infiltration (29). In human PTC, the density of lymphocytes is correlated with improved overall survival and lower recurrences (30,31). In our study, the rate of VAP-1 staining was higher in patients with PTC than in healthy tissues. This may be because the VAP-1 protein has a significant lymphocyte infiltration and migration effect, and the expression is increased to prevent the progression of the disease in cancer tissue.

FTC comprises between 10% and 15% of all differentiated thyroid cancers. This cancer usually presents later in life and is more aggressive than PTC (32,33). It is a malignant epithelial tumor showing follicular cell differentiation and lacking the nuclear diagnostic features of PTC (34). In this study, VAP-1 protein staining was found in half of the patients with FTC. However, statistical results could not be obtained to support the use of VAP-1 protein as a biomarker in this cancer type. Also, less VAP-1 staining in FTC than PTC may explain that the prognosis of FTC is worse than papillary cancer. Further studies are needed to investigate the effects of tissue VAP-1 protein expression on prognosis in FTC.

In this study, tissue VAP-1 protein staining was detected in six of ten patients with benign thyroid nodules. VAP-1 is upregulated at sites of inflammation, and it mediates lymphocyte binding to inflamed endothelium (35). Increased serum VAP-1 levels in chronic liver disease and multiple sclerosis compared to healthy individuals has been reported previously (36,37). Since VAP-1 levels can be detected in inflammatory tissues besides cancers, we thought that positivity detected in benign nodules might be related to inflammation at the tissue level. New data are needed to explain the pathophysiology of VAP-1 staining in benign thyroid nodules at the tissue level and the importance of this staining.

FNAC results may not yield accurate results in thyroid nodules, so positive or negative staining of FNAC material with VAP-1 protein may be helpful in the differential diagnosis. As a result, positive staining of VAP-1 protein in FNAC results may stimulate us for the diagnosis of papillary cancer. It can also help reduce the patient's follow-up or additional cost tests. To demonstrate that VAP-1 protein can be used as an important biomarker in thyroid cancer, studies may be needed in larger series. Further studies are needed to investigate the prognostic effects of VAP-1 on TPC.

Conclusions

Different amounts of VAP-1 staining were obtained in different thyroid pathologies. VAP-1 protein can be used as an important biomarker in TPC.

Ethics Committee Approval: Our study was approved by the Ethics Committee of Ümraniye Training and Research Hospital (decision no: 165, date: 24.11.2017).

Informed Consent: For the study, approval was obtained from the patients.

Peer-review: Externally peer-reviewed.

Author Contributions: Surgical and Medical Practices - İ.T.; Concept - A.B.; Design - A.B.; Data Collection and/or Processing - A.B., İ.T.; Analysis and/or Interpretation - A.B., İ.T., M.Y.; Literature Search - A.B., M.Y.; Writing Manuscript - A.B., M.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: Our special thanks Ser-med medical and Meders-Maxton corporation for their financial support.

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