ER-α and ER-β Expression in Thyroid Gland Lesions (Immunohistochemical and Histopathological Study)

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Abstract

Background: Thyroid diseases (clinical and subclinical) are estimated to affect 10% of the population and they are more prevalent in females. This suggest a role of estrogen in the pathogenesis of thyroid diseases.

Objective: To evaluate the presence of estrogen receptors alpha and beta in different thyroid lesions and to detect if there is benign-malignant differences in their expression.

Material and Methods: 60 previously diagnosed specimens of various thyroid lesions were reviewed in this retrospective and immunohistochemical study. All were obtained from Pathology Department, Faculty of Medicine, Cairo University between January 2013 and December 2015. Formalin-fixed Paraffin-embedded tissue sections were used. Three sections were prepared from each paraffin block; one of them stained with Hematoxylin and Eosin (H & E) for histological re-evaluation, while others were subjected to the two immunohistochemical markers (ER-α and ER-β). The (Allred) score method was used to evaluate immunohistochemical staining. The cases were considered positive if their score was more than 2, and showing nuclear immunoreactivity.

Results: Cases were 52 females and 8 males ranging in age from 17 to 72 years with mean 42.1 ± 10.88 years. They were as follows: 8 cases (13.3%) non neoplastic, 11 cases (18.3%) benign, and 41 cases (68.3%) malignant. The ER-α positive cases were four cases (4/60; 6.7%) with non significant statistical correlations between ER-α immunostaining and patient age, sex, menopausal state or pathological diagnoses. The ER-β positive cases were 51/60 cases with significant correlation between both ER-β positivity and Allred scoring and different malignant pathological diagnoses with p-value (<0.05) for both. The expression is observed more in non neoplastic cases followed by malignant and at last benign cases. On contrary there were no significant correlation between ER-β expression and age, sex or menopausal status. On studying the expression of both ER-α and ER-β simultaneously, we revealed that all positive ER-α cases were positive for ER-β but not the reverse but by using chi square, p-value was 0.384 which was statistically not significant.

Conclusion: ER-β is the more detectable estrogen receptor in thyroid tissue different lesions than ER-α and with more expression in non neoplastic cases followed by malignant cases.

Key Words: Estrogen receptors – Thyroid gland – Tumors–ER-α–ER-β.

Introduction

BENIGN and malignant thyroid lesions are more common in women and different thyroid lesions occur predominantly in the premenopausal age group [1]. These epidemiologic associations have led to investigations to detect the presence of Estrogen Receptors (ER) in both neoplastic and non-neoplastic thyroid lesions [2].

Action of estrogen is mediated classically through binding of two soluble intracellular nuclear receptors, Estrogen Receptor (ER) alpha, and Estrogen Receptor (ER) beta [3]. Attempts to prove the presence of the Estrogen Receptor (ER) in the nuclei of thyroid lesion cells [4], is not only to prove their dependence on estrogen for development or progression but also hope that anti-estrogenic drugs may help in stopping growth and proliferation of the neoplastic cells [8].

Material and Methods

60 previously diagnosed specimens of various thyroid lesions were reviewed in this retrospective and immunohistochemical study. All were obtained from Pathology Department, Faculty of Medicine, Cairo University between January 2013 and December 2015. Selection was both consecutively and according to the availability of adequate tissue and clinical data.
Immunohistochemistry:

Paraffin-embedded tissue sections fixed in formalin were used. Three (5 microns thick) sections were prepared from each paraffin block; one of them stained with Hematoxylin and Eosin (H & E) for histological re-evaluation, while others were subjected to the two immunohistochemical markers (ER-α and ER-β) and immunohistochemical staining in this study was automated.

Positive control: Breast cancer was used as a positive control for ER-α, and normal breast was used as a positive control for ER-β.

Assessment of ER-α and ER-β: The (Allred) score method was used to evaluate immunohistochemical staining for the estrogen receptors. The Allred score is the sum of two component scores; proportion and intensity of staining, gives us a score in the range 0-8 (excluding score=1) [6]. Intensity score: No staining of any nuclei at high magnification=0, weak (only visible at high magnification)=1, moderate (readily visible at low magnification)=2, strong (strikingly positive at low magnification)=3. While proportion score: 0% =0 , <1%=1, 1-10%=2, 11-33%=3, 34-66%=4, 67-100%=5.

Scoring of ER-α and ER-β in tumor cells was performed on high power field (400X) using a standard light microscope. The cases were considered positive if their Allred score was more than 2, and showing nuclear immunoreactivity.

Statistical analysis: Data were statistically described in terms of mean ± Standard Deviation (±SD), or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Mann Whitney U-test for independent samples for comparing two groups and Kruskal Wallis test for comparing more than two groups. For comparing categorical data, Chi-square ($\chi^2$) test was performed. Exact test was used instead when the expected frequency is less than 5. Correlation between various variables was done using Spearman rank correlation equation. $p$-values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows (2006).

Results

The cases were 52 females and 8 males ranging in age from 17 to 72 years with mean 42.1 ± 10.88 years. Most of the cases were females (86.6%) ranging in age from 17 to 72 years with mean 41.11 ± 10.77 years and according to menopausal state 40 females were premenopausal (76.2%) and 12 were postmenopausal (23.8%). While male age ranged from 27 to 57 years with mean 48.5 ±9.89 years.

According to pathological diagnoses; the cases were as follows: 8 cases (13.3%) (goiter) representing non neoplastic cases, 11 cases (18.3%) benign tumors (follicular adenoma), and 41 cases (68.3%) malignant tumors (carcinoma). The carcinoma cases were 12 (29.3%) follicular carcinomas, 16 (39%) papillary carcinomas, 3 (7.3%) poorly differentiated carcinomas, 3 (7.3%) anaplastic carcinomas and 7 (17.1%) medullary carcinomas (Table 1).

ER-α immunostaining:

The ER-α-positive cases were four cases (4/60; 6.7%). The cases were ranging in age from 36 to 56 years with mean age 45.75 ± 11.26 years. They were 3 females (5.8% of total female cases) and 1 male (12.5% of total male cases).

As regards positivity in relation to sex using Fisher Exact Test, the $p$-value=0.445 while in relation to age mean using Spearman rank correlation, the $p$-value=0.361 and within female groups (premenopausal or postmenopausal) using Fisher Exact Test was 0.553; all were statistically non significant.

Positivity for ER-α in relation to diagnoses (Table 2) (one benign case (9.1% of all benign cases) and three malignant cases (7.3% of all malignant cases) using Pearson Chi-Square revealed $p$-value=0.704 while the Allred scoring in relation to different pathological diagnoses using Kruskal-Wallis Test revealed $p$-value=0.714; both were statistically non significant.
So there were no significant statistical correlations between ER-α immunostaining and patient age, sex, menopausal state or pathological diagnoses.

**ER-β immunostaining:**

The ER-β positive cases were fifty one cases (51/60; 85%). The cases ranged in age from 17 to 72 years with mean age 41.56±10.61 years. They were 44 females (84.6% of total female cases) ranging in age from 17 to 72 years with mean 40.10±10.50 years; 35 premenopausal and 9 postmenopausal and 7 males (87.5% of total male cases) ranging in age from 45 to 57 years with mean 51.57±5.12 years. Allred scoring of positive cases were as follows: Three cases=3, eight cases=4, two cases=5, eleven cases=6, sixteen=7 and eleven cases=8.

As regards positivity in relation to sex using Fisher Exact Test, the \( p \)-value=1.0 while Allred scoring in relation to sex using Mann-Whitney Test revealed \( p \)-value=0.790, both were statistically non significant. As regards positivity in relation to age using Spearman rank correlation, the \( r \)-value=0.361 and within female groups (premenopausal or postmenopausal) in relation to positivity using Exact Test, \( p \)-value was=0.366 and in relation to Allred score using Mann-Whitney Test \( p \)-value=0.493 all were statistically non significant.

As regards pathological diagnoses, ER-β positive cases were as follows: 8 adenomatous goiter (100% of goiters), 6 follicular adenoma (54.5% of adenomas) and 37 carcinoma (90.2% of carcinomas) (Chart 1).

As regards positivity for ER-β in relation to diagnoses category (non neoplastic, benign and malignant) using Pearson Chi-Square, \( p \)-value=0.006 and on correlation between Allred scoring and the pathological diagnoses categories using Kruskal-Wallis Test revealed \( p \)-value=0.001, both were statistically significant and the expression is observed more in non neoplastic cases followed by malignant and at last in benign lesions.

For adenomatous goiter 8/8 (100%) (Chart 2) were ER-β positive (15.7% out of all positive ER-β cases), they were 7 females (four premenopausal and three postmenopausal), ranging in age from 25 to 55 years with mean age 43.8±10.6 years. And one male 57 years old. According to Allred scoring the cases were as follows: Four cases=4, two cases=6 and two cases=7. Most of cases were females (7 out of 8) (87.5%), four were premenopausal (57.1%).

For follicular adenoma 6/11 (54.5%) were positive (11.8% out of all positive ER-β cases) (Chart 2), all were females (four premenopausal and two postmenopausal), ranging in age from 27 to 55 years with mean age 43.7±8.5 years. According to Allred scoring the cases were as follows: Two cases=3, one case=5 and three cases=6. All positive cases were females, two thirds of them (66.7%) were premenopausal.

In carcinoma cases 37/41 (90.2%) were ER-β positive, they were 31 females (27 premenopausal and four postmenopausal) ranging in age from 17 to 72 years with mean age 38.3±10.58 years, and 6 males ranging in age from 45 to 57 years with mean age 50.7±4.96 years. According to Allred scoring the cases were as follows: One case=3, four cases=4, one case=5, six cases=6, fourteen cases=7, eleven cases=8. Most of positive cases were females (31 out of 37) (83.8%), and most of them were premenopausal 27 (87%).

Analysis of different malignant diagnoses revealed: Positive papillary carcinoma cases were 16/16 (100%) (Chart 2), they were thirteen females (all were premenopausal) ranging in age from 26 to 47 years with mean age 33.3±5.92 years and three males ranging in age from 45 to 57 years with mean age 49.3±6.65 years. Allred score were: three cases=4, one case=5, six cases=6, four cases =7 and two cases=8. Mean age of females were less than of males (33.3 years compared to 49.3 years). Positive Follicular carcinoma cases were 12/12 (100%) (Chart 2); ten females (7 premenopausal and 3 postmenopausal) ranging in age from 34 to 72 years with mean age 46±11.33 years and two males 50 and 56 years with mean age 53±4.24 years. Allred score were: One case=3, nine cases=7 and two cases=8. Females were more than males (10/12), seven were premenopausal (70% of females). Positive poorly differentiated carcinoma cases were 1/3 (33.3%) (Chart 2); one female 33 years old (premenopausal). Allred score=8. Anaplastic carcinoma cases were 1/3 (33.3%) (Chart 2); one female 17 years old (premenopausal). Allred score=4. Medullary carcinoma cases were 7/7 (100%) (Chart 2); six females (5 premenopausal and one postmenopausal) ranging in age from 31 to 55 years with mean age 41.16±8.20 years and one male (50 years old). Allred score were: One case=7 and six cases=8. Most of cases were females (6/7, 85.7%), most of them were premenopausal (5/6, 83.3%).
On analysis of age of males and females in ER-\(\beta\) positive cases; mean of age of females was lower than males in all types of carcinomas. But by using Spearman rank correlation, the \(p\)-value was 0.834 which was insignificant.

On comparing positivity in the different malignant pathological diagnoses by using Pearson Chi-Square, \(p\)-value was 0.001. and on correlation between Allred scoring and different malignant pathological diagnoses using Kruskal-Wallis Test revealed \(p\)-value=0.001 . Both were statistically significant and ER-\(\beta\) expression is observed more in well differentiated malignant tumors than higher grade tumors. While there was no significant correlation between ER-\(\beta\) expression and age, sex or menopausal status.

Expression of both ER-\(\alpha\) and ER-\(\beta\) simultaneously revealed \(p\)-value=0.384 which was statistically insignificant.

![Fig. (1): Papillary thyroid carcinoma (H & E X1000).](image1)

![Figs. (2,3): Papillary thyroid carcinoma (ER-\(\alpha\), negative X100 & ER-\(\beta\), positive cytoplasmic and nuclear membrane X100).](image2)

![Fig. (4): Medullary thyroid carcinoma; amyloid deposits (H & E X100).](image3)

![Fig. (5): Medullary thyroid carcinoma (ER-\(\beta\), positive, cytoplasmic and nuclear X400).](image4)

![Fig. (6): Undifferentiated thyroid carcinoma (ER-\(\alpha\), negative X400).](image5)

![Fig. (7): Undifferentiated thyroid carcinoma (ER-\(\beta\), positive nuclear and cytoplasmic X1000).](image6)
Adenomatous goiter
Thyroid adenoma
Thyroid carcinoma

Number of cases
18
16
14
12
10
8
6
4
2
0

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Discussion

The aim of this study is to detect the expression of ER-α and ER-β in different thyroid lesions. In the current study females with thyroid lesions mean age was 41.11 years and according to menopausal state 40 were premenopausal (76.2%) and 12 were postmenopausal (23.8%). While mean male age was 48.5 years. In Vaiman et al., study [7] females age mean=29 years and according to menopausal state were 189 premenopausal (81.81%) and 42 postmenopausal (18.18%), while male age mean was 37 years. In another study done by Huang et al., 2014 [8] cases were all females, 80 were premenopausal (67.2%) and 39 were postmenopausal (32.8%). In our study the age means for both females and males were higher but distribution of cases according to menopausal status were close.

In this study ER-α positive cases were four cases (4/60; 6.7%), one case follicular adenoma and three cases were follicular carcinomas. In studies done by Vaiman et al., [7] and Bléchet et al., [9] none of the histologic samples showed immunoreactivity for ER-α. While in Huang et al., study [8] positive cases were thirty two (32/119;
26.9%) cases, 30 papillary carcinoma and two goiters. So there is discrepancy between different studies that might be explained by technical factors such as use of monoclonal or polyclonal antibodies or various staining procedures.

In this study there is no significant statistical correlations between ER-α immunostaining and patient's age, sex, menopausal state or pathological diagnoses. While in Huang et al., study [8] both the positive percentage and total Allred score of ER-α expression were significantly increased (p-value = 0.000) in premenopausal female papillary thyroid carcinoma patients as compared with that of age- and gender-matched goiter patients.

In Bléchet et al., study [9] as well as in this study ER-β immunoreactivity was detected in the nuclei, nuclear membrane and cytoplasm of various thyroid lesions. In Vaiman et al., study [7] as in this study positive immunoreactivity results for ER-β were found in tissue samples from benign, malignant and non neoplastic lesions. In this study ER-β positive cases were fifty one cases (51/60; 85%). They were 44 females (84.6% of total female cases); 35 premenopausal and 9 postmenopausal and 7 males (87.5% of total male cases). In Vaiman et al., study [7]; as in this study on correlation between ER-β expression and patient's age, sex and menopausal state; all were found to be insignificant (p>0.05).

ER-β expression was detected in all 8/8 adenomatous goiter (100% of goiters), 6/11 follicular adenoma (54.5% of adenomas) and 37/41 carcinoma (90.2% of carcinomas). While in Vaiman et al., study [7] ER-β was detected in goiters (126/150; 84% of goiters), follicular adenoma (17/19; 89.47% of adenomas) and carcinoma (70/103; 67.96% of carcinomas); the incidence was lower than that in our study as regards malignant cases. In Bléchet et al., study [9] ER-β was detected in 26/28 medullary carcinoma (92.85%) which is almost the same result of our study where all medullary carcinoma cases were 7/7 (100%) expressing ER-β.

As regards positivity for ER-β in this study in relation to different diagnoses categories (non neoplastic, benign and malignant), p-value = 0.006 which was statistically significant. The expression is more prevalent in non neoplastic followed by malignant followed by benign conditions. And on comparing positivity between different malignant pathological diagnoses (papillary carcinoma, follicular carcinoma, poorly differentiated carcinoma, anaplastic carcinoma and medullary carcinoma), p-value = 0.001 which also statistically highly significant and it reveals that expression is observed in well differentiated malignant tumors more than higher grade tumors. On contrary in Vaiman et al., study [7] no significant difference was found between the various thyroid lesions (p-value >0.05).

In this study expression of both ER-α and ER-β simultaneously revealed p-value=0.384 which was statistically not significant.

References


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دراسة مناعية نسيجية كيميائية لتعبير مستقبل الإستروجين ألفا وبيتا
في أمراض الغدة الدرقية المختلفة

في هذه الدراسة المناعية النسيجية الكيميائية، تم دراسة تعبير مستقبل الإستروجين ألفا وبيتا في ستين حالة من حالات أمراض الغدة الدرقية المختلفة، والتي وردت ضمن الباحثي، كلية الطب، جامعة القاهرة في الفترة من يناير 2012 إلى ديسمبر 2015.

تم تحضير ثلاثة مقطع من كل مكعب شعري ادراستها من الناحية الهيستولوجية كما تم صياغتها بصفتي مناعتين كيميائيتين، وحما مستقبل الإستروجين ألفا وبيتا. وتتم استخدام نظام الأليل لتقييم الصبغة المناعية، واعتبار الحالة إيجابية إذا سجلت أكثر من 2 ومكان التعبير في التواجد.

وجاء توزيع الحالات كالآتي: 24 إناث و8 ذكور، تتراوح أعمارهم بين 17 و76 سنة بمتوسط 42 سنة. معظم الحالات من الإناث (68.8٪) ومن الملاحظ ارتفاع مستوى عمر الذكور عن الإناث.

بالنسبة لتوزيع الحالات على حسب التشخيص فهي كالتالي: 8 حالات (13.3٪) تضخم الغدة الدرقية، و11 حالة (18.3٪) ورم حميد، و41 حالة (68.3٪) ورم سرطاني.

حالات الأورام السرطانية تتوزع كالتالي: 12 حالة سرطان غدي حليمي و2 حالات سرطان سبيتة التماسيح
و2 حالات غير متماشية و7 حالات سرطان نخاعي.

اربعة حالات فقط وجد بها مستقبل الإستروجين ألفا (100٪). 3 إناث (50٪ من إجمالي الإناث)، حالة واحدة ورم غدي جريبجي حميد في عمر 61 سنة، وحالتين بسرطان غدي جريبجي في عمر ما قبل إنقطاع الطمث، والحالة الأخيرة ذكر في (12.5٪) من إجمالي الذكور، بسرطان غدي جريبجي.

لم توجد أي إحصائية عند مقارنة تعبير مستقل الإستروجين ألفا بالعمر أو الجنس أو التشخيص للحالات المشاركة في هذه الدراسة.

أما بالنسبة لمستقبل الإستروجين بيتا فقد وجد في 61 حالة (100٪) 51/85٪. تتوزع الحالات كالتالي: 44 حالة (84.6٪ من إجمالي الإناث) بمتوسط عمر 41 سنة. 33 حالة منهن في سن ما قبل إنقطاع الطمث و8 في سن ما بعد إنقطاع الطمث. أما الذكور فهم 7 حالات (87.5٪ من إجمالي الذكور) بمتوسط عمر 51 سنة. ومن الملاحظ انخفاض مستوى عمر الإناث عن الذكور.

بالتالي توزيع الحالات على حسب التشخيص فهي كالتالي: 8 حالات تضخم الغدة الدرقية (100٪ من حالات تضخم الغدة الدرقية)، 6 حالات ورم غدي جريبجي حميد (50٪)، و37 حالة سرطان سبيتة التماسيح.

حالات الأورام السرطانية تتوزع كالتالي: 12 حالة (100٪) سرطان غدي حليمي وحالة واحدة (33٪) سرطان سبيتة التماسيح وحالة واحدة (33٪) سرطان غدي حليمي وحالة واحدة (33٪) سرطان سبيتة التماسيح.

لم توجد أي إحصائيات عند مقارنة تعبير مستقل الإستروجين بيتا بالعمر أو الجنس، ولكن وجدت أهمية إحصائية كبيرة عند المقارنة بالتشخيصات المختلفة. الحالات الأكثر تعبيراً كانت حالات تضخم الغدة الدرقية بلبلاء الأورام السرطانية، الأكثر ثمايراً أكثر من الأقل تماساً.