Malays J Pathol 2020; 42(3): 415 – 422

ORIGINAL ARTICLE

Expression of oestrogen-α receptor in papillary thyroid carcinoma and its association with metastasis

Siti Atiqah AB HALIM[1,2], Suk Kam LEE[2], Muaatamarulain MUSTANGIN[1], Muhammad Fakhri MOHD SALEH[1], Shamsul Azhar SHAH[3] and Nurismah MD ISA[1]*

[1]Department of Pathology, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia, [2]Department of Pathology, Hospital Pulau Pinang, Malaysia, [3]Department of Community Health Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia.

Abstract

Introduction: Papillary thyroid carcinoma (PTC) is the ninth most common malignancy among women. Although the disease prognosis is good, less favourable outcomes are predicted in those with higher disease stages and nodal metastasis. Oestrogen- α (ER-α) expression has been associated with aggressive presentation and greater disease progression and has been proposed as a predictor for lymph node metastases. The objective of this study was to evaluate the association between ER expression and clinicopathological features i.e. lymph node metastasis, tumour size, extrathyroidal extension, histological variants of PTC , age groups , ethnic and gender. *Methods:* We studied ER-α expression in 84 cases of PTC obtained within an eight-year period (2011-2018) by immunohistochemical technique (IHC). Associations between ER-α expression and clinicopathological features were evaluated using Fisher's exact test. The statistical significance was set at $p < 0.05$. *Results:* ER-α was expressed in 13.1% of all the PTC cases examined (n=11/84). There were no associations observed between ER-α expression and lymph node metastasis (p=1.000), tumour size (p=0.970), extrathyroidal extension (p=0.677), variants of PTC (p=1.000), age groups (p=0.188), gender (p=0.725) or race (p=0.920). *Conclusion:* There was no evidence in this study to support the application of ER-α as prediction marker for lymph node metastasis or disease aggressiveness in PTC. Given that the scope of this study was limited to the protein expression of ER- α, we also propose the inclusion of molecular analysis of ESR1 gene expression, as well as inclusion of detailed clinical and radiological findings in future research investigating the role of ER-α in prognostication of PTC.

Keywords: Papillary thyroid carcinoma, immunohistochemistry, disease progression, prognosis

INTRODUCTION

Papillary thyroid carcinoma (PTC) is the ninth most common malignancy among women and occurs about three times more frequently in women than men.[1,2] The incidence of thyroid cancer tripled over the last 30 years.[3] Although more than 95% of patients diagnosed with PTC are expected to have good outcomes, less favourable outcomes are predicted in those with higher disease stages and those with nodal metastasis.[4] Cervical lymph node metastasis in PTC is very common and is found in 20% to 50% of PTC patients.[5] Nodal metastasis is associated with increased risk of locoregional recurrences (LRR), which affects quality of life and alters disease prognosis.[5–8]

Oestradiol (E2) is the main physiological oestrogen in mammals which mediates physiological response by binding to oestrogen receptors (ER).[9] As there is strong female predilection for thyroid cancers, it is reasonable to postulate an association between E2 and thyroid neoplasms. A review of various in vitro studies on E2 in thyroid cancers showed that there is evidence to suggest a strong relationship between E2 and pathogenesis of thyroid cancers in females.[10] A recent study found that Hsp27, which is a type of heat shock protein (Hsps) that enables cells to survive and recover from stress conditions, can be upregulated by E2.[11,12] This

**Address for correspondence*: Nurismah Md Isa, Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, Cheras 36000 Kuala Lumpur, Malaysia. Tel: +60 19-2687199; Fax: +60 3-91737340 Email: *nuris@ppukm.ukm.edu.my.*

upregulation was shown to facilitate proliferation and confer apoptosis in human papillary thyroid cancer cells.[12]

ER is a hormone nuclear receptor that exists in two forms, namely the oestrogen-α receptor (ER-α) and oestrogen-β receptor (ER-β).[9,13,14] ER-α and ER-β are encoded by oestrogen receptor 1 (ESR1) and oestrogen receptor 2 (ESR2) genes, respectively, and are found in many tissues with variable proportions and distinct distributions.[9,15] Molteni *et al*. first reported an ER-α expression in the human thyroid[16], and subsequent studies have observed its expression in both neoplastic and non-neoplastic human thyroid tissues.[17-21]

ER-α expression in PTC has been associated with aggressive presentation and disease progression and has been proposed as predictor for lymph node metastasis and poor outcomes in various studies.[18,23-26] In contrast to ER-α, various studies have suggested that ER-β has inhibitory effects on the growth and progression of PTC.[27,28] One study reported an association between loss of ER-β expression and PTC recurrence, notably within women of younger age group.[17]

Higher ESR1 expression and higher ESR ratios (ESR1:ESR2) were associated with aggressive prognostic factors.[29] Despite the growing evidence to support ER-α expression association with greater disease progression, Sturniolo *et al*. found a significant association between ER-α expression and remission in PTC.[20]

The objective of this study was to evaluate the association between ER expression and clinicopathological features i.e. lymph node metastasis, tumour size, extrathyroidal extension, histological variants of PTC , age groups , ethnic and gender.

MATERIALS AND METHODS

Patients and tissue specimens

A total of 84 cases of primary PTC diagnosed between 2011 and 2019 at Hospital Pulau Pinang were included in the study. Clinicopathological data were retrieved from the hospital pathology information system, which included age, gender, race, variants, tumour size, extrathyroidal extension and nodal status. Tumour size and age were grouped according to TNM staging 8th edition and American Joint Committee on Cancer (AJCC) prognostic stage grouping respectively.[30,31] The specimens were comprised of 61 total thyroidectomy and 23 hemithyroidectomy specimens. All the slides were examined to select the most representative paraffin tissue blocks of the tumours for ER- α immunostaining. Cases with equivocal features, indefinite diagnoses, or suboptimal tissue blocks were excluded from the study.

Primary antibodies

Monoclonal, rabbit, anti-human, Oestrogen Receptor-α, Ready-To-Use, Clone EP1 (Code M3643, Dako Denmark) breast carcinoma was used as the positive control tissue.

Immunohistochemistry staining method

Immunohistochemistry (IHC) staining was performed on tissue sections using the protocol from EnVision™ FLEX+, Mouse, High pH (Code No. K8012, Dako Denmark). The primary antibody was diluted to an optimal concentration using the Antibody Diluent, Dako REALTM (Code No. S2022, Dako Denmark). Washing steps between each reagent were performed using EnVisionTM FLEX Wash Buffer 20x (Code No. DM831, Dako Denmark) by diluting to a 1X working solution with deionized water. The 1X DAB-containing Substrate Working Solution was prepared by diluting the 50X concentrated EnVisionTM FLEX DAB+ Chromogen (Code No. DM827, Dako Denmark) with EnvisionTM FLEX TM Substrate Buffer (Code No. DM823, Dako Denmark).

Tissue blocks were sectioned approximately 3 μm thickness and mounted on adhesive glass slide, Platinum Pro White (Product No: PRO-01, Matsunami Japan). The slides were left to be air-dried in room temperature overnight. The tissue slides were then incubated on a hot plate at 60ºC for one hour. An initial deparaffinisation and pre-treatment step was performed in the Decloaking Chamber™ NxGen (Ref. No: DC2012-220V, Biocare Medical California) using the EnVision™ FLEX Target Retrieval Solution, High pH (Code No. DM828, Dako Denmark) with the conditions of temperature 110°C and time 30 minutes, followed by cooling at room temperature for 30 minutes and rinsing with running tap water for three minutes. The slides were subsequently incubated with EnVisionTM FLEX Peroxidase-Blocking Reagent (Code No. DM821, Dako Denmark) for five minutes followed by a washing step.

Slides were then incubated with the primary antibody for 30 minutes at room temperature, followed by incubation with EnVisionTM FLEX/HRP (Code No. DM822, Dako Denmark) for 30 minutes. Sections were then incubated with 1X DAB-containing Substrate Working

Solution for five minutes. The slides were then counterstained with Hematoxylin 2 (REF 7231, ThermoScientific USA) for five seconds after the procedures had been completed, followed by dehydration with increasing alcohol solutions (80%, 90%, 100% and 100%) and 2-times Xylene. Finally, the slides were mounted using CoverSealTM-X xylene-based mounting medium (Cat. No.: FX2176, Cancer Diagnostics, USA).

Evaluation of immunostaining

Each ER-α stained slide was evaluated for the proportion of positive cells (scored 0-5) and the intensity of staining (scored 0-3).[32] The scoring / assessment for ER-α expression is based on Allred scoring, as used by previously published research.[19,32,33] Both the proportion and intensity score were summed up to get the final Allred scores, which were used to interpret the results.[32] Final Allred scores of three and above were considered positive. Details of the scoring system are summarised in Table 1.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 24 was used to analyse the data. The distribution of variables (clinicopathologic characteristics) was studied by descriptive statistics. Fisher's exact test was used to study the association between ER-α expression and clinicopathological characteristics. The statistical significance was set at $p < 0.05$.

RESULTS

Clinicopathological features

Table 2 summarises the clinicopathological characteristics in all PTC cases studied (n=84). There is an obvious female predominance in PTC, with a female to male ratio of 2:1. The patients' age ranged from 21 to 85 years old, with a mean age of 50 years. The number of patients that fall into the two age-group (<55 years old and ⩾ 55 years old) categories were almost equally distributed. There were 45 (53.6%) cases of classical type, which made up the most prevalent form of PTC. Tumour size ranged from 2 mm to 140 mm in diameter, with a median of 25 mm. There were 46 (54.8%) cases reported without lymph node excision, which were excluded from the analysis of nodal metastasis. A total of 31 out of 38 cases (81.6%), with histological examination of lymph nodes, showed evidence of metastasis. None of the cases had any evidence of distant metastasis.

ER-α expression in PTC and its association with clinicopathological features

A total of 11 (13.1%) out of 84 PTC cases studied expressed ER-α as depicted in Figure 1. Overall, there is no association between ER-α expression and lymph node metastasis and the other clinicopathological characteristics. Associations between ER-α expression in PTC and clinicopathological features are as illustrated in Table 3.

DISCUSSION

PTC is more prevalent in women[1,34], leading to various studies that have looked into oestrogen and oestrogen receptors in papillary thyroid carcinomas to understand its role in disease pathogenesis.[22,35] In recent years, researchers have employed molecular techniques to understand the role of oestrogen in pathogenesis of PTC by quantification of ESR1 and ESR2 gene expression, or measuring ER-α mRNA through PCR or Western blot technique.[26,29] Nevertheless, the immunohistochemistry method was still used in recent studies of ER-α expression

TABLE 1: Allred score for evaluation of ER-α immunohistochemistry[32]

Proportion score (PS)			**Intensity score (IS)**		**Final score (PS+IS)**	
Score	**Positive cells (%)**		**Score**	**Staining intensity**	**IHC score**	**Interpretation ER- α status**
0	No staining	(0%)	0	No staining	0 - 2	Negative
1	<1/100	(<1%)	1	Weak	3 - 8	Positive
2	1/100 –1/10	(1% - 10%)	2	Intermediate		
3	1/10 – 1/3	(10% - 33%)	3	Strong		
4	1/3 – 2/3	(33% - 66%)				
5	>2/3	(>66%)				

TABLE 2: Clinicopathological details of all the cases studied

Clinicopathological details	PTC (n=84)	
	No.	%
Gender		
Male	25	29.8
Female	59	70.2
Age group (years)		
< 55	49	58.3
$\geqslant 55$	35	41.7
Race		
Malay	37	44.0
Chinese	30	35.7
Indian	11	13.1
Others	6	7.1
Variants		
Classical	45	53.6
Follicular	19	22.6
Microcarcinoma	20	23.8
Tumour size (mm)		
$\leqslant 10$	20	23.8
11 – 20	19	22.6
21 – 40	33	39.3
> 40	12	14.3
Extrathyroidal extension		
Yes	13	15.5
No	71	84.5
Lymph node metastasis		
Yes	31	81.6
No	7	18.4

and PTC.[18,20,21,24] In this study, we found that ER-α was expressed in 13.1% of all PTC cases examined (n=11/84). This is comparable to other studies which reported ER-α expression in PTC ranging from 9.9% to 66.5%.[17-21] The wide range of ER-α expression observed in different studies are most likely due to the nature of the study, i.e. retrospective vs prospective, different IHC staining procedures and scoring system employed and also inter-observer variability. We have also noted that the immunoreactivity for ER-α is observed only in cases where the tissue blocks have been stored for less than three years. We hypothesised that the actual percentage of ER-α expression in the cases studied is higher than what was observed. The loss of antigenicity in some of the archival tissue blocks leading to false negative immunostaining were possible due to the retrospective nature of this study.[8]

ER-α expression has been proposed as a marker for predicting lymph node metastasis and poor outcome in PTCs, because of its association with more aggressive disease progression and nodal metastasis.[18,23-25] However, in our study, we did not find any significant association between ER-α expression, lymph node metastasis and clinicopathological features, which implied greater disease progression, i.e. tumour size and extrathyroidal extension. In some studies, tumour size was associated with ER-α expression.[18,20,23,26] Vannucchi *et al*. found that ER-α expression is significantly correlated with tumour size and was frequently expressed in clinically evident tumours as compared to incidental cases.[18] Nevertheless, these findings are not universal, as there are still various studies, including this study, that did not find any association between ER-α expression and tumour size.[17,37] A number of studies also did not find any association between ER-α expression and lymph node metastasis and extrathyroidal extension.[18,20,37] Although there is no statistically significant

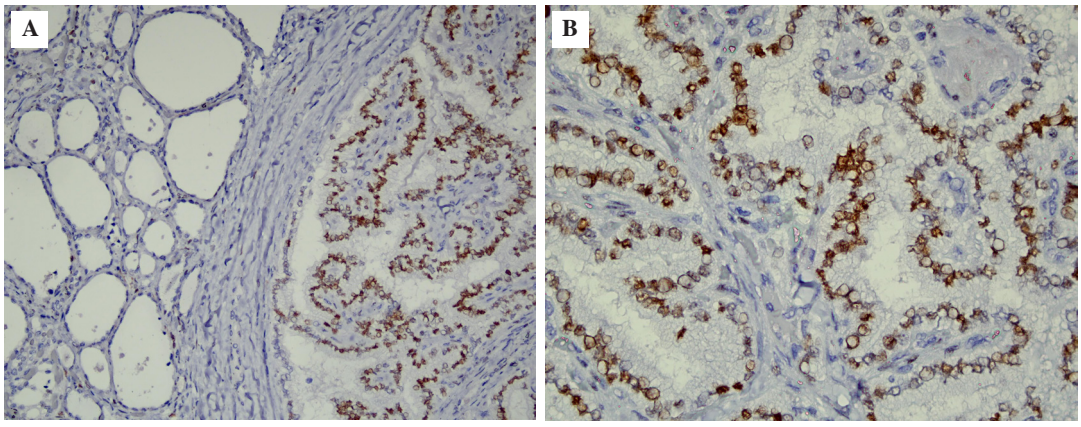

FIG. 1: Expression of ER-α in PTC by IHC. (A) The neoplastic cells of the classical PTC (on the right) show reactivity for ER-α. The non-neoplastic follicles on the left do not express ER-α (x100). (B) These malignant cells demonstrate strong nuclear reactivity for ER-α (x400).

TABLE 3: Associations between ER-α expression in PTC and its clinicopathological characteristics

Variables	ER-α status				p-value
	Positive (n=11)		Negative (n=73)		
	No.	%	No	%	
Gender					0.725
Male	4	16.0	21	84.0	
Female	7	11.9	52	88.1	
Age group (years)					0.188
< 55	4	8.2	45	91.8	
⩾ 55	7	20.0	28	80.0	
Race					
Malay	5	13.5	32	86.5	0.920
Chinese	5	16.7	25	83.3	
Indian	1	9.1	10	90.9	
Others	0	0.0	6	100.0	
Variants					1.000
Classical	6	13.3	39	86.7	
Follicular	2	10.5	17	89.5	
Microcarcinoma	3	15.0	17	85.0	
Tumour size (mm)					0.970
⩽ 10	3	15.0	17	85.0	
11 – 20	3	15.8	16	84.2	
21 – 40	4	12.1	29	87.9	
> 40	1	9.1	11	91.7	
Extrathyroidal extension					0.677
Yes	2	15.4	11	84.6	
No	9	12.7	62	87.3	
Lymph node metastasis					1.000
Yes	5	16.1	26	83.9	
No	1	14.3	6	85.7	

association between ER-α expression and the clinicopathological parameters that indicate disease aggressiveness, following up with these patients would provide an additional insight into their disease progression. Sturniolo *et al*. found that tumours with ER-α expression are significantly associated with disease remission based on the follow-up data.[20] This association was not reflected in their statistical analysis of the correlation between clinicopathological features and ER-α expression.[20]

In general, PTC shows a consistent and strong female predilection.[1,2] There are studies that showed increased ER-α expressions in female patients, which implied the role of E2 in pathogenesis of PTC, but separate studies failed to show similar results.[18,20,23,37] In our study, although the female to male ratio is 2:1, the ER-α expression in female and male patients was not significantly different.

Kilfoy *et al*. reported that PTC incidence rates rise sharply at the beginning of the reproductive years, and the age-specific female-to-male rate ratios did not show to be significantly different around menopausal years.[34] Yet, a number of studies found no significant difference in ER-α expression among younger and older age groups, and some looked at pre- and post-menopausal groups with no significant difference noted.[18,20,23,25,26,37] Tang *et al*. studied the expression of the G protein-coupled oestrogen receptor 1 (GPER1), which binds to oestrogen and functions alongside oestrogen receptors (ER-α and ER-β).[36] In that particular study, no significant association was found between GPER1 expression and age, gender and subgroup.[36] Similarly, in our study, there was no significant difference in ER-α expression between older patients (55 years old) and younger patients (<55 years old).

There are six histopathological variants of PTC listed in the WHO classification of tumours of the thyroid glands (8th edition), which include the classical papillary carcinoma, follicular variant, encapsulated variant, papillary microcarcinoma, columnar cell variant and oncocytic variant.[30] In the present study, conventional PTC made up the majority of the cases (53.6%), followed by papillary microcarcinoma (23.8%) and follicular variant (22.6%). No other variants were observed. Although the majority of the cases that express ER-α were classical PTC, there were no associations noted between ER-α expression and different variants of PTC. This finding is similar to a few studies that did not find any association between ER-α expression or GPER1 expression and histologic subtypes.[20,36]

The majority of the patients in this research were of Malay ethnicity (44.0%), followed by Chinese (35.7%), Indian (13.1%) and other ethnic groups (7.1%). Other research within a similar local setting also showed relatively similar ethnic distributions of patients diagnosed with PTC, although the sample size is smaller.[38] The multiracial nature of the studied population allowed us to examine differences of ER-α expressions across different ethnic backgrounds. It is worth noting that to the best of our knowledge, ER-α expressions in PTC across different ethnic groups were not investigated in previous studies.[18,20,23,37] Our study demonstrates that the expressions of ER-α are almost equally distributed across the ethnic groups (p=0.920). Although our data suggests that ethnicity variation does not influence ER-α expression in PTC, others have shown evidence of racial influences to the overall survival in PTC, and its incidence.[39,40]

Deli *et al*. suggested that the receptor expression is insufficient to predict the effect of oestrogen and progesterone on tumour cells.[41] The lack of evidence to support the role of ER-α expression to predict poor prognosis in PTC is probably due to its complex interplay with other receptors and proteins, such as CXCR1 (chemokine receptors), epidermal growth factor receptor (EGFR), GPER1 and most recently pescadillo ribosomal biogenesis factor 1 (PES1).[21,36,42] PES1, a protein which contains breast cancer–associated gene 1 (BRCA1) C-terminal (BRCT) domains has been shown to modulate ER-α and ER-β protein ratios in PTC.[21]

In conclusion, there is no evidence in this study to support the application of ER-α as prediction marker for lymph node metastasis or disease aggressiveness in PTC. However, given that the scope of this study was limited to the protein expression of ER- α, we propose the integration of molecular analysis of ESR1 gene expression, as well as inclusion of detailed clinical and radiological findings in future research investigating the role of ER-α in prognostication of PTC.

Acknowledgement: We would like to thank the Director General of Health Malaysia for his permission to publish this article. The authors declare no conflict of interest. This research was supported by UKMMC Fundamental Research

Fund (FF-2018-02) and was approved by the Medical Research and Ethics Committee, Ministry of Health Malaysia (NMRR-17-3416-39178).

Authors' contribution: SAAH: Preparation of proposal, data collection and analysis, preparation of manuscript. SKL: Editing and supervising. MM and MFMS: Technical support SAS: Statistical analysis. NMI: Overall planning and budget procurement, supervising, editing and final approval.

Conflicts of interest: The authors have no conflicts of interest to declare.

REFERENCES

1. Pellegriti G, Frasca F, Regalbuto C, Squatrito S, Vigneri R. Worldwide Increasing Incidence of Thyroid Cancer: Update on Epidemiology and Risk Factors. J Cancer Epidemiol. 2013; 2013: 1-10.
2. Kilfoy BA, Devesa SS, Ward MH, *et al*. Gender is an Age-Specific Effect Modifier for Papillary Cancers of the Thyroid Gland. Cancer Epidemiol Biomarkers Prev. 2009; 18(4): 1092-100.
3. Lubitz CC, Sosa JA. The changing landscape of papillary thyroid cancer: Epidemiology, management, and the implications for patients. Cancer. 2016; 122(24): 3754-9.
4. Vollmer RT. Defining Papillary Carcinoma of the Thyroid. Am J Clin Pathol. 2017; 148(2): 100-7.
5. Sellers M, Beenken S, Blankenship A, *et al*. Prognostic significance of cervical lymph node metastases in differentiated thyroid cancer. Am J Surg. 1992; 164(6): 578-81.
6. Park JY, Koo BS. Individualized optimal surgical extent of the lateral neck in papillary thyroid cancer with lateral cervical metastasis. Eur Arch Oto-Rhino-Laryngology 2014; 271(6): 1355-60.
7. Kim SK, Park I, Hur N, Choe JH, Kim JH, Kim JS. Patterns, predictive factors and prognostic impact of multilevel metastasis in N1b papillary thyroid carcinoma. Br J Surg. 2017; 104(7): 857-67.
8. Fergenbaum JH, Garcia-Closas M, Hewitt SM, Lissowska J, Sakoda LC, Sherman ME. Loss of antigenicity in stored sections of breast cancer tissue microarrays. Cancer Epidemiol Biomarkers Prev. 2004; 13(4): 667-72.
9. Burns KA, Korach KS. Estrogen receptors and human disease: an update. Arch Toxicol. 2012; 86(10): 1491-504.
10. Derwahl M, Nicula D. Estrogen and its role in thyroid cancer. Endocr Relat Cancer. 2014 ;21(5): T273-83.
11. Ciocca DR, Oesterreich S, Chamness GC, McGuire WL, Fuqua SA. Biological and clinical implications of heat shock protein 27,000 (Hsp27): a review. J Natl Cancer Inst. 1993; 85(19): 1558-70.
12. Mo XM, Li L, Zhu P, *et al*. Up-regulation of Hsp27 by ERα/Sp1 facilitates proliferation and confers resistance to apoptosis in human papillary thyroid cancer cells. Mol Cell Endocrinol. 2016; 431: 71-87.
13. Baker ME. What are the physiological estrogens? Steroids. 2013; 78(3): 337-40.
14. Deroo BJ. Estrogen receptors and human disease. J Clin Invest. 2006; 116(3): 561-70.
15. Dahlman-Wright K, Cavailles V, Fuqua SA, *et al*. International Union of Pharmacology. LXIV. Estrogen Receptors. Pharmacol Rev. 2006; 58(4): 773-81.
16. Molteni A. Estradiol Receptor-binding Protein in Head and Neck Neoplastic and Normal Tissue. Arch Surg. 1981; 116(2): 207.
17. Ahn HY, Kim MS, Kim MJ, *et al*. Loss of ERβ expression in papillary thyroid carcinoma is associated with recurrence in young female. Clin Endocrinol (Oxf). 2015; 82(2): 300-6.
18. Vannucchi G, De Leo S, Perrino M, *et al*. Impact of estrogen and progesterone receptor expression on the clinical and molecular features of papillary thyroid cancer. Eur J Endocrinol. 2015; 173(1): 29-36.
19. Huang Y, Dong W, Li J, Zhang H, Shan Z, Teng W. Differential expression patterns and clinical significance of estrogen receptor-α and β in papillary thyroid carcinoma. BMC Cancer. 2014; 14: 383.
20. Sturniolo G, Zafon C, Moleti M, Castellví J, Vermiglio F, Mesa J. Immunohistochemical Expression of Estrogen Receptor-α and Progesterone Receptor in Patients with Papillary Thyroid Cancer. Eur Thyroid J. 2016; 5(4): 224-30.
21. Qiu Y-B, Liao L-Y, Jiang R, *et al*. PES1 promotes the occurrence and development of papillary thyroid cancer by upregulating the ERα/ERβ protein ratio. Sci Rep. 2019; 9(1): 1032.
22. Santin AP, Furlanetto TW. Role of estrogen in thyroid function and growth regulation. J Thyroid Res. 2011; 2011: 875125.
23. Ahmed RA, Aboelnaga EM. Thyroid Cancer in Egypt: Histopathological Criteria, Correlation With Survival and Oestrogen Receptor Protein Expression. Pathol Oncol Res. 2015; 21(3): 793-802.
24. Eldien MMS, Abdou AG, Rageh T, Abdelrazek E, Elkholy E. Immunohistochemical expression of ER-α and PR in papillary thyroid carcinoma. Ecancermedicalscience. 2017; 11: 748.
25. Rubio GA, Catanuto P, Glassberg MK, Lew JI, Elliot SJ. Estrogen receptor subtype expression and regulation is altered in papillary thyroid cancer after menopause. Surg (United States). 2018; 163(1): 143-9.
26. Di Vito M, De Santis E, Perrone GA, *et al*. Overexpression of estrogen receptor-α in human papillary thyroid carcinomas studied by laser-capture microdissection and molecular biology. Cancer Sci. 2011; 102(10): 1921-7.
27. Dong W, Li J, Huang Y, Zhang H, Shan Z, Teng W. Differential expression patterns of estrogen receptor (ER)-β splice variants between papillary thyroid cancer and nodular thyroid goiter. Med Sci Monit. 2012; 18(9): BR351-5.
28. Magri F, Capelli V, Rotondi M, *et al*. Expression of estrogen and androgen receptors in differentiated

thyroid cancer: an additional criterion to assess the patient's risk. Endocr Relat Cancer. 2012; 19(4): 463-71.
29. Yi JW, Kim S jin, Kim JK, *et al*. Upregulation of the ESR1 Gene and ESR Ratio (ESR1/ESR2) is Associated with a Worse Prognosis in Papillary Thyroid Carcinoma: The Impact of the Estrogen Receptor α/β Expression on Clinical Outcomes in Papillary Thyroid Carcinoma Patients. Ann Surg Oncol. 2017; 24(12): 3754-62.
30. Llyod R, Osamura R, Klöppel G, Rosai J. WHO Classification of Tumours of Endocrine Organs 4th Edition. 4th ed. World Health Organization; 2017.
31. Amin M, Edge S, Greene F, *et al*. AJCC Cancer Staging Manual. 8th edition. Springer International Publishing; 2017.
32. Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol. 1998; 11(2): 155-68.
33. Chen D, Qi W, Zhang P, Guan H, Wang L. Expression of the estrogen receptor α, progesterone receptor and epidermal growth factor receptor in papillary thyroid carcinoma tissues. Oncol Lett. 2015; 10(1): 317-20.
34. Rahbari R, Zhang L, Kebebew E. Thyroid cancer gender disparity. Futur Oncol. 2010; 6(11): 1771-9.
35. Dong W, Zhang H, Li J, *et al*. Estrogen induces metastatic potential of papillary thyroid cancer cells through estrogen receptor α and β. Int J Endocrinol. 2013; 2013: 941568.
36. Tang C, Yang L, Wang N, *et al*. High expression of GPER1, EGFR and CXCR1 is associated with lymph node metastasis in papillary thyroid carcinoma. Int J Clin Exp Pathol. 2014; 7(6): 3213-23.
37. Jalali-Nadoushan M-R, Amirtouri R, Davati A, Askari S, Siadati S. Expression of estrogen and progesterone receptors in papillary thyroid carcinoma. Casp J Intern Med. 2016; 7(3): 183-7.
38. Muthusamy S, Azhar Shah S, Abdullah Suhaimi SN, *et al*. CD56 expression in benign and malignant thyroid lesions. Malays J Pathol. 2018; 40(2): 111-9.
39. Tang J, Kong D, Cui Q, *et al*. Racial disparities of differentiated thyroid carcinoma: clinical behavior, treatments, and long-term outcomes. World J Surg Oncol. 2018; 16(1): 45.
40. Horn-Ross PL, McClure LA, Chang ET, *et al*. Papillary thyroid cancer incidence rates vary significantly by birthplace in Asian American women. Cancer Causes Control. 2011; 22(3): 479-85.
41. Deli T, Orosz M, Jakab A. Hormone Replacement Therapy in Cancer Survivors - Review of the Literature. Pathol Oncol Res. 2019; 26(1): 63-78.
42. Coperchini F, Croce L, Marinò M, Chiovato L, Rotondi M. Role of chemokine receptors in thyroid cancer and immunotherapy. Endocr Relat Cancer. 2019; 26(8): R465-78.