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HEAD AND NECK

Lipocalin-2 expression in papillary thyroid carcinoma and its association with clinicopathological characteristics

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Abstract

Purpose The present study aimed to assess Lipocalin-2 (LCN2) expression in patients with papillary thyroid cancer (PTC) and to compare it with multinodular goitre (MNG). We also investigated the correlation between LCN2 expression and clinicopathologic characteristics.

Methods This retrospective study included 63 surgically treated adult patients with papillary carcinoma and 65 adult patients with a MNG. Age, gender, physical, radiological and histopathological examinations, and surgical data of the patients were extracted from the hospital records. Size, histological subtype, capsule invasion, multifocality, extrathyroidal extension (ETE), lymph node metastasis (LNM), and immunohistochemical (IHC) studies of the tumour were recorded from the final histopathological reports of patients with PTC. The patient groups were compared in terms of LCN2 expression. The relationships between LCN2 expression and clinicopathological and other IHC parameters were also evaluated in patients with PTC.

Results LCN2 expression was significantly higher in the PTC group than in the control group. No significant correlation was demonstrated between LCN2 expression and the presence of multifocal disease, capsular invasion, vascular invasion, ETE, and LNM. There was a moderate positive correlation between LCN2 and human bone marrow endothelial cell marker-1 (HBME-1) expressions, however, no correlation was found between LCN2 and cytokeratin-19 (CK19), CD56, and galectin-3.

Conclusion LCN2 expression may be a useful biomarker in differentiating benign and malignant lesions of the thyroid gland; however, its expression pattern may not be associated with clinicopathologic characteristics of the PTC and should be investigated in further studies with larger clinical samples.

Keywords Thyroid neoplasms · Immunohistochemistry · Lipocalin-2 · Differential diagnosis

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Introduction

Over the past few decades, there has been a notable rise in the incidence of papillary thyroid cancer (PTC), which now accounts for 85–90% of all cases of thyroid cancer [1]. Patients with PTC generally exhibit good prognostic behaviour, with a 5-year survival rate of over 93% [2]. However, the rates of tumour recurrence and disease-specific deaths have increased according to studies with long-term follow-ups [3, 4]. Although the diagnostic criteria for PTC were established more than fifty years ago, with the gold standard of diagnosis being the examination of tissue samples using haematoxylin and eosin staining [5], several biomarkers have been investigated in the literature as auxiliary tools for the diagnosis of the thyroid cancers and to predict their clinical behaviour [6].

Lipocalin-2 (LCN2), also known as neutrophil gelatinase-associated lipocalin (NGAL), is a secreted 25-kDa glycoprotein from the lipocalins family proteins that participates in promoting survival, growth, and metastasis during tumourigenesis. It is involved in transporting iron from the extracellular space to inner cells, which is crucial for tumour cell multiplication [5]. LCN2 expression has been demonstrated to be overexpressed in various histological types of cancer [7–9]. It has also been reported that LCN2 expression may be a useful diagnostic biomarker of differentiated thyroid carcinoma [6] but its relationship with prognosis remains unclear in the literature [10]. The present study aimed to evaluate LCN2 expression in patients with PTC and to compare it with patients with multinodular goitre (MNG). We also investigated the relationship between LCN2 expression and clinicopathologic parameters in patients with PTC.

Methods

The ethical approval for the study was obtained from the institutional review board of Kayseri City Training and Research Hospital (01.09.2022/698). Sixty-three adult patients with PTC who underwent surgical treatment between June 2018 and June 2022 in our department were assigned as a study group (PTC group). Sixty-five age- and sex-matched patients who had surgery for MNG were recruited as the control group. Age, gender, physical, radiological and histopathological examinations, and surgical data of the patients were extracted from the hospital records. In patients with PTC, the size, histological subtype, capsule invasion, multifocality, extrathyroidal extension (ETE), lymph node metastasis (LNM), distant metastasis, and IHC studies of the tumour were extracted from patient files and the final histopathological reports.

For the IHC study, formalin-fixed, paraffin-embedded lesion-representative tissue sections of 4 µm were analysed using the biotin-free, HRP multimer-based, hydrogen peroxide substrate and 3, 3'-diaminobenzidine tetrahydrochloride (DAB) chromogen (ultraView ™ Universal DAB Detection Kit, Catalog number 760 – 500, Ventana Medical Systems, Tucson, USA) on a fully automatic immunohistochemical staining device (Ventana BenchMark XT, Ventana Medical Systems, Tucson, USA). IHC staining was performed using a mouse monoclonal LCN2 antibody (NGAL Monoclonal Antibody [HYB 211-01-02], Thermo Fisher Scientific, Rockford, USA). The slides were evaluated by an experienced pathologist blinded to the clinical and pathological data of the patients in terms of the presence of cytoplasmic, membranous, and focal nuclear staining in tumour cells, using a binocular microscope (Olympus BX46, Olympus Corporation, Tokyo, Japan). The distribution and intensity of IHC staining of LCN2 were scored using a semi-quantitative scoring system. The intensity of staining (IS) was graded as (0) negative, (1) weak, (2) moderate, and (3) strong, whereas the distribution of staining (DS), recorded as the percentage of positive cells, was recorded as the following values: 0 ($\leq$ 10%), 1 (11–25%), 2 (26–50%), 3 (51–75%), and 4 ($>$ 75%). The values obtained from these two parameters were multiplied to determine a final score. A score of less than 1 was considered negative, while a score ranging from 1 to 12 was regarded as positive for LCN2 expression [9]. The procedure included appropriate negative and positive controls. The PTC and the control groups were compared by LCN2 expression (Figs. 1 and 2). Also, the relationship between LCN2 expression and clinicopathological characteristics including age, gender, tumour diameter, tumour subtypes, vascular invasion, capsular invasion, ETE, multifocality, LNM, and distant metastasis was evaluated in patients with PTC. The correlations between LCN2 expression and molecular markers utilised during the routine histopathologic examination of PTC, including galectin-3, human bone marrow endothelial cell marker-1 (HBME-1), cytokeratin-19 (CK19), and CD56, were assessed in the PTC group.

Statistical analysis was performed using Statistical Package for Social Sciences (v. 26; SPSS Inc.; Armonk, New York). Data were expressed as mean $\pm$ SD and a p-value of less than 0.05 was considered significant for all comparisons. Data were tested for a normal distribution using the Kolmogorov–Smirnov test and the Chi-square test was performed to compare the categorical variables. The Mann-Whitney U and the Kruskal Wallis tests were used to compare the variables of the groups. The correlation between clinicopathological characteristics and LCN2 expression was evaluated with the Pearson correlation coefficient. The correlation was determined with ρ values and the strength of

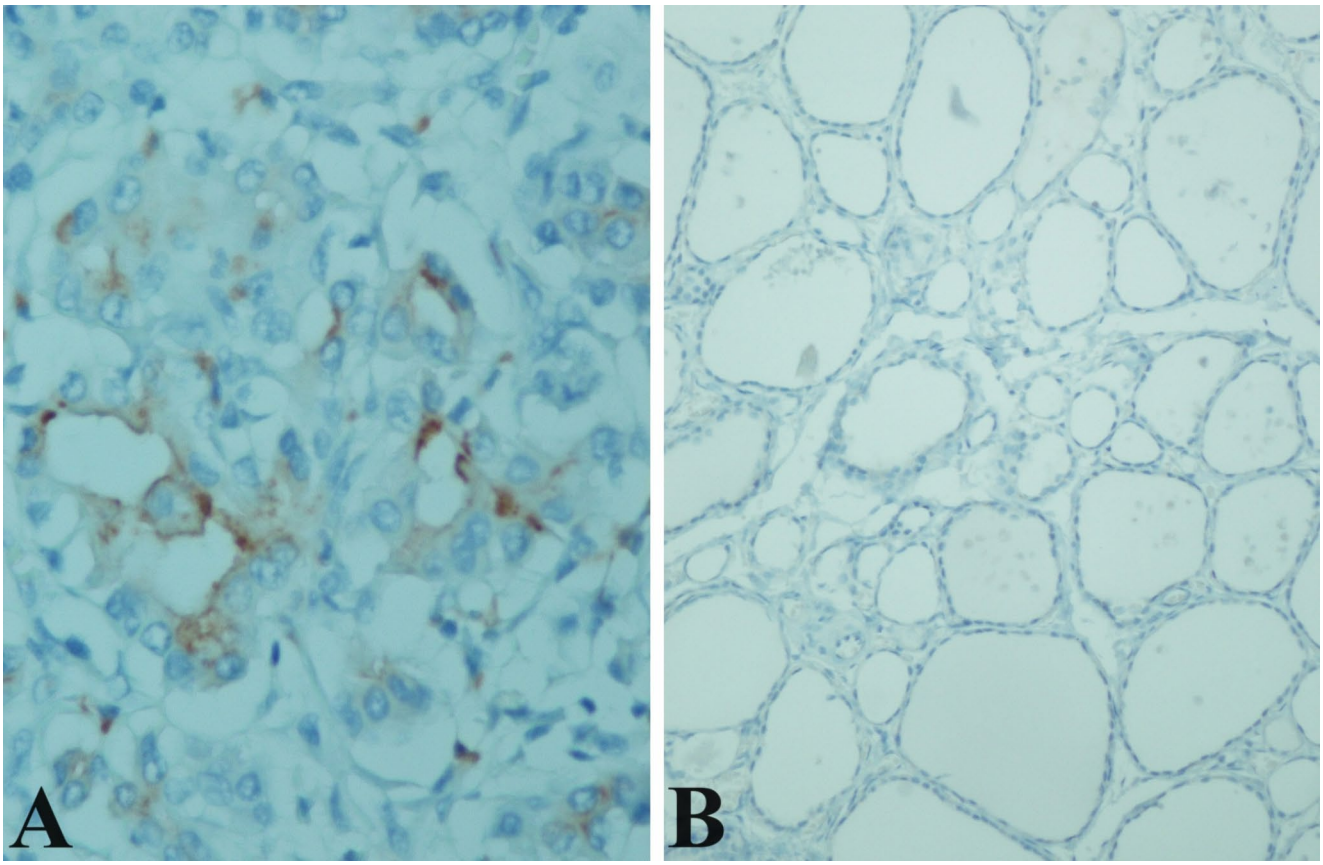

Fig. 1 (**A**) LCN2 expression in pancreatic tissue as a positive control (x400 magnification) (**B**) Benign follicular nodule with no LCN2 expression (x200 magnification)

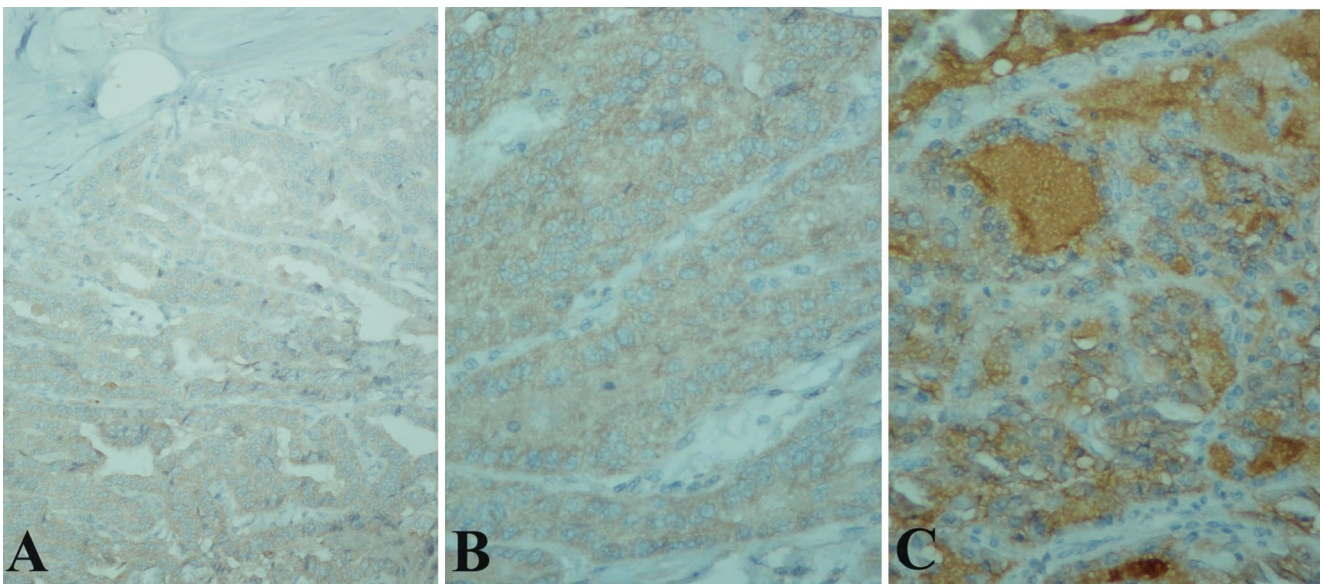

Fig. 2 Representative figures of LCN2 expression in papillary thyroid carcinoma tissues according to the intensity of staining. (**A**) weak (x200 magnification); (**B**) moderate (x400 magnification); C: strong (x400 magnification)

the correlation was described as follows: 0.00 to 0.29, weak; 0.30 to 0.49, moderate; and 0.50 to 1.0, strong correlation.

Results

There was no significant difference in terms of age and gender between the groups. The mean tumour size was 16.7 ± 14.9 mm and five patients had a tumour larger than 4 cm (7.9%) in the PTC group. Multifocal disease, capsular invasion, ETE, LNM, and distant metastasis were observed in 29 patients (46%), 25 patients (39.6%), eight patients (12.7%), 16 patients (25.4%), and five patients (7.9%), respectively. The classical variant was present in 21 patients (33.3%), the follicular variant in 14 patients (20.6%), microcarcinoma in 22 patients (34.9%), the columnar variant in two patients (3.1%), the oncocytic variant in two patients (3.1%), tall cell variant in one patient (1.5%), and the cribriform-morular variant in one patient (1.5%). LCN2 expression was determined in 56 (88.8%) of 63 patients in the PTC group, whereas there were four patients (6.1%) with LCN2 expression in the control group. The number of patients with LCN2 expression was significantly higher in the PTC group than in the control group ($p < 0.001$). The IS and DS of LCN2 scores were also significantly higher in the PTC group than in the control group ($p < 0.001$ and $p < 0.001$, respectively). Demographic and LCN2 expression characteristics of the groups are presented in Table 1.

There was no significant correlation between LCN2 expression and the presence of multifocal disease, capsular invasion, vascular invasion, ETE, or LNM, with r values of 0.124 ($p = 0.333$), 0.184 ($p = 0.150$), 0.135 ($p = 0.292$), 0.154 ($p = 0.229$) and 0.206 ($p = 0.105$), respectively. There were 39 patients (61.9%) aged ≤ 55 years old, while 24 patients (38.1%) were > 55 years of age according to the age cut-off of 55 years old for risk stratification proposed by the 8th Edition of American Joint Committee on Cancer [11]. No significant difference was found in terms of LCN2 expression according to the age risk stratification ($p = 0.543$). No significant difference was found in terms of LCN2 expression between male and female patients ($p = 0.085$) and between patients with a tumour larger than 4 cm and those with a smaller tumour ($p = 0.457$). The seven patients with negative LCN2 expression in the PTC group consisted of four with microcarcinomas and three with follicular carcinomas. There was no significant difference in terms of LCN2 expression between patients with the classical variant, follicular variant, and microcarcinoma ($p = 0.094$). All three patients with aggressive variants, including two with columnar and one with tall cell variants, exhibited LCN2 expression.

In the PTC group, an IHC study of HMBE-1, CK19, CD56, and galectin-3 was performed during routine histopathological examination in 42, 59, 60, and 47 patients, respectively. There was a moderate positive correlation between LCN2 and HMBE-1 expressions ($r = 0.358$, $p = 0.005$), however, no correlation was found between LCN2 and other molecular markers, including CK19, CD56 and galectin-3 ($p = 0.498$ for CK19; $p = 0.454$ for CD56; and $p = 0.102$ for galectin-3).

Discussion

Several epidemiological studies have demonstrated that LCN2 is overexpressed in a variety of malignant tumours and have suggested it as a potential biomarker and therapeutic target for malignancy. It is reported to be associated with tumour size, stage, and invasiveness, thereby enhancing cancer aggressiveness [12]. LCN2 expression has also been investigated in thyroid gland pathologies including differentiated thyroid malignancies; however, studies evaluating its association with clinicopathological parameters are rare in the literature [5, 6, 8, 13].

Several biomarkers have been investigated to potentially assist in the diagnosis of PTC in the literature, with some

Table 1 Demographic and LCN2 expression characteristics of the groups

	PTC Group (*n*:63)	Control Group (*n*:65)	*p value*
Age (years)	49.2 ± 12.9	46.2 ± 11.5	0.169
Gender (F/M)	44/19	48/17	0.274
LCN2 (−/+)	7/56	61/4	< 0.001
LCN2 (IS_{mean})	1.95 ± 1.14	0.09 ± 0.38	< 0.001
0	7 (11.1%)	61 (93.8%)	
1	29 (46%)	2 (3.1%)	
2	22 (34.9%)	2 (3.1%)	
3	5 (7.9%)	0	
LCN2 (DS_{mean})	1.44 ± 0.8	0.08 ± 0.32	< 0.001
0	7 (11.1%)	61 (93.8%)	
1	15 (23.8%)	2 (3.1%)	
2	21 (33.3%)	2 (3.1%)	
3	14 (22.2%)	0	
4	6 (9.5%)	0	

DS: distribution of staining, F: female, IS: intensity of staining, LCN2: lipocalin-2, M: male, n: number of patients, PTC: papillary thyroid carcinoma

yielding promising results. Galectin-3, mesothelial antigen HBME-1, and CK19 are widely utilised for PTC diagnosis. Also, CD56 has been demonstrated to be commonly expressed in normal thyroid tissues, but it is not present in malignant thyroid tumours, particularly in PTC [5]. Similarly, LCN2 was suggested to be useful in differentiating malignant follicular cell-derived thyroid tumours from follicular benign lesions [8]. Rosignolo et al. [14] reported that LCN2 was undetectable in all 12 normal thyroid tissues studied and 31 of the 38 PTCs. Celestino et al. [6] indicated that LCN2 expression may be able to improve the differential diagnosis of benign versus malignant thyroid nodules. Similarly, in the present study, LCN2 expression was found in 56 (88.8%) of 63 patients with PTC, which is significantly higher than in the control patients operated on for MNG. Ma et al. [5] studied CK19, galectin-3, HBME-1, CD56, claudin-1, and LCN2 in PTC and peritumoural normal thyroid tissues, and reported that a thyroid lesion can be diagnosed as PTC if four of these markers are positive (CD56 [-]). In the present study, we evaluated the correlation of these tumour markers with LCN2 and found a moderate positive correlation between the expressions of LCN2 and HMBE-1. However, no significant correlation was found between LCN2 and CK19, CD56, or galectin-3 expressions in PTC tissues. Further studies with more participants may provide additional data regarding the association between these markers.

LCN2 overexpression has been suggested as an unfavourable prognostic indicator in several types of cancer [15, 16]. The relationship between LCN2 expression and clinicopathologic features, including prognostic factors in thyroid carcinoma, has been reported with conflicting results in the literature. According to in vitro experimental studies, LCN2 overexpression has been reported as a survival factor for thyroid neoplastic cells and it enhances the metastatic potential of the tumour [17, 18]. Rosignolo et al. [14] reported that there was no significant association between LCN2 mRNA levels and American Thyroid Association risk groups. Celestino et al. [6] reported that LCN2 expression in differentiated thyroid carcinomas was significantly associated with the presence of ETE, oncocytic features, and larger tumour size. In a recent review, Roli et al. [10] reported that LCN2 appeared to be a useful diagnostic marker for thyroid cancer, although its prognostic accuracy remained undetermined. Celepli et al. [13] demonstrated that ETE, LNM, and vascular invasion were significantly higher in PTC patients with high LCN2 expressions and indicated that LCN2 may play a role in cancer progression. In the present study, no correlation was found between LCN2 expression and vascular invasion, capsular invasion, ETE, multifocality, and LNM. There were five patients with distant metastasis and all of them expressed LCN; however, distant metastasis was not considered for statistical evaluation due to the low number of patients. The discrepancies between the studies that investigated the association between LCN2 expression and clinicopathologic features of thyroid cancers, including ours, may be due to the different methodologies and techniques utilised for assessing LCN2, the small sample size of the studies with heterogenous patient groups, and different sample matrices used such as cancer tissue or plasma.

In the literature, LCN2 expression has been reported to be frequently correlated with tumour size in a variety of malignant tumours [12]. However, studies that investigated the association between LCN2 expression and age, gender, and tumour size in thyroid cancers are rare. Barresi et al. [9] determined no significant associations between LCN2 expression and the age and gender of their patients. Celestino et al. [6] analysed the relationship between LCN2 expression and prognostic factors including age and tumour size (> 4 cm) and didn't find any significant correlation. In the present study, there was no significant correlation between LCN2 expression and age, gender, or tumour size (> 4 cm) which was similar to previous reports in the literature. LCN2 expression pattern didn't significantly differ between the classical variant, follicular variant, and microcarcinoma. Celepli et al. [13] reported that aggressive variants of PTC had high LCN2 levels. In their study, a total of five patients consisting of three tall cell and two hobnail variants had a stronger LCN2 expression. Similarly, all three patients with aggressive subtypes, including two columnar and one tall cell variant, expressed LCN2 in the present study.

This study has some limitations. Firstly, a third group of patients with normal thyroid tissue may reflect the expression pattern discrepancies of LCN2 between the normal gland and the thyroid lesions including the cancers. Secondly, due to the retrospective nature of the study, the PTC group was heterogeneous in terms of clinicopathologic parameters. Also, the relatively small sample size of the present study may limit the strength of the statistical analysis. Investigating the association between LCN2 expression and the clinicopathologic features of PTC in a larger patient series is essential.

Conclusion

Although LCN2 expression may be a useful biomarker for differentiating benign and malignant lesions of the thyroid gland, its expression pattern may not be associated with the clinicopathologic characteristics of the PTC. The utility of LCN2 as a prognostic indicator should be investigated in further studies with larger clinical samples.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were

performed by Mustafa Alkaya, Ali Bayram, Mehmet Yaşar, Merve Doğan, Hümeyra Gençer. The first draft of the manuscript was written by Ali Bayram and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability None.

Declarations

Conflict of interest The authors declare that they have no competing interests relevant to the content of this article.

Ethics approval The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study protocol was approved by the institutional review board of xxx City Training and Research Hospital (01.09.2022/698).

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