RESEARCH ARTICLE

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Expression of Long Non-Coding RNA-NEAT-1 in Papillary Thyroid Carcinoma in Relation to Immunohistochemical expression of CD8 and PD-L1 as Indicators of Tumor Immune Microenvironment

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Abstract

Objective: Investigation of *CD8* and *PD-L1* immunohistochemical expression, as indicators of tumor microenvironment in PTC, and their correlation with *lncRNA-NEAT1* expression. **Methods:** Quantitative determination of relative expression level of *lncRNA-NEAT1* using the real-time PCR (RT-qPCR) and evaluation of CD8 and PD-L1 immunohistochemical expression in 47 specimens of PTC tissue compared to 5 controls of normal thyroid tissue. The results were analyzed for their association with the clinicopathological parameters of the studied cases. **Results:** Compared to control specimens, *LncRNA-NEAT1* expression levels were considerably higher in PTC cases (p=0.000), and they were also significantly higher in patients with nodal metastases and lymphovascular emboli (LVE) (p=0.003 and 0.002 respectively). There were significant associations between *PD-L1* positive expression and extra-thyroid extension (p=0.02), nodal metastasis (p=0.00), and LVE (p=0.00). In contrast, significant associations of increased *CD8* expression with Hashimoto's thyroiditis (HT) (p=0.007), absent nodal metastasis (p=0.02), and negative LVE (p=0.01) were detected. There was a significant negative correlation between *CD8* and *PD-L1* expression levels (p=0.014). **Conclusion:** *LncRNA-NEAT1* and *PD-L1* expression levels are associated with more aggressive PTC. In contrast, high *CD8* expression is associated with favorable parameters. These findings suggest the possible diagnostic and prognostic role of these biomarkers in PTC.

Keywords: *lncRNA-NEAT1- CD8- PD-L1-* Papillary thyroid carcinoma

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Introduction

Among endocrine system cancers, thyroid cancer (TC) is the most prevalent. It is further classified into four entities: anaplastic, medullary, follicular, and papillary. Papillary subtype is the most prevalent kind, with an incidence rate of 80–85%, a survival ratio of >90%, and a low risk of metastasis [1] .

LncRNAs, which comprise transcripts with more than 200 nucleotides, have been associated with various cancer types. They have the ability to regulate a wide range of biological processes even though they are not translated into proteins. NEAT1 is a lncRNA that is present in a number of malignancies and can initiate thyroid carcinogenesis. *NEAT1* overexpression in TC causes inhibition of cell apoptosis and increased tumor cell motility, survival, proliferation, invasion, and migration. Growth arrest and the prevention of metastasis were the outcomes of NEAT1 knockdown in papillary thyroid cancer (PTC) cell lines. According to these results, NEAT1 may be used in PTC as a diagnostic and treatment tool [2, 3].

In turn, the tumor microenvironment (TME) generates a variety of elements that support tumor angiogenesis. The surrounding extracellular matrix, immune cells, fibroblasts, macrophages, endothelial cells, tumor cells, and soluble materials including the released cytokines and growth factors make up this environment. In order to get rid the body of tumor cells and damaged cells, immune cells are crucial. CD4+ T cells (helper T cells), and CD8+ T cells (cytotoxic T lymphocytes) (CTLs), are two varieties of tumor-infiltrating T cells that work against tumors by releasing cytokines including tumor necrosis factor-alpha. The growth, invasion, and metastasis of

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tumors are significantly influenced by these cytokines [4] .

A higher density of lymphocytes is linked to improved survival and lesser recurrences in human PTC. There was a favorable association between decreased tumor size and CD8+ and CD4+ T cells, and B cells [5]. The main function of CTLs is to kill tumor cells by specifically identifying endogenous antigen peptide-MHC I molecular complexes. This is now a crucial marker for assessing the prognosis of tumors. Greater *CD8+ T* cell expression in PTC patients results in reduced tumor stages and improved survival, whereas decreased *CD8+ T* cell expression impairs the capacity of immune system to eliminate tumor cells, increasing the aggressiveness of tumors [6].

There is growing evidence that lncRNAs alter the TME, which explains the emergence and spread of tumors [7]. NEAT1 inhibition has been shown to enhance cytolytic activity and reduce CD8+ T-cell apoptosis. NEAT1's regression suggests a useful target for enhancing immunotherapy results [8].

Inflammation linked to TC is a key target for both new therapeutic approaches and diagnostic techniques. PD-1 and PD-L1 are two examples of molecules that are linked with membranes. A synonym for them is "immune checkpoints" [5]. Immunological checkpoints serve as regulators that preserve self-tolerance, inhibit immunological hyperactivity, and regulate the onset and severity of immune responses. These regulators promote immune evasion in TME by suppressing immunological responses, which prevents the body from initiating a successful immune defense against cancer. Indoleamine 2,3-dioxygenase (IDO), cytotoxic T lymphocyte antigen 4, PD-1, and PD-L1 are common immunological checkpoints in PTC [6].

By binding PD-1, PD-L1 suppresses the immunological response of antitumor T lymphocytes. Poor prognosis and tumor progression are linked to *PD-L1* overexpression [8]. *PD-L1* expression has been found to be elevated in PTC patients, with higher expression in those with lymph node metastases [9].

The objective of this study is to investigate *CD8* and *PD-L1* immunohistochemical expression, as indicators of tumor microenvironment in PTC, and their correlation with PTC clinopathological parameters, each others and *lncRNA-NEAT1* expression.

Materials and Methods

Retrieval of Cases

This is a retrospective observational cross-sectional study. A total of 52 specimens (47 specimens of PTC tissue and 5 controls of normal thyroid) were obtained from the pathology department archives at Kasr Al-Ainy Hospital and stored as formalin-fixed, paraffin-embedded blocks. Inclusion criteria included cases of PTC of both males and females above 18 years (non-childhood age) and any stage of PTC subjected to thyroidectomy with complete clinicopathological data. Cases with missing data were excluded.

To determine the sample size, G*Power 3.1.9.7 was used [10, 11]. After reviewing the literature for relevant studies investigating similar markers [9, 12 , 13], at the level of confidence of 95% and power of 80%, an effect size of 0.8, and a case/ control ratio of 1. The calculated minimum total sample size was 47 specimens and we agreed on a total of 52 specimens to be included in the present study, 47 specimens of PTC tissue, and 5 specimens of normal thyroid as healthy controls.

The Faculty of Medicine's Ethical Committee at Cairo University approved this work (Ethical approval code: N-461-2023). An ID number was used in place of the study participants' names to protect patient privacy. Only this ID number was utilized in the data sheet and statistical analysis, as well as on the paraffin blocks and glass slides.

The collected clinic-pathological parameters for each case including age, sex, laterality, multicentricity and tumor size were obtained from the pathology request form and pathology report.

Histopathological examination

Hematoxylin-eosin stained tissue sections were prepared for histological examination, to confirm the diagnosis, and the cases were categorized according to Tumor stage (T stage), Nodal metastases, +/- extra thyroid extension, +/- LVE, and +/- associated HT.

RNA extraction and real-time polymerase chain reaction (RT-PCR)

The collected blocks were sectioned at 10 µm thickness. Each sample included 3 sections and was stored at -80◦C for RNA extraction followed by reverse transcription into complementary DNA (cDNA) then quantitative determination of *lncRNA NEAT1* relative expression levels using quantitative real-time polymerase chain reaction (qRT-PCR).

RNA extraction: The guidelines provided by the manufacturer were followed utilising the miRNeasy mini kit for extracting total RNA from tissue (Qiagen, Valencia, CA, USA). The NanoDrop®-1000 with the use of a spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA), the purity of the RNA samples was assessed.

cDNA reverse transcription: Reverse transcription of extracted RNA was performed using RT2 first strand kit (the miScript II RT kit, Qiagen, Valencia, CA, USA) to produce cDNA following the manufacturer's guidelines.

Quantitect SYBR green PCR master mix was used for quantitative real-time PCR for lncRNA NEAT1 detection (Qiagen, Valencia, CA, USA) and NEAT1 primer assay (Qiagen, Valencia, CA, USA) in a 25 µl per well reaction volume. Additionally, as an internal control for NEAT1, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was applied (Qiagen, Germany, Cat. No. 330701, GeneGlobe ID- QT00079247).

The expression levels were assessed using the ΔCt method. The fold change in *NEAT1* expression levels was measured using the equation $2^{-\Delta\Delta Ct}$ [14].

PD-L1 and CD8 immunohistochemical staining

The paraffin blocks were serially sectioned at 4 µm in thickness on positively charged slides and immunostained using a Ventana Benchmark Ultra machine automated staining system. Rabbit monoclonal antibodies against

PD-L1 (QR001) [quartett GmbH- Germany] and CD8A rabbit polyclonal antibody [GeneID 925, Elabscience, USA] were the primary antibodies adopted. Positive controls prepared from tonsil for both antibodies were used. Negative controls were also performed by using phosphate buffered saline (PBS) instead of primary antibody.

Evaluation of immunohistochemical results

PD-L1 scoring: To calculate the combined positivity score (CPS), the total number of PD-L1 positive cells-including tumor cells (cell membrane), lymphocytes, and macrophages (cell membrane and cytoplasm)-was counted, divided by the total number of viable tumor cells, and then multiplied by 100. A score of ≥1 was considered positive [15].

CD8 scoring: The DAB reaction revealed a brownish membrane coloring, which was indicative of positive cells. Additionally, the staining intensity for each cell was reported and evaluated on a scale of 0 to 3 (0; negative, 1; mild, 2; moderate, and 3; strong); the staining intensity 2 or 3 was considered positive. Staining intensity levels of two and three were categorized as positive. Cases were split into two groups: low expression (0–20%) and high expression (21–100%) [16].

Statistical analysis

IBM's statistical package for the social sciences (SPSS) software package, version 20 (Armonk, NY: IBM Corp.), was used to analyze the data. The statistical analysis included:

- Descriptive statistics of clinicopathological parameters of the studied cases (Frequency and percentages for categorical variables, Mean ± Standard Deviation (SD) for numerical continuous parametrical variables).

- The expression pattern of *lncRNA NEAT1* by RT-PCR (Fold change: Mean ± SD), CD8 and PD-L1 by immunohistochemistry (Expression score: Frequency, %).

- Investigating the association of *lncRNA NEAT1* expression, *CD8* and *PD-L1* with clinicopathological parameters (Chi-square, Mann Whitney U and Kruskal–Wallis test).

- The diagnostic accuracy of NEAT1 in PTC was evaluated by analyzing the receiver-operating-characteristic (ROC) and calculating the area under the curve (AUC). An AUC of >0.5 was considered a significant discriminator. Detection of the cutoff point with the highest sensitivity and specificity was calculated from the coordinate points of the ROC curve.

- Correlating the expression of *lncRNA NEAT1* by RT-PCR with CD8 and PD-L1 by immunohistochemistry using Spearman correlation tests.

- The statistical significance was determined at p value <0.05 for all values.

Results

This study included 47 cases of PTC. The mean age at diagnosis was 41.04±12.40; 37 cases were females (79%). Unilateral tumors represented 72 % (34 cases) of the studied cases. Tumor multicentricity was detected in 57% (27 cases) of the studied cases. The mean tumor size was 2.44±1.32 cm.

The T stage was divided into 2 groups: 1st was T1, 2nd was (T2+T3). 24 cases (51%) were T1. Nodal metastasis was detected in 14 cases only (30%). LVE was present in 15 cases (32%), while extra-thyroid extension was detected in 3 cases (6%). Associated HT was detected in 3 cases only (6%) of the studied cases (Table 1).

The mean *lncRNA NEAT-1* expression detected in our cases was 36.250±48.379. Positive *PD-L1* expression was detected in 15 cases (32%), while high *CD8* expression was detected in 10 cases (22%) (Table 1) (Figure 1).

As shown in Table 2, the relative expression levels of *lncRNA-NEAT1* were found to be significantly over-expressed in PTC cases compared to the control specimens; p=0.000). Distribution of the fold change values of lncRNA-NEAT1 in PTC cases and control specimens is demonstrated in Figure 2.

Regarding the association of *lncRNA-NEAT1* expression with the clinicopathological features of the studied cases using Mann Whitney U and Kruskal–Wallis tests as illustrated in Table 4, patients with lymph node metastasis and LVE had significantly higher expression levels of *lncRNA-NEAT1* compared to patients without lymph node metastasis or LVE (p=0.003 and 0.002 respectively). Meanwhile, no statistically significant

Table 1. The Clinicopathological Characteristics, Immunohistochemical *PD-L1* and *CD8* Expression, as well as, *lncRNA-NEAT1* Expression of Studied PTC Cases

Number 47 (100)		
Age (years)	Mean± SD	41.04±12.40
Sex	Female	37 (79%)
	Male	10 (21%)
Laterality	Unilateral	34 (72%)
	Bilateral	13 (28%)
Multicentricity	Single	20 (43%)
	Multiple	27 (57%)
T size	Mean± SD	2.44±1.32
Extra-thyroid extension	Absent	44 (94%)
	Present	3 (6%)
Hashimoto's thyroiditis	Absent	44 (94%)
	Present	3 (6%)
T. stage	T1	24 (51%)
	T2,3	23 (49%)
Nodal metastasis	Absent	33 (70%)
	Present	14 (30%)
LVE	Absent	32 (68%)
	Present	15 (32%)
PDL1	-ve	32 (68%)
	+ve	15 (32%)
CD8	≤20%	37 (78%)
	>20%	10(22%)
LncRNA-NEAT1	Mean± SD	36.250±48.379

T. Stage, Tumor size; LVE, Lympho-vascular emboli.

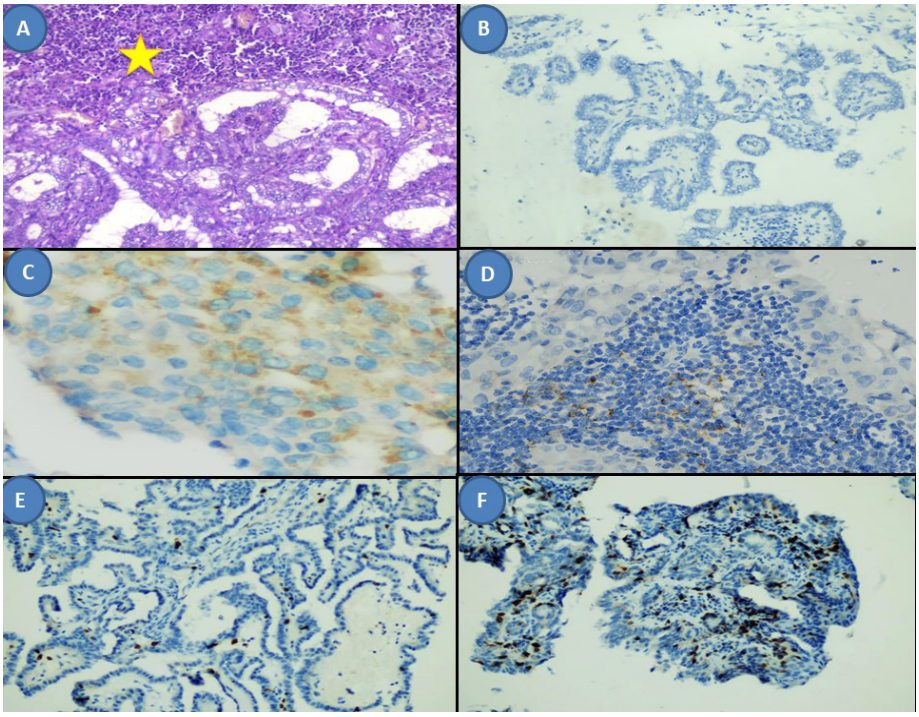

Figure 1. *PD-L1 & CD8* Expression among Studied Papillary Thyroid Carcinoma Cases. (A) Papillary thyroid carcinoma (the yellow star refers to tumor infiltrating lymphocytes) by routine hematoxylin and eosin stain (x100 original magnification). (B) Negative *PDL-1* expression in both tumor cell membrane and tumor infiltrating lymphocytes (x100 original magnification). (C) Positive *PDL-1* expression in tumor cell membrane (x200 original magnification). (D) Positive *PDL-1* expression in tumor infiltrating lymphocytes (x200 original magnification). (E) Low *CD8* expression in tumor infiltrating lymphocytes (x100 original magnification). (F) High *CD8* expression in tumor infiltrating lymphocytes (x100 original magnification).

Table 2. *LncRNA-NEAT1* Expression in the Studied Cases of PTC versus Control

		N	Mean Rank	Sum of Ranks	P
NEAT1	Control	5	6	30	0.000**
	Case	47	27.67	1245	
	Total	52			

Mann-Whitney U test; P** ≤ 0.01

difference in *NEAT1* expression was found based on other clinicopathological parameters.

The ROC curve of *lncRNA-NEAT1* expression levels was plotted between sensitivity (y-axis) and 1–specificity (x-axis). Every point on the ROC curve represented a chosen cutoff (Figure 3). As shown in Table 3, ROC analysis revealed that lncRNA-NEAT1 discriminated PTC from healthy thyroid tissue with AUC=0.933, sensitivity=93%, and specificity=100% at cutoff=1.865 (P=0.002).

There were significant associations between *PD-L1* positive expression and the presence of extra thyroid extension (p=0.02), nodal metastasis (p=0.00), and LVE (p=0.00) (Table 5). The three cases of HT were associated with *PD-L1* negative expression. There were no significant associations of *PD-L1* with the rest of the studied parameters (Table 5).

There were significant associations of increased *CD8* expression with HT (p=0.007), absent nodal metastasis (p=0.02), and negative LVE (p=0.01). There were no significant associations of *CD8* expression with the rest of the studied parameters (Table 6).

The Spearman Bivariate correlation test was used to examine the relationship between the immunohistochemical expression patterns of *PD L1, CD8*, and *lncRNA NEAT-1* expression levels. The results showed a significant negative correlation between *CD8* and *PD-L1* expression levels (P value = 0.014, correlation coefficient = -0.356). Additionally, neither of these two biomarkers and lncRNA-NEAT1 showed a statistically significant

Table 3. ROC Curve Analysis of *lncRNA-NEAT1* Relative Expression Levels in PTC Cases

Area Under the Curve	P value	95% Confidence Interval				
		Lower Bound	Upper Bound	Cut off	Sensitivity %	Specificity %
0.933	0.002**	0.86	1	1.865	93%	100%

P** ≤ 0.01

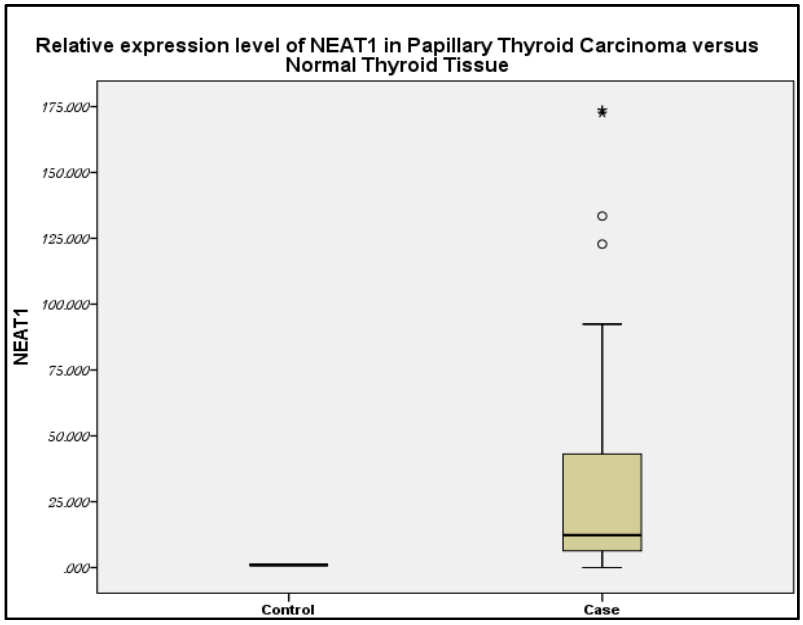

Figure 2. Simple Box Plot of *lncRNA-NEAT1* Expression in PTC Cases versus Control

Table 4. Association of *lncRNA-NEAT1* Expression with the Clinicopathological Parameters of PTC Cases

		LnRNA-NEAT1 expressions status		
		N	Mean± SD	P
Sex	F	37	33.9±40.7	0.2
	M	10	42.5±69.7	
Laterality	Unilateral	34	25.4±27.5	0.1
	Bilateral	13	32.7±21.5	
Multicentricity	Single	27	30.06±25.55	0.4
	Multiple	20	23.9±26.8	
Extra-thyroid extension	Absent	44	28.6±26.4	0.4
	Present	3	10.27±6.3	
Hashimoto's thyroiditis	Absent	44	27.34±25.6	1
	Present	3	29.21±37.40	
T. stage	T1	24	30.7±29.27	0.8
	T2,3	23	24.07±22.17	
Nodal metastasis	Absent	33	19.97±22.64	0.003**
	Present	14	45.11±25.47	
LVE	Absent	32	19.25±22.62	0.002**
	Present	15	44.97±24.55	

Mann-Whitney U test; P** ≤ 0.01; T. Stage, Tumor size; LVE, Lympho vascular emboli.

association (Table 7).

Discussion

Papillary thyroid carcinoma is the most prevalent endocrine cancer, and throughout the past 20 years, its morbidity has increased. According to estimates, up to 67% of people have the diagnosis, and the prevalence is between 3 and 7% of the general population. The rising incidence has highlighted the inadequacy of the current methods for making an accurate diagnosis of thyroid cancer. Therefore, updated knowledge about the pathophysiology of this malignant disease is required including studies on genetic markers, biomarkers, and prognostic markers [17].

LncRNAs play a number of regulatory roles, such as in metastasis and oncogenesis. While *LncRNA NEAT1* plays a pro-oncogenic role in a number of malignancies, its underlying regulatory mechanism in the progression of PTC is yet not understood [13]. *NEAT1* expression has been shown in numerous studies to be a unique prognostic and diagnostic marker for prostate, colorectal, gastric, and esophageal squamous cell carcinoma cancers [18].

There is mounting evidence that lncRNAs alter the tumor microenvironment, which explains the genesis and spread of tumors. However, the discovery of lncRNAs in immune identification and evasion raises questions about immuno-oncology [7].

The current study demonstrated that PTC specimens had significantly higher relative expression levels of *lncRNA-NEAT1* (P value=0.000) contrasting with the control specimens. This finding is consistent with previous studies that found that lncRNA-NEAT1 was highly expressed in PTC tissues and cells in contrast to healthy controls or normal tissue [13, 17]. Additionally, NEAT1 has been reported in a preceding study as a promoter in the malignant progression of thyroid carcinoma [19].

Cases with nodal metastasis and LVE had significantly higher expression levels of *lncRNA-NEAT1* compared to patients without nodal metastasis or LVE (P value=0.003 and 0.002 respectively). These results align with prior work that identified NEAT1 as an oncogenic long noncoding RNA in PTC via regulating micro-RNA (miR)-524-5p/ inhibitor of DNA binding 1 (ID1) axis [13].

Using the ROC curve, the most widely used method of reporting the diagnostic accuracy of dysregulated ncRNAs [20], NEAT1 has an AUC value of 0.933, sensitivity=93% and specificity=100% at cutoff=1.865. This finding is in line with Azadeh et al. [21] findings which showed that ROC analysis revealed that *NEAT1* can be a novel factor

Table 5. Association of *PDL1* Expression Status and Clinicopathological Parameters of PTC Cases

			PDL1 expression status		
		N	-ve	+ve	P
Age (y)	Mean± SD		42±12	38±13	0.4
Sex	F	37	27	10	0.2
	M	10	5	5	
Laterality	Unilateral	34	25	9	0.2
	Bilateral	13	7	6	
Multicentricity	Single	27	17	10	0.3
	Multiple	20	15	5	
T size	Mean± SD		2.3±1.3	2.6±1.4	0.3
Extra-thyroid extension	Absent	44	32	12	0.02*
	Present	3	0	3	
Hashimoto's thyroiditis	Absent	44	29	15	0.3
	Present	3	3	0	
T. stage	T1	24	6	2	0.09
	T2,3	23	13	3	
Nodal metastasis	Absent	33	30	3	0.00**
	Present	14	2	12	
LVE	Absent	32	29	3	0.00**
	Present	15	3	12	

Chi-Square, Mann-Whitney U test P*< 0.05, P** ≤ 0.01; T. Stage, Tumor size; LVE, Lympho-vascular emboli.

for distinguishing the tumor from control samples.

Numerous human malignancies express *PD-L1*, which has been demonstrated to strongly suppress T-cell-mediated anti-tumoral immunity after binding to *PD-1*. In several cancer types, elevated PD-L1 levels have been linked to a poor prognosis. The likelihood of a lower disease-free survival was three times higher for TC patients with positive *PD-L1* expression than for those without [22].

Our study detected significant associations between *PD-L1* expressions and aggressive clinicopathological characteristics of PTC such as extra-thyroid extension, nodal metastasis, and lymphovascular emboli. There was non-significant association between its expression and the

Table 6. Association of *CD8* Expression Status and Clinicopathological Parameters of PTC Cases

			CD8 expression status		
		N	low	high	P
Age (y)	Mean± SD		41±13	42±11	0.8
Sex	F	37	27	10	0.06
	M	10	10	0	
laterality	Unilateral	34	27	7	0.5
	Bilateral	13	10	3	
Multicentricity	Single	27	23	4	0.2
	Multiple	20	14	6	
T size	Mean± SD		2.5±1.3	2.3±1.3	0.6
Extra-thyroid extension	Absent	44	34	10	0.3
	Present	3	3	0	
Hashimoto's thyroiditis	Absent	44	37	7	0.007**
	Present	3	0	3	
T. stage	T1	24	19	5	0.09
	T2,3	23	13	10	
Nodal metastasis	Absent	33	23	10	0.02*
	Present	14	14	0	
LVE	Absent	32	22	10	0.01*
	Present	15	15	0	

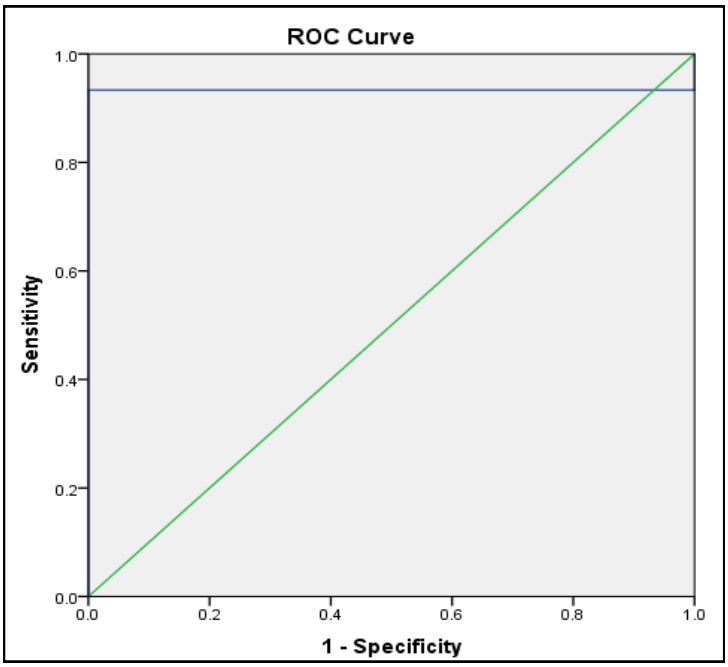

Figure 3. The ROC Curve of *lncRNA-NEAT1* Relative Expression Levels in PTC Cases

Table 7. Association of *lncRNA-NEAT1, PDL1* and *CD8* Expressions in PTC Cases

			NEAT1	*PDL1*	*CD8*
Spearman's rho	*NEAT1*	Correlation Coefficient	1	0.224	-0.128
		Sig. (2-tailed)	.	0.131	0.39
		N	47	47	47
	PDL1	Correlation Coefficient	0.224	1	-.356*
		Sig. (2-tailed)	0.131	.	0.014
		N	47	47	47
	CD8	Correlation Coefficient	-0.128	-.356*	1
		Sig. (2-tailed)	0.39	0.014	.
		N	47	47	47

* Correlation is significant at the 0.05 level (2-tailed).

rest of clinicopathological features.

All cases of extrathyroidal extension were positive for *PD-L1* expression (P= 0.02*) and 12 out of 14 PTC cases with nodal metastasis were also positive for *PD-L1* expression (P = 0.00**). This agreed with Siraj et al. [23] study in which a significant association was noted between *PD-L1* over-expression and aggressive clinicopathological features as extrathyroidal extension (p = 0.0203) and nodal metastasis (p = 0.0466). Shi et al. [24] conclude in their study that positive PD-L1 staining of tumor tissue was associated with extra-thyroidal extension (p=0.012), and lymph node metastasis (p=0.004).

As regards lymphovascular emboli, 12 cases out of 14 PTC cases with LVE were positive for PD-L1 (P = 0.00**). This agreed with Kocaoz and Turan, [9] study which detected a significant relationship between the increased *PD-L1* expression and presence of LVE in patients with PTC (p=0.047).

Aghajani et al. [25] also illustrated that PD-L1 expression was significantly correlated with higher incidence of lymphovascular invasion (P=0.038), and extra-thyroidal extension (P=0.026).

Higher lymphocyte density is associated with better overall survival and lower recurrences in human PTC. A higher disease-free survival rate in children and young adults may be predicted by proliferating lymphocytes. Increased disease-free survival was linked to CD8+ T cell infiltration in TCs [5].

In our study, all PTC cases with nodal metastasis and LVE showed low *CD8* expression (P= 0.02* and 0.01* respectively). Aghajani et al. [25] published similar results, showing that individuals with low *CD8+* expression had a significantly increased frequency of lymph node metastases (P = 0.042). Nodal metastases was substantially more common in the subgroup of cases who had lower CD8+ T cell infiltration and positive *PD-L1* expression (P = 0.031).

Xie et al. [26], study reported also that CD8+ T cells were higher in N0 stage of PTC. Zheng et al. [6] concluded that PTC patients with a higher expression of *CD8* show lower tumor stages and higher survival rates. Despite the low number of cases in our study associated with HT,

all of them showed high *CD8* expression (P= 0.007**). Sulaievaa et al. [27] found that PTC with HT was linked to a higher number of CD8+ cells (P<0.001), indicating the immune system's capacity to produce and attract T-cytotoxic cells in the tumor site, which may account for HT's protective effect on PTC growth. Similarly, in Wang et al. [28], study CD8+ lymphocytes in PTC with HT were significantly higher than those without HT (P<0.001).

In summary, our study documented that more aggressive tumor features of PTC are linked to *lncRNA-NEAT1* and *PD-L1* expression. Conversely, favorable characteristics are linked to CD8 expression. These results point to the potential diagnostic function of *lncRNA-NEAT1* in PTC as well as the potential predictive function of *lncRNA-NEAT1, PD-L1*, and *CD8* in predicting the aggressiveness of PTC.

The predictive importance of *lncRNA-NEAT1, PD-L1*, and *CD8* expression has to be established by much larger study samples and long-term follow-up. Controlled trials are required to validate the usefulness of *PD-L1* immunohistochemistry in identifying PTC candidates for immunotherapy.

Author Contribution Statement

All authors shared in research design and approval of the manuscript.

Research design: AME, RTA, NAS, LMM, HMA and KHA; data collection: AME and HMA; data interpretation: AME, RTA, NAS, LMM and KHA; statistical analysis: NAS; writing of the manuscript: LMM and KHA; work revision and final approval: AME, RTA, NAS, LMM, HMA and KHA.

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Ethical approval

The Faculty of Medicine's Ethical Committee at Cairo University approved this work (Ethical approval code: N-461-2023).

Availability of data and material

The dataset generated in the current study is available from the corresponding author on demand.

Conflict of interest

The authors declare that they have no conflict of interest.

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