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Programmed Death – Ligand 1 Expression in Malignancy of Thyroid Follicular Epithelial Cell Origin

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Abstract

Several previous studies reported the fact that expression of Programmed Death – Ligan 1 (PD-L1) in various types of histopathology of thyroid cancer showed varied results and had predictive value and prognosis that were expected to be targeted for anti PD-1/PD-L1 immunotherapy. The aim of this study was to evaluate comparison expression of PD-L1 one to each other group between papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), poorly differentiated carcinoma (PDTC) and anaplastic thyroid carcinoma (ATC). This study was an observational analytic with a cross sectional design using paraffin block samples from three anatomic pathology laboratories in Makassar during the periode of July 2015 – February 2019. PD-L1 expression was evaluated using *Rabbit Monoclonal Antibody* (28-8) and data were analysed using The Mann – Whitney Test. There was a significant difference of PD-L1 expression score between PTC with PDTC (p = 0,045 ; p < 0,05) and ATC (p = 0,046 ; p < 0,05), whereas there was no significant difference of PD-L1 expression score between PTC with FTC (p = 0,371 ; p > 0,05), between FTC with PDTC (p = 0,147 ; p > 0,05) and ATC (p = 0,069 ; p > 0,05), also between PDTC with ATC (p = 0,483 ; p > 0,05). But overall, PD-L1 expression showed higher expression in a subset of advanced thyroid cancers such as poorly differentiated thyroid carcinoma and anaplastic thyroid carcinoma.

Keywords: PD-L1; thyroid cancer; papillary thyroid carcinoma; follicular thyroid carcinoma; poorly differentiated thyroid carcinoma; anaplastic thyroid carcinoma.

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1. Introduction

Throughout the world, thyroid carcinoma, although relatively rare, is the most common malignancy of the endocrine system [1,2], with increasing dramatically in the last three decade [3,4]. The incidence rate of thyroid carcinoma between women and men is quite consistent between three to one which is attended by all geographical regions and ethnic groups [1]. In Indonesia, based on Estimated Data on Number of New Cases and Number of Deaths due to Cancer in Dharmais Cancer Hospital in 2010-2013, thyroid carcinoma is processed in the 6th position [5]. Most thyroid neoplasms are derived from epithelial follicular cells, It can be distinguished into differentiated (PTC and FTC) and undifferentiated (PDTC and ATC). Comprising the papillary thyroid carcinoma (PTC) and the follicular thyroid carcinoma (FTC) histotypes, which may progress towards the poorly differentiated thyroid carcinoma (PDTC) and the anaplastic thyroid carcinoma (ATC). Although originating from the same cell type, thyroid cancers display different morphological features, functional behavior, and grade of differentiation as a result of heterogeneous genetic alterations [6,7,8,9]. In 1863, Virchow for the first time showed a relationship between the immune response and carcinoma, one of which was by binding to pathways that inhibit T cell activation through ligand expression to inhibit receptors on T cells such as PD-1 receptors When PD-1 binds to its ligand in tumor cells, it triggers two mechanisms: increasing apoptosis (programmed cell death) on specific antigen T cells and simultaneously reducing apoptosis in regulatory T cells (anti-inflammatory, suppressive T-cells). Several previous studies, especially in patients with lung cancer and melanoma with high expression of PD-L1, showed an improved response during anti-PD-L1 therapy [10,11,12,13,14,15,16,17,18,19,20]. Cunha and his colleagues 2013 found positive PD-L1 results expressed in the cytoplasm of 82.3% (209 of 254 samples) in thyroid papillary carcinoma and 87.5% (35 out of 40 samples) in thyroid follicular carcinoma. Bastman and his colleagues 2016 obtained positive PD-L1 results expressed in membranes of 58.3% (7 of 12 samples) in differentiated carcinomas and 75% (6 of 8 samples) in thyroid anaplastic carcinomas. Whereas Soomin Ahn and his colleagues 2017 obtained positive PD-L1 results expressed in membranes of 6.1% (20 of 326 samples) in thyroid papillary carcinoma, 7.6% (5 of 66 samples) in thyroid follicular carcinoma and 22.2% (2 of 9 samples) in anaplastic carcinoma. Although the published data shows different predictive values of PD-L1 expression, overall the data shows PD-L1 is strongly expressed in patients with advanced thyroid carcinoma, such as thyroid anaplastic carcinoma [12,21,22].

2. Materials and Methods

2.1. Case Selection

This study evaluated the expression of PD-L1 in tumor cells by identifying a total of 92 samples of patients with thyroid malignancy according to inclusion criteria. Each formalin-fixed paraffin-embedded of the block samples was cut with a microtome. A 3-μm sections were stained haematoxylin and eosin (H&E) and then re-evaluated by two Anatomical Pathologists to establish a consistent diagnosis. The Re-evaluation results obtained 40 samples of papillary thyroid carcinoma (PTC), 40 samples of follicular thyroid carcinoma (FTC), 9 samples of poorly differentiated thyroid carcinoma (PDTC) and 3 samples of thyroid anaplastic carcinoma (ATC).

2.2. PD-L1 Immunohistochemistry

All paraffin block samples were cut again to a thickness of 5 μm, deparaffinized and hydrated with xylene and alcohol, blocking endogenous peroxidase activity using 3% Hydrogen Peroxide then followed by antigen retrieval and protein block. Tissue sections were incubate with primary antibody using PD-L1 (28-8) *Rabbit Monoclonal Antibody*, then followed by washes and incubation with HRP conjugated secondary antibody and detection of antibody complex by adding DAB chromogen. PD-L1 immunohistochemistry microscopy result was independently scored manually by two experienced pathologists with a double-blind method. Interpretation of PD-L1 positive immunoexpression in tumor cells was defined as complete and/or partial circumferential linear plasma membrane staining at any intensity that can be differentiated from the background and diffuse cytoplasmic staining (Phillips and his colleagues 2015). Stains were initially scored in semi-quantitative estimates using Allred-Like Score for intensity of staining (0-3) as following : negative (0), weak (1), intermediate (2), strong (3), and the percentage of tumor cell staining area (0-100%) as following : no tumor cell area was stained (0), <1% (1), 1-10% (2), 11-33% (3), 34-66% (4), and 67-100% (5) [23]. The score of intensity and the percentage area of tumor cells stained were added to get the final score/total score. Samples were considered positive if Allred-like score was ≥ 3, with the interpretation of score levels : negative (0) if total score 0-2, weak (+1) if total score 3-4, intermediate (+2) if total score 5-6 and strong (+3) if total score 7-8.

2.3. Data analysis

The data were processed and analyzed statistically with The Mann-Whitney and Chi-Square Test using the SPSS 20.0 program

3. Result

3.1. Control Sample

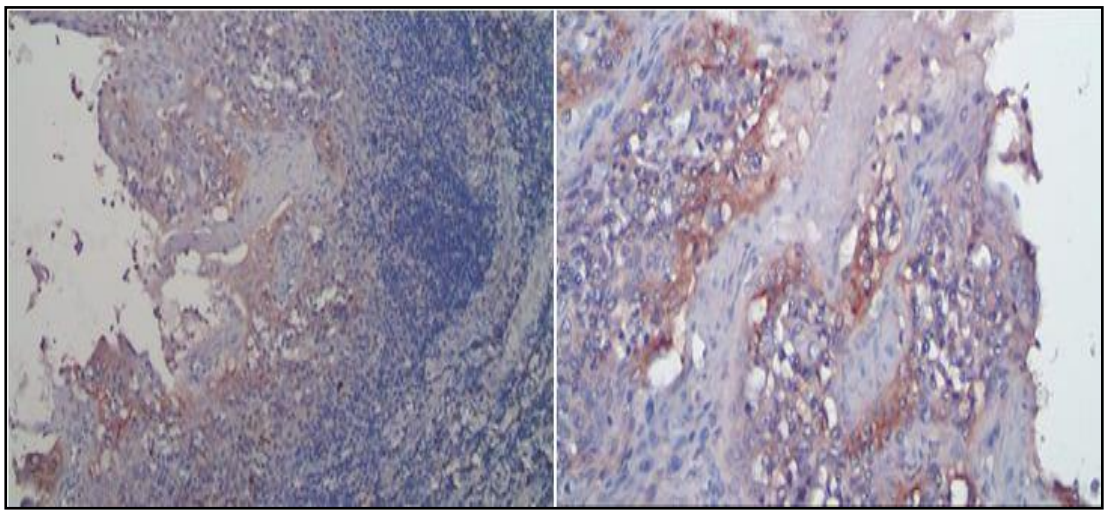

Figure 1: The epithelium in the tonsillar crypt used as a positive control for PD-L1 staining (obj.x4, obj.x20)

3.2. The intensity of PD-L1 staining

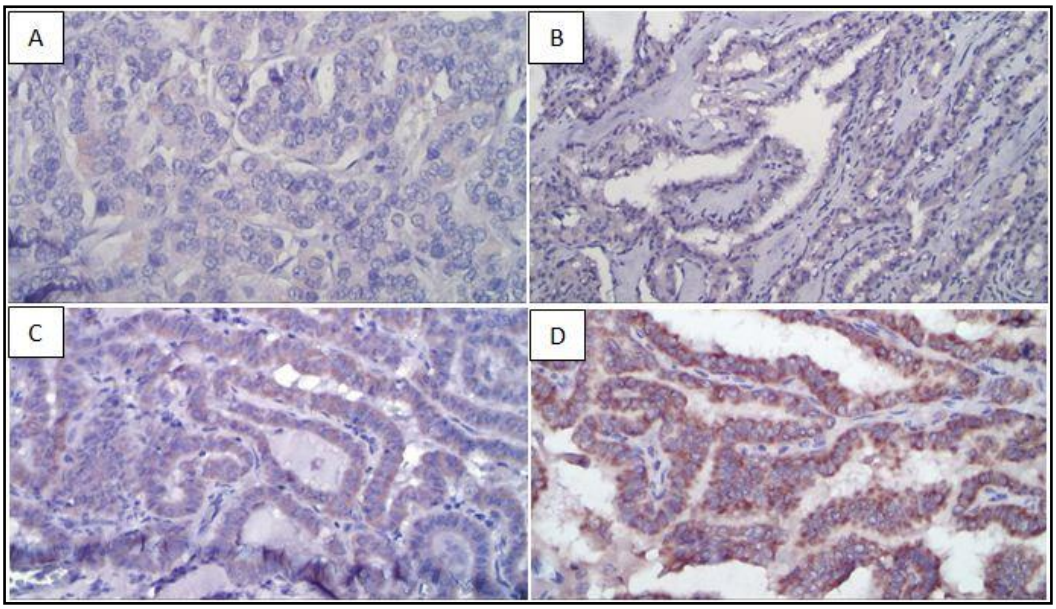

Figure 2: The intensity of PD-L1 staining in papillary thyroid carcinoma (A-D).

PD-L1 negative staining, only diffuse in sitoplasm (A). PD-L1 positive staining in tumor cells : weak 1+ (B. Obj.x20), intermediate 2+ (C. Obj.x20), and strong 3+ (D. Obj.x20)

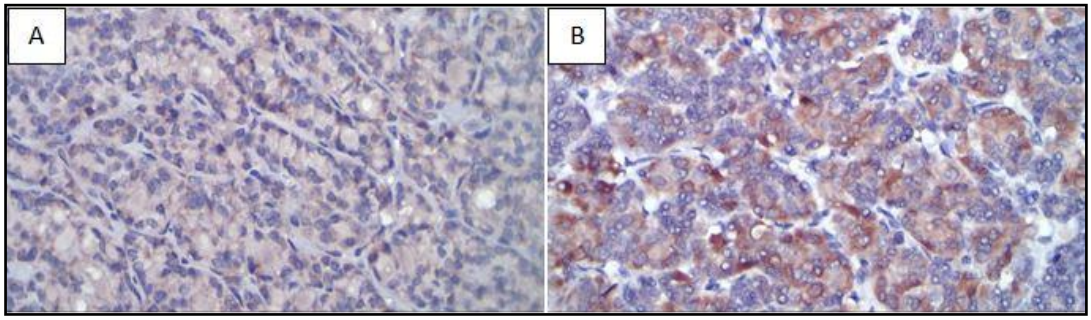

Figure 3: The intensity of PD-L1 staining in follicular thyroid carcinoma (A-B).

PD-L1 positive staining in tumor cells : intermediate 2+ (A. Obj.x40), and strong intensity 3+ (B. Obj.x40)

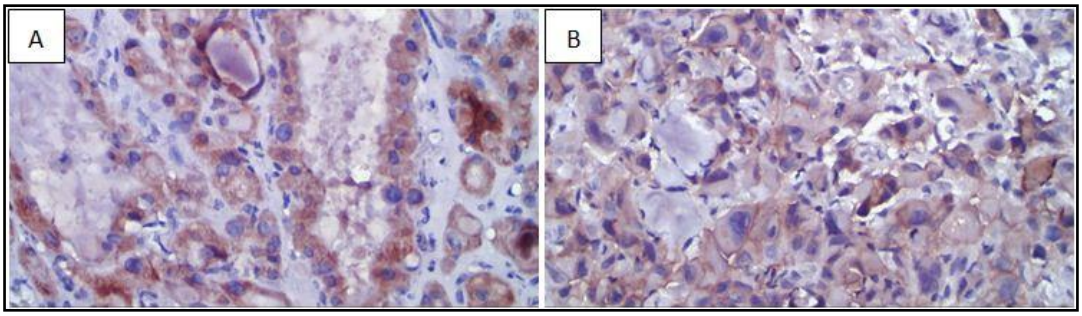

Figure 4: PD-L1 positive staining with strong intensity 3+ in poorly differentiated thyroid (A. Obj.x40) and anaplastic thyroid carcinoma (B. Obj.x40)

3.3. The percentage score of tumor cells staining area

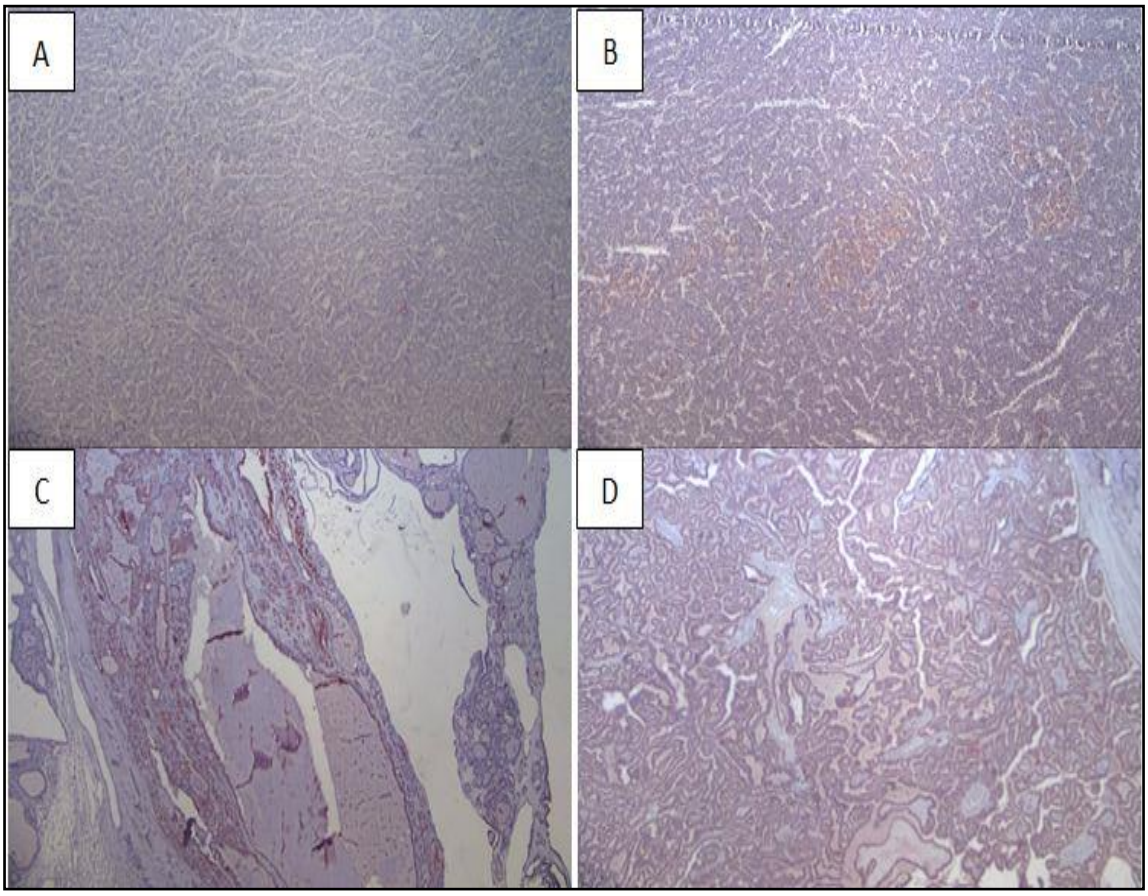

Figure 5: The percentage score of tumor cells staining area. No tumor cell area was stained, score 0 (A. Obj.x4), area of tumor cells stained 10%, score 2 (B). 34% - 66%, score 4 (C). 67% - 100%, score 5 (D).

The characteristics of 92 samples of thyroid carcinoma patients are presented in Table 1. The age of the youngest and oldest patients at the time of diagnosis was 12 and 75 years old (median 45.00 years). Among patients, 19 (20.7%) were males and 73 (79.3%) were females. The proportions based on histopathological diagnosis were 40 (43.5%) of PTC, 40 (43.5%) of FTC, 9 (9.8) of PDTC and 3 (3.20) of ATC. After going through PD-L1 staining and assessed based on the intensity and percentage of tumor cell area that was stained, obtained negative staining were count for 9 samples (9.8%), weak intensity were 27 samples (29.3%), intermediate intensity were 14 samples (15.2%) and strong intensity were 42 samples (45.7%). The percentage of tumor cell area obtained 29 samples (31.5%) were score 4 (34% -66%) and some samples were not stained by PD-L1 antibodies (9 samples, 9.8%).

Table 1: Characteristics of 92 samples of thyroid carcinoma patients

Characteristic	n	Minimum	maximum	mean	median
Age	92	12	75	43.71	45.00
Characteristic			n (%)		
Sex					
Male			19 (20.7)		
Female			73 (79.3)		
Histopathological Diagnosis					
Papillary Thyroid Carcinoma (PTC)			40 (43.5)		
Follicular Thyroid Carcinoma (FTC)			40 (43.5)		
Poorly Differentiated Thyroid Carcinoma (PDTC)			9 (9.8)		
Anaplastic Thyroid Carcinoma (ATC**)**			3 (3.20)		
Intensity of staining					
Negative			9 (9.8)		
Weak			27 (29.3)		
Intermediate			14 (15.2)		
Strong			42 (45.7)		
The percentage of tumor cell areas staining					
No tumor cell area was stained			9 (9.8)		
<1%			5 (5.4)		
1 - 10%			20 (21.7)		
11 - 33%			11 (12.0)		
34 - 66%			29 (31.5)		
67 - 100%			18 (19.6)		

Table 2: The correlation between the interpretation score of PD-L1 expression with histopathological diagnosis

Histopathological diagnosis	**The interpretation score of PD-L1 expression**				
	Negative (score 0-2)	**Weak (score 3-4)**	**Intermediate (score 5-6)**	**Strong (score 7-8)**	**n**
PTC	10	8	10	12	40
FTC	3	10	13	14	40
PDTC	0	3	0	6	9
ATC	0	0	1	2	3
TOTAL	13	21	24	34	**92**

Chi-Square 14.116[a], df = 9, p = 0.118 (p > 0,05)

According to the data in table 2, PD-L1 expression score was positive in the majority of samples, including weak positive values (n = 21), intermediate (n = 24) and the strong positive with the highest number of positive samples (n = 34). Negative samples were also identified (n = 13).

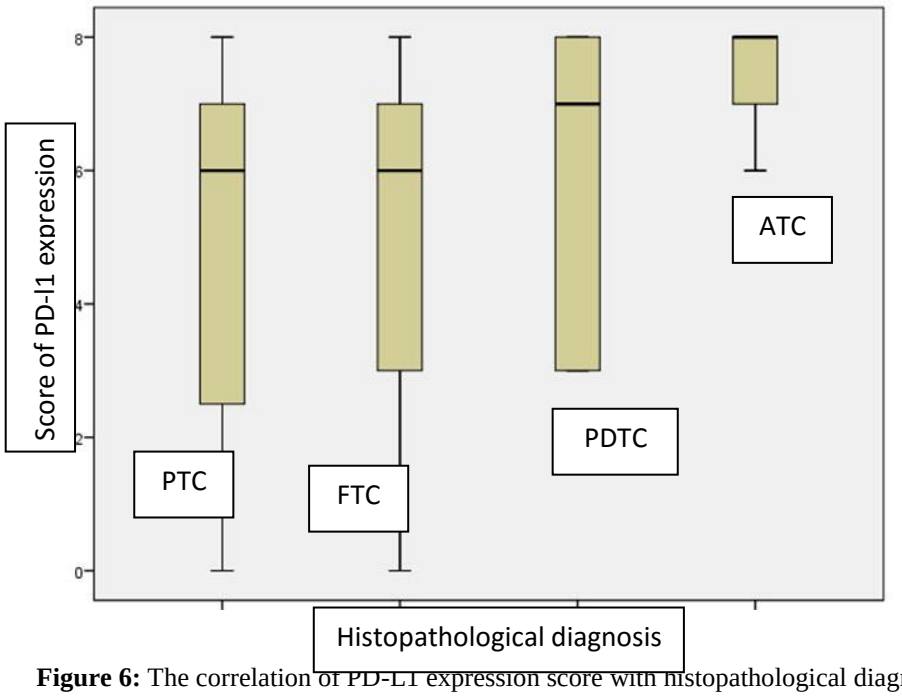

Figure 6: The correlation of PD-L1 expression score with histopathological diagnosis

The chart above shows that the midline at each box is a median value of the PD-L1 expression score. In well-differentiated thyroid carcinoma (PTC and FTC) shows the same median value. Whereas in undifferentiated thyroid carcinoma (PDTC and ATC) there is an increase in median value along with the increasing degree of malignancy differentiation in the thyroid. In general, there was an increase PD-L1 expression scores in undifferentiated thyroid carcinoma compared with well-differentiated thyroid carcinoma.

Table 3: Analysis score of PD-L1 expression between 2 histopathological diagnosis groups : Mann-Whitney Test

Histopathological diognosis	**Score of PD-L1 expression**			
	n	Mean	Total	Asymp. Sig. (2-tailed)
PTC **FTC**	40 40	38.21 42.79	80	p = 0.371 > 0.05
PTC **PDTC**	40 9	23.09 33.50	49	p = 0.045 < 0.05
PTC **ATC**	40 3	20.98 35.67	43	p = 0.046 < 0.05
FTC **PDTC**	40 9	23.63 31.11	49	p = 0.147 > 0.05
FTC **ATC**	40 3	21.06 34.50	43	p = 0.069 > 0.05
PDTC **ATC**	9 3	6.11 7.67	12	p = 0.485 > 0.05

The data in table 3 show there was a significant difference of PD-L1 expression score between PTC with PDTC

($p = 0,045$; $p < 0,05$) and ATC ($p = 0,046$; $p < 0,05$), whereas there was no significant difference of PD-L1 expression score between PTC with FTC ($p = 0,371$; $p > 0,05$), between FTC with PDTC ($p = 0,147$; $p > 0,05$) and ATC ($p = 0,069$; $p > 0,05$), also between PDTC with ATC ($p = 0,483$; $p > 0,05$).

4. Discussion

Based on epidemiological data, thyroid malignancy worldwide has increased dramatically in the last few decades [3,4]. The recurrence rate that reaches 15 - 30% of cases and the mortality rate of thyroid malignancy has not shown a decrease, although an increase in the ability to detect small thyroid nodules at a preclinical stage makes it possible to diagnose thyroid cancer earlier and provide a variety of better treatment modalities [8]. The latest therapy for cancer patients is the administration of immunotherapy, which shows good results in cases of cancer with high mutations [23].

Through immunohistochemical examination, the results studies of patients with cases of lung cancer and malignant melanoma with higher PD-L1 expression results showed an increase in good response during the administration of anti-PD-L1 therapy [21]. For the case of thyroid cancer, various studies have also been conducted to investigate the possibility of giving immunotherapy using an inhibitory pathway on the immune checkpoint [23,27,28]. Despite, their role in determining prognosis or as a predictive marker is still lacking. This study attempted to assess the correlation between the degree of differentiation of thyroid carcinoma and expression of PD-L1 in thyroid cancer patients in our center and obtain data that is expected to be a prognosis and predictive factor associated with the development of immune targeting therapy. Based on the data in table 2, we identified that PD-L1 was expressed in the most samples (79 of 92). In PTC obtained an almost equal number of samples between negative samples and those expressing PD-L1. In the FTC, PD-L1 expression was found positive in most samples, but there were still negative samples. All PD-L1 positive in PDTC and ATC, mostly showing strong intensity (3+). Anaplastic thyroid carcinoma is known as a malignancy with high aggressiveness, composed of undifferentiated thyroid follicle cells with the average 1-year survival rate is only in 10-20% of cases [24,25]. The expression of PD-L1 in this study was positive if there was a complete and/or partial circumferential linear plasma membrane staining at any intensity in the tumor cell membrane [21]. However, in this study, PD-L1 was expressed not only in tumor cell membranes but also in some diffuse expression in the cytoplasm. These results were also obtained from previous studies which conducted studies to investigate the expression of PD-L1 in thyroid carcinoma [10,12,14,22,26].

This is according to the structure of PD-L1 which acts as a transmembrane protein consisting of one transmembrane region and two extracellular domains, Ig-C and Ig-V. PD-L1 also has a short cytoplasmic domain and transmits intracellular signals [13]. Based on the data in table 3, the results in this study must be assessed more carefully because it is different from previous studies which found that the distribution of positive PD-L1 differed significantly according to the histological type/degree of malignancy differentiation. Although there was no significant correlation between PD-L1 expression with the degree of differentiation in the four groups of thyroid carcinoma, there were results that showed a significant correlation between PD-L1 expression scores between PTC with PDTC and ATC ($p < 0,05$). This shows that PD-L1 was strongly expressed in undifferentiated thyroid carcinoma compared to differentiated thyroid carcinoma. A similar result is also found

in the research by S Ahn and his colleagues that PD-L1 is strongly expressed in thyroid carcinoma advanced or undifferentiated thyroid, i.e 22.2% thyroid anaplastic carcinoma compared with follicular thyroid carcinoma 7.6% and thyroid papillary carcinoma 6.1 % [21]. Similarly, research data by Rosenbaum and his colleagues conclude that PD-L1 expression in tumor cells can be associated with more aggressive tumor behavior [26]. Although originating from the same cell type, i.e thyroid follicular epithelial cells, thyroid carcinoma displays a different morphological, functional behavior and level of differentiation as a result of heterogeneous genetic changes, where the most frequent mutations are mutations of activation points from BRAF and RAS oncogene, translocation of chromosomes from RET (Rearranged during Transformation) and NTRK1 (Neurotropic Tyrosine Kinase Receptor 1) gene, which leads to activation of a common carcinogenic pathway, namely the MAPK / ERK signaling pathway [20].

The difference in results with previous studies can be caused by various factors, including the use of different antibody clones, interpretations, and sample characteristics both in terms of the number and distribution of samples that are not normal and other clinicopathological factors. limitations of this study include the small sample size and the inclusion of a uniform assessment of patient characteristics; Strengths include data processed through various analytical tests to ascertain whether there is a relationship between the degree of differentiation of thyroid carcinoma and PD-L1 expression using samples of Makassar.

5. Conclusion

Although there was no significant correlation between the degree of differentiation of thyroid carcinoma with PD-L1 expression in this study, however, it was found that the expression of PD-L1 in undifferentiated thyroid carcinoma was higher than well-differentiated thyroid carcinoma.

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