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The Correlation of CD31 Immunohistochemical Expression with Histopathological Subtypes, Lymphovascular Invasion, and Angiogenesis in Papillary Thyroid Carcinoma

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ABSTRACT

Background
Thyroid carcinoma is a malignancy of the thyroid gland originating from thyroid follicular cells or parafollicles. The incidence increases slowly and is rare, occurring in approximately 1% of all-body malignancies. Papillary thyroid carcinoma (PTC) is influenced by radiation factors, geographic variations, age, and gender. Lymphovascular invasion (LVI) is an independent predictor of lymph node metastases. Angiogenesis is the process of forming new blood vessels and estimating angiogenesis is carried out using a microscopic approach to tumor tissue microvessel density (MVD through CD31 immunohistochemistry. Increased MVD is associated with poor prognosis as an endothelial marker used in calculating MVD using CD31.

Method
There is a cross-sectional analytic study with 32 samples diagnosed with PTC from the Anatomic Pathology Unit of H. Adam Malik General Hospital, Medan. MVD is the number of small blood vessels in terms of size and diameter (arterioles and venules) with a round lumen stained positively with CD31 which are categorized as low (1-9 blood vessels/10 LPB), moderate (10-19 blood vessels/10 LPB) and high (>20 blood vessels/10 LPB). The relationship of CD31 immunohistochemical expression with histopathological subtypes, LVI and angiogenesis was analyzed by Eta and Somers'd tests.

Results
There was a significant relationship between CD31 and histopathological subtype (p=0.05), lymphovascular invasion and angiogenesis with a p=0.01 (p-value<0.05).

Conclusion
MVD is significantly associated with histopathological and lymphovascular subtypes.

Keywords: CD31, PTC, LVI, MVD

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INTRODUCTION

Thyroid carcinoma is a malignancy of the thyroid gland originating from thyroid follicular cells or parafollicles. This carcinoma is relatively rare, which is only about 1% of all malignancies throughout the body.[1] The incidence of thyroid cancer appears to be increasing gradually. It has slow growth and disease course, as well as low morbidity and mortality.[2] A small proportion are fast growing and very malignant with a fatal prognosis. The death rate from thyroid carcinoma is only about 0.4% of all cancer deaths or around five per one million population per year. Geographic conditions are known to influence the prevalence of thyroid carcinoma types. In coastal areas such as Iceland where iodine consumption is sufficient or sometimes excessive, papillary thyroid carcinoma (PTC) is more predominant.[3-6]

Thyroid carcinoma is mostly diagnosed in the 3rd to 6th decades of life, more in women than men.[4] The median age of patients with PTC at diagnosis is 50 years old, with 91% of patients at diagnosis aged 20-74 years old.[1,6,7] The risk of recurrence and death increases with age, especially after the age of 40 years old. Children under 10 years old have a higher risk of recurrence than older children or adolescents.[2] Environmental, genetic and hormonal factors. PTC is most closely related to radiation.[8-13] The incidence of thyroid cancer is strongly influenced by many factors including geographic variations, age and gender.[14]

Lymphovascular invasion (LVI) has been known to be an independent predictor of lymph node metastasis and LVI has been an independent predictor of disease-free survival as well as overall survival. LVI was assessed in carcinoma tissue on hematoxylin and eosin-stained sections (H&E), this is defined as carcinoma cells that are present in endothelial (lymphatic or vascular) bounded spaces.[15]

Angiogenesis plays an important role in the spread of cancer cells. Cancer cells can penetrate into the blood or lymph vessels, circulate through the vascular bed, and then proliferate elsewhere or metastasize.[16] Pathologically, to estimate the presence of an angiogenesis, an approach is carried out by microscopically estimating the microvessel density (MVD) of tumor tissue through immunohistochemical examination.[17] MVD estimation is the technique most commonly used to quantify intratumoral angiogenesis in cancer. It was originally developed by Weidner et al in 1991 and used imonuhistochemical penandothelial staining on microvessels. Increased MVD has been associated in general with a poorer prognosis in several cancers including breast, colon, melanoma and genitourinary tract cancers in women.[18]

Tumor neovascularization was assessed quantitatively by immunohistochemical examination using endothelial markers. Common markers used in calculating MVD are CD31, CD34, and the von Willebrand factor (vWF). A number of studies have demonstrated the significance of MVD as a prognostic factor and demonstrated that MVD is a prognostic factor independent of other conventional pathological prognostic factors.[17] This study used CD31 monoclonal antibody immunohistochemistry. The selection of CD31 antibodies for MVD assessment as a prognostic factor is more accurate than other endothelial cell markers such as CD34 and von Willebrend factor (vWF). CD31 is said to be more sensitive as an endothelial cell marker. Immunohistochemical staining can be used to determine whether benign and malignant neoplastic lesions originate from endothelial cells.[9] The aim of this study was to determine the relationship between CD31 immunohistochemical expression and histological subtypes, lymphovascular invasion and angiogenesis in papillary thyroid carcinoma.

METHOD

analytical study with a cross-sectional approach conducted at the Department of Anatomic Pathology, Faculty of Medicine, University of North Sumatra, Medan and the Anatomic Pathology Unit, RSUPH Adam Malik Medan by using a paraffin block which has been diagnosed histopathologically as papillary thyroid carcinoma. Sample calculation was carried out with a total of 32 samples including inclusion and exclusion criteria. Samples were taken using consecutive sampling technique . Inclusion criteria were medical record data of papillary thyroid carcinoma patients diagnosed histopathologically at the Laboratory of Anatomic Pathology Unit H. Adam Malik General Hospital Medan with clinical data (age, sex, and tumor size) and all paraffin blocks derived from postoperative tissue. who had been diagnosed as papillary thyroid carcinoma histopathologically adequate after staining with H&E. Exclusion criteria for this study were

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incomplete patient data and paraffin slides/blocks that after being reviewed were inadequate/didn't meet standards (damaged, etc.) so they couldn't be assessed. The dependent variable is the Cluster of differentiation 31 expression (CD 31) and the independent variable are histopathological subtypes, lymphovascular invasion and angiogenesis. Each sample was stained with HE and CD31 primary antibody diluent K004 immunohistochemistry (Diagnostic BioSystem, IgG1 isotype kappa, clone JC/70A, dilution 1:10-1:25).

PTC histopathological subtype with cellular morphological features consists of malignant epithelial cells, follicular cell differentiation, branching, enlarged nuclei, oval and elongated shapes, overlapping with a clearing or ground-glass appearance, or Orphan-Annie eye features, with irregular contours, grooves, and nuclear pseudoinclusions.[1,3,4] Lymphovascular invasion is the spread of cancer cells to local lymph vessels and blood vessels. Expression CD31 immunohistochemistry is an assessment of endothelial cell protein. Stain pattern of positive expression was on the vascular endothelial cell membrane. In the examination results, this study has an ordinal scale that is categorized into positive and negative. Microvessel density (MVD) is the number of small vessels as measured by size and diameter (arterioles and vanules) with a spherical lumen, counted visually with an Olympus CX23 light microscope. It is performed using the hot spot method where the slide is initially viewed at 100x magnification to identify areas with high microvessels.[19] Microvessel density was calculated at 400x magnification in the clockwise area and the total number of microvessels expressed by CD31 was accumulated, categorized as low (1-9 vessels/10 large visual fields), medium (10-19 vessels/10 large visual fields), tall (>20 vessels/10 large visual fields).[20]

The data obtained in this study will be analyzed using a statistical program using tests ETA and Somers'd test.

RESULTS

In this study, 32 papillary thyroid carcinoma samples were obtained. The mean age of the samples from papillary thyroid carcinoma in the study was 44.56 years old, with the lowest age being 11 years old and the highest being 70 years old. The largest group was found at the age of <55 years old, namely 22 people (68.8%), and aged ≥55 years old, 10 people (32.2%). Meanwhile, from the gender distribution, there were 25 women (78.1%) and 7 men (21.9%). Classification of tumor sizes based on the TNM system found mostly T3 with 28 samples (87.4%) and T1 and T2 with the same number of samples as many as 2 samples (6.3%). Based on data on the involvement of the KGB (N), there were 26 N0 samples (81.2%) and 6 N1 samples (18.8%). Based on metastatic data, the most found were M0, 31 (96.9%) and M1, 1 (3.1%), and based on clinical stage, the most were found in stage I, 22 samples (68.8%), followed by stage II, 10 samples (31.3%), and stage III and stage IV were not found.

Based on histopathological subtypes, the highest number were follicular variant of PTCwhich was 15 samples (46.9%), classic PTC 14 samples (43.8%), columnar cell variant 2 samples (6.2%) and oncocytic variant 1 sample (3,1%). While the results obtained based on Lymphovascular invasion (LVI) obtained 16 positive samples (50%) and also 16 negative samples (50%).

CD31 expression obtained a p-value <0.05 (significant) indicating that there is a relationship between high CD31 expression based on prognosis, where the worse the prognosis, the higher the expression of CD31 in papillary thyroid carcinoma histopathological subtypes which were analyzed statistically using Eta test with a value of p=0.05. From the analysis of the relationship between CD31 immunohistochemical expression in lymphovascular invasion, the statistical test results obtained a p-value <0.05 (significant) indicating a strong relationship (p-value=0.001). It can be concluded that there is a significant relationship between CD31 immunohistochemical expression and lymphovascular Invasion was analyzed statistically using the Somers'd test with a value of p=0.001.

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Table 1. The frequency distribution of the characteristics of papillary thyroid carcinoma patients includes age, tumor size , KGB involvement , metastases, stage, subtype, lymphovascular invasion

Characteristics	Total (n=32)	Percentage (%)
Age		
<55 years old	22	68.8
≥55 years old	10	32.2
Gender		
Man	7	21.9
Woman	25	78.1
Tumor size		
T1	2	6.3
T2	2	6.3
T3	28	87.4
T4	0	0.0
Nodule		
N0	26	81.2
N1	6	18.8
Metastases		
M0	31	96.9
M1	1	3.1
Clinical stage		
Stadium I	22	68.7
Stadium II	10	31.3
Stadium III	0	0.0
Stadium IV	0	0.0
Histology subtype		
Follicular variant of PTC	15	46.9
Papillary microcarcinoma	0	0
Encapsulated variant	0	0
Diffuse sclerosing variant	0	0
Tall cell variant	0	0
Oncocytic variant	1	3.1
Columnar cell variant	2	6.2
Warthin-like variant	0	0
Hobnail variant	0	0
Papillary thyroid carcinoma with fibromasis/fasciitis-like stroma	0	0
Solid/trabecular variant	0	0
Spindle cell variant	0	0
Clear cell variant	0	0
PTC classic	14	43.8
Lymphovascular invasion		
Positive	16	50.0
Negative	16	50.0

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Table 2. Relationship of CD31 immunohistochemical expression in papillary thyroid carcinoma against subtypes histopathology, lymphovascular invasion, and angiogenesis.

Characteristics	Microvessel density			Total (%)	p-Value
	Low	Currently	Tall		
Histological subtypes					
Follicular variant of PTC	1(3,1)	14(43.8)	0(0,0)	15(46.9)	
Papillary microcarcinoma	0(0,0)	0(0,0)	0(0,0)	0(0,0)	
Encapsulated variant	0(0,0)	0(0,0)	0(0,0)	0(0,0)	
Diffuse sclerosing variant	0(0,0)	0(0,0)	0(0,0)	0(0,0)	
Tall cell variant	0(0,0)	0(0,0)	0(0,0)	0(0,0)	
Oncocytic variant	1(3,1)	0(0,0)	0(0,0)	1(3,1)	
Columnar cell variant	0(0,0)	0(0,0)	2(6,2)	2(6,2)	<0,05*
Warthin-like variant	0(0,0)	0(0,0)	0(0,0)	0(0,0)	
Hobnail variant	0(0,0)	0(0,0)	0(0,0)	0(0,0)	
Papillary thyroid carcinoma wiith fibromasis/fasciitis-like stroma	0(0,0)	0(0,0)	0(0,0)	0(0,0)	
Solid/trabecular variant	0(0,0)	0(0,0)	0(0,0)	0(0,0)	
Spindle cell variant	0(0,0)	0(0,0)	0(0,0)	0(0,0)	
Clear cell variant	0(0,0)	0(0,0)	0(0,0)	0(0,0)	
PTC classic	13(40.6)	1(3,1)	0(0,0)	14(43.8)	
Lymphovascular invasion					
Positive	2(6,3)	14(43.8)	0(0,0)	16(50.0)	0.001**
Negative	13(40.6)	1(3,1)	0(0,0)	16(50.0)	

*Eta test, **Somers'd test

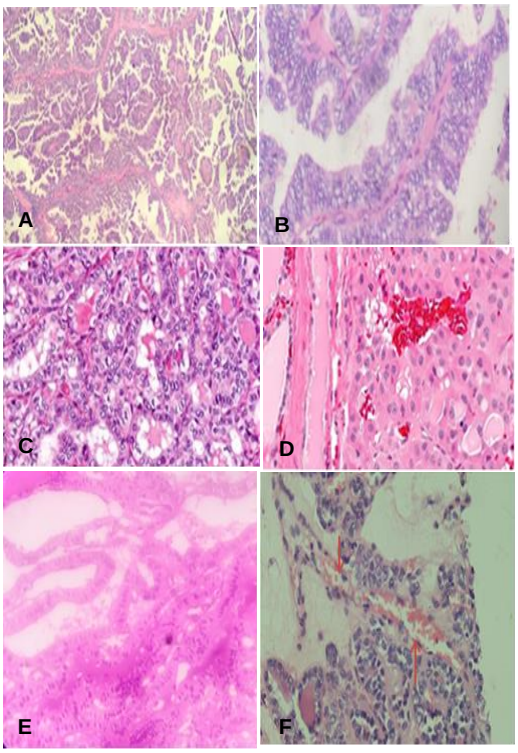

Figure 1. Microscopic subtipe histopatologi papillary thyroid carcinoma dan lymphovascular invasion. A. Papillary thyroid carcinoma classic, H&E 40 times. B. Papillary thyroid carcinoma classic , H&E 100 times. C. Oncocytic variant, H&E 100 times PTC. D. Follicular variant H&E 100 times PTC. E. Columnar variant, H&E 100 times PTC. F. H&E 40 times Lymphovascular Invasion.

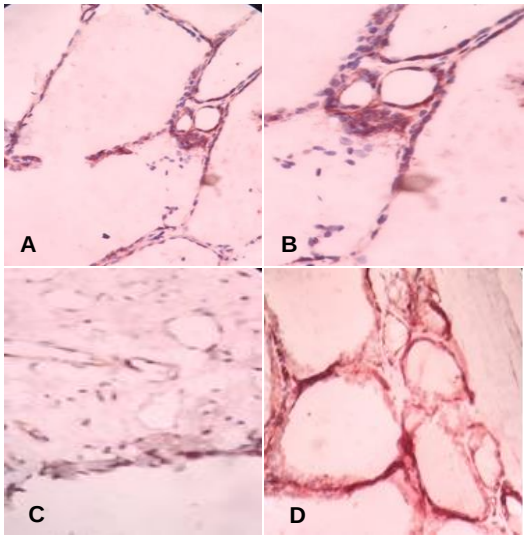

Figure 2. CD31 immunohistochemical expression. A Low 100 times. B. Low 400 times. C. Medium 400 times. D. High 400 times.

DISCUSSION

There were 32 samples in this study and most were aged <55 years old (68.8%) with an average age of 44.56 years old, the lowest age was 11 years old and the highest was 70 years old. The age-related increase in incidence is still not fully understood, at least partly due to advances in diagnostic methods such as ultrasound, thyroid scan, fine needle biopsy (Sibajah) and increased recognition of cancer.[21]

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The results of this study contradict Polyzoz and Ye et al, namely 22.2% of thyroid carcinoma patients aged ≥70 years old and patients aged at least 50-59 years old (5.9%) with a value of p=0.022). Meanwhile, Ye et al stated that most thyroid cancer patients were aged between 30-44 years old (n=449 sample, 43.8%).[17]

Patients with papillary thyroid carcinoma are more common in women than men. This is in accordance with the research of Ye et al. Ye et al also found that the rate of malignancy was significantly increased in female patients (82.3%) (p-value=0.014). However, the Polyzoz study stated that male patients had a significantly higher increase in malignancy (28.3%) compared to female patients (6.8%) (p-value=<0.001). This high female gender indicates that changes during pregnancy can increase the risk of thyroid cancer. Ponniah et al stated that the highest PTC incidence was found in women. Likewise, the period of early menarche, young parous with a pregnancy that has just occurred.[22] In some literature it can also occur in adults and rarely in children.[12,13]

The clinical stage of thyroid carcinoma was classified based on WHO TNM system. From the results of the study, it was found that there were 28 samples (87.4%) of T3, followed by T1 and T2 with the same sample, namely 2 samples (6.3%) while T4 was not found. For the most Nodules (N) at N0 were 26 samples (81.2%) and N1, 6 samples (18.8%). In the metastases found the most M0 with a total sample of 31 (96.9) and M1, 1 sample (3.1%). The results of the TNM study are in accordance with the study conducted by Ort and Goldenberg which states that central lymph node involvement is more common in the pretracheal (43%) and ipsilateral paratracheal (36%). When on the lateral side, mid-lower level KGB or levels III to IV (38%) are more common. Lateral skip metastases may occur. Most papillary thyroid carcinoma patients did not experience distant metastases and only 1 sample (3.1%) had distant metastases, namely to the lungs. The patients who have distant metastases are female. [23]

In accordance with the research of Fiore and Vitti, Ye et al stated that most cases of thyroid malignancy were found at T3.[23,33] This increase in the incidence of thyroid carcinoma is possibly related to the increasing prevalence of early detection methods through ultrasound and Sibajah examinations and because the typical nuclear changes have been recognized as PTC morphological criteria.[24]

In a study conducted by Gulobova et al in 2013, the most thyroid cancer was in the papillary thyroid carcinoma type compared to other types of thyroid cancer, namely in a study that compared FTC with 28.3% less than PTC. This is also consistent with the results of this study where the most common histopathological subtype in papillary thyroid carcinoma was the follicular variant of PTC, which was 46.9%.[25]

In the study lymphovascular invasion (LVI) is determining metastasis and prognosis in papillary thyroid carcinoma. In this study lymphovascular invasion was found in the same percentage of 50%. This result is different from the study by Leni et al which stated that the number of patients with negative lymphovascular invasion was higher than positive lymphovascular invasion with 63 patients with LVI (+) and 615 patients with LVI (-). The difference from the results of this study may lie in the sampling technique.[26]

CD31 immunohistochemical expression for histopathological subtypes was found to be the most with moderate microvessel density, which was 43.8%. The data were analyzed statistically using the Eta test and obtained a value of p<0.05. Based on the statistics, it can be concluded that there is a relationship between CD31 immunohistochemical expression and histopathological subtypes in patients with papillary thyroid carcinoma. The results of this study are not much different from previous studies. Gulobova et al. in a 2013 study reported that positive expression of CD31 immunohistochemistry was 59.3%, analysis of all tumor specimens showed that tumors with capsule formation had significantly more positive blood vessels compared to tumors without capsule.[25]

As for the results obtained in this study, the percentage showing positive lymphovascular invasion (LVI) was 50%, as well as 50% negative lymphovascular invasion. After statistical tests, it was found that there was a relationship between CD31 immunohistochemical expression and lymphovascular invasion in patients with papillary thyroid carcinoma, namely p=0.001 (<0.05) using the Somers'd test. This result is different from the study by Leni et al which stated that the number of negative lymphovascular invasion sufferers was higher.[26]

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CONCLUSION

The most common histopathological subtype in this study was follicular variant of PTC 46.9%, positive and negative Lymphovascular invasion (LVI) were found with the same number of 50%. The highest expression of CD31 in the age group <55 years old with moderate microvessel density was 34.4%, female sex 43.8%, T3 tumor size 43.7%, stage I 34.4%. Correlation between CD31 immunohistochemical expression in papillary thyroid carcinoma with histopathological subtypes, lymphovascular invasion and angiogenesis was found.

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