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Comparision of CD56 & Claudin-1 Expression in Papillary Thyroid Carcinoma and Thyroid Follicular Nodules

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Abstract

Background : Thyroid carcinomas are the most common of all endocrine system malignancies.The pathological diagnosis of papillary thyroid carcinoma(PTC) is usually easily achieved if the histological structure and nuclear features are present, but in the absence of these features the diagnosis can be difficult .We evaluated the diagnostic value of CD56 and claudin-1 expression in PTC , follicular lesions and follicular neoplasms.**Methods** : Seventy five cases diagnosed as PTC and 75 cases diagnosed as follicular lesions(follicular adenoma,follicular carcinoma, hyperplastic nodule) were stained immunohistochemically with both CD56 and claudin-1 antibodies. A positive membranous immunostaining with or without cytoplasmic reactivity in more than 10% of the neoplastic cells with CD56 antibody qualified the case as positive .A positive membranous immunostaining in more than 5% of the neoplastic cells with claudin-1 antibody was considered positive. **Results**: CD56 expression was positive in 69 (92%) cases of follicular lesions. On the other hand CD56 expression was negative in 62(82.7%) cases of PTC (p value<0.001). CD56 as a negative marker for diagnosing PTC had the 82.7% sensitivity, 92% specificity, PPV OF 91.2% and NPV of 84.1%. Claudin-1 expression was positive in 72 cases (96%) of PTC and 56 (74.7%) cases of thyroid follicular lesions (p value<0.001). Claudin-1 as a positive marker for diagnosing PTC had a 96% sensitivity,74.7% specificity,79.1% of PPV and 94.9% of NPV. The panel of (claudin-1+/CD56-) had the specificity and PPV of 100%. In other words a thyroid nodule with positive claudin-1 and negative CD56 reactivity is definitely PTC.The panel of(claudin1-/CD56+) had the specificity and PPV of 100%.A thyroid nodule with positive CD56 and negative claudin-1 reactivity is definitely a Non- PTC lesion. **Conclusion**: claudin-1 expression is a sensitive immunohistochemical marker for differentiating PTC from other thyroid follicular lesions.CD56 is a less sensitive but more specific marker for identification of PTC. Combination of these two antibodies as a panel has the specificity and PPV of 100%.

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Introduction

Thyroid nodules are common findings, and in 7% of general population, palpable nodules are found during routine examinations. Using ultrasonographic methods, discovery of thyroid nodules have increased by 20%. (Gharib and et al., 2007; Topliss, 2004) Most of the thyroid nodules are benign and malignancy is detected in 5 to 24 percent of the thyroid nodules. (Hundahl and et al., 1998) However, an increase in malignancy in thyroid nodules has been reported in several studies over the past thirty years. Papillary carcinoma accounts for about 80% of all thyroid malignancies. (Finely and et al., 2004) The diagnosis of papillary thyroid carcinoma from other follicular thyroid nodules is based on the nuclear morphological characteristics of malignant cells. A set of cell nucleus characteristics in hematoxylin-eosin staining includes nuclear enlargement, nuclear overlapping, nuclear transparency, increased nucleus wall thickness, nuclear grooves, and intrinsic pseudo-inclusions for differentiation of papillary thyroid carcinoma from other useful thyroid follicular nodules. (Chan, 2002) The quality of the aforementioned nuclear characteristics is strongly dependent on the quality of the sample preparation, including type of stabilizer, duration of fixation and thickness of the incisions. Morphological characters between benign and malignant thyroid lesions are common. Follicular and papillary structures, nuclear alterations and dislocations are seen in benign and malignant thyroid lesions. (El demellawy and et al., 2008; Parsad,and et al., 2005) In addition, inflammatory conditions can lead to reactive changes in the nuclei, including nuclear enlargement, nuclear transparency, and even nuclear grooves. (Baloch Zw and Livolsi, 2004; Albores-Saavedra and Wu, 2006) In contrast, the subgroup of follicular papillary carcinoma and its other variants, in case of doubtful and incomplete nuclear characteristics, can cause differential diagnosis problems with follicular carcinoma, follicular adenoma and even nodular thyroid hyperplasia. (Schmid and Farid, 2006) Therefore, diagnosis of encapsulated follicular variant of papillary carcinoma from adenoma or follicular carcinoma can be subjected to a diagnostic disagreement among different pathologists. (Chan, 2002; Lloyd and et al., 2004) Diagnostic agreement among the Norwegian pathologists in 696 thyroid nodules was about 58%, and in the remaining 42% of the cases, there were major diagnostic problems. (Saxen and et al., 1987) Diagnostic agreement between eight American and Japanese pathologists in the case of 21

thyroid nodules was also achieved only in 62% of the cases. (Hirokawa and et sl., 2002) We aimed in this study to investigate the expression of claudin-1 and CD56 markers in the papillary thyroid carcinoma and other follicular thyroid nodules including follicular adenomas, follicular carcinoma and hyperplastic nodules in isolation and in combination with each other. Although the expression of these markers has been studied individually in a number of studies, the combined panel of these two markers has not been considered. Our aim is to investigate the difference between the two markers between papillary carcinoma and other follicular thyroid nodules in order to determine probable role of these markers in differentiation of papillary thyroid carcinoma from other follicular thyroid nodules.

Research Methodology

In this study, paraffin slides and blocks of thyroid samples (thyroidectomy and lobectomy) transferred to the pathology laboratory of Kashan Shahid Beheshti Hospital were studied during 2009 to 2016. Sampling was done census-based and all the identified samples were evaluated during this period based on input and output indices. According to the results of various studies, CD56 expressions were respectively 95% and 1.3% in the group of patients with papillary carcinoma and in the follicular nodule group (Mokhtari and et al., 2013), and the level of Claudin-1 in the group of patients with papillary carcinoma was 70% and this amount was 1% in the follicular nodule group. (Nemeth and et al., 2010) With a power = 10% and a confidence level of 95%, the sample size was calculated 10 people in each group. But since the comparison between the expression levels of the two above factors within the groups of different types of follicular nodules was also considered by the researchers, the minimum difference in follicular carcinoma and in the follicular adenoma was respectively corrected to 67% and 87% in Claudin-1 expression of the sample size (Tzelepi and et al., 2008) and increased to 66 cases in each group. Accounting for 10% of the sample defect, this volume reached to 75 in each group. This retrospective case-control study was performed on thyroidectomy and lobectomy specimens sent to pathology laboratory of Shahid Beheshti Hospital during 2009-2016. By referring to the archives of pathology laboratory of Shahid Beheshti Hospital, patients who were diagnosed with papillary carcinoma, follicular adenoma, follicular carcinoma and multinodular goiter were selected by census method. Among these samples, 75 samples with papillary carcinoma as the case group and 75 samples with the other diagnosis mentioned above under generalized title of follicular nodules were selected as the sham group. Paraffin blocks were then separated by referring to the block archive. Five- micron -thick sections were prepared from blocks with conventional methods and stained with H & E method. The stained slides by H & E method were reviewed by two pathologists. In order to detect papillary carcinoma, the histological specification proposed by Chan (Suren and et sl, 2017) and to diagnosis carcinoma and follicular adenoma, the histological specification proposed by Livolsi and Baloch (Park and et al, 2009) were used. Demographic characteristics and histologic diagnosis were recorded. To describe the data in this study, central tendency and dispersion criteria such as mean, standard deviation, median, and domain for quantitative data as well as frequency and percentage for qualitative data were used. The Cohen's Kappa agreement coefficient was used to determine the agreement between the two methods. To evaluate the performance of these two methods in diagnosis of PTC from Non PTC cases, sensitivity, specificity, positive predictive value and negative predictive value were reported. The relationship between qualitative variables was done through chi-squared test. It should be noted that the normality of distribution of quantitative variables such as age was performed using the Shapiro-Wilk-Chek test and where their distribution was not normal, then appropriate nonparametric tests and otherwise the related parametric test were used. The significance level in this study was considered 0.05. All analyzes were performed using SPSS software version 22.

Research Findings

Of 150 studied samples, 30 cases (20%) were male and 120 cases (80%) were female. The patients ranged in age from 20 to 69 years old with a mean age of 39.5 years old. The mean age in the sham group (Non PTC) was about 43 years and in the case group (PTC) was about 36 years. The results of the Mann-Whitney U test showed that there is a significant difference between the age of patients in the PTC and non PTC groups. (Table 1).

Table 1- Distribution of the studied people according to histopathology

Group	Mean age	Standard deviation	Median	Minimum	Maximum	P value
Non PTC	43.33	11.56	42	20	69	*<0.001
PTC	35.68	10.11	34	20	61	
Total	39.51	11.48	38	20	69	

*Mann-Whitney U test

As can be seen, using the logistic regression, it was found that the effect of age on PTC was significant. For increase of one year of age, the chance of PTC decreased by about 6% (Table 2).

Table 2- Regression analysis of age relationship with those samples with PTC

	Regression coefficient	Standard deviation	Test statistic	Odds ratio (OR)	P value
Age	-0.064	0.016	15.297	0.938	<0.001

In 150 studied samples, 80% and 20% of the cases were female and male, respectively. In the case (PTC) group, about 11% of the cases were male and in the sham group (Non-PTC) group approximately 30% of the cases were male. Chi-square test showed a significant relationship between gender and having PTC (P = 0.004) (Table 3).

Table 3- Distribution of the samples' gender according to the histopathologic status

Gender	Non PTC		PTC		Total number	Percentage	P value
	Number	Percentage	Number	Percentage			
Male	22	29.33%	8	10.7%	30	20.0%	*0.004
Female	53	70.67%	67	89.3%	120	80.0%	

*Chi-square test

As can be seen, using logistic regression, it was found that the effect of gender on PTC is significant. The chance of having PTC in female is about 3.5 times that of male (Table 4).

Table 4- Regression analysis of the samples' gender on having PTC

	Regression coefficient	Standard deviation	Test statistic	Odds ratio (OR)	P value
Gender	1.246	0.452	7.601	3.476	0.006

In the case group, 39 specimens (52%) were classic papillary carcinoma, 21 specimens (28%) were papillary microcarcinoma and 15 specimens (20%) were follicular variant papillary thyroid carcinoma. In the sham group, 37 specimens (49.3%) were hyperplastic nodules, 24 specimens (32%) were follicular adenomas and 14 specimens (18.7%) were follicular carcinoma. Using Chi-square test, there was a significant relationship between Claudin-1 expression and PTC detection (P <0.001). In most cases of non-PTC group (74.7%), less than 5% of the cells were stained, while in the PTC group, the majority of the cases (68%) had more than 50% of their sample cells stained. Gamma statistic value was obtained 0.97, which indicates that the ratio of those who had higher grades in the Claudin-1 test in the PTC group was greater than those in the Non PTC group (Table 5).

Table 5- Comparison of expression levels of claudin-1 markers in both groups of papillary carcinoma with individual follicular nodules

Claudin-1 expression level	Non PTC		PTC		Total number	Percentage	Gama coefficient	P value
	Number	Percentage	Number	Percentage				
0	56	74.7%	3	4.0%	59	39.3%	0.97	*<0.001
1	6	8.0%	0	0.0%	6	4%		
2	12	16.0%	21	28.0%	33	22%		
3	1	1.3%	51	68.0%	52	34.7%		

It was shown using Chi-square test that there was a significant negative correlation between CD56 expression and PTC detection (P <0.001). The gamma statistic value was -0.875, which indicates that the ratio of those who had lower grad in CD56 test was higher in the PTC group than in the Non PTC group. The negative sign in this test indicates a reverse relationship between having PTC and CD56 grading (Table 6).

Table 6- Comparison of CD56 marker expression levels in the two groups of papillary carcinoma with individual follicular nodules

CD56 expression level	Non PTC		PTC		Total number	Percentage	Gama coefficient	P value
	Number	Percentage	Number	Percentage				
0	6	8.0%	62	82.7%	68	45.3%	-0.875	<0.001
1	25	33.3%	5	6.7%	30	20%		
2	29	38.7%	7	9.3%	36	24%		
3	15	20.0%	1	1.3%	16	10.7%		

Chi-square test was used to examine the relationship between CD56 and having PTC expression or absence of PTC in the sample. There was a significant relationship between lack of expression of CD56 and PTC lesion (P <0.001). 62 cases (82.7%) in the case (PTC) group had negative CD56 expression, while 6 cases (8%) of the sham (Non-PTC) group had a negative CD56 expression (Table 7).

Table 7- Comparison of expression distribution of positive and negative CD56 markers in the two groups of papillary carcinoma and follicular nodules of thyroid

CD56 expression	Non PTC		PTC		P value
	Number	Percentage	Number	Percentage	
Positive	69	92%	13	17.3%	<0.001
Negative	6	8%	62	82.7%	

Of the 150 examined cases of both the case and sham groups by CD56, 131 cases (87.3%) from the total number of specimens were correctly identified by CD56. The rate of Kappa agreement coefficient was 0.747, which shows a high agreement between morphological diagnosis and CD56 marker results. It was also significant (P <0.001). This indicates that CD56 was a good predictor in PTC detection (Table 8).

Table 8 - CD56 marker diagnostic value for identification of PTC from Non-PTC

Correctness results of CD56 marker expression	Number	Percentage (%)	Kappa agreement Coefficient	P value

Correct detection cases	131	87.3%	0.747	<0.001
Incorrect detection cases	19	12.7%		

The sensitivity of the CD56 marker for differentiating the papillary thyroid carcinoma from the thyroid follicular nodules was 82.7%, meaning that 82.7% of the samples that were truly PTC had negative CD56 result and therefore they were placed in the PTC group. The specificity of this marker was 92%, meaning that CD56 result of 92% of the specimens that were truly follicular thyroid nodule (non-FTD) was positive, thus they were placed in this group. The positive predictive value of this marker was 91.2%, meaning that among the samples that were placed in the PTC group as a result of their CD56 marker results, 91.2% of the cases were truly PTC. The negative predictive value of this marker was 84.1%, meaning that 84.1% of the samples that were placed in the Non-PTC group as a result of this marker results were really Non-PTC (Table 9).

Table 9- Sensitivity, specificity, positive predictive value and negative predictive value of CD56 marker for differentiation of papillary thyroid carcinoma from individual follicular nodules

Sensitivity	Specificity	Positive predictive value	Negative predictive value
82.7%	92%	91.2%	84.1%

Chi-square test was used to examine the relationship between the result of Claudin-1 expression and the samples with and without PTC. There was a significant relationship between the positive expression of this marker and the samples with PTC (P <0.001). 72 cases (96%) of the case (PTC) group had positive Claudin-1 expression, while 19 cases (25.3%) of the sham (non-PTC) group had a positive expression of Claudin-1 (Table 10).

Table 10- Comparison of the positive and the negative expression of Claudin-1 marker in the two groups of papillary thyroid carcinoma and individual follicular nodules

Claudin-1 expression	Non PTC		PTC		P value
	Number	Percentage	Number	Percentage	
Negative	56	74.7%	3	4%	<0.001
Positive	19	25.3%	72	96%	

Of the 150 studied cases of the both case and sham groups by Claudin-1 marker, 128 (85.3%) cases of the specimens were correctly identified by this marker. The Kapa agreement coefficient was 0.707, which showed a significant agreement between morphological diagnosis and Claudin-1 marker results that was significant (P <0.001). So Claudin-1 was a good predictor in PTC detection (Table 11).

Table 11 - Claudin-1 Marker Diagnostic Value in identification of PTC from Non-PTC specimens

Correctness results	Number	Percentage	Kappa	P value
of Claudin-1 marker expression		(%)	agreement Coefficient	
Correct detection cases	128	85.3%	0.707	<0.001
Incorrect detection cases	22	14.7%		

The sensitivity of the Claudin-1 marker in differentiation of the papillary thyroid carcinoma from the follicular thyroid nodules is 96%, meaning that 96% of the samples that were truly PTC also had positive result in Claudin-1 marker expression. The specificity of this marker was 74.7%, meaning that 74.7% of the cases that were really follicular thyroid nodules (Non-PTC) had negative result for Claudin-1 marker expression. The positive predictive value of this marker was 79.1%, which meant that among the cases that the results of Claudin-1 marker expression put them in the PTC group, 79.1% of the cases were truly PTC. The negative predictive value in this test was 94.9%, meaning that among the samples that the results of Claudin-1 marker expression placed them in the Non PTC group, 94.9% of the cases were truly Non PTC (Table 12).

Table 12- Sensitivity, specificity, positive predictive value and negative predictive value of the claudin-1 marker for differentiation of papillary thyroid carcinoma from individual follicular nodules

Sensitivity	Specificity	Positive predictive value	Negative predictive value
96%	74.7%	79.1%	94.9%

Of 150 samples in both case (PTC) and sham (Non PTC) groups, the two examined markers had agreement in diagnosis in 109 cases (72.6%), and the rate of diagnostic disagreement between the markers was 27.4%. The Kappa agreement coefficient was 0.464, which showed a moderate agreement between the two markers (P <0.001). (Table 13)

Table 13- Comparison of the level of agreement or disagreement of CD56 and Claudin-1 diagnostic tests in the diagnosis of papillary thyroid carcinoma from the follicular thyroid nodules

Level of agreement between CD56 and Claudin-1 markers	Number	Percentage (%)	Kappa agreement Coefficient	P value
Agreeing cases	109	72.6%	0.464	<0.001
Not agreeing cases	41	27.4%		

Chi-square test was used to examine the relationship between Claudin-1 marker expression and follicular adenoma (F.A) or papillary carcinoma (PTC) in the samples. 96% of papillary carcinoma specimens had a positive marker expression, but follicular adenomas in 25% of the cases showed this marker. Significant relationship was observed between them (P <0.001). (Table 14)

Table 14- Comparison of the positive and the negative expression of Claudin-1 marker in the two groups of papillary carcinoma and follicular adenoma

Claudin-1expression	F.A		PTC		P value
	Number	Percentage	Number	Percentage	
Positive	18	75%	3	4%	<0.001
Negative	6	25%	72	96%	

*Chi-square test

The sensitivity of the Claudin-1 marker in the diagnosis of follicular adenoma from the papillary carcinoma was 96%, meaning that 96% of the samples that were truly PTC were also positive for Claudin-1. The specificity of this method was 75%, meaning that 75% of the specimens that were really follicular adenomas (FA) were also negative for Claudin-1. The positive predictive value in this method was 92.3%, meaning that among the samples with positive results in the Claudin-1 marker expression, 92.3% of the cases were truly PTC. The negative predictive value of this marker was 85.7%, meaning that 85.7% of the people whose marker expression was negative were really follicular adenoma (FA) (Table 15).

Table 15- Sensitivity, specificity, positive predictive value and negative predictive value of the claudin-1 marker in differentiating papillary thyroid carcinoma from follicular adenoma

F.A detection from PTC by using Claudin-1			
Sensitivity	Specificity	Positive predictive value	Negative predictive value
96%	74.7%	79.1%	94.9%

Of 99 cases with papillary carcinoma or follicular adenoma, 90 (90.9%) cases were correctly diagnosed by Claudin-1 markers. The Kappa agreement rate was obtained 0.742, which showed a significant agreement between the histopathologic diagnosis and the results of the expression of this marker. It was also significant (P <0.001). (Table 16)

Table 16 - Comparison of the correct and incorrect detection of PTC from F.A using the expression of the Claudin-1 marker in comparison with the H & E staining

Correctness results of Claudin-1 marker expression	Number	Percentage (%)	Kappa agreement Coefficient	P value
Correct detection cases	90	90.9%	0.742	<0.001
Incorrect detection cases	9	9.1%		

Chi-square test was used to examine the relationship between CD56 marker expression and follicular adenoma (F.A) or papillary carcinoma (PTC) in the samples. There was a significant relationship between them (P <0.001). 82.7% of the samples with papillary carcinoma had negative CD56, while 12.5% of the follicular adenoma (F.A) samples had a negative CD56 result (Table 17).

Table 17- Comparison of distribution of positive and negative expression of CD56 marker in the two groups of papillary carcinoma and follicular adenoma

CD56 expression	F.A		PTC		P value
	Number	Percentage	Number	Percentage	
Positive	21	87.5%	13	17.3%	<0.001
Negative	3	12.5%	62	82.7%	

The sensitivity of CD56 marker was 82.7% in detection of papillary carcinoma and follicular adenoma, meaning that 82.7% of the samples with truly PTC and the result of the expression of the CD56 marker also put them in the PTC group. The specificity of this method was 87.5%, meaning that 87.5% of the cases that were truly follicular adenoma (F.A) were also placed in this group as a result of their result of the expression of the CD56 marker. The positive predictive value of this marker was 95.4%, meaning that among 95.5% of the samples that were placed in the PTC group as a result of their CD56 marker results, 95.4% of the cases were truly PTC. The negative predictive value in this test was 61.8%, which meant 61.8% of the samples that the results of the expression of the CD56 marker were placed them in the follicular adenoma group was actually a follicular adenoma. (Table 18).

Table 18 - Sensitivity, specificity, positive predictive value and negative predictive value of CD56 marker for differentiation of papillary thyroid carcinoma from follicular adenoma

Sensitivity	Specificity	Positive predictive value	Negative predictive value
82.7%	87.5%	95.4%	61.8%

Of 99 cases of papillary carcinoma or follicular adenoma, 83.8% of the cases were correctly diagnosed with CD56. The Kappa agreement rate of 0.615 was found to provide a significant agreement between the histopathologic diagnosis and the results of this marker expression (P <0.001) (Table 19).

Table 19- Diagnostic Value of CD56 Marker in identification of PTC from F.A

Correctness results of CD56 marker expression	Number	Percentage (%)	Kappa agreement Coefficient	P value
Correct detection cases	83	83.8%	0.615	<0.001
Incorrect detection cases	16	16.2%		

Using Fischer's exact test, a relationship between the expression of the Claudin-1 marker and the follicular carcinoma (F.C) or papillary carcinoma (PTC) of the cases was investigated. There was a significant relationship between them (P <0.001). 96% of the cases with papillary carcinoma (PTC) had a positive Claudin-

1 result, while 42.9% of the cases with follicular carcinoma (F.C) had positive result for Claudin-1 (Table 20).

Table 20- Comparison of positive expression of Claudin-1 marker expression in the two groups of papillary thyroid carcinoma and follicular carcinoma

Claudin-1 expression	F.C		PTC		P value
	Number	Percentage	Number	Percentage	
Negative	8	57.1%	3	4%	<0.001
Positive	6	42.9%	72	96%	

*Fisher exact test

Sensitivity of the Claudin-1 marker for detection of papillary carcinoma and follicular carcinoma was 96%, meaning that 96% of the specimens that were really papillary carcinoma were also positive for Claudin-1 result. The specificity of this marker was 57.1%, meaning that 57.1% of people who had truly follicular carcinoma (F.C) were also negative for Claudin-1 result. The positive predictive value of this marker was 92.3%, meaning that among the samples that had positive results for the Claudin-1 marker, 92.3% were truly PTC cases. The negative predictive value of this marker was 72.7%, meaning that 72.7% of the samples with negative test results, really had follicular carcinoma (F.C) (Table 21).

Table 21- Sensitivity, specificity, positive predictive value and negative predictive value of Claudin-1 in detecting papillary carcinoma from follicular carcinoma

Sensitivity	Specificity	Positive predictive value	Negative predictive value
96%	57.1%	92.3%	72.7%

Of 89 cases of papillary carcinoma or follicular carcinoma, 89.9% of the cases were correctly diagnosed by Claudin-1. The rate of Kappa agreement coefficient was 0.582 which showed a moderate agreement between histopathological diagnosis and the results of expression of this marker (P <0.001) (Table 22).

Table 22- Diagnostic value of Claudin-1 marker in identification of PTC from F.C

Correctness results of Claudin-1 marker expression	Number	Percentage (%)	Kappa agreement Coefficient	P value
Correct detection cases	80	89.9%	0.582	<0.001
Incorrect detection cases	9	10.1%		

Using Fisher's exact test, a relationship between the expression of CD56 marker and follicular carcinoma (F.C) or papillary carcinoma (PTC) in the samples was investigated. There was a significant relationship between them (P <0.001). 82.7% of the papillary carcinoma cases had a negative CD56 result, while none of the follicular carcinoma (F.C) specimens had a negative CD56 (Table 23).

Table 23- Comparison of distribution of CD56 positive and negative expression in the two groups of papillary carcinoma and follicular carcinoma

CD56 expression	F.C		PTC		P value
	Number	Percentage	Number	Percentage	
Positive	14	100%	13	17.3%	<0.001
Negative	0	0.0%	62	82.7%	

The CD56 marker sensitivity was 82.7% in diagnosis of papillary carcinoma and follicular carcinoma, meaning that 82.7% of the specimens that were truly papillary carcinoma (PTC) were also placed in the PTC group as a result of their negative CD56 result. The specificity of this method was 100%, meaning that all samples that were truly follicular carcinoma (F.C) were also placed in this group as a result of their positive CD56 result. The positive predictive value of this marker was 100%, meaning that among all samples that were placed in the papillary carcinoma (PTC) group as a result of their negative results of the CD56 marker, they all were truly PTC. The negative predictive value in this test was 51.9%, meaning that 51.9% of the samples that were placed in the follicular carcinoma (F.C) group as a result of their positive CD56 results were truly follicular carcinoma. (Table 24).

Table 24 - Sensitivity, specificity, positive predictive value and negative predictive value of CD56 in identification of papillary carcinoma from follicular carcinoma

Sensitivity	Specificity	Positive predictive value	Negative predictive value
82.7%	100%	100%	51.9%

Of 89 cases with papillary carcinoma or follicular carcinoma, 85.4% of the samples were correctly diagnosed with CD56. The Kappa Coefficient was obtained 0.6 which showed a moderate agreement between the histopathologic diagnosis and the results of the expression of this marker (P <0.001) (Table 25).

Table 25- Diagnostic Value of CD56 Marker in identification of PTC from F.C

Correctness results of CD56 marker expression	Number	Percentage (%)	Kappa agreement Coefficient	P value
Correct detection cases	76	85.4%	0.6	<0.001
Incorrect detection cases	13	14.6%		

To evaluate the diagnostic power of combination of both Claudin-1 and CD56 markers in diagnosis of follicular thyroid nodules (non-PTC) from papillary carcinoma, a combination of negative Claudin-1 and positive CD56 expressions was used simultaneously and named a negative panel for papillary carcinoma. 50 cases (66.7%) of the follicular nodules (non-PTC) group had negative Claudin-1 and positive CD56 expressions (negative panel), and 25 cases (33.3%) showed an expression other than this combination. On the opposite side, none of the PTC group samples concurrently had CD56 and Claudin-1 results according to the negative panel and showed a combination other

than it. Chi-square test was used to determine a relationship between concurrent results of positive CD56 test, negative Claudin1 and non PTC lesion. A significant relationship was observed between them (P <0.001) (Table 26).

Table 26- Distribution of expression of positive CD56 marker and negative Claudin1 (negative panel) in both groups of papillary thyroid carcinoma and individual follicular nodules

Claudin1 & CD56 Combined panel	PTC		Non PTC		P value
	Number	Percentage (%)	Number	Percentage (%)	
Negative panel (Claudin 1-/CD56+)	0	0%	50	66.7%	<0.001
Non negative panel	75	100%	25	33.3%	

Sensitivity of the negative panel (+claudin1/- CD56) in diagnosing follicular thyroid nodules (Non PTC) was 66.7%, its specificity was 100%, its positive predictive value was 100%, and its negative predictive value was 75% (Table 27).

Table 27- Sensitivity, specificity, positive predictive value and negative predictive value of the negative panel (+Claudin1 / -CD56) in identification of individual follicular nodules

Sensitivity	Specificity	Positive predictive value	Negative predictive value
66.7%	100%	100%	75%

Logistic regression analysis was used to investigate the effect of CD56 by considering simultaneous effect of age and gender variables on the cases with PTC. As can be seen, people who had negative expression of CD56 had PTC chance approximately 42 times more than those with positive CD56 (Table 28).

Table 28 - Logistic regression analysis of CD56 effect by taking into account the simultaneous effects of age and gender variables on the samples with PTC

	Regression coefficient	Standard deviation	Test statistic	Odds ratio (OR)	P value
Age	-0.035	0.023	2.282	0.966	0.131
Gender	0.58	0.654	0.787	1.787	0.375
CD56	3.732	0.537	48.329	41.772	<0.001

Using the logistic regression analysis and considering the simultaneous effects of gender and age variables, the effect of Claudin-1 on the samples with PTC was studied. As can be seen, Claudin-1 positive people had PTC chance 83 times more than those with negative Claudin-1 (Table 29).

Table 29- Logistic regression analysis of Claudin-1 effect by taking into account the simultaneous effects of gender and age variables on the samples with PTC

	Regression coefficient	Standard deviation	Test statistic	Odds ratio (OR)	P value
Age	-0.075	0.023	10.84	0.927	0.001
Gender	4.421	0.707	39.123	5.892	0.012
Claudin 1	1.774	0.704	6.347	83.198	<0.001

Discussion and Conclusion

The microscopic diagnosis of papillary thyroid carcinoma is usually possible based on papillary structures and characteristics of the nucleus of the malignant cells in H & E staining; however, in the absence of a papillary structure or prominent papillary characteristics, differentiation between follicular type and other follicular thyroid nodules can be difficult. In recent years, immunohistology has been used to solve diagnostic problems and the expression of CD56 and Claudin-1 markers have been investigated for diagnosis of papillary thyroid carcinoma. (Etem, and et al., 2010; Abd El and et al., 2012; Nemeth and et al., 2010) Considering the different results of previous studies, we decided to investigate the role of these markers in differentiation of papillary thyroid carcinoma from other follicular thyroid nodules. Based on the results of this study, CD56 membrane expression was positive in 92% of the follicular thyroid nodules. This positive expression was observed in 100% of follicular carcinoma cases, 87.5% of follicular adenoma cases and 91.5% of hyperplastic nodules cases. On the other hand, 82.7% of the studied cases with papillary carcinoma did not express the CD56 marker. The sensitivity of negative expression of CD56 marker to detect papillary thyroid carcinoma from other follicular thyroid nodules was 82.7% and its negative predictive value was 84.1%, its specificity was 92%, and its positive predictive value was 91.2%. In a study by Golu.l et al., the negative expression of the CD56 marker had a sensitivity of 76%, specificity of 89%, positive predictive value of 86%, and negative predictive value of 81% for the detection of papillary carcinoma from follicular thyroid nodules. (Golu and et al., 2017) These results are consistent with the results of the recent study. In Nehal S. Abouhashem et al., 81.8% of papillary carcinoma cases showed negative CD56 marker, which is similar to the results of this study. (Abouhashem and Talat, 2017) Contrary to the results of this study, Etem et al. did not report a significant difference in the expression of CD56 between follicular nodules and papillary thyroid carcinoma. Of course, in this study, the membrane or cytoplasmic expression of CD56 was considered positively in any ratio of examined neoplasm cells. (Etem and et al., 2010) In the present study, a total of 150 samples examined in both papillary carcinoma and follicular thyroid nodules groups, positive or negative expression of CD56 marker in immunohistochemistry samples were consistent with histopathology diagnosis in 87.3% of the cases and the Kappa agreement coefficient of the expression of CD56 marker was obtained 0.747 by histopathological examination, which indicates the proper predictive power of this marker for the diagnosis of papillary carcinoma. In the study of Abd El Atti et al., the CD56 marker correctly recognized the papillary carcinoma in 87% of the studied cases from the follicular thyroid nodule group (Abd and et al., 2012), which is similar to the results of this study. Positive expression of CD56 in some cases of papillary carcinoma may be

related to more aggressive phenotypes and more metastatic potency, as Scarpino et al., showed that decreasing in the expression of CD56 in papillary carcinoma leads to decreased expression of vascular growth factors (VEGF-C and VEGF-D), which these factors can stimulate the lymphatic migration of malignant cells. (Scarpino and et al., 2007) Role of the Claudin family, including Claudin-1, has been studied extensively as a protein component of inter-cellular tight junctions at the onset and progression of malignancies. Reduced expression, increased surface area, or subcellular relocalization of tight junction proteins have been reported in a variety of human malignancies, which have been linked in different ways to differentiation and prognosis. In this study, 96% of the cases of papillary carcinoma expressed Claudin-1 marker with membrane pattern, and only 4% of the expression of this marker was negative. On the other hand, 74.7% of the follicular thyroid nodules did not express this marker. The negative expression of Claudin-1 was separately in the follicular nodule group, containing 81.1% of hyperplastic nodules, 57.1% of follicular carcinoma and 75% of follicular adenoma. Positive expression of Claudin-1 with membrane pattern for diagnosis of papillary carcinoma had sensitivity of 96%, negative predictive value of 94.9%, specificity of 74.7% and positive predictive value of 79.1%. In the present study, from 150 samples examined in both groups of papillary carcinoma and follicular thyroid nodules, the interpretation of positive or negative expression of Claudin-1 in immunohistochemistry was consistent with histopathology diagnosis in 85.3% of the cases, and Kappa agreement coefficient of Claudin-1 was found to be 0.707 with histopathologic examination, which was significant ($p < 0.001$) and showed suitable predictive power of this marker for the diagnosis of papillary carcinoma. In the study of Abd El Atti et al., Claudin-1 marker correctly recognized papillary carcinoma from the follicular thyroid nodule in 86% of the studied cases. (Abd and et al., 2012) In the study of Dink Suren et al., there was a significant difference in Claudin-1 expression between papillary carcinoma, follicular carcinoma, follicular adenoma and multinodular goiter nodules groups, so that 97% of the papillary carcinoma cases were expressed this marker, while 12.8% of benign follicular nodules cases and 10% of malignant follicular cases expressed this marker. (Suren and et al., 2017) The results of Claudin-1 expression in papillary carcinoma are consistent with the results of the recent study. In the study of Abd El Atti et al., Claudin-1 expression in 80.9% of the thyroid follicular nodule cases were negative and positive expression of this marker was found in 100% of papillary carcinoma cases. (Abd and et al., 2012) Also, in the study of Nemeth. J et al., a significant difference in Claudin-1 expression between papillary carcinoma and non-papillary follicular thyroid nodules was reported. (Nemeth and et al., 2010) Due to the role of Claudin-1 in tight intercellular junctions, increased expression of this protein in malignancy seems to be far from the mind. However, this expression may be related to other roles of this protein, which is irrelevant to tight intercellular junctions. In a study on nano-fibrillated cellulose (NFC) culture, Claudin-1 expression was associated with a reduction in apoptosis in cell lines following fluorouracil treatment. A possible mechanism for Claudin role in malignant transformation may occur from the matrix metalloproteinases (MMPs) pathway.

Increasing Claudin-1 expression in oral squamous cell carcinoma enhances invasion by activating MMP-1 and MMP-2 and increasing Claudin-4 expression in surface epithelial ovarian carcinoma enhances invasion by activating MMP-1 and MMP-2. (Oku et al., 2006) Based on the results of this study, the compound panel with the result (+Claudin-1/-CD56) had a specificity and positive predictive value of 100%, sensitivity of 78.7% and negative predictive value of 82.4% for identification of papillary thyroid carcinoma. This means that if a nodule has a positive result of Claudin-1 expression and a negative result of the expression of CD56, then papillary carcinoma is definitely confirmed. On the other hand, the compound panel with the result (-Claudin-1/+CD56) had a specificity and positive predictive value of 100%, sensitivity of 66.7%, and negative predictive value of 75% for identification of Non PTC lesions, which means that if a nodule has a positive result of CD56 expression and a negative result of Claudin-1 expression the lesion is definitely a lesion other than papillary thyroid carcinoma. In the only study with a similar panel with the compound panel of the present study by Rasha M. Abd El Atti., the compound panel (-CD56/+claudin-1) had a sensitivity of 81.3%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 94%. (Abd El Atti & Shash, 2012).

References

Abd El Atti RM, Shash LS. Potential diagnostic utility of CD56 and claudin-1 in papillary thyroid carcinoma and solitary follicular thyroid nodules. J Egypt Natl Canc Inst. 2012;24:175-184.

Abouhashem N,m.Talat s. Diagnostic utility of CK19 and CD56 in the differentiation of PTC from its mimics.Pathology, Research and practice.2017;213:509-517.

Albores-Saavedra J, Wu J. The many faces and mimics of PTC . Endocr Pathol 2006;17:1-18.

Baloch Zw, Livolsi Va. FNA of thyroid nodules: past, present and future. Endocr Pract 2004;10:234-41.

Chan J. Strict criteria should be applied in the diagnosis of papillary carcinoma .Am j Clin Pathol 2002;117:16-8.

El demellawy D, Nasr A, Alowami S. Application of CD56, p63, CK19 IHC in the diagnosis of PTC .Digan Pathol 2008;3:5.

Etem H,Ozekinci S,Mizrak B,Senturk S. The Role of CD56,HBME-1 and p63 in follicular neoplasms of the thyroid.Turk Patoloji Derg 2010;26:238-242.

Finely Dj, Arora N, Zhu B, Gallagher l, Fahey Tj. Molecular proliferating distinguishes papillary carcinoma from benign thyroid nodules. J Clin Endocrinol Metab 2004;89:3214-23

Gharib H Papini E. Thyroid nodules: clinical importance, assessment and treatment. Endocrinol Metab clin north am 2007;36:707-35.

Golu l, Vlad Mm, Dema A, Moleriu lc, Tudor A, Lacob M, Popa O, Corniau M. The absence of CD56 expression can differentiate PTC from other thyroid lesions.Indian J Pathol Microbiol 2017;60:161-6.

Hirokawa M, Carney JA, Goellner JR, DeLellis RA, Heffess CS, Katoh R, et al. Observer variation inencapsulated follicular lesions of the thyroid. Am J Surg Pathol. 2002;26:1508-1514

Hundahl Sa , Fleming id, Fergmen Am,Menck Hr. A national cancer data base report on 53856 cases of thyroid carcinoma treated in the US 1985-1995 .Cancer 1998;83:2638-48

Lloyd rv, Erickson la, Casey Mb, Lam Ky , Lohse Cm, Asa Sl, et al. Observer variation in diagnosis of FV-PTC . Am J Surg Pathol 2004;28:1336-40.

Mokhtari M , Eftekhari M , Tahririan R . Absent CD56 expression in papillary thyroid carcinoma : A finding of potential diagnostic value in problematic cases of thyroid pathology. J Res Med Sci. 2013;18(12):1046-1050

Nemeth J, Nemeth Z, Tatrai P, Peter I, Somoracz A, Szasz AM,et al. High expression of claudin-1 protein in papillary thyroid tumor and its regional lymph node metastasis. Pathol Oncol Res. 2010;16:19–27.

Park WY, Jeong SM, Lee JH, Kang HJ, Sin DH, Choi KU et al. Diagnostic value of decreased expression of CD56 protein in papillary carcinoma of the thyroid gland. Basic Appl Pathol,.2009;2:63–8.

Parsad Ml, Pellegasta Ns, Huang Y, Nagaraja Hn, de la Chapelle a, Kloos Rt. Galectin-3 ,fibronectin-1, CITED-1,HBME-1 andCK-19 IHC is useful for D.Dx of thyroid tumors. Mod Pathol 2005;18:48-57

Saxen E, Franssila K, Bjarnason O, Normann T, Ringertz N. Observer variation in histologic classification of thyroid cancer.Acta Pathol Microbiol Scand a 1978;86a: 483-6.

Scarpino S, di Napoli a, Melotti F, Talerico C, Cancrini A, Rucco l .PTC: low expression of NCAM is associated with downregulation of VEGF –d production by tumor cells.J Pathol 2007;212:411-9.

Schmid Kw, Farid Nr. How to define follicular thyroid carcinoma? Virchows Arch 2006;448:385-93.

Suren D, Yildrim M, Sayiner A, et al.Expression of claudin-1,4 and 7 in thyroid neoplasms.Oncol let. 2017;13(5):3722-3726.

Topliss D. Thyroid incidentaloma: the ignorant in pursuit of the impalpable. Clin Endocrinol 2004;60:18-20

Tzelepi VN , Tsamandas AC , Vlotinou H , Vagianos C , Scopa Ch . Tight junction in thyroid carcinogenesis:diverse expression of claudin-1 claudin-4 claudin-7 and occludin in thyroid neoplasms.modern path.2008;21:22-30.