Original Research Article

Increased thyroglobulin and receptor activator of nuclear factor kappa B ligand expression is a risk factor of bone metastasis on patients with thyroid cancer

Gede Ketut Alit Satria Nugraha[1]*, Putu Astawa[1], Made Bramantya Karna[1], I. Gede Eka Wiratnaya[1], I. Wayan Juli Sumadi[2]

[1]Department of Orthopaedic and Traumatology, Sanglah Hospital, University of Udayana, Bali, Indonesia
[2]Department of Pathology Anatomy, Udayana University, Sanglah General Hospital Denpasar, Bali, Indonesia

*Correspondence:
Gede Ketut Alit Satria Nugraha,
E-mail: alit.orthobali.unud@gmail.com

ABSTRACT

Background: Metastatic bone disease (MBD) of thyroid cancer poses increased risk of morbidity and mortality and significant decrease of quality of life of the patient, with 10 years survival rate of 40-70%. This study aims to find correlation between increased expression of thyroglobulin and receptor activator of nuclear factor kappa B ligand (RANKL) on thyroid cancer biopsy and increased risk of bony metastasis.

Methods: This study use case control design to analyze the histopathologic preparation taken from biopsy of the patients with thyroid cancer from 2015 until 2020. The histopathology preparation was cut with 4 µm thickness, then analyzed through immunohistochemistry assay using thyroglobulin antibody cocktail and anti-RANKL polyclonal antibody by a pathologic anatomy consultant. Analysis and correlation between the high thyroglobulin and RANKL expression with the incidence of bony metastasis using chi-squared test and odd ratio calculation.

Results: There is a significant difference of thyroglobulin and RANKL expression between the group with metastasis and without metastasis (p=0.05 and p=0.02, respectively). ROC curve analysis of thyroglobulin and RANKL expression resulted in the optimal cutoff value of both parameters. Thyroglobulin cutoff value was 1.70 and RANKL cutoff value was 1.95. The analysis showed significant correlation between high expression of thyroglobulin with the incidence of bony metastasis (p=0.044). Similar result was also found in the high expression of RANKL (p=0.02).

Conclusions: Increased thyroglobulin and RANKL expression are a risk factor of bone metastasis on patients with thyroid cancer.

Keywords: Thyroid cancer, Metastatic bone disease, Thyroglobulin, RANKL

INTRODUCTION

Thyroid cancer is an endocrine malignancy whose global incidence has increased rapidly in recent decades, where it reaches 164 cases on 100.000 people.[1] There are several types of cytologic variation such as follicular, papillary and medullary, where follicular type is the most common type.[2] This type is the most common cause of bony metastasis in thyroid cancer patients.[3,4] The incidence of MBD in thyroid cancer patients reaches 4% of all patients.[2,3] MBD of thyroid cancer poses increased risk of morbidity and mortality and significant decrease of quality of life of the patient, with 10-years survival rate of 40-70%.[2,3]

The high expression of thyroglobulin and RANKL is thought to be one of the risk factors for MBD.[5-8] Several

research has shown increased serum concentration of thyroglobulin and RANKL is correlated with MBD of thyroid cancer.[8,9] However, there is still few studies that analyze the expression of thyroglobulin and RANKL on patients with thyroid cancer and its correlation with increased risk of bone metastasis.[10-12]

This paper aims to find correlation between increased expression of thyroglobulin and RANKL on thyroid cancer biopsy and increased risk of bony metastasis.

METHODS

This research used case control study design, where we analyzed the histopathologic preparation taken from biopsy of the patients with thyroid cancer from 2015 until 2020. This research was conducted at Sanglah general hospital Denpasar. The research protocol for ethical clearance from the research ethics commission at the faculty of medicine, UNUD/Sanglah hospital Denpasar was submitted before the research is carried out. Subjects were explained the purpose of the study and were asked to fill out written informed consent.

From the medical record of the patients, we gathered the information regarding the bony metastasis status of the patients. The histopathology preparation was then cut with thickness of 4 µm and was then analyzed through immunohistochemistry assay using thyroglobulin antibody cocktail from BIOCARE medical and anti-RANKL polyclonal antibody from BIOENZY by a pathologic anatomy consultant. The resultant thyroglobulin and RANKL expression were then recorded and analyzed for the cutoff value using ROC curve analysis and correlation between the high thyroglobulin and RANKL expression with the incidence of bony metastasis using chi-squared test and odd ratio

calculation. Statistical package for social sciences (SPSS) for windows version 26 program is used for data processing.

The sample size in this study was determined by consecutive sampling in accordance with the inclusion and exclusion criteria to meet the number according to the requirements of the analysis. The sample size was calculated using case controls analytic comparative categorical unpaired formulas. The inclusion criteria are patient with thyroid cancer, data of the presence of bony metastasis and available paraffin histopathology block. The exclusion criteria are incomplete clinical data of the patient, no paraffin histopathology block and non-interpretable immunohistochemistry assay result.

RESULTS

The characteristic of subject from each group can be seen on Table 1. From the demographic data gathered, mean age of the patients with bony metastasis is 51.63±18.3 years old, while the non-metastasis group is 48.87±15.7 years old. There are 45.5% male on the metastasis group and 54.5% on non-metastasis group. There are no significant difference of age and gender between two groups with p value of 0.656 and 0.611 respectively.

The immunohistochemistry assay of thyroglobulin and RANKL expression from paraffin preparation result can be seen on Table 2. There is a significant difference of thyroglobulin and RANKL expression between the group with metastasis and without metastasis with p value of 0.05 and 0.02, respectively.

Table 3 and 4 showed ROC curve analysis of thyroglobulin and RANKL expression resulted in the optimal cutoff value of both parameters.

Table 1: Research subject characteristic based on group.

Variables	Group		P value
	With metastasis (n=16)	Without metastasis (n=15)	
Age (in years), mean ±SD	51.63±18.3	48.87±15.7	0.656[a]
Sex, number (%)			
Male	5 (45.5)	6 (54.5)	0.611[b]
Female	11 (55)	9 (45)	

SD=standard deviation; a=student t-test; b=chi-square test.

Table 2: Thyroglobulin and RANKL expression on the metastasis and non-metastasis group.

Variables	Group		Mean difference (95% CI)	P value
	With metastasis (n=16)	Without metastasis (n=15)		
Mean thyroglobulin expression ±SD	2.07±0.39	1.64±0.39	0.43 (0.14-0.72)	0.05
Mean RANKL expression ±SD	2.29±0.41	1.73±0.49	0.55 (0.23-0.88)	0.02

Table 3: ROC curve analysis of thyroglobulin variable.

Thyroglobulin expressions	Sensitivity (%)	Specificity (%)
1.35	100	267
1.45	100	33.3
1.55	100	46.7
1.70	81.3	53.3
1.85	56.3	73.3
1.95	56.3	80
2.05	43.8	86.7

Table 4: ROC curve analysis of RANKL variable.

RANKL expressions	Sensitivity (%)	Specificity (%)
1.65	93.8	40
1.75	93.8	46.7
1.85	93.8	60
1.95	75	66.7
2.10	56.3	80
2.25	50	80
2.35	50	86.7

Table 5: High and low thyroglobulin and RANKL expression correlation with the incidence of bony metastasis.

Variables	Group		P value
	With metastasis (n=16) (%)	Without metastasis (n=15) (%)	
Thyroglobulin expression			
High	13 (81.3)	7 (46.7)	0.044
Low	3 (18.8)	8 (72.7)	
RANKL expression			
High	12 (75)	5 (33.3)	0.020
Low	4 (25)	10 (66.7)	

Thyroglobulin odd ratio=5.0 (p=0.050, CI 95%: 0.99-24.88); RANKL odd ratio=6.0 (p=0.024; CI 95%: 1.26-28.55).

The thyroglobulin cutoff value was 1.70 with sensitivity and specificity of 81.3% and 53.3% respectively and area under curve (AOC) of 0.775. RANKL cutoff value was 1.95 with sensitivity and specificity of 75% and 66.7% respectively and AOC of 0.810. After being classified into high and low expression, both thyroglobulin and RANKL samples were analyzed for its correlation with the presence of bony metastasis. The analysis showed significant correlation between high expression of thyroglobulin with the incidence of bony metastasis. Similar result was also found in the high expression of RANKL. The summary of the analysis result can be seen in Table 5.

DISCUSSION

This research showed a significant relationship between increased thyroglobulin expression and bone metastases in patients with thyroid cancer. Thyroglobulin (Tg) is excreted by thyroid cells and is one of the key factors in the follow-up of patients with follicular differentiated thyroid cancer (FDTC). Metastasis in bone showed the highest Tg expression, whereas lymph node metastasis was associated with lower Tg expression. The sensitivity of Tg is very high for detecting bone metastases especially after stimulation of thyroid stimulating hormone (TSH). Low Tg associated with normal neck ultrasound has been reported to have a very high negative predictive value for persistent disease and is used in many clinical trials as evidence of patient response to treatment.[5,6,13]

The tendency of increased thyroglobulin expression in patients with metastatic thyroid cancer is similar to that obtained by Indrasena et al that increased Tg expression in blood was significantly correlated with the incidence of bone metastases and postoperative recurrence rates in patients with thyroid cancer.[9] Another study by Whitley et al demonstrated that increased thyroglobulin expression is associated with metastatic incidence in thyroid cancer and is useful for determining the origin of a metastatic focus.[14]

Besides in blood plasma, thyroglobulin expression can also be assessed in tissues by immunohistochemical examinations. In general, it is a useful modality in the

diagnosis of MBD, especially for determining the primary tumor of these metastases.[15] Study by Ramos-Vara et al regarding the detection of thyroid transcription factor-1 (TTF-1), thyroglobulin and calcitonin by histochemical methods in normal, hyperplastic and neoplastic canine thyroid glands shows that detection of Tg and calcitonin can be used in conjunction with detection of TTF-1 to improve the ability to detect the presence and differentiation of thyroid tumors and their metastases.[16]

On the other hand, research by Zakavi et al showed that thyroglobulin expression is not directly related to the incidence rate of MBD in the case of bone metastases of patients with thyroid cancer. Thus, it is argued that low thyroglobulin expression cannot be used as an exclusion for bone metastases in patients with thyroid cancer.[17]

Study about the relationship of RANKL expression in thyroid cancer patients and the incidences of bone metastases showed a significant relationship. The process of increasing RANKL is associated with increased osteoclast activity, which increase bone reabsorption and cause lytic lesions in bone metastases of thyroid cancer.[18,19] It has been shown that parathyroid hormone-related protein (PTHrP) cannot directly induce osteoclast activation but can mediate its effect through transactivation of the RANKL gene in stromal cells and osteoblast cells. Therefore, upregulation of RANKL has been found in almost all conditions of bone destruction caused by cancer.[20-23]

Regarding the detection of RANKL through immunohistochemical examination, a study conducted by Heymann et al showed that RANKL together with osteoprotegerin and RANK are expressed by pathologic thyroid gland tissue, malignant parafollicular cells, as well as in the microenvironment of the lymph nodes where the metastasis of the thyroid gland tumor occurs.[10] Hence, it can be concluded that RANKL together with RANK and osteoprotegerin may be involved in the pathogenesis of thyroid cancer, especially follicular and parafollicular tumor types as well as its metastatic processes.[10,24,25]

Research by Deligiorgi et al demonstrated an increase in RANKL expression in patients with metastatic thyroid cancer to the lymph nodes.[26] Meanwhile, a study by Ma et al found an increase in RANKL expression due to an increase in TSH production in patients with thyroid cancer.[27] However, expression pattern of receptor activator of NFκB (RANK) in primary tumor and bone metastatic cancer may show different process. Study of Santini et al stated that there is no significant difference of RANKL levels from cases of thyroid cancer with MBD compared with thyroid cancer without MBD.[28]

Limitations of this study including the small number of samples and short period of research. In the future, we hoped that research can be carried out with a larger number of research subjects and longer period of research to get stronger research power.

CONCLUSION

In conclusion, there is a significant difference of thyroglobulin and RANKL expression between thyroid cancer patient with metastasis and without metastasis. It is proven that increased thyroglobulin and RANKL expression is a risk factor of bone metastasis on patients with thyroid cancer.

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