Original Article

Examination of CD10 expression in the differentiation of benign and malignant thyroid tumors

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ABSTRACT

CD10 is expressed as a cell surface antigen in acute lymphoblastic leukemias. Few and conflicting studies have been conducted to determine the role of CD10 in thyroid cancer prognosis. Therefore, the present study was conducted with the aim of investigating the expression of CD10 in the differentiation of benign and malignant thyroid tumors. In this retrospective study, 80 malignant tumors and 25 benign thyroid blockes were collected from the histopathological laboratory of Golestan Hospital in Ahvaz between 2021 and 2023. The specific staining for the CD10 marker was performed using an immunohistochemical method using paraffin blocks. In the present study, 87.5% of malignant cases showed positive CD10 expression and 12.5% of remaining cases showed negative CD10 expression, while 44% of benign cases showed positive CD10 expression and 56% of remaining cases showed negative CD10 expression showed. The results showed that there is a statistically significant relationship between the pathological tumor type and CD10 expression ($P < 0.0001$). According to the results, the highest positive percentage of CD10 expression was related to the type of follicular papillary malignant tumor with a frequency of 96%. Finally, in the present study, there was no significant correlation between the expression intensity of the CD10 marker and demographic and clinicopathological characteristics ($p > 0.05$). Overall, the present study showed that CD10 marker can be used to differentiate between benign and malignant thyroid tumors, but it is not associated with prognosis.

Keywords: Malignant thyroid neoplasm, Benign thyroid lesions, CD10, Clinicopathological factors

Introduction

Thyroid cancer is the most common endocrine malignancy. Thyroid carcinoma accounts for about 1% of all cancers, and its incidence varies significantly geographically. Papillary thyroid carcinoma (PTC) is the most common form of thyroid cancer, accounting for 75-85% of all thyroid cancers. The prevalence of thyroid cancer in Iran is given as 3.5% [1]. There are two main obstacles in diagnosing thyroid lesions, particularly follicular pattern lesions. These lesions include four types of adenomatous nodules, follicular adenomas, follicular carcinomas, and follicular-type papillary thyroid carcinomas. Follicular carcinoma is distinguished from follicular adenoma when there is vascular or extrathyroid invasion or nodal or distant metastases [2]. In addition, the differential diagnosis of hyperplastic and neoplastic lesions originating from follicular cells can be very problematic, and this problem is rarely solved by immunohistochemistry. In addition, some encapsulated thyroid nodules that present a follicular morphology may have diffuse nuclear or intermediate focal features of papillary carcinoma. Therefore, it can pose a challenge in diagnosing different types of malignant thyroid tumors [3]. As long as the lesion shows capsular and/or vascular invasion, the diagnosis of a well-differentiated carcinoma is possible without further subtyping. Conversely, in the absence of invasion, follicular adenoma and the follicular variant of papillary carcinoma should be considered in the diagnosis. This important problem cannot be solved for some nodules only by morphology and even immunohistochemistry. In this context, these cases are described as well-differentiated tumors with uncertain malignant potential [4]. Several immunohistochemical markers such as HBME1, CK19 and galectin 3 have been used to solve this problem. These antibodies can be valuable, especially when used together, but they all have some disadvantages and limitations. Therefore, even more reliable markers are needed to distinguish between benign and malignant thyroid neoplasia [5]. Structurally, CD10 is known to be a 90–110 kDa single-chain

zinc-dependent metalloprotease that degrades many biologically active peptides in the extracellular matrix. In addition to enzymatic degradation, it affects several intracellular signal transduction pathways [6]. CD10 expression has been extensively studied in human epithelial cancers from numerous organs and locations. Recently, immunohistochemical expression of CD10 has been used for diagnostic purposes in lymphoma, leukemia and solid tumors including clear cell renal cell carcinoma, pancreatic neoplasia and pseudopapillary tumors, skin tumors, urinary tumors, endometrial stromal tumors and mesonephric tumors [7]. Conflicting results have been reported for the association of CD10 expression changes with poor prognosis in various tumors including lung cancer, prostate cancer, head and neck cancer, colorectal cancer, melanoma and ovarian cancer. Several previous studies have also reported selective expression of CD10 in differentiated thyroid cancers and not in normal thyroid tissue and benign thyroid nodules [8]. It was recently reported that CD10 is highly expressed in anaplastic thyroid carcinoma, but the PTC is low and absent in follicular thyroid carcinoma and medullary thyroid carcinoma. Also, expression of CD10 in thyroid pathology was positive in non-Hodgkin's lymphoma of the thyroid gland. However, these studies were performed on a relatively small number of cases. Little is known about the diagnostic and prognostic values of CD10 expression in PTC [9]. Therefore, the present study was carried out with the aim of examining the expression of CD10 in the differentiation of benign and malignant thyroid tumors.

Materials and Methods

Case selection and tissue samples

This retrospective descriptive-analytical study was performed on paraffin tissue samples from patients with benign and malignant thyroid lesions referred to Imam Khomeini Hospital in Ahvaz between 1401 and 1399. This study was conducted following approval by the Ethics Committee of Jundishapur University of Medical Sciences, Ahvaz. Inclusion criteria included only patients whose benign and malignant thyroid lesions were initially confirmed by a pathologist. In addition, patients who are undergoing treatment will be excluded from the study. The demographic and clinicopathological characteristics of each patient, including patient age and gender, type of thyroid tumor, benign and malignant, size, number of involved lymph nodes, and tumor grade in the malignant group, were extracted from the patient record and recorded in a checklist.

Immunohistochemical staining assay

The 5-µm paraffinized sections were soaked in water-alcohol solution for 5 minutes. Slides were placed in microwave oven for 30 minutes at 60°C. Deparaffinization was performed by soaking the slides in xylene (Merck, Germany) and, then, alcohol (from 100% to 75% concentration) for 5 to 10 minutes. Sections were rinsed with 10% phosphate-buffered saline (PBS; Yekta Tajhiz Azma, Iran), followed by H2O2/methanol (1:9) and 10% PBS for 10 minutes. Then, the slides were heated in microwave oven for 10 minutes in ethylenediaminetetraacetic acid (EDTA; Yekta Tajhiz Azma, Iran). The samples were left to reach the room temperature; then, were rinsed with PBS.. Then Monoclonal anti-CD10 Clone GM003 primary antibody (Genemed, South San Francisco, CA, USA) was added to the sections and incubated at 4°C under a humidified chamber overnight. Afterwards, sections were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody for 30 min and visualized with diaminobenzidine (DAB) substrate at room temperature. Counterstaining was performed using hematoxylin in space for 2 minutes and placebo antibody was used as a negative control. Tumor tissue sections were examined and evaluated under a Leica ICC50HD microscope at low to high magnification by two pathologists who were unaware of the histological diagnosis. Sections were considered positive if staining occurred in the cytoplasm and cell membrane. Staining was scored 0 (negative) when less than 10% of the tumor cells were positive, 1 (weak) when 10-49% of the tumor cells were positive, and 2 (strong) when 50% or more of the tumor cells were positive [9].

Statistical Analysis

The data analysis was carried out with SPSS Version 22 (IBM). The normal distribution of the quantitative data was checked with the Kolmogorov-Smirnov test. The relationship between qualitative variables was assessed by chi-square or Fisher's exact test. P value<0.05 was considered statistically significant.

Results and Discussion

In the present study, the expression of CD10 was examined in the differentiation of 80 thyroid tumors, including 80 malignant tumors and 25 benign tumors. In the present study, the mean age in the malignant and benign groups was 41.67 ± 12.18 and 40.21 ±10.18, respectively. The studied cases were divided into benign and malignant groups. 87.5% of malignant cases showed positive CD10 expression and 12.5% of remaining cases showed negative CD10 expression, while 44% of benign cases showed positive CD10 expression and 56% of remaining cases showed negative CD10 expression showed. The results showed that there is a statistically significant relationship between the pathological tumor type and CD10 expression (P < 0.0001).

Table 1. Evaluation of the expression frequency and intensity of CD10 expression according to the pathological type of benign and malignant thyroid tumors

Pathological type of tumor	Total	Negative expression CD10	Weak expression CD10	Strong expression CD10	p-value
Benign	25	14 (56)	5 (20)	6 (24)	

P<0.0001	50 (62.5)	20 (25)	10 (12.5)	80	Malignant

In the present study, the expression frequency and intensity of CD10 expression according to the pathological type of benign and malignant thyroid tumors are shown in **Table 2-4**. Based on the results of the present study, CD10 immunohistochemical staining was found in 22 of 25 (88%) normal papillary carcinomas, 31 of 32 (96%) follicular-type papillary carcinomas, and 6 of 8 (75%) low-invasive follicular carcinomas, 4 of 5 cases (80%) were highly invasive follicular carcinomas, 5 of 13 (39%) follicular adenomas and 7 of 12 (50%) adenomatous nodules were positive. Based on the results of the study, the highest percentage of strong CD10 expression was associated with follicular-type papillary carcinoma (78%), and the lowest percentage of strong CD10 expression was associated with adenomatous nodule (16.67%).

Table 2. Expression frequency and intensity of CD10 expression according to the pathological type of benign and malignant thyroid tumors

Strong expression CD10	Weak expression CD10	Negative expression CD10	Total	Type	Pathological type of tumor
15 (60)	7 (28)	3 (12)	25	Papillary thyroid carcinoma	Malignant
25 (78)	6 (18)	1 (4)	32	Follicular papillary carcinoma	
5 (62.5)	1 (12.5)	2 (25)	8	Follicular carcinomas with low invasiveness	
1 (20)	3 (60)	1 (20)	5	Follicular carcinomas with high invasiveness	
7 (70)	2 (20)	1 (10)	10	Undifferentiated carcinoma	
4 (30)	1 (9)	8 (61)	13	Follicular adenoma	Benign
3 (16.67)	4 (33.33)	6 (50)	12	Adenomatous nodule	

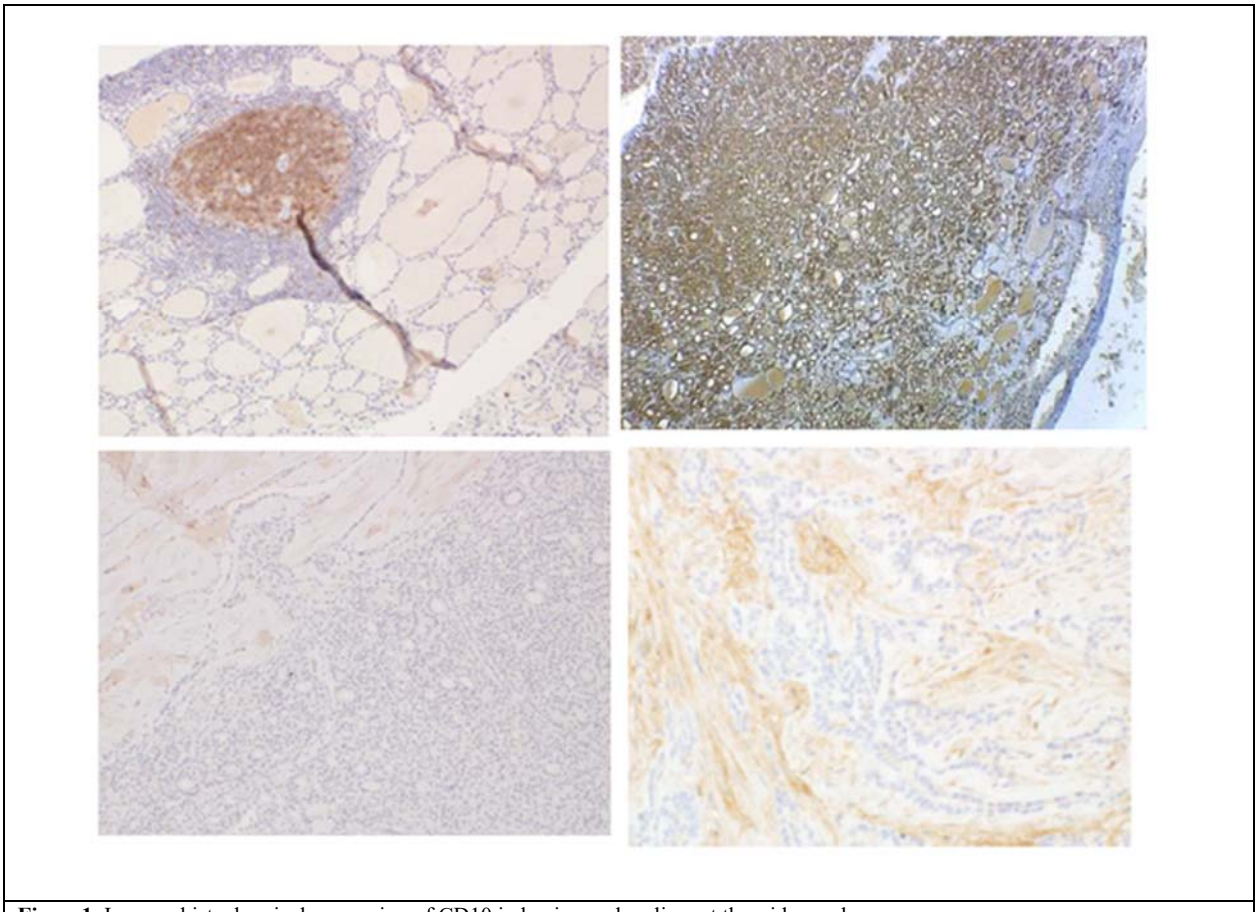

Figure1. Immunohistochemical expression of CD10 in benign and malignant thyroid samples.

Based on the results of the present study, there was no significant association between the intensity of CD10 marker expression and demographic and clinicopathological characteristics, including age, gender, initial tumor stage, lymph node metastasis, angioinvasion, lymphatic invasion and extrathyroidal invasion ($p > 0.05$).

Table 3- Expression frequency and intensity of CD10 expression according to the pathological type of benign and malignant thyroid tumors

p-value	Strong expression	Weak expression CD10	Negative expression CD10	Total		
0.751	36 (64.29)	14 (25)	6 (10.71)	56 (70)	Under 50 years	**Age**
	14 (58.34)	6 (25)	4 (16.66)	24 (30)	Over 50 years old	
0.34	12 (54.34)	8 (36.66)	2 (9)	22 (27)	Male	**Gender**
	38 (65.53)	12 (20.68)	8 (13.79)	58 (73)	Female	
0.79	15 (60)	5 (20)	5 (20)	25 (31)	T1	**Early stage of the tumor**
	23 (77)	4 (13)	3 (10)	30 (37)	T2	
	9 (45)	10 (50)	1 (5)	20 (24)	T3	
	3 (60)	1 (20)	1 (20)	5 (7)	T4	
0.34	35 (61.42)	13 (22.8)	9 (15.78)	57(71.25)	N0	**Lymph node metastasis**
	15 (65.27)	7 (30.43)	1 (4.3)	23 (28.75)	N1	
0.8	41 (64.1)	15 (23.4)	8 (12.5)	64 (80)	No	**Angioinvasion**
	9 (56.25)	5 (31.25)	2 (12.5)	16 (20)	Yes	
0.38	28 (70)	8 (20)	4 (10)	40 (50)	No	**Lymphatic invasion**
	22 (55)	12 (30)	6 (15)	40 (50)	Yes	
0.2	45 (66.3)	16 (23.5)	7 (10.2)	68 (85)	No	**Extra thyroidal invasion**
	5 (41.67)	4 (33.33)	3 (25)	12 (15)	Yes	

Conclusion

In the present study, examining CD10 expression in the differentiation of thyroid tumors, including 80 malignant tumors and 25 benign tumors, it was shown that 87.5% of malignant cases had positive CD10 expression, while 44% of benign ones Cases have positive CD10 expression CD10 expression. A statistically significant association was found between the pathological tumor types with respect to CD10 expression ($P < 0.0001$). According to the results, the highest positive percentage of CD10 expression was related to the type of follicular papillary malignant tumor with a frequency of 96%. Finally, in the present study, there was no significant correlation between the expression intensity of the CD10 marker and demographic and clinicopathological characteristics ($p > 0.05$). Differentiation between benign and malignant thyroid tumors, especially the follicular type, can sometimes be problematic as it depends primarily on the presence of capsular and/or vascular invasion. If the focus of invasion is small or not obvious, it becomes difficult to make a correct diagnosis, which is why the use of markers is particularly important in differentiating these two types of tumors [10]. CD10 was originally thought to be a tumor-specific antigen, but studies have shown that it is expressed by a variety of cell types, including bronchial epithelial cells, renal proximal tubule epithelial cells, cultured fibroblasts, bone marrow stromal cells, and mammary myoepithelium. , biliary tract, fetal intestine and certain solid tumors are expressed [11]. In the study by Chisato Tomoda and his colleagues in Japan in 2003, it was shown that CD10 was not detected in normal thyroid tissue, benign lesions (15 follicular lesions and 15 adenomatous goiters) and pure papillary carcinoma except follicular types. However, its expression was observed in 80% of follicular carcinomas and 77% of follicular papillary carcinomas, suggesting that the expression status of CD10 is lower in this study compared to the present study. However, they concluded that CD10 immunohistochemical staining may be a useful marker for follicular carcinoma to differentiate it from follicular adenoma and benign hyperplastic nodules and to differentiate follicular types from papillary thyroid carcinoma. which is consistent with the results of the present study [12]. In the study of 515 patients with papillary thyroid cancer by Mr. Eun Ji Oh and colleagues (Korea, 2020), CD10 expression was observed in 295 (57.3%) but not in the adjacent benign thyroid tissue. which is consistent with the results of the present study. and the frequency of CD10 expression is lower compared to the present study [9]. According to the results of the present study, the expression of the CD10 marker was observed in 29.9% of papillary carcinoma cases in the Mokhtari study, but not in any of the benign thyroid lesions (0%), so the results are comparable with the results the present study. A significant association between the expression of CD10 and benign and malignant thyroid lesions was reported ($P < 0.001$) [13]. However, in contrast to the results of the present study and the studies mentioned, the study by Yegen and colleagues concluded that the CD10 marker is not effective in distinguishing between benign and malignant lesions. However, there is a strong association between CD10 expression and papillary thyroid cancer. Furthermore, in this study, they reported that CD10 expression

was negative in adenomatous nodules, minimally invasive follicular carcinomas, and well-differentiated carcinomas. However, it was positive in normal papillary carcinomas (64.2%), follicular types of papillary carcinomas (16.6%), papillary microcarcinomas (50%), highly invasive follicular carcinomas (11.1%) and follicular adenomas (30%) [14]. In the study by Chu and colleagues, the expression of CD10 was negative in all thyroid tumors, which is not consistent with the results of the present study [15]. In the study by Yasuda and colleagues, they reported that CD10 is absent in thyroid tumors and has no diagnostic value [16]. Reasons for the different results in different studies may include geographical and genetic diversity between patients, different sample sizes, technical changes and different antibodies, clones and brands, differences in sample size and differences in the examination of CD10 expression with respect to CD10 -Expression belong to pathological type and the numerical similarity of pathological types. A well-known fact in immunohistochemistry is that the specificity and sensitivity of antibodies can vary depending on the clone and brand. In addition, some studies did not evaluate various malignant thyroid lesions and did not determine the sensitivity and specificity of CD10.

In the study by Heshmati and his colleagues in 2016, the increased expression of CD10 was related to tumor size, lymph node involvement, and capsular invasion, but there is no significant relationship between the expression of this biomarker and the age and gender of patients with papillary thyroid carcinoma [17]. Also, in the study of Mokhtari and his colleagues, there was no significant relationship between the expression of CD10 and age and gender, which is why there is a similarity in the results [13]. The study of Mr. Gabal and his colleagues in Egypt in 2018 in malignant thyroid neoplasms stated that there is no statistically significant relationship between CD10 expression and age, gender, metastasis to lymph nodes, tumor stage and capsule invasion, which is consistent with the results of the present study [18]. The study of Eun Ji Oh and his colleagues in Korea in 2020 indicates that CD10 expression is not related to clinical outcomes [9].

Authors Contribution

Study concept and design: P. K.; analysis and interpretation of data: M. T; drafting of the manuscript: M. G.; critical revision of the manuscript for important intellectual content: S. J., P. K., and M. G.; statistical analysis: M. T.

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Conflict of interest: None

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