

# AN IMMUNOHISTOCHEMICAL STUDY OF THE RELATIONSHIP BETWEEN ESTROGEN RECEPTOR ALPHA (ER $\alpha$ ) AND ESTROGEN RECEPTOR BETA (ER $\beta$ ) IN PAPILLARY THYROID CARCINOMA (PTC) IN GENERAL HOSPITAL HAJI ADAM MALIK MEDAN

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DOI: 10.29322/IJSRP.11.06.2021.p11448  
<http://dx.doi.org/10.29322/IJSRP.11.06.2021.p11448>

**Abstract:** Thyroid carcinoma is malignancy in thyroid gland showing differentiation of epithelial cells, namely follicular or parafollicular cells. Papillary thyroid carcinoma (PTC) is a thyroid carcinoma that is most often found in about 80% of all thyroid carcinomas. ER $\alpha$  contributes to tumor growth primarily by stimulating aberrant cell proliferation and inhibiting cell apoptosis, whereas ER $\beta$  suppresses tumorigenesis through cell proliferation, maintains cell differentiation and induces apoptosis. Based on contradictory nature of ER $\alpha$  and ER $\beta$  contradictory and several studies linking ER expression with PTC have conflicting results, the researchers are interested in assessing the relationship between ER $\alpha$  and ER $\beta$  immunohistochemical expression in PTC. Objective: To analyse the relationship between ER $\alpha$  and ER $\beta$  immunohistochemical expression in PTC. Materials and Methods: A cross-sectional study was performed using formalin-fixed tissue paraffin blocks from 32 PTC patients. Each PTC case was stained with ER $\alpha$  and ER $\beta$  immunohistochemistry. Then, the relationship between ER $\alpha$  and ER $\beta$  expression in PTC was assessed and analyzed with statistical software by using the chi-square and Mann-Whitney U test. Results and Discussion: In this study found that there is a relationship statistically significant between the expression of ER $\alpha$  with ER $\beta$  in PTC patients with p value of 0.004. ER $\alpha$  expression seemed to walk in line with ER $\beta$ , for example, when a positive ER $\alpha$  ER $\beta$  then more likely to be positive, and vice versa. Qiu et al. the research also found that there is a relationship between the expression of ER $\alpha$  with ER $\beta$  in patients with PTC with p value <0.001 and r -0.293. This indicates that the relationship between the expression ER $\alpha$  and ER $\beta$  is inversely related. The results of the study by Qiu et al. are incompatible with this study. Based on theory, the expression of ER $\alpha$  and ER $\beta$  should be inversely proportional because ER $\alpha$  contributes to tumor growth while ER $\beta$  suppresses tumorigenesis. Conclusion: There is a statistically significant relationship between ER $\alpha$  and ER $\beta$  expression in PTC patients (p value of 0.004). It seems that the relationship between the two expressions is parallel.

**Keywords:** Papillary thyroid carcinoma, ER $\alpha$ , ER $\beta$

## I. INTRODUCTION

Thyroid carcinoma is a malignancy in thyroid gland showing differentiation of epithelial cells, namely follicular or parafollicular cells.<sup>1</sup> Based on Global Burden of Cancer (GLOBOCAN) data in 2018, the incidence of this disease ranked in ninth place of all carcinoma around the world, which accounts for 567,000 cases.<sup>2,3</sup> From Registration of Indonesia Association of Anatomical Pathology Specialists were obtained that thyroid carcinoma ranked in 9<sup>th</sup> out of 10 malignancies.<sup>4,5</sup> In previous research, the researchers found about 25 PTC cases in General Hospital Haji Adam Malik Medan in 2018.

Based on World Health Organization/ WHO 2017, papillary thyroid carcinoma (PTC) is included one of the thyroid malignancy.<sup>5,7</sup> PTC is the most commonly found thyroid cancer which is about 80% of all thyroid malignancy.<sup>1,8-10</sup> The main cause of this disease is exposure to ionizing radiation, especially during childhood.<sup>1</sup> The incidence rate of thyroid carcinoma in women is three times higher than men, dan the peak of the incident is earlier in women.<sup>2,3,11</sup> This epidemiological data gives impression that there is a role for estrogen in pathogenesis of thyroid diseases.<sup>11</sup>

Signaling cellular of estrogen is simply mediated by the binding of two intracellular nucleus receptor, which are estrogen receptor (ER) alpha and ER beta.<sup>11,12</sup>  $\beta$  isoform is smaller than  $\alpha$ . The presence of this ER is very important so that estrogen can work directly in certain cells. ER has been studied in neoplastic and nonneoplastic human thyroid tissue, but the results were conflicting. It has been stated that there is important role of different distribution and ER subtype expression pattern in thyroid carcinoma.<sup>11</sup> The binding of estrogen to ER $\alpha$  will promote cell proliferation and antiapoptosis, meanwhile ER $\beta$  will support pro-apoptosis activity, other suppressive function and differentiative effects.<sup>9,11,13-15</sup>

Based on contradictory nature of ER $\alpha$  and ER $\beta$  and PTC ranked in 9<sup>th</sup> in Indonesia, then researchers were interested in assessing the relationship between ER $\alpha$  and ER $\beta$  immunohistochemical expression in PTC post thyroidectomy tissues, by staining slides with immunohistochemistry. This study aimed to analyze the relationship between the characteristics of PTC and ER $\alpha$  and ER $\beta$  immunohistochemical expression and also relationship between ER $\alpha$  and ER $\beta$  immunohistochemical expression in PTC.

## II. MATERIAL AND METHODS

### Sample selection

This analytic research with cross-sectional approach was conducted in Department of Anatomic Pathology, Faculty of Medicine, Universitas Sumatera Utara and Anatomic Pathology Unit, General Hospital Haji Adam Malik Medan. This study was done from May 2020 until May 2021, after receiving approval from Health Research Ethics Committee, Medical Faculty, Universitas Sumatera Utara.

These study samples were slides and paraffin blocks from postoperative tissues histopathologically diagnosed as PTC fulfilling inclusion and exclusion criteria. Samples were gathered by using consecutive sampling technique. The inclusion criteria were all adequate and representative slides and paraffin blocks from postoperative tissues diagnosed as PTC after stained with hematoxylin-eosin as well as having medical record data such as age/ date of birth, gender, and tumour size (macroscopically). Exclusion criteria for this study was diminished or damaged paraffin blocks which can't be cut back. Each sample was stained with hematoxylin-eosin and immunohistochemistry ER alpha polyclonal antibody and ER beta polyclonal antibody (Elabscience, with dilution 1:100).

ER $\alpha$  and ER $\beta$  expression were interpreted using Allred scoring system. Interpretation was done by adding proportion score and staining intensity. Proportion score was done as follows: score 0 = 0, score 1 (<1% of positive stained nuclei and/or cytoplasm cells), score 2 (1 - 10% of positive stained cells), score 3 (11 - 33% of positive stained cells), score 4 (33-66% of positive stained cells), and score 5 ( $\geq$  67% of positive stained cells). Meanwhile, staining intensity was scored as follows: 0 (none), 1 (weak intensity), 2 (moderate), 3 (strong). After that, the total score of ER $\alpha$  and ER $\beta$  immunohistochemical expression was categorized as negative (if total score 0-2) and positive (total score 3-8).<sup>12</sup>

### Data analysis

Data collected in this research were processed by using statistical software and presented in tables. Statistical tests used in this study were chi-square or Mann-Whitney U test.

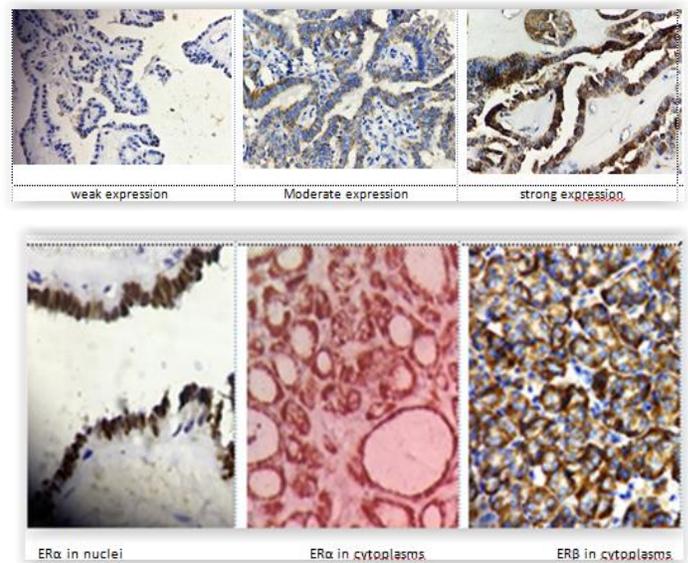


Figure 1. ER $\alpha$  and ER $\beta$  immunohistochemical expression.

## III. RESULTS

In this study, 32 samples with PTC diagnosis in Department of Anatomic Pathology, Faculty of Medicine, Universitas Sumatera Utara and Anatomic Pathology Unit, General Hospital Haji Adam Malik Medan were obtained. In this study, analysis about the relationships between the characteristics of PTC samples and each ER $\alpha$  and ER $\beta$  was done. Statistical tests showed that there were no significant relationships between age, gender, and tumour size with ER $\alpha$  and ER $\beta$  in PTC patients (Table 1 and 2).

Besides that, analysis about the relationship between ER $\alpha$  and ER $\beta$  immunohistochemical expression in PTC was also done (Table 3). From chi square test analysis, the researchers found that p value =0.004, indicating that there was a significant relationship between ER $\alpha$  and ER $\beta$  expression in PTC samples. We also concluded that it seems that the relationship between two expression is in line. It meant that if ER $\alpha$  positive, then ER $\beta$  would also be positive.

**Table 1 The relationships between the characteristics of PTC samples and ER $\alpha$**

Variables	Expression ER $\alpha$				p-value
	Positive		Negative (%)		
	N	%	n	%	
Age (years old)					
• $\leq$ 20	1	50	1	50	0.100**
• 21-30	2	40	3	60	
• 31-40	0	0	4	100	
• 41-50	3	42.9	4	57.1	
• 51-60	9	100	0	0	
• 61-70	2	40	3	60	
Gender					
• Men	7	63.6	4	36.4	0.388*
• Women	10	47.6	11	57.3	
Tumour size					
• <2cm	2	100	0	0	0.416**
• 2-4cm	1	50	1	50	
• >4cm	14	50	14	50	

NB: \* Chi Square test  
\*\* Mann-Whitney U test

**Table 2 The relationships between the characteristics of PTC samples and ERβ**

Variables	Expression ERβ				p-value
	Positive		Negative (%)		
	N	%	n	%	
Age (years old)					0.217**
• ≤20	1	50	1	50	
• 21-30	2	40	3	60	
• 31-40	1	25	3	75	
• 41-50	1	14.3	6	85.7	
• 51-60	8	88.9	1	11.1	
• 61-70	2	40	3	60	
Gender					0.529*
• Men	6	54.6	5	45.5	
• Women	9	42.9	12	57.1	
Tumour size					0.291**
• <2cm	2	100	0	0	
• 2-4cm	1	50.0	1	50	
• >4cm	12	42.9	16	57.1	

NB: \* Chi Square test  
\*\* Mann-Whitney U test

**Table 3 The relationships between the ERα and ERβ immunohistochemical expression in PTC**

ERα	ERβ				P-value*
	Positive		Negative		
	n	%	n	%	
Positive	12	70.6	5	29.4	0.004
Negative	3	20.0	12	80.0	
<b>Total</b>	<b>15</b>	<b>46.9</b>	<b>17</b>	<b>53.1</b>	

NB: \* Chi square test

#### IV. DISCUSSION

Both ER subtypes function contradicting each other. ERα contributes to tumour growth particularly by stimulating aberrant cell proliferation and inhibiting cell apoptosis.<sup>12</sup> ERβ suppresses tumorigenesis by cell proliferation, maintaining cell differentiation and inducing apoptosis.<sup>12,13</sup> There were few previous studies about ER in neoplastic and nonneoplastic human thyroid tissues, but the results were conflicting.<sup>11</sup> ERα expression in PTC with positive percentage ranged from 9.9% to 66.5%.<sup>14-17</sup> Some researchers found ERα upregulation in PTC by using immunohistochemistry such as Vannuchi et al, Dai et al and Huang et al.<sup>15-17</sup> However, ERβ expression was more often found with positive percentage ranging from 44.4% to 97.5%.<sup>16,18,19</sup> In this study, some of PTC samples stained with positive ERα (53.1%) and positive ERβ (46.9%).

Huang et al revealed that ER subtypes has three expression pattern in PTC lesion, which combination of nuclei and cytoplasm, only nuclei or only cytoplasm localization.<sup>16</sup> This study found that of 17 samples with positive ERα, there were 16 samples stained in cytoplasm (94.1%) and only one in nuclei (5.9%). All ERβ were stained positive in cytoplasm (100%). This study results was much different from Huang et al. In their researches, they proved that localization of ERα is absolutely different between PTC (extranuclei expression 26.7%) and nodular thyroid goiter (NTG) (don't have extranuclei expression) (p value 0.000). In reproductive women with PTC, extrathyroidal extension was more often found in nuclei ERα expression than extranuclei. Besides that, ERβ1 expression levels in this study was markedly lower in PTC than NTG. Extranuclei ERβ1 expression was also apparently more often seen in women PTC

patients than in NTG control group (17.9%) in reproductive age (p value=0.000).<sup>16</sup> This study is similar with Ceresini et al, who also found that ERβ was more expressed in cytoplasm than in nuclei in PTC.<sup>20</sup> Whereas, Dong et al has found more positive ERβ1 expression in nuclei and cytoplasm (65.7%). Positive expression in cytoplasm were only found about 14.3%.<sup>21</sup>

The presence of these ERs is a fundamental principle so that estrogen hormone can work directly in certain cells, which is translocating from cytoplasm to nuclei after activated by hormone. Membrane ER may be present as cytoplasmic pool which is tethered to inner bilayer plasma membrane through palmitoylation activity and binding with protein, such as caveolin-1. After binding in membrane, ERα and ERβ trigger some extranuclear response. After activating signaling G protein, estrogen also induces rapid kinase signaling through ERα located in membrane. This binding promotes the activation of growth factor receptors by releasing ligand of growth factor receptors mediated by MMP. Specific growth factor receptors that have been proven to be activated were epidermal growth factor receptor (EGFR) and insulin-like growth factor receptor (IGFR). After activated, these growth factor receptors trigger the activation of Akt, ERK, and other kinase signals. Besides that, the activation of PI3K and MAPK promote the phosphorylation of coregulator, and maybe directly phosphorylation of ERα, thus leading to increase protein transcription and synthesis mediated by ERα.<sup>22</sup> Non-genomic signaling by ERβ may be needed for its tumour suppressor effect and this effect might involve the interaction with and activation of p38 MAPK in plasma membrane. This effect is different from ERα that mainly activating ERK and PI3K-AKT through rapid non-genomic signaling.<sup>23</sup>

Just like in breast carcinoma, estradiol and cholesterol metabolites (27 HC) may induce nongenomic and genomic ERα activity seen from immunohistochemical staining seen in cytoplasm and nuclei cell. Nongenomic signaling can modulate nuclei transcription through many pathways, including nuclear localization from classical nuclear receptor or alteration in phosphorylation of important molecular nuclear signaling.<sup>22</sup>

This study obtained that there was no significant relationship between age and ERα and ERβ expression in PTC (respectively p=0.100 and p=0.217 (Table 1 and 2)). This study supported researches from Eldien et al, Sturniolo et al, Jalali-Nadoushan et al, Mishra et al, and Qiu et al.<sup>14,24-27</sup> Eldien et al, Sturniolo et al and Jalali-Nadoushan et al together didn't find any significant relationship between age and ERα expression in PTC.<sup>14,24,27</sup> Mishra et al didn't reveal any significant relationship between age and ERβ expression in PTC (p value 0.371).<sup>25</sup> Meanwhile, Qiu et al didn't find any difference in ERβ expression in age <45 years old and ≥45 years old in PTC.<sup>26</sup>

From this study, the researchers also found that both ERα and ERβ were more expressed positive in men than in women. However, after analysis with chi-square test was done, we found that there was no significant relationship between gender and ERα and ERβ expression in PTC (respectively p value= 0.388 and 0.529) (Table 1 and 2). This research is in accordance with the one done by Eldien et al, Sturniolo et al, Mishra et al, and Qiu et al.<sup>14,24-26</sup> Both Mishra et al and Qiu et al in their study didn't reveal any significant relationship between gender and ERβ expression in PTC (p value> 0.05).<sup>25,26</sup> Eldien et al and

Sturniolo et al also revealed that ER $\alpha$  expression in PTC was similar in both gender (p value>0.05).<sup>14,24</sup> Meanwhile, Jalali-Nadoushan *et al* found that there was significant relationship between gender and ER $\alpha$  expression in PTC (p=0.014).<sup>27</sup>

From this study, there was no significant relationship between tumour size and ER $\alpha$  and ER $\beta$  expression in PTC (respectively p value 0.416 and 0.291) (Table 1 and 2). This study is in line with Huang et al and Mishra et al.<sup>16,25</sup> Huang et al discovered that if compared tumour size in reproductive or advanced aged PTC women ( $\leq 20$ mm vs  $>20$ mm), no significant difference in positive percentages or both ER subtypes expression level was found.<sup>16</sup> Mishra et al didn't discover any significant relationship between tumour size and ER $\beta$  expression in PTC.<sup>25</sup> Whereas, this study was in contrary with Sturniolo et al and Qiu et al.<sup>24,26</sup> Sturniolo et al found that ER $\alpha$  expression was significant correlated with tumour size (p=0.02).<sup>24</sup> Qiu et al discovered that there was significant relationship between tumour size and ER $\beta$  expression.<sup>26</sup>

Many variations in immunohistochemistry results may be occurred due to diversity in methodology, the use of antibodies to estrogen with different sensitivity and specificity, or diversification in the use of criteria for interpreting immunohistochemical ER $\alpha$  and ER $\beta$  expression results.

Beside that, in this study, the researchers also found significant relationship between ER $\alpha$  and ER $\beta$  expression in PTC samples with p value 0.004. From Table 3, they can obtained that ER $\alpha$  expression seemed to go parallel with ER $\beta$ , for example if ER $\alpha$  positive, then ER $\beta$  more likely to be positive, and vice versa. Qiu et al in their study also found that there was relationship between ER $\alpha$  and ER $\beta$  in PTC patients with p value<0.001 and r-0.293. This indicated that the relationship between ER $\alpha$  and ER $\beta$  was inversely proportional. Results from Qiu et al was not in accordance with this study. Based on theory, both of these ER $\alpha$  and ER $\beta$  should be inversely related because ER $\alpha$  contributes to tumour growth, while ER $\beta$  inhibits tumorigenesis.<sup>12,13</sup>

Rubio et al said that PTC can develop in patients with regard to increasing ER $\alpha$  level but ER $\beta$  remains unchanged.<sup>28</sup> Both this study and Spirina et al showed increased ER $\beta$  level. According to Spirina, the oncosuppressive role of ER $\beta$  is not proven in thyroid carcinoma, because of biological peculiarity of this tumour. The presence of ER $\beta$  is not only determine the prognosis of this disease but also the efficiency of antitumoural therapy and patient survival rate.<sup>29</sup>

Based on this study results and others, we can believe that ER $\alpha$  expression in PTC may be a potential prognostic marker and show more aggressive behavior because recurrence, extrathyroidal extension and metastasis to lymph node usually occur in PTC with ER $\alpha$  positive.<sup>14,16</sup> Furthermore, in women ptc with ER $\alpha$  negative, diminished ER $\beta$ 1 expression is correlated with tumour invasion and metastasis.<sup>21</sup> Therefore, if PTC is expressed positive with ER $\alpha$ , then the patients can be recommended for tamoxifen.

Eventhough there has been solid evidence that estrogen is involved in pathogenesis of PTC, the results are often conflicting in various ER expression pattern. Considering that how complex ER expression is, multiple variants, epigenetic modification, different subcellular localization and various phosphorylation location, the comparison of ER expression patterns alone may be

not sufficient to draw conclusions regarding the relevance ER in PTC. Involved pathway is very complex and not yet understood clearly. Therefore, further researches are needed.<sup>30</sup>

Unfortunately, the researchers can't differentiate how ER $\alpha$  and ER $\beta$  are, compared to benign tumour because increase in ER $\alpha$  expression and decrease in ER $\beta$  expression may be found in PTC. Besides that, in this study, medical record data were limited regarding menopausal status so the researchers could not ensure whether premenopausal women with PTC would have more ER $\alpha$  and ER $\beta$  positive expression. Furthermore, this study only determined whether PTC were expressed positive ER $\alpha$  and ER $\beta$  or not. From immunohistochemistry, we could know how much exactly are the ER $\alpha$  and ER $\beta$  expression. Therefore, further researches about ratio of ER $\alpha$  and ER $\beta$  expression in PTC by using western blot analysis is need.

During this study, there was difficulty experienced by researchers. In interpreting results of immunohistochemical ER $\alpha$  and ER $\beta$  staining, it was difficult to calculate the area of positive tumour cells because it was subjective.

## V. CONCLUSION

In this study, some conclusion could be emphasized. There were no significant relationship between age, gender, and tumour size with ER $\alpha$  and ER $\beta$  expression in PTC. Besides that, there was significant relationship between ER $\alpha$  and ER $\beta$  expression in PTC samples (p value= 0.004). The relationship between these two was parallel.

## VI. COMPETING INTERESTS

The author has no financial interests relevant to the product or company described in this article.

## VI. ACKNOWLEDGMENT

We would like to thank all staff of the Department of Anatomical Pathology, University of North Sumatra, Private hospitals and private clinics in Medan, Indonesia for all their assistance and cooperation.

## VII. ETHICAL APPROVAL

Health Research Ethical Committee, Universitas Sumatera Utara, Medan, Indonesia approved this study.

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