Original paper

Investigation of ZIP4, ZO-1, and CLAUDIN-1 expression in thyroid tumours by immunohistochemistry and real-time polymerase chain reaction methods

Mustafa Nacir[1], ibrahim İbiloğlu[2], Ulaş Alabalik[2]

[1]Department of Pathology, University of Health Sciences Gazi Yaşargil Trainning and Research Hospital, Diyarbakır, Turkey
[2]Department of Pathology, Dicle University, Medical School, Diyarbakır, Turkey

Thyroid neoplasms are the most common endocrine malignancies. ZIP4 is an intramembranous zinc trans membrane protein. Zinc plays a central role in the activation of transcription factors, and zinc transporters. This affects tumour migration, invasion, and cell proliferation. ZO-1 and Claudin-1 are important tight junction proteins whose amounts increase and decrease in various cancers.
In this study, we aimed to investigate the expression of ZIP4, ZO-1, and Claudin-1 in thyroid tumours and the relationship of this expression with tumour types and prognostic parameters.
ZIP4, ZO-1, and Claudin-1 were studied in all cases by immunohistochemical and Real-Time PCR methods.
ZIP4 and Claudin-1 tended to be expressed more in cases with tumours, while ZO-1 in cases with and without tumours. Expression of ZIP4 and Claudin-1 by real-time polymerase chain reaction showed a significant difference between histological subtypes, and this difference was not observed with ZO-1. It was observed that the presence of metastasis increased with the expression of ZIP4 and Claudin-1, and there was no significant change with ZO-1.
We think that Claudin-1 and ZIP4 expression can be used as an important marker in terms of showing poor prognosis and susceptibility to metastasis in thyroid tumours, and in developing targeted therapy.

Key words: ZIP4, ZO-1, Claudin-1, thyroid carcinoma.

Introduction

Thyroid cancers are the most common endocrine malignancies, and their frequency of occurrence is increasing rapidly [1]. The widespread use of fine needle aspiration biopsy and advances in radiological diagnostic tools constitute the main reason for this increase [2]. Papillary, follicular, and anaplastic cancers originate from the thyroid follicular epithelium. Other neoplasms of the thyroid include medullary, primary lymphoma, sarcoma, and metastasis [3].

Zinc (Zn) plays a vital role in many biological processes in the human body. The ZIP family (encoded by SLC39) in Zn uptake and the ZnT family (encoded by SLC30) in Zn release are transporters that function in cellular Zn homeostasis. While the ZIPs increase cellular Zn content by taking Zn into the cytoplasm from the cell membrane, the ZnTs reduce intracellular Zn levels by facilitating the flow of Zn out of cells or into intracellular organelles [4, 5]. Dysregulation of Zn and Zn-carriers has been reported to be associated with various types of cancer such

as glial tumours, prostate, breast, pancreatic, and lung cancers, and brain disorders such as Alzheimer's disease, Parkinson's disease, and epilepsy [6]. It has been shown that ZIP4, a member of the ZIP family and a plasma membrane localized protein, plays an important role in the development and progression of carcinomas of various organs [4]. Although ZIP4 is generally associated with poor prognosis and invasive behaviour in the literature, different organ-specific behavioural patterns have also been reported [7].

Epithelial tissues in various organs (lungs, intestines, skin, etc.) form special structures that separate the biological compartments in the body with different internal environments. The intercellular junction complex between epithelial cells consists of tight junctions, interconnections, and desmosomes and is essential for the maintenance of epithelial cell structure and cellular polarity [8].

Tight junction proteins are very important structures for forming a barrier between different parts of the body, providing selective paracellular diffusion, and maintaining balance in tissues [9]. Tight junctions consist of 4 types of transmembrane proteins consisting of occludin, claudins, junctional adhesion molecules, and tricellulin, as well as numerous cytosolic proteins, including ZO-1 [8]. It has been reported in the literature that the tight junctional mechanisms of ZO-1 and Claudin-1, which are expressed on the cell surface, are frequently disrupted and their expression decreases during tumour progression [10, 11]. However, there are also publications in which intracellular localizations of ZO-1 and Claudin-1 are associated with tumour progression [11].

The aim of our study was to reveal the diagnostic and prognostic values of ZIP4, ZO-1, and Claudin-1, which have been proven to play an effective role in the metastatic process in different tumours, in metastatic and aggressive thyroid cancers, by evaluating the expression status of ZIP4, ZO-1, and Claudin-1 in patients with thyroid neoplasia by immunohistochemical (IHC) and real-time polymerase chain reaction (RT-PCR) methods.

Material and methods

Necessary research permission (135–23.05.19) was obtained from Dicle University Faculty of Medicine Ethics Committee. Thyroidectomy materials received by Dicle University Faculty of Medicine, Department of Pathology between January 2010 and April 2019 were examined.

Diagnosed as "multinodular goitre (MNG), papillary thyroid carcinoma (PTC), medullary thyroid carcinoma (MTC), follicular thyroid carcinoma (FTC, minimally invasive-widely invasive), and anaplastic thyroid carcinoma (ATC)", appropriate fixation procedure was performed, and 66 cases selected from thyroidectomy materials were included in the study. Of these 66 cases, 20 were diagnosed with MNG, 18 with PTC, 11 with MTC, 11 with FTC (8 minimally invasive, 3 widely invasive), and 6 with ATC.

Immunohistochemical studies

Three 5 μm thick sections were taken on adhesive slides (Isotherm, Germany) for each case. The slides were kept in an oven at 65°C for 30 minutes and deparaffinized. In the IHC study, ZIP4 (Lifespan Biosciences, USA, polyclonal rabbit/IgG antibody, catalogue number: LS-C676512), ZO-1 (Thermo Fisher, USA, polyclonal rabbit/IgG antibody, catalogue number: PA5-28858) and Claudin-1 (Cell Signalling Technology, USA, D5H1D clone, monoclonal rabbit/IgG antibody, catalogue no. 13255) primary antibodies in ready-to-use form were used. The immunohistochemical study was run automatically on a Ventana BenchMark Ultra instrument (Roche Diagnostics, USA).

Preparations with IHC staining were evaluated semiquantitatively and subjectively by 2 pathologists under a light microscope. Cytoplasmic for ZIP4, membranous for ZO-1, and membranous and cytoplasmic-nuclear staining were evaluated separately for Claudin-1, and they were considered positive. The percentage (extension) and staining intensity (intensity) of positively stained areas were scored in all cases, and the total score was obtained.

The staining score was set to 0 in 0–5% of tumour cells, 1 in 6–25%, 2 in 26–50%, 3 in 51–75%, and 4 in 76–100%. Staining intensity of cells: if staining was not present, the score was 0; in the presence of weak staining, the score was 1; moderate staining was determined as 2; and strong staining was determined as 3. If there was no staining in the total score, it was considered negative, 1 and 2 weakly positive, 3 and 4 moderately positive, and 5–7 strong positive [12].

Polymerase chain reaction analysis

To investigate the expression of ZIP4, ZO-1, and Claudin-1 proteins by PCR, two 5 μm thick sections were cut into 1.5 ml reaction tubes. RNA isolation procedure was performed using Roche High Pure RNA Paraffin Kit (Roche, Germany, Ref no: 06650775001), centrifuge (Beckman Coulter, Microfuge 16, USA), vortex (IKA, Germany), and digital drying bath (Labnet AccuBlock, USA). β-Actin was used as a reference gene [13].

The Transcriptor First Strand cDNA Synthesis Kit (Roche, Germany, Ref no: 04896866001) and thermal cycler (SensoQuest, Germany) were used for cDNA synthesis from RNA. In RT-PCR analysis the Roche FastStart Essential DNA Probes Master (Roche, Germany, Ref no: 06402682001) kit, Molbiol ZIP4 (NM_130849), ZO-1 (NM_03257), Claudin-1 (NM_021101) as primer and

probe, and β-Actin (NM_001101, Molbiol, Germany) as positive control were used.

Statistical analysis

IBM SPSS 25 for Windows (Statistical Package for Social Sciences) was used for the statistical evaluation of our research data. Frequency of variables, and means and standard deviations of numerical data were found. The measured variables were presented as mean ± standard deviation, and categorical variables were presented as numbers and percentages (%). Conformity of continuous variables to normal distribution was measured by Kolmogorov-Smirnov test. In the statistical evaluations, the independent *t*-test was used to evaluate the averages of the continuous variables with normal distribution between the two groups, and those that did not fit the normal distribution were analysed with the Kruskal-Wallis and Mann-Whitney *U* test. The χ^2 test was used in the statistical evaluation of categorical variables. The hypotheses were 2-sided, and $p \leq 0.05$ was accepted as a statistically significant result.

Table I. Clinical characteristics of 66 patients with thyroidectomy who participated in this study

Factors	
Age, years	
Average	45.97 ±15.86
Range	18–75
Sex, *n* (%)	
Male	48 (72.7)
Female	18 (27.3)
Histological subtype, *n* (%)	
PTC	18 (27.3)
MTC	11 (16.7)
FTC	11 (16.7)
ATC	6 (9.1)
MNG	20 (30.3)

ATC – anaplastic thyroid carcinoma, FTC – follicular thyroid carcinoma, MNG – multi-nodular goitre, MTC – medullary thyroid carcinoma, PTC – papillary thyroid carcinoma

Results

The clinical characteristics of the patients in our study are given in Table I. Metastasis status, IHC staining characteristics, and molecular analysis results are given in Table II.

Of the 66 cases in our study, 48 (72.7%) were female and 18 (27.3%) were male. Statistically no significant difference was observed between the IHC and molecular expressions of ZIP4, ZO-1, and Claudin-1 according to gender with the χ^2 test ($p > 0.05$). The youngest of the cases was 18 years old and the oldest was 75 years old, and the mean age was 45.97 ±15.86 years. There was no significant difference between IHC and molecular expressions of ZIP4, ZO-1, and Claudin-1 according to age ($p > 0.05$). In our study, 9 patients had regional lymph node metastases (LNM) and 2 patients had distant metastases, and these 2 groups were considered as the 'metastatic group'. There was no metastasis in 55 patients.

ZIP-4 expression

In our study, 39 (95%) of 46 tumours with IHC showed ZIP4 positivity (Fig. 1A). We observed ZIP4 positivity in only 2 (10%) of 20 cases diagnosed with MNG. ZIP4 expression was significantly correlated with histological subtype ($p = 0.000$*). All MTC cases expressed ZIP4, followed respectively by PTC, ATC, and FTC.

We detected ZIP4 gene expression by RT-PCR in 20 (30.3%) of our cases. Eighteen (39.1%) of the tumour cases and 2 (10%) of the tumour-free cases exhibited ZIP4 gene expression. Eleven (61.1%) of the PTC cases and 5 (45.5%) of the FTC cases showed molecular ZIP4 expression. Molecular and IHC studies for ZIP4 expression showed that both methods were compatible with each other ($p = 0.014$).

Table II. Immunohistochemical staining and real-time polymerase chain reaction positivity table according to histological subtype and metastasis status

Histological Subtype	ZIP-4		ZO-1		Claudin-1		Metastasis	Total
	IHC	PCR	IHC	PCR	IHC	PCR	–	
PTC, *n* (%)	15 (83.3)	11 (61.1)	14 (77.7)	15 (83.3)	16 (88.8)	14 (77.7)	4	18
FTC, *n* (%)	8 (72.7)	5 (45.4)	10 (90.9)	11 (100)	7 (63.6)	3 (27.2)	1	11
ATC, *n* (%)	5 (83.3)	0 (0)	4 (66.6)	6 (100)	5 (83.3)	1 (16.6)	1	6
MTC, *n* (%)	11 (100)	2 (18.1)	5 (45.4)	11 (100)	11 (100)	0 (0)	5	11
MNG, *n* (%)	2 (10)	2 (10)	19 (95)	19 (95)	2 (10)	0 (0)	0	20

ATC – anaplastic thyroid carcinoma, FTC – follicular thyroid carcinoma, IHC – immunohistochemical, MNG – multi-nodular goitre, MTC – medullary thyroid carcinoma, PCR – polymerase chain reaction, PTC – papillary thyroid carcinoma

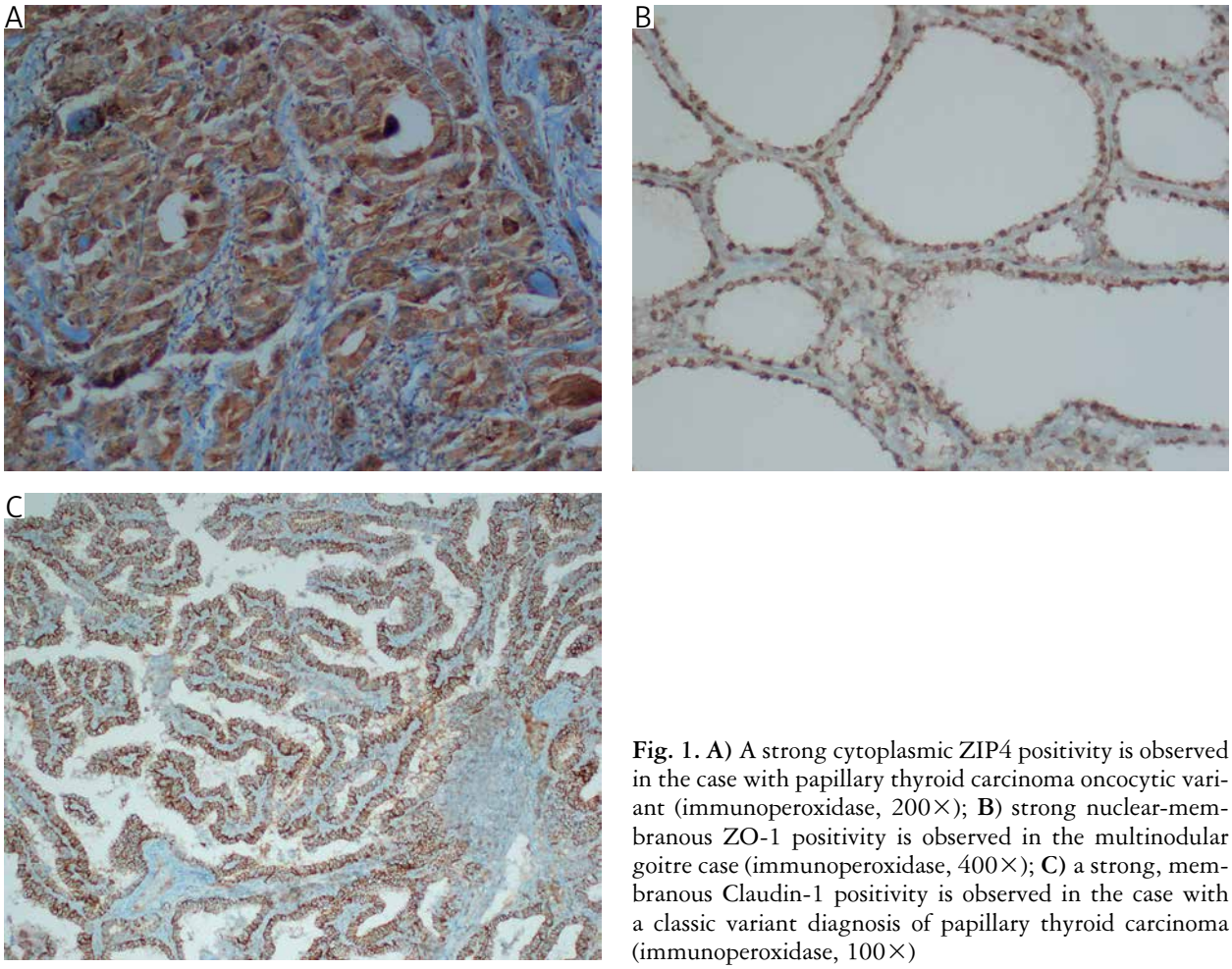

Fig. 1. A) A strong cytoplasmic ZIP4 positivity is observed in the case with papillary thyroid carcinoma oncocytic variant (immunoperoxidase, 200×); **B)** strong nuclear-membranous ZO-1 positivity is observed in the multinodular goitre case (immunoperoxidase, 400×); **C)** a strong, membranous Claudin-1 positivity is observed in the case with a classic variant diagnosis of papillary thyroid carcinoma (immunoperoxidase, 100×)

While all metastatic cases expressed ZIP4 by IHC method, the ZIP4 expression rate was 54.5% in cases without metastasis. A significant correlation was found between IHC ZIP4 positivity and metastasis ($p = 0.005$). No significant correlation was found between ZIP4 expression and metastasis by RT-PCR method ($p > 0.05$).

ZO-1 expression

In our study, 33 (71.7%) of our tumoural cases and 19 (95%) of our MNG cases showed ZO-1 IHC positivity (Fig. 1B). The highest positivity rate among tumour cases was observed in FTC cases (10 cases [90.9%]). A significant difference was found between histological subtypes and ZO-1 IHC staining ($p = 0.018$). We detected ZO-1 gene expression by PCR in 43 (93.5%) of our tumour cases and 19 (95%) of the MNG cases. There was no significant relationship between the histological subtypes in our study and RT-PCR results. No significant correlation was found between IHC and RT-PCR studies in terms of ZO-1 ($p > 0.5$).

Claudin-1 expression

In our study, 39 (95.1%) of the cases with IHC positivity for Claudin-1 were tumoural cases (Fig. 1C). Claudin-1 expression was significantly correlated according to histological subtypes. All MTC cases expressed Claudin-1, followed by PTC 16 (88.2%), ATC 5 (83.3%), and FTC 7 (63.6%), respectively. Claudin-1 expression was observed in 2 (10%) MNG cases. Claudin-1 IHC positivity was membranous in 48.7% and intracellular (cytoplasmic, nuclear granular) in 51.3% of cases (Fig. 2).

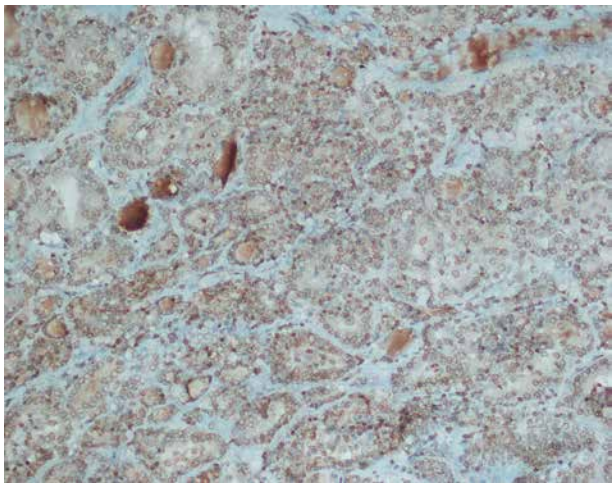

Fig. 2. Nuclear-granular Claudin-1 positivity is observed in the case with follicular thyroid carcinoma (immunoperoxidase, 200×)

Table III. Evaluation of the staining pattern of Claudin-1 with histological subtypes

	Claudin-1 staining pattern			
Histological subtype	Negative	Membranous	Intracellular	Total
PTC, *n* (%)	2 (11.1)	16 (88.9)	0 (0)	18
FTC, *n* (%)	4 (36.4)	2 (18.2)	5 (45.5)	11
ATC, *n* (%)	1 (16.7)	1 (16.7)	4 (66.7)	6
MTC, *n* (%)	0 (0.0)	0 (27.3)	11 (100)	11
MNG, *n* (%)	18 (90.0)	0 (0)	2 (10)	20
p-value		0.000***		

**p ≤ 0.05 was accepted as statistically significant.*

ATC – anaplastic thyroid carcinoma, FTC – follicular thyroid carcinoma, MNG – multi-nodular goitre, MTC – medullary thyroid carcinoma, PTC – papillary thyroid carcinoma

Intracellular staining was observed in 11 cases (100%) in MTC, and intracellular staining pattern was observed in 4 cases (66.2%) with ATC. All of the cases showing Claudin-1 positivity in PTC showed membranous staining (Table III).

We detected Claudin-1 gene expression in 18 (27.2%) of the cases by RT-PCR. All of the positive cases were tumour cases and Claudin-1 expression was not observed in MNG cases. Fourteen (77.8%) of the PTC cases, 3 (27.3%) of the FTC cases, and one (16.7%) of the ATC cases expressed Claudin-1 molecularly. Contrary to the IHC study, none of the MTC cases were positive by RT-PCR, while all MNG cases were negative, consistent with IHC.

When all cases were examined in terms of Claudin-1 positivity and metastasis, a significant correlation was found between the presence of metastasis and IHC Claudin-1 expression ($p = 0.005$). While all metastatic cases expressed Claudin-1, the expression rate was 54.5% in tumour cases without metastasis. The relationship of Claudin-1 with the presence of metastases is similar to that of ZIP4.

Discussion

Over the past 3 decades, there has been a dramatic increase in the number of people diagnosed with thyroid cancer. In 2019, the incidence of thyroid cancer in the United States was reported as 52,070, making it the sixth most common cancer in women. Together, PTC and FTC are known as "well-differentiated thyroid carcinomas" and account for approximately 90% of thyroid malignancies [14]. Management of well-differentiated thyroid carcinomas and PTC consists largely of resection and radioiodine therapy, with an excellent 5-year overall survival of 98.2%. Although well-differentiated thyroid carcinomas may remain silent for a long time and have a high survival rate, the rates of LNM and recurrence are approximately 50% and 20%, respectively [15]. Cervical LNM are considered an independent risk factor for local recurrence and increased morbidity [16]. Although BRAF V600E status is recommended as a genetic and prognostic marker in patients with well-differentiated thyroid carcinoma, conflicting results have also been published, indicating the need for reliable new markers [14, 16]. Cabanillas *et al.* [17] stated in their study that there was 70% LNM in the MTC at the time of application with a prominent nodule. Brassard *et al.* [15] reported approximately 50% of LNM in PTC at the time of initial diagnosis and with subsequent recurrences. Among our cases, LNM is 45.5% in MTC and 22.5% in PTC, and long-term follow-up of the patients may increase these results.

In the literature, there are studies investigating the expression of ZIP4 in tumours of non-thyroid tissues. Fan *et al.* [18] observed strong ZIP4 expression at a rate of 75% in high-grade serous ovarian tumours and 25% in low-grade serous ovarian carcinomas. Ishida *et al.* [19] compared oral SCC cases and normal tissues with IHC methods and found 4.2-fold higher ZIP4 expression in cancerous tissues. In the same study, they found the ZIP4 ratio in oral SCC-derived cell lines to be significantly higher than normal oral keratinocytes by RT-PCR method.

Chen *et al.* [7] investigated ZIP4 expression in human tissues and cell lines by RT-PCR and Western blot methods in prostate cancer tissues. Contrary to previous studies, they found that ZIP4 expression was reduced (approximately 49-fold) in prostate cancer tissues by both methods. In the study, it was suggested that ZIP4 reduced metastasis in prostate cancer but had no effect on the stage of the tumour. Chen *et al.* explained that the reason why the results of their study of MD contrast with other studies were that healthy prostate cells physiologically contain approximately 10 times more Zn than other soft tissues and that the intracellular Zn level decreases by 70% during the cancer process [20, 21]. This supports the

idea that ZIP4 has a tissue-specific proliferative effect by changing the Zn level.

Although there are studies in the literature investigating the relationship between ZIP4 expression and metastasis in various tumours, such as prostate, gliomas, ovarian carcinomas, and pancreatic carcinomas, there is no study about this subject in thyroid tissue.

In our study, we detected significant IHC ZIP4 expression in the tumour group. In addition, although it is not as high as IHC, we obtained significant gene expression in the tumour group by RT-PCR. Because all our metastatic cases expressed IHC ZIP4, we thought that IHC ZIP4 expression could be used as a marker that may show poor prognosis and predisposition to metastasis in thyroid tumours.

There are few studies investigating ZO-1 in thyroid tissue. Fluge *et al.* [22] reported that ZO-1 expression occurs in the apical and weak staining pattern in normal thyroid tissue, whereas it occurs more intensely, at a higher rate, and in its intracellular localization in PTC. Rebuffat *et al.* [23] investigated the expression of ZO-1 IHC in non-tumour tissues of the thyroid. In their study, they compared ZO-1 expression in Graves' and Hashimoto's thyroiditis and observed higher ZO-1 expression in Graves' patients compared to Hashimoto's thyroiditis patients.

Bai *et al.* [24] reported a decrease in the expression of N-cadherin and Vimentin, and an increase in the expression of E-cadherin and ZO-1 with miR-376c-3p transfer, which reduces tumour aggressiveness and invasiveness in MTC cell lines. Thus, they demonstrated the protective effect of ZO-1 against tumour invasiveness.

There are also studies investigating ZO-1 expression in non-thyroid organs in terms of tumour development. Du *et al.* [25] demonstrated the correlation of increased miR-103 level with decreased expression of ZO-1 in endometrial carcinoma. They also reported that ZO-1 expression decreased significantly in endometrial carcinoma cells compared to their tumour-free counterparts.

Babkair *et al.* [10], in a study they conducted with Claudin-1 and ZO-1, observed ZO-1 expression as membranous and cytoplasmic, while Claudin-1 expression was not observed in the normal oral mucosa. In oral squamous cell carcinoma, membranous Claudin-1 positivity was observed in almost all cases, while ZO-1 expression was observed in both membranous and nuclear patterns. Liu *et al.* [26] found that the DCLK1 gene, which was found to be effective in the metastasis process in breast cancer, decreased epithelial cell markers such as ZO-1, increased mesenchymal markers such as Vimentin and ZEB1, and revealed that ZO-1 expression decreased when cancer invasiveness increased.

In our study, no significant difference was observed between the tumour group and the non-tumoural group in terms of ZO-1 expression by IHC and RT-PCR methods. Tight junction proteins such as ZO-1 can also be found in normal follicle epithelial structure. High rates of IHC and RT-PCR positivity in our MNG cases suggest that ZO-1 is expressed outside of neoplastic processes. As stated in the literature, non-tumour events such as thyroiditis also affect ZO-1 expression in thyroid tissue [23]. In our series, it was shown that there is no relationship between metastasis and ZO-1 expression ($p > 0.05$), suggesting that ZO-1 expression is affected by environmental factors, including thyroiditis-like clinical conditions, and therefore does not clearly reflect the situation related to metastasis and prognosis.

There are few studies in the literature investigating the Claudin-1 marker in thyroid tissue. Zwanziger *et al.* [11] found increased intracellular expression in follicular thyroid tumours and increased nuclear localization in metastases of FTC in their study on tissues and cell lines of FTC. In addition, they observed 67% higher Claudin-1 protein expression in cell lines of distant metastasis compared to cell lines with regional LNM. Süren *et al.* [27] showed that Claudin-1 was not expressed in any of the cases diagnosed with MNG using the IHC method, and it was expressed in 97% of the cases diagnosed with PTC. In addition, they showed expression in only 10% of cases diagnosed with FTC and stated that Claudin-1 can be used in the differential diagnosis of FTC and PTC. Abd el Atti *et al.* [28] emphasized that Claudin-1 is expressed in all cases of follicular variant PTC and will help in the differential diagnosis with other follicular tumours.

In the literature, there are many studies on Claudin-1 expression in non-thyroid tumours. Hoellen *et al.* [29] described a significantly higher expression level in squamous cell cervical carcinoma tissues compared to the peritumoral stroma. Some studies suggest that Claudin-1 should be a tumour marker equivalent to p16INK4a or a marker of precancerous lesions [30, 31]. Yamamoto *et al.* [32] reported that in cases of tongue localized SCC, the rate of LNM increased significantly in cases where Claudin-1 had nuclear staining, and that Claudin-1, which was stained membranous, decreased the mobility and metastasis in cell lines. Szasz *et al.* [33] reported that loss of Claudin-1 plays an important role in LNM in patients with breast carcinoma and is associated with poor prognosis.

In our study, most of the cases (95.1%) with Claudin-1 positivity were in the tumour group. Intracellular staining pattern was observed in all the cases in MTC and in most of the cases of ATC. All the PTC cases exhibited a membranous staining pattern. The expression of Claudin-1 in tumoural cases and the histologically different staining pattern according to tumour subtypes suggest that its use for diagnostic purposes will be useful. We think that the membra-

nous staining pattern can be used to support the diagnosis of PTC and intracellular staining can be used to support the diagnosis of MTC and ATC.

All the cases with Claudin-1 expression in the evaluation performed by RT-PCR were in the tumour group. When all the cases were examined in terms of Claudin-1 positivity and metastasis, all the metastatic cases expressed Claudin-1 by IHC, while Claudin-1 expression rate was 54.5% in tumour cases without metastasis. The relationship of Claudin-1 with the presence of metastases is similar to that of ZIP4.

While some publications in the literature emphasized the limiting role of invasion and metastasis of Claudin-1 [32], some publications emphasized its increased expression in tumour lesions and metastatic cases compared to normal tissues [29, 33]. In our study, its expression also showed an increasing effect in terms of metastasis, and a significant expression was noted in tumoural cases with both IHC and RT-PCR method.

The fact that tight junction proteins tighten the cell junction on the surface and reduce the metastasis tendency is predictable, as can be seen from the example of E-cadherin, and this is supported by various studies [34]. In the literature, it is also stated that there is an effect of Claudin-1 localization, which has been transported from the cell membrane into the cell, on cell proliferation. In our study, intracellular staining pattern was observed in 54.5% of metastatic cases. In MTC with the highest rate of metastasis, all the cases showed intracellular Claudin-1 positivity. Further studies are needed to elucidate the mechanism of action of Claudin-1, which migrates from the cell membrane to the subcellular space.

There is only one study in the literature examining ZIP4, ZO-1, and Claudin-1 together. Liu *et al.* [35] found ZIP4 to be higher in cancerous tissues compared to normal tissues in IHC and molecular research in pancreatic cancer cell lines and pancreatic cancer cell lines, and they also found that there was a reverse correlation with ZO-1 and Claudin-1. ZIP4 expression was increased in tumour tissues and tissues with metastasis tendency, while ZO-1 and Claudin-1 expression were decreased. It has been suggested that ZEB1 is effective in this mechanism. In our study, a correlation was observed between ZIP4 and Claudin-1 expression by both IHC and RT-PCR methods ($p = 0.000$**). However, ZO-1 did not show significant correlation with either marker.

Thyroid cancers exhibit a wide spectrum of clinical behaviour, from tumours with low mortality in most cases to aggressive malignancies such as anaplastic thyroid cancer. Considering the mortality and morbidity of patients with lymph node and distant metastasis rates of up to 50%, and the burdens they impose on health systems, there is a need for targeted therapies that will affect the biological behaviour of tumours.

We suggest that the expression of Claudin-1 and ZIP4 can be used as an important marker for thyroid tumours to show poor prognosis and predisposition to metastasis, and to develop target therapy. We think that the use of ZO-1 marker, which is highly expressed in non-tumour tissue and is open to changes in clinical conditions such as thyroiditis, is not beneficial for thyroid.

This study was presented as an oral presentation at the 8th International Hippocrates Congress On Medical And Health Sciences, 4-5 March, 2022.

The authors declare no conflict of interest.

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Address for correspondence:

Ulaş Alabalık
Department of Pathology
Dicle University Faculty of Medicine
21280, Sur/Diyarbakır, Turkey
e-mail: ulasalabalik@gmail.com