ISSN: 2476-2377

Research Article

International Journal of Cancer Research & Therapy

E-Cadherin Expression as risk factor Lymph Nodes Metastatic of Paediatric Thyroid Carcinoma

Yohana Azhar*, Dimyati Achmad, Kiki Lukman, Dany Hilmanto

[1]*Division Oncology, Head and Neck Surgery, Department of Surgery, Hasan Sadikin Hospital, Universitas Padjadjaran, Bandung, West Java-Indonesia*

[2]*Department of Paediatric, Hasan Sadikin Hospital, Universitas Padjadjaran, Bandung, West Java-Indonesia*

***Corresponding author**

Yohana Azhar, Department of Surgery, Hasan Sadikin General Hospital/ Universitas Padjadjaran Bandung Indonesia, Tel: +62 22 2034655; Fax: +62 22 2034655; E-mail: r.yohana@unpad.ac.id

Submitted: 27 May 2019; **Accepted**: 31 May 2019; **Published**: 10 June 2019

Abstract

Background: *Although Paediatric Thyroid Carcinoma (PTC) has excellent prognosis, certain cases exhibit aggressive clinical manifestations. Treatment of PTC patient is therefore littered with controversies.*

Purpose: *We assessed the expression of RET/PTC1, TGFβRII and E-cadherin in PTC and metastatic lymph nodes, in order to investigate the mechanism underlying the mainly indolent but potentially aggressive of PTC.*

Methods: *A retrospective study included 93 PTC patients treated in our hospital were included in this study. All primary tumours and metastatic lymph nodes were immunohistochemically stained to evaluate the expression of RET/PTC1, TGF βRII and E-cadherin. Clinical characteristics also evaluated to predict the metastasis.*

Results: *A total of 77 (82.3%) were positive for RET/PTC1, 61(65.6%) positive for TGF βRII and 63 (67.7%) positive for E-cadherin. Even though Capsular Invasion, Size of tumours, TGF βRII and RET/PTC1 expression were significantly correlated with metastasis with multivariate analysis E-cadherin is the protein which was strongly correlated with metastasis (P<0.001 OR = 263.53; 95% CI (21.88-3172.72).*

Conclusion: *Our results suggest that the decreased expression of E-cadherin in primary lesion is correlated with regional lymph node for metastasis in PTC. E-cadherin may be useful as a marker for metastatic potential in PTC. The expression of transforming growth factor βRII in PTC was shown to be associated with clinically aggressive characteristics. RET/PTC1 rearrangement still remains the cause of spontaneous PTC in Indonesia*

Keywords: RET/PTC1, TGFβRII, E-cadherin, Paediatric Thyroid Cancer, Lymph Node Metastatic

Introduction

Although Paediatric Thyroid Carcinoma (PTC) has a good prognosis, certain cases reveal aggressive clinical manifestations. As paediatric PTC is similarly rare diseases, most treatment concepts for this patient populations are derived from experiences in the adult PTC population. While not necessarily false, those adaptations are often based on empiric derivation rather than on a solid basis evidence – as there is a little, if any, of the latter in the literature. Many questions, therefore, remain open regarding the optimal management of paediatric PTC, and many challenges remain unsolved [1,2].

Several studies reported that the presence of clinical lymph node metastasis in PTC was one of the most important prognostic indicators, whereas others revealed that pathological lymph node metastasis identified following prophylactic dissection did not significantly affect patient prognosis. Pathological lymph node metastasis from PTC was found in range up 25-56% event in the small size of tumours [3-8].

In several country such as Japan, thyroid function-preserving surgery with prophylactic lymph node dissection is deliberated to be standard procedure for PTC. Postoperative followup with surgeon-performed US, without radioactive iodine ablation (RIA) or thyroid stimulating hormone (TSH) suppression is widely used as a standard management, due to limited facilitation and regulation on radioisotope use, the shortage of institutes that perform radioiodine therapy and the difficulties in restricting iodine intake in the daily diet [9].

RET rearrangements were subsequently identified in a subset of spontaneous PTC and in these PTC-1 was predominant. The required step for cancer cells to form metastases are escape from

the primary tumours, active migration toward the vasculature and survival within the systemic circulation. To successfully undertake this steps, cancer cells may alter their characteristics from epithelial-to-mesenchymal-like form (epithelial-to mesenchymal transition; EMT). The expression of Transforming Growth Factor β RII (TGF-β RII) was shown to be associated with high proliferative potential and clinically aggressive characteristics through EMT by the stimulation of TGF-β and in the end of process E-cadherin is well-known cellular adhesion molecule in epithelial cells and is known to be lost during the process of EMT. E-cadherin expression is commonly observed in differentiated thyroid cancer and loss of its expression was reported to be independent prognostic factor for these tumours [10,11].

Methods

Patients

A consecutive series of 93 PTC who were surgically treated in our institute between 2015-2017 was investigated. The patients with multiple lesions were excluded from this study. All patients were diagnosed with PTC prior to surgery by fine-needle aspiration cytology. The post-operative pathological examination confirmed the absence of a poorly differentiated carcinoma component. The cases included 9 boys and 84 girl's patients, with a median age 11 years (range 7-18 years). A total 93, 61 patients underwent total thyroidectomy and 32 patients also underwent therapeutic lateral lymph node dissection. This study was approved by ethic committee Universitas Padjadjaran No 1075/UN6.C1.3.2/KEPK/PN/2016.

Immunohistochemistry

The 93 primary tumours and the metastatic lesions in lymph nodes from 32 patients were immunohistochemically stained as described previously. Briefly, the specimens were fixed in 10% formaldehyde solution and embedded in paraffin. The specimen were then cut in 4-µm section and deparaffinised in xylene. The tissue was heated for 20 min at 105 by autoclave in Target Retrieval solution (Dako, Carpintaria CA, USA). Following blocking of the endogenous peroxidase activity, the sections were incubated in normal goat serum. The specific immune reactivity of RET/PTC1, TGF-ßRII and E-cadherin was detected on the membranous surface of the cancer cells. The membranous staining of the follicular cells in adjacent normal thyroid tissue was used as (+) control. Positive staining was defined as > 10% of the cancer cells showing specific immune reactivity at the centre of the tumour. The invasive front was defined as the interface (<0,1 cm) between the tumour & the adjacent non-neoplastic tissue. Mutant of RET/PTC1 was determined by used RET/PTC1 antibody EPR2871 (ab134100) rabbit monoclonal Abcam United Kingdom. TGFßRII expression determined by used TGFßRII (ab78419) mouse monoclonal Abcam United Kingdom. E-cadherin expression determined by used (ab15148) rabbit polyclonal Abcam United Kingdom [12,13].

Statistical Analysis

Statistical analysis was performed using SPSS 23.0 statistical software. The Chi-square test was used to compare the differences in the positivity rate for immunostaining and the clinic pathological factors and $p < 0.05$ was considered to indicate a statistically significant difference.

Results

A total of 93 tumours of PTC were included in membrous staining by Immunohistochemistry. RET/PTC1 was detected in 77 patients and only 18 patients were correlated with nodal involvement. (P<0.001, OR 0.04 CI (0.01-0.21) TGFβ RII was detected in 32 patients and chi-square analysis show positivity rate in immunohistochemistry staining with nodal involvement (P<0.001). E-Cadherin was detected in 63 patients and there was a strong correlation between expression of E-cadherin with nodal involvement. Only 4 cases PTC with lymph node metastasis were expressed E-cadherin ($P < 0.001$, OR 206.5 CI (35.76-1195.43))

Discussion

Previous guidelines for the management of thyroid cancers were geared toward adults. Compared to thyroid neoplasm in adults, those in the paediatric population exhibit differences in pathophysiology, clinical presentation and long-term outcomes. Furthermore, therapy that may be recommended for an adult may not be appropriate for children who are at low risk for death but at higher risk for long-term harm cause by excessively aggressive treatment. For these reason, specific guidelines for children are needed.

Many previous research show that the nodal involvement is the strong predictor factor to indicate recurrence. Even though we support the aggressive therapy for PTC which have already came with lymph node metastatic, the PTC which come in early stage can be manage less aggressive. However, we feel that this should be supported at least by molecular biology marker to seek predictor that can be used as reference for the aggressive therapy application especially since management of PTC is quite difficult to handle. The needed to adjustment the Levothyroxine dosage for example, become more challenging.

Compared to adults, differentiated thyroid cancer in children is characterized by higher prevalence of gene rearrangements and lower frequency of point mutations in protooncogenes. Recent molecular studies have shown that BRAF mutation is the most common molecular abnormality in adults (35-63% of cases), while this is rare in children [14-18].

In contrast to the adults, papillary thyroid carcinoma on children's molecular pathogenesis occur sporadically on which 80% of them related to RET gene mutation, following the process of realignment with other genes, i.e., H4 and Elei that formed oncogenes RET/PTC. These genes encode proteins that play a role in that is the kinase tyrosine pathway in cells of the thyroid gland that is the path of Mitogen-Activated Protein Kinase (MAPK). Until now the RET/PTC oncogenes family have been found to be 11, but the most commonly associated with the incidence of PTC are RET/PTC1 and RET/PTC3 oncogenes. PTC that is caused by mutations un RET/PTC1 is more in the age group above 20 years with sub-type classic PTC, with tumour grows relatively slowly and occurs sporadically, where's mutation in RET/PTC3 are more common in the age group under 20-year-old and have aggressive biological characteristic usually as tall cell variant PTC and a history of radiation exposure associated with head and neck area as happened in Chernobyl and Nagasaki-Hiroshima [14].

In general, the aggressiveness of tumours is characterized by increasing proliferation and the ability tumour cells to migrate out of the primary tumours to the other organs. The process is known as metastasis. Children with PTC have increased proliferation allegedly because of gene mutations and realignment of RET/PTC that will activate the MAPK pathway. RET/PTC, respectively phosphorylate proteins that work in the MAPK pathway, ranging from Ras, Raf,

MEK and ERK. The active ERK proteins undergo translocation into the cell nucleus to activate the transcription process by promoters of genes that play a role in the proliferation [17].

The ability of tumours cells to migrate begins with the unchain of bonds with neighbouring cells and change in the cell skeleton or framework. Change in the framework of the cell causes the cell to penetrate the extracellular matrix and induced the transcription factors that alter epithelial cell into mesenchymal transition (EMT). Integrity between cells in maintained by E-cadherin and E-cadherin strong bond relating to the actin framework of cell. E-cadherin bonding loose between these cells is maintained by E-cadherin and E-cadherin strong bond relating to actin framework of the cell. E-cadherin bonding loose between these cells that will cause disruption of desmosomes that maintain ties inner filaments order that prevents the cells to penetrate the extraseluler matrices such as matrix metalloproteinase (MMP) [15-17].

This theory supports our research, we observed a loss of E-cadherin expression at PTC with lymph node involvement, suggesting clinical characteristics of EMT. The loss of E-cadherin expression was one of important factor that indicated phenotypical characteristic suggesting EMT in PTC. Several other factors are also involved in this process. The expression of Transforming Growth Factor β (TGF-β) was shown to be associated with high proliferative potential and clinically aggressive characteristics through EMT by the stimulation of TGF-β and overexpression of TGFβ R-II as we observed in our research [10,17]. Although RET/PTC 1 rearrangement still the most common cause of spontaneous PTC in Indonesia, our research didn't show that one as a predictor factor for PTC aggressiveness.

Table 1: Characteristic Patients

Characteristics	Number (n=93)	Percentage (%)
Gender		
Boys	9	9.7
Girls	84	90.3
Age		
<10 years' old	12	12.9
10-20 years old	81	87.1
Size Tumour		
<20 mm	17	18.3
20-40 mm	51	54.8
>40 mm	25	26.9
Capsular Invasion		
Negative	75	80.6
Positive	18	19.4
Tumour Size		
T1b	20	21.5
T2	48	51.6
T3a	23	24.7
T3b	2	2.2
Lymph Node Metastasis		
N0	61	65.6
N1	32	34.4
Extend of Surgery		
Hemi thyroidectomy	12	12.9
Total Thyroidectomy	49	52.7
Total Thyroidectomy + Neck Dissection	32	34.4
RET/PTC1 Expression		
Positive	77	82.8
Negative	16	17.2
TGF β RII		
Positive	32	34.4
Negative	61	65.6
E-Cadherin		
Positive	63	67.7
Negative	30	32.3

Data presented as median with interquartile range for continuous variables and n (%) for categorical variables.

Primary Tumour (pT) AJCC Cancer Staging Manual 8th Edition,T1b: The tumour is larger than 1 cm but less than 2 cm ; T2 : Tumour > 2 cm but 4 cm in greatest dimension limited to the thyroid ; T3a: Tumour > 4 cm limited to the thyroid; T3b : Gross extrathyroid extension invading only strap musles (sternohyoid, sternothyroid, thyrohyoid or omohyoid muscles) from a tumour of any size.

Table 2: Univariate analysis of risk factors PTC for the development metastatic

Category (n = 93)	Non Metastasis (%)	Metastasis (%)	P Value	OR(CI 95%)
Gender				
Male	7 (77.8 %)	2 (22.2%)	0.403	1.94 (0.380-9.960)
Female	54(64.3%)	30 (35.7%)		
Age				
years 6-19 (12.76 ±2.87)	8(66.7%)	4 (33.3%)	0.993	1.06 (0.292-3.818)
< 10 years' old 10 – 20 years old	53(65.4%)	28(34.6%0		
Capsular Invasion				
Negative	58(77.3%)	17 (22.7%)	<0.001*	17.06 (4.41-65.96)
Positive	3(16.7%)	15 (83.3%)		
Size Nodule (mm)				
< 20 mm	17(100%)	0 (0%)	<0.001*	
20 – 40 mm	42(82.4%)	9 (17.6%)		
40 mm	2 (8%)	23 (92%)		
E-cadherin				
Positive	59 (93.7%)	4 (6.3%)	<0.001*	206.5 (35.67-1195.43)
Negative	2 (6.7%)	28 (93.3%)		
TGF βRII				
Negative	61(100%)	0 (0%)	<0.001*	
Positive	0 (0%)	32 (100%)		

RET/PTC1				
Negative	2 (12.5%)	14(87.5%)	<0.001*	0.04 (0.009-0.210)
Positive	59(76.6%)	18 (23.4%)		

Data presented as median with interquartile range for countinuous variables and n (%) for categorical variables.
*Significant association using Chi-square

Table 3: Multivariate Analysis Factors might have influenced to lymph node metastatic

Independent Variable	Odds Ratio	95 % CI		*P value*
		Upper	Lower	
Age	0.439	0.033	5.758	0.531
Capsular Invasion	28.004	2.006	391.029	0.013
RET/PTC1	0.026	0.002	0.415	0.010
E-cadherin	263.530	21.889	3172.723	<0.001
Constanta	1.053873			0.971

Conclusions

In conclusions, low expression of E-cadherin in primary lesion is correlated with regional lymph node for metastasis in PTC. E-cadherin may be useful as a marker for metastatic potensial in PTC. The expression of Transforming Growth Factor β RII in PTC was shown to be associated with clinically aggressive characteristics. RET/PTC1 rearrangement still remains the cause of spontaneous PTC in Indonesia.

References

1. Boltz MM, Enomoto L, Ornstein R, Saunder B, Hollenbeck C (2013) Incidence and survival differences of differentiated thyroid cancer among younger women. Clinical Oncologic Adolescent Young Adults 3: 79-88.
2. Vecchia C, Malvezzi M, Bosetti C, Garavello W, Bortaccio P, et al. (2015) Thyroid cancer mortality and incidence : a global overview. International Journal of Cancer 136: 3187-3195.
3. Zhang L, Wei WJ, Ji QH, Zhu YX, Wang Y, et al. (2012) Risk factor for neck nodal metastasis in papillary thyroid micro carcinoma : a study of 1066 patients. J Clin Endocrinol Metab 97: 1250-1257.
4. Hyun SM, Song HY, Kim S, Nam SY, Roh JL, et al. (2012) Impact of combined prophylactic unilateral central neck dissection and hemi thyroidectomy in patients with papillary thyroid micro carcinoma. Ann Surg Oncol 19: 59-56.
5. Caliskan M, Park JH, Jeong JS, Lee CR, Park SK, et al. (2012) "Role of prophylactic ipsilateral central compartment lymph node dissection in papillary thyroid micro carcinoma. Endocr J 59: 305-311.
6. Zeng RC, Li Q, Lin KL, Zhang W, Gao EL, et al. (2012) Predicting the factors of lateral lymph node metastasis in papillary microcarcinoma of the thyroid in eastern china. Clin Transl Oncol 14: 842-847.
7. Zhou YL, Gao EL, Zhang W, Yang H, Guo GL, et al. (2012) Factors predictive of papillary thyroid microcarcinoma with bilateral involvement and central lymph node metastasis : a retrospective study. World J Surg Oncol 10: 67.
8. Kim BY, Jung CH, Kim JW, Lee SW, Kim CH, et al. (2012) Impact of clinicopathologic factors on subclinical central lymph node metastasis in papillary thyroid micro carcinoma. Yonsei Med J 53: 924-930.
9. A Miyauchi, Ito Y, Oda H (2018) Insights into the Management of papillary Microcarcinoma of Thyroid. Thyroid 28: 23-21.
10. Chapnick DA, Warner L, Bernet J, Rao T, Liu X (2011) Partners in crime: the TGF β and MAPK pathways in cancer progression. Cell & Bioscience 1: 42.
11. Buehler D, Hardin H, Shan W, Montemayor-Garcia C, Rush PS, et al. (2013) Expression of epithelial mesenchymal transition regulators SNAI2 nd TWIST1 in thyroid carcinomas. Modern Pathology 1: 54-61.
12. Puxeddu E, Knauf JA, Sartor MA, Mitsutake N, Smith EP, et al. (2005) RET/PTC induced gene expression thyroid PCCL3 cells reveals early activation of genes involved in regulation of the immune response. Endocr Relat Cancer 2: 319-334.
13. Bay Y, Kakudo K, Nakamura M, Ozaki T, Li Y, et al. (2011) Loss of cellular polarity/cohesiveness in the invasive front of papillary thyroid carcinoma, a novel predictor for lymph node metastasis: possible morphological indicator of epithelial mesenchymal trasi-tion. J Clin Pathol 64: 325-329.
14. Romei C, Elisei R, RET/PTC Translocations and Clinico-Pathological Features in Human Papillary Thyroid Carcinoma. Front Endocrinol (Lausanne) 3: 54.
15. Zheng X, Wei S, Han Y, Li Y, Yu Y, et al. (2013) Papillary micro carcinoma of the thyroid: clinical characteristics BRAF (V600E) mutational status of 977 cases. Ann Surg Oncol 20: 2266-2273.
16. Virk RK, Van Dyke AL, Finkelstein A, Prasad A, Gibson J, et al. (2013) BRAFV600E mutation in papillary thyroid micro carcinoma : a genotype-phenotype correlation. Mod Pathol 26: 62-70.
17. Knauf JA, Sartor MA, Madvedovic M, Lundsmith E, Ryder M, et al. (2011) Progression of BRAF-induced thyroid cancer in associated with epithelial-mesenchymal trasition requiring concomitant MAP kinase and TGFβ signalling. Oncogene 30: 3153-3162.
18. Givens DJ, Buchmann LO, Agarwal AM, Grimmer JF, Hunt JP (2014) BRAF V600E does not predict aggressive features of Pediatric Papillary Thyroid Carcinoma. Laryngoscope 124: E389-E393.