Online ISSN: 2682-2628
Print ISSN: 2682-261X

# IJCBR

## International Journal of Cancer and Biomedical Research

https://jcbr.journals.ekb.eg



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PUBLISHED BY
EACR EGYPTIAN ASSOCIAN FOR CANCER RESEARCH
Since 2014

INTERNATIONAL JOURNAL OF CANCER AND BIOMEDICAL RESEARCH (IJCBR)

RESEARCH ARTICLE

# Possible prognostic significance of Her-2/neu, estrogen, progesterone and androgen receptors expression in papillary thyroid carcinoma

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## ABSTRACT

**Background**: Most of thyroid carcinomas are of papillary type. Gender difference in incidence suggests that such tumor might be influenced by sex hormones. HER 2/neu is known to play a role in different tumor types. **Aim**: This study aimed at assessing immunohistochemical expression of estrogen receptors (ERs), progesterone receptors (PRs), androgen receptors (ARs) and Her 2/neu receptors in papillary thyroid carcinoma (PTC) and its correlation with clinic-pathological parameters. **Methods**: This retrospective study included 42 cases of PTC. Clinic-pathological data were revised with calculation of overall survival (OS) and disease-free survival (DFS). Immunohistochemical staining for ERs, PRs, ARs and HER 2/neu were performed and interpreted in double blind by two expert pathologists. **Results**: PRs expression was significantly associated with existence of residual activity in the post-operative whole-body iodine scan (P=0.03). Meanwhile, ERs and ARs expression were insignificantly correlated with patients' clinic-pathologic features. HER-2/neu expression was significantly associated with tumor size (P=.04), extra-thyroid extension (P=0.003) and nodal recurrence (p=0.009). Logistic regression analysis confirmed a significant effect of HER2 expression as independent predictor of nodal recurrence (P=0.017). Significant difference between HER2 positive and HER2 negative cases regarding DFS and OAS was found. **Conclusion**: PRs and HER-2/neu, but not ERs and Ars, expression was associated with tumor aggression.. Larger multicenter studies are needed to confirm these observations that could define high risk group of patients with nodal recurrence, and the value of hormonal therapy.

**Keywords**: Androgen receptors, Estrogen receptors, HER2 neu, Papillary thyroid carcinoma, Progesterone receptors

Editor-in-Chief: Prof. M.L. Salem, PhD - Article DOI: 10.21608/JCBR.2022.141478.1261

## ARTICLE INFO

**Article history**
Received: May 29, 2022
Revised: December 03, 2022
Accepted: December 28, 2022

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## INTRODUCTION

Thyroid gland neoplasms account for 1% of all cancers (1.5% of malignancies in females and 0.5% in males) and represent the most common endocrine malignancy. During the past decades, its incidence has increased. Eighty percent of these carcinomas are papillary thyroid carcinoma (PTC) (Jalali-Nadoushan et al., 2016).

Most benign thyroid diseases such as Grave's disease, endemic goiter, and Hashimoto thyroiditis are significantly more frequent in women of childbearing age (Vanderpump 2011). PTC prevalence is nearly 3 times higher in females than in males. This gender difference suggests that growth and progression of PTC might be influenced by female sex hormones. It is known that estrogens can regulate thyroid cell proliferation by binding to both types of estrogen receptors (ERs) alpha and beta, displaying different effects on cell survival and proliferation (Dong et al., 2013, Vannucchi et al., 2015, Sturniolo et al., 2016).

An increased risk of thyroid cancer has also been documented with estrogen therapy for gynecological reasons and use of oral contraceptive pills in females. Interestingly, estrogen metabolites and conjugates have been found to be significantly higher in women with

well-differentiated thyroid cancer as compared to age-matched control women (Zahid et al., 2014). However, results are not consistent because of the existence of confounding factors in the populations selected (Derwahl Nicula 2014).

The presence of androgen receptors (ARs) was demonstrated both in normal and neoplastic thyroid tissues, with variable degrees of expression. Few data exist regarding expression of both Progesterone receptors (PRs) and ARs (Zhai et al., 2003, Vannucchi et al., 2015). Stanley et al. (Stanley et al., 2012) found a varying pattern of testosterone level and ARs status in thyroid tissues of men and women possibly predisposing to the gender-specific incidence of thyroid tumors.

Proto-oncogene HER-2/neu (C-erbB2), known as CD340, is located on the 17q chromosome. Its amplification or overexpression plays an important part in tumor development and progression through direct effects on the cell cycle, angiogenesis, cellular motility and apoptosis (Yarden 2001). HER-2/neu overexpression is associated with poorly differentiated phenotype, high metastasis capacity and poor overall survival. The role that could be played by targeted therapies such as Herceptin is a fruitful field of research (Bilici 2014, Iqbal Iqbal 2014, May 2014).

This study aimed at assessing immunohistochemical (IHC) expression of ERs, PRs, ARs and Her 2/neu receptors in PTC and its correlation with clinic-pathological parameters.

## MATERIALS AND METHODS

This retrospective study included 42 eligible cases (38 cases of classic variant and 4 cases of follicular variant) out of a total of 70 cases pathologically diagnosed as PTCs during the period between January 2014 and December 2016 in the surgical pathology laboratory in our Oncology Center and attended at the Clinical Oncology &Nuclear Medicine Department of our University Hospitals. Inclusion criteria were the availability of tissue blocks and complete follow up data. Exclusion criteria were presence of severe co-morbidities or other associated malignancies. The medical electronic records of patients who underwent surgery for PTC were reviewed. Data regarding age, gender, tumor size, extra-thyroid extension, lymph node status, TNM staging according to (AJCC) 8th edition, treatment and follow up were with calculation of overall and disease-free survivals.

All included patients performed pre-operative neck ultrasound and underwent fine needle aspiration cytology (FNAC) from thyroid lesion. Patients underwent thyroidectomy by an onco-surgeon. Lateral and/or central neck dissection was performed in indicated cases. The ablative doses of radioactive iodine (I 131) and the suppressive doses of L-thyroxine were recorded. Clinical examination, a whole-body scan with I 131 and serum thyroglobulin measurements were performed at least once every year. Re-examination of hematoxylin and eosin (H&E) stained slides was performed in double blind by two expert pathologists.

**Tissue microarray (TMA) construction:**

First, the region of interest is identified on a donor tissue block guided by H&E-stained slides. Next, a small punch (0.6 mm) is taken from the donor tissue block and seated into a recipient paraffin block. Three TMA blocks were made by Beecher manual microarrayer. Six cores were punched from each case and inserted in the recipient blocks according to a designed map for each block. Normal tissue and carcinomas from different organs were used as a guide control to the TMA technique.

**Immunohistochemical staining**

The primary antibodies used were mouse monoclonal anti-human estrogen receptor (ER), clone EP1 (ready to use) (Dako, Copenhagen, Denmark), mouse monoclonal anti-human progesterone receptor (PR), clone PgR 636 (ready to use) (Dako, Copenhagen, Denmark), mouse monoclonal anti-human androgen receptor (AR), and rabbit polyclonal anti-human c-erbB-2 oncoprotein (Dako, Copenhagen, Denmark).

Immune reaction for ER, PR, AR and Her2 neu was visualized using Envision system Dako, Copenhagen, Denmark) with DAB chromogen as substrate and Mayer's hematoxylin as counterstain. ERs, PRs and Her2 neu positive breast carcinoma and ARs positive prostatic tissue were used as positive control for ERs, PRs,

Her2 neu and ARs, respectively. Negative controls were prepared by substituting the primary antibodies with cross-matched isotopes. The IHC staining was performed using the automated Dako Cytomation Auto-Stainer platform. The optimal dilution for each antibody was determined using positive and negative control tissues such as breast and prostate carcinomas. Interpretation of immunoreactivity for ERs, PRs, and ARs expressions in tumor cells was performed on high-power field (x400) using a standard light microscope. The examination field was evaluated by two pathologists. Interobserver differences were less than 1%. Only cells showing nuclear staining of any intensity in any number of tumor cells is required to assign ERs, PRs or ARs positivity.

The expression of HER-2/neu was evaluated by an adapted semiquantitative score proposed for thyroid carcinomas (Kremser et al., 2003; Wu et al., 2013), with assessment of not only membranous, but also cytoplasmic staining. This score was based on the percent of positive tumor cells (P), classified as 0 for less than 10%, 1 for 10% to 25%, 2 for 25% to 50%, 3 for 50% to 75% and 4 for more than 75% positive cells, respectively, and the staining intensity (I), categorized as 1 weak, 2 moderate, and 3 strong. The final score was obtained as a product between P and I, with the following values: 0 negative, 1–4 weakly positive, 5–8 moderately positive, 9–12 strongly positive. These values were extrapolated to the scoring system usually applied for Her2/neu in breast and gastric cancer (Rakha et al., 2014, Ciobanu Apostol et al., 2017). Consequently, taking into account both cytoplasmic and/or membranous Her2/neu staining, we considered the values between 0 and 4 as negative (corresponding to HER-2/neu scores 0 and 1+) and those between 5 and 12 as positive (corresponding to HER-2/neu scores 2+ and 3+).

**Statistical analysis of the data**

Data were analyzed using the Statistical Package of Social Science (SPSS) program for Windows (Standard version 21). The normality of data was first tested with one-sample Kolmogorov-Smirnov test. Qualitative data were described using number and percent. Association between categorical variables was tested using Chi-square test while Fischer exact test was used when expected cell count less than 5. Continuous variables were presented as Median (min-max) for non-parametric data. The two groups were compared with Mann–Whitney test. Significant variables on univariate analysis of the predictors of death entered into cox regression model using then enter statistical technique to predict the most significant determinants and to control for possible interactions and confounding effects. Kaplan-Meier test was used for survival analysis and statistical significance of differences among curves was determined by Log-Rank test. Disease –free survival (DFS) was calculated from the date of surgery to date of recurrence while the overall survival (OAS) from date of diagnosis to the date of death or last follow up. For all above mentioned statistical tests done, the threshold of significance is fixed at 5% level (p-value). The results were considered as non-significant when the probability of error is more than 5% ($p > 0.05$) and significant when the probability of error is 5% or less ($p \leq 0.05$). The smaller the p-value obtained, the more significant are the results.

## RESULTS

This study included 42 cases of PTC, 61.9% of them were females. Table 1 shows the patients and tumor characteristics, while Table 2 shows the treatment modalities and follow up results. The mean age was 46.4 ±14.08 years. None of the patients had previous radiotherapy on head and neck region or a family history of thyroid cancers. Total thyroidectomy was performed in 39 cases (93%). Postoperative severe hypocalcemia that necessitated hospitalization was encountered in 1 case only. Stage I was the commonest stage encountered in 26 cases (61.9%) while 10 cases (23.8%) were stage IV (7%). Fifteen cases (36%) showed extra-thyroid extension, which was reported only by microscopic examination in 6 cases while it was detected by both gross and microscopic examination in the remaining 9 cases. Lymph-vascular invasion was detected in 6 cases (14%). As regard expression of hormone receptors, positive expression for ERs, PRs and ARs was detected in 81%, 74% and 57% respectively (Fig.1-3). More than 90% of the positive ERs and PRs cases showed weak expression.

**Table 1.** Patients and tumor characteristics of the studied cases.

| Patients' characteristics | The study cases (n=42) | |
|---|---|---|
| | No | % |
| Age/ years | | |
| <55 | 28 | 66.7 |
| ≥55 | 14 | 33.3 |
| Mean ± SD (46.45±14.08) | | |
| Gender | | |
| Male | 16 | 38.1 |
| Female | 26 | 61.9 |
| T-size | | |
| T1 | 16 | 38.1 |
| T2 | 9 | 21.4 |
| T3 | 6 | 14.3 |
| T4 | 11 | 26.2 |
| LN status | | |
| N0 | 15 | 35.7 |
| N1 | 27 | 64.3 |
| Stage | | |
| I | 26 | 61.9 |
| II | 4 | 9.5 |
| III | 2 | 4.8 |
| IV | 10 | 23.8 |
| Extra-thyroid extension | 15 | 35.7 |
| Lymph-vascular invasion | 6 | 14.3 |
| ER expression | | |
| Positive | 34 | 81.0 |
| Negative | 8 | 19.0 |
| PR expression | | |
| Positive | 31 | 73.8 |
| Negative | 11 | 26.2 |
| AR expression | | |
| Positive | 24 | 57.1 |
| Negative | 18 | 42.9 |
| HER-2/neu expression | | |
| Positive | 11 | 26.2 |
| Negative | 31 | 73.8 |

ER: Estrogen receptors; PR: Progesterone receptors; AR: Androgen receptors

**Table 2.** Treatment modalities and follow up results of the studied cases.

| Treatment and follow up results | The study cases (n=42) | |
|---|---|---|
| | No | % |
| Surgery | | |
| Total thyroidectomy | 39 | 92.9 |
| Hemi thyroidectomy | 3 | 7.1 |
| RAI | | |
| No | 6 | 14.3 |
| Once | 26 | 61.9 |
| Twice | 10 | 23.8 |
| Residual tumor in whole body iodine scan | 27 | 64.3 |
| Nodal Recurrence | 8 | 19.0 |
| Mortality | 3 | 7.1 |
| DFS Median (Min-Max) | 35 months (4-76) | |
| OAS Median (Min-Max) | 47 months (25 -130) | |

RAI: Radioactive iodine; DFS: Disease free survival; OAS: Overall survival

Strong androgen expression was present in 29% of ARs positive cases. HER-2/neu, positive expression was detected in 26% of cases (Figures 4-6).

Ablative I131 (80-100 mCi) was given for 39 patients (93%). The indications of ablative iodine treatment were tumors 2-4cm, high risk histology, lymph-vascular invasion, macroscopic multifocality, elevated postoperative thyroglobulin, microscopic positive margins, remaining thyroid tissue in the postoperative neck sonar and gross extra-thyroid extension. Ablative doses were given at 6weeks to 13 weeks from surgery. Postoperative residual tumor on whole body iodine scan was recorded in 27 patients (64%).

On follow up, nodal recurrence occurred in 8 cases (19%). Therapeutic I131(with doses ranging from 100-150mCi) was given for all patients with recurrence except 2 who were treated by surgery only. The early side effects of I131 were mild in all cases in the form of sialadenitis and bone marrow suppression. No late side effects were recorded. External beam radiotherapy was used for 2 patients, one with bone metastasis and the other with brain metastasis respectively. Three cases (7%) died from their disease complications. DFS Median (Min-Max), 35 months (4-76), and OAS Median (Min-Max) 47 months (25 -130).

Regarding the clinic-pathological parameters, we found a significant association between nodal recurrence and older age(P=.041), larger tumor size (P=0.003), extra-thyroid extension (P=0.01) and distant metastasis (P=0.04). Also, distant metastasis showed statistically significant relation with extra-thyroid extension (P=0.047) as well as larger tumor size (P=0.03) (Table 3).

Regarding the relation between the receptor's expression and the different demographic and tumor factors. The expression of hormone receptors did not significantly differ in pre- and postmenopausal age. ERs and ARs expression were not significantly associated with any of other studied factors. Meanwhile, PRs expression was significantly associated with post-operative residual tumor (p=0.03) (Table 4-6). But, on univariate analysis PRs expression was not found to be an independent predictor of post-operative residual tumor.

Concerning HER-2/neu expression, it was significantly associated with tumor size (P=0.04), extra-thyroid extension (P=0.003) and nodal recurrence (p=0.009) (Table 7). No significant association was detected between HER-2/neu expression and any of hormone receptors positivity (Table 8). As regard survival analysis, we found a significant difference between HER2 positive and HER2 negative cases regarding DFS (P=0.05) and OAS (P=0.035) with shorter DFS and OAS in HER-2/neu positive cases (Figures 7 and 8).

For further investigation, we analyzed the data using cox logistic regression model. Using parameters that were significantly associated with HER-2/neu expression during univariate testing as input variables confirmed a significant effect of HER-2/neu expression as independent predictor of nodal recurrence, $\beta = 2.05$, $p= 0.017$, OR=7.8, 95% CI= 1.4-41. In other words, PTC patients with positive HER-2/neu expression more frequently develop nodal recurrence when compared to patients with negative HER-2/neu expression. Concerning the clinical parameters, we found that tumor size and subsequently extra-thyroid extension also act as independent predictors of nodal recurrence (Table 9). However, on multivariate analysis none of these factors were statistically significant.

## DISCUSSION

Patients with differentiated thyroid carcinoma have an excellent prognosis. However, minority of patients show a dismal course with tumor recurrence and mortality rate less than 5% (Elsayed et al., 2020; Lingli Wang et al., 2022). The treatment includes surgery usually followed by radioiodine ablation and hormone treatment (TSH suppression therapy) according to the guidelines of the American Thyroid Association (ATA) and European Association of Nuclear Medicine (EANM) (Schmidbauer et al., 2017).

Our clinic-epidemiologic data were generally homogenous with literature. The incidence in females exceeded that of males (1.5:1).

**Table 3.** Relation between nodal recurrence and clinic-pathological features.

| Patients' and tumor characteristics | Nodal Recurrence | | | | Value |
|---|---|---|---|---|---|
| | Positive (n=8) | | Negative (n=34) | | |
| | No | % | No | % | |
| Age/ years | | | | | .041* |
| <55 | 2 | 25.0 | 22 | 64.7 | |
| ≥55 | 6 | 75.0 | 12 | 35.3 | |
| Gender | | | | | 1.00 |
| Male | 3 | 37.5 | 13 | 38.2 | |
| Female | 5 | 62.5 | 21 | 61.8 | |
| T-size | | | | | .003* |
| T1 | 0 | 0 | 16 | 47.1 | |
| T2 | 1 | 12.5 | 8 | 23.5 | |
| T3 | 1 | 12.5 | 5 | 14.7 | |
| T4 | 6 | 75.0 | 5 | 14.7 | |
| LN status | | | | | .128 |
| N0 | 1 | 12.5 | 14 | 41.2 | |
| N1 | 7 | 87.5 | 20 | 58.8 | |
| Stage | | | | | .064 |
| I | 2 | 25.0 | 24 | 70.6 | |
| II | 2 | 25.0 | 2 | 5.9 | |
| III | 0 | 0 | 2 | 5.9 | |
| IV | 4 | 50.0 | 6 | 17.6 | |
| RAI | | | | | .080 |
| No | 3 | 37.5 | 3 | 8.8 | |
| Once | 3 | 37.5 | 23 | 67.6 | |
| Twice | 2 | 25.0 | 8 | 23.5 | |
| Surgery | | | | | 1.00 |
| Total | 8 | 100 | 31 | 91.2 | |
| hemi thyroidectomy | 0 | 0 | 3 | 8.8 | |
| Extra-thyroid extension | 6 | 75.0 | 9 | 26.5 | .01* |
| Lymph-vascular invasion | 2 | 25.0 | 4 | 11.8 | .319 |
| Residual tumor in whole body iodine scan | 4 | 50.0 | 23 | 67.6 | .349 |
| Distant metastasis | 3 | 37.5 | 2 | 5.9 | .04* |
| ERs expression | | | | | |
| Positive | 6 | 75.0 | 28 | 82.4 | .634 |
| Negative | 2 | 25.0 | 6 | 17.6 | |
| PRs expression | | | | | .932 |
| Positive | 6 | 75.0 | 25 | 73.5 | |
| Negative | 2 | 25.0 | 9 | 26.5 | |
| ARs expression | | | | | .706 |
| Positive | 4 | 50.0 | 20 | 58.8 | |
| Negative | 4 | 50.0 | 14 | 41.2 | |
| HER-2/neu expression | | | | | .009* |
| Positive | 5 | 62.5 | 6 | 17.6 | |
| Negative | 3 | 37.5 | 28 | 82.4 | |

ERs: Estrogen receptors; PRs: Progesterone receptors; ARs: Androgen receptors; * Statistically significant

Other studies recorded larger ratios as that of Arianpoor, *et al*. (Arianpoor et al., 2020).

The surgical policy in our cases justified total thyroidectomy aiming at better results. Such preference is coincident with the literature (Hirsch et al., 2014). Following surgery, the definitive staging revealed predominance of early stages while distant metastasis represented 7% only. Such results are in harmony with literature (Shaha et al., 2001, Farahati et al., 2019) and clarify the early diagnosis of thyroid carcinoma.

Extra-thyroid extension was reported in 36% of our cases. However, variability existed in the literature, some reported lesser percentage (31%) as Shin, *et al*. (Shin et al., 2013), while other reported higher figures up to 50% as Park *et al*. (Park et al., 2017). Lymph-vascular invasion was not found extensively in the present study (14% of cases) which is an incidence less than that reported by Sezer *et al* (26%) (Sezer et al., 2017).

**Table 4.** Correlation of estrogen receptors expression with tumor characteristics in the studied cases.

| Variables | Total N=42 | ERs | | Test of significance |
|---|---|---|---|---|
| | | Negative n=8 | Positive n=34 | |
| Age/years | | | | |
| <55 | 28 | 7(87.5) | 21(61.8) | $\chi^2$=1.93 |
| ≥55 | 14 | 1(12.5) | 13(38.2) | P=0.165 |
| Sex | | | | |
| Male | 16 | 1(12.5) | 15(44.1) | χ2=2.75 |
| Female | 26 | 7(87.5) | 19(55.9) | P=0.09 |
| Tumor size | | | | |
| Tx | 1 | 1(12.5) | 0(0.0) | MC |
| T1 | 16 | 4(50.0) | 12(35.3) | *P*=.24 |
| T2 | 9 | 1(12.5) | 8(23.5) | |
| T3 | 6 | 1(12.5) | 5(14.7) | |
| T4 | 10 | 1(12.5) | 9(26.5) | |
| Lymph node | | | | |
| N0 | 15 | 4(50.0) | 11(32.4) | MC |
| N1A | 2 | 0(0.0) | 2(5.9) | *P*=.55 |
| N1B | 25 | 4(50.0) | 21(61.8) | |
| Stage | | | | |
| I | 26 | 7(87.5) | 19(55.9) | MC |
| II | 4 | 0(0.0) | 4(11.8) | *P*=.34 |
| III | 2 | 0(0.0) | 2(5.9) | |
| IV | 10 | 1(12.5) | 9(26.5) | |
| Extra-thyroid extension | 15 | 3(37.5) | 12(35.3) | χ2=0.01 *P*=.91 |
| Lymph-vascular invasion | 6 | 0(0.0) | 6(17.6) | χ2=1.65 *P*=.19 |
| Residual tumor in whole body iodine scan | 27 | 7(87.5) | 20(58.8) | χ2=2.32 *P*=.13 |
| Nodal recurrence | 8 | 2(25.0) | 6(17.6) | χ2=0.23 *P*=.63 |
| Distant metastasis | 5 | 1(12.5) | 4(11.8) | FET *P*=1.0 |

ERs: Estrogen receptors; FET: Fischer exact test; χ2=Chi-Square test; MC: Monte Carlo test

**Table 5.** Correlation of progesterone receptors expression with tumor characteristics in the studied cases.

| Variables | Total N=42 | PRs | | Test of significance |
|---|---|---|---|---|
| | | Negative n=11(%) | Positive n=31(%) | |
| Age/years | | | | |
| <55 | 28 | 9(81.8) | 19(61.3) | $\chi^2$=1.54 |
| ≥55 | 14 | 2(18.2) | 12(38.7) | P=0.215 |
| Sex | | | | |
| Male | 16 | 3(27.3) | 13(41.9) | χ2=0.74 |
| Female | 26 | 8(72.7) | 18(58.1) | P=0.39 |
| Tumor size | | | | |
| Tx | 1 | 0(0.0) | 1(3.2) | MC |
| T1 | 16 | 6(54.5) | 10(32.3) | *P*=.58 |
| T2 | 9 | 1(9.1) | 8(25.8) | |
| T3 | 6 | 2(18.2) | 4(12.9) | |
| T4 | 10 | 2(18.2) | 8(25.8) | |
| Lymph node | | | | |
| N0 | 15 | 3(27.3) | 12(38.7) | MC |
| N1A | 2 | 0(0.0) | 2(6.5) | *P*=.48 |
| N1B | 25 | 8(72.7) | 17(54.8) | |
| Stage | | | | |
| I | 26 | 9(81.8) | 17(54.8) | MC |
| II | 4 | 0(0.0) | 4(12.9) | *P*=.45 |
| III | 2 | 0(0.0) | 2(6.5) | |
| IV | 10 | 2(18.2) | 8(25.9) | |
| Extra-thyroid extension | 15 | 3(27.3) | 12(38.7) | χ2=0.46 *P*=.49 |
| Lymph-vascular invasion | 6 | 1(9.1) | 5(16.1) | χ2=0.33 *P*=.57 |
| Residual tumor in whole body iodine scan | 27 | 10(90.9) | 17(54.8) | χ2=4.60 *P*=.03* |
| Nodal recurrence | 8 | 2(18.2) | 6(19.4) | χ2=0.01 *P*=.93 |
| Distant metastasis | 5 | 1(9.1) | 4(12.9) | FET *P*=1.0 |

PRs: progesterone receptors; FET: Fischer exact test; χ2=Chi-Square test; MC: Monte Carlo test; * Statistically significant

**Table 6.** Correlation of androgen receptors expression with tumor characteristics in the studied cases.

| Variables | Total N=42 | ARs | | Test of significance |
|---|---|---|---|---|
| | | Negative n=18(%) | Positive n=24(%) | |
| Age/years | | | | |
| <55 | 28 | 13(72.2) | 15(62.5) | $\chi^2$=0.438 |
| ≥55 | 14 | 5(27.8) | 9(37.5) | P=0.51 |
| Sex | | | | |
| Male | 16 | 5(27.8) | 11(45.8) | χ2=1.42 |
| Female | 26 | 13(72.2) | 13(54.2) | P=0.23 |
| Tumor size | | | | |
| Tx | 1 | 1(5.6) | 0(0.0) | MC |
| T1 | 16 | 8(44.4) | 8(33.3) | *P*=.24 |
| T2 | 9 | 3(16.7) | 6(25.0) | |
| T3 | 6 | 4(22.2) | 2(8.3) | |
| T4 | 10 | 2(11.1) | 8(33.3) | |
| Lymph node | | | | |
| N0 | 15 | 8(44.4) | 7(29.2) | MC |
| N1A | 2 | 1(5.6) | 1(4.2) | *P*=.55 |
| N1B | 25 | 9(50.0) | 16(66.7) | |
| Stage | | | | |
| I | 26 | 13(72.2) | 13(54.2) | MC |
| II | 4 | 2(11.1) | 2(8.3) | *P*=.16 |
| III | 2 | 1(5.6) | 1(4.2) | |
| IV | 10 | 2(11.1) | 8(33.3) | |
| Extra-thyroid extension | 15 | 5(27.8) | 10(41.7) | χ2=0.86 *P*=.35 |
| Lymph-vascular invasion | 6 | 1(5.6) | 5(20.8) | χ2=1.96 *P*=.16 |
| Residual tumor in whole body iodine scan | 27 | 13(72.2) | 14(58.3) | χ2=0.86 *P*=.35 |
| Nodal recurrence | 8 | 4(50.0) | 4(50.0) | FET *P*=.65 |
| Distant metastasis | 5 | 2(11.1) | 3(12.5) | FET *P*=1.0 |

ARs: Androgen receptors; FET: Fischer exact test; χ2=Chi-Square test; MC: Monte Carlo test; * Statistically significant

**Table 7.** Correlation of HER-2/neu receptors expression with tumor characteristics in the studied cases.

| Variables | Total N=42 | HER-2/neu | | Test of significance |
|---|---|---|---|---|
| | | Negative n=31(%) | Positive n=11(%) | |
| Age/years | | | | |
| <55 | 28 | 22(71.0) | 6(54.5) | $\chi^2$=3.08 |
| ≥55 | 14 | 9(29.0) | 5(45.5) | P=0.08 |
| Sex | | | | |
| Male | 16 | 2(22.2) | 14(42.4) | χ2=1.22 |
| Female | 26 | 7(77.8) | 19(57.6) | P=0.27 |
| Tumor size | | | | |
| Tx | 1 | 0(0.0) | 1(9.1) | MC |
| T1 | 16 | 14(45.2) | 2(18.2) | *P*=.04* |
| T2 | 9 | 6(19.4) | 3(27.3) | |
| T3 | 6 | 6(19.4) | 0(0.0) | |
| T4 | 10 | 5(16.1) | 5(45.5) | |
| Lymph node | | | | MC |
| N0 | 15 | 9(29.0) | 6(54.5) | *P*=-.26 |
| N1A | 2 | 2(6.5) | 0(0.00 | |
| N1B | 25 | 20(64.5) | 5(45.5) | |
| Stage | | | | |
| I | 26 | 21(67.7) | 5(45.5) | MC |
| II | 4 | 3(9.7) | 1(9.1) | *P*=.29 |
| III | 2 | 2(6.5) | 0(0.0) | |
| IV | 10 | 5(16.2) | 5(45.5) | |
| Extra-thyroid extension | 15 | 7(22.6) | 8(72.7) | χ2=8.89 *P*=.003* |
| Lymph-vascular invasion | 6 | 3(9.7) | 3(27.3) | FET *P*=.31 |
| Residual tumor in whole body iodine scan | 27 | 22(71.0) | 5(45.5) | χ2=2.3 *P*=.13 |
| Nodal recurrence | 8 | 3(9.7) | 5(45.5) | χ2=6.74 *P*=.009* |
| Metastasis | 5 | 4(12.9) | 1(9.1) | FET *P*=1.0 |

FET: Fischer exact test; χ2=Chi-Square test; MC: Monte Carlo test; * Statistically significant

**Table 8.** Correlation between HER-2/neu and hormone receptors expression.

| Variables | Total N=42 | HER-2/neu | | Test of significance |
|---|---|---|---|---|
| | | Negative n=38 (%) | Positive n=4(%) | |
| ERs | 34 | 24(77.4) | 10 (90.9) | $\chi 2=0.96$ $p=.33$ |
| PRs | 31 | 21(67.7) | 10(90.9) | $\chi 2=2.25$ $p=.13$ |
| ARs | 24 | 16(51.6) | 8(72.7) | $\chi 2=1.48$ $p=.22$ |

ERs: Estrogen receptors; PRs: Progesterone receptors; ARs: Androgen receptors; χ2=Chi-Square test; *Statistically significant

**Table 9** Logistic regression analysis of independent predictors of nodal recurrence

| Independent predictor | β | *P* - value | OR (95%CI) |
|---|---|---|---|
| Age/ years<br>≤50 y (r)<br>> 50 y | 1.70 | .06 | 5.5 (0.9-31) |
| T-size<br>T1,2 (reference group)<br>T3,4 | 2.82 | .013* | 16.8 (1.8-54.9) |
| Extra-thyroid extension | 2.12 | .019* | 8.3 (1.4-49) |
| Metastasis | 1.65 | .152 | 5.2 (0.54-48) |
| HER-2/neu<br>Positive<br>Negative (reference group) | 2.05 | .017* | 7.8 (1.4-41) |

OR: Odds ratio; CI: Confidence interval; * Statistically significant

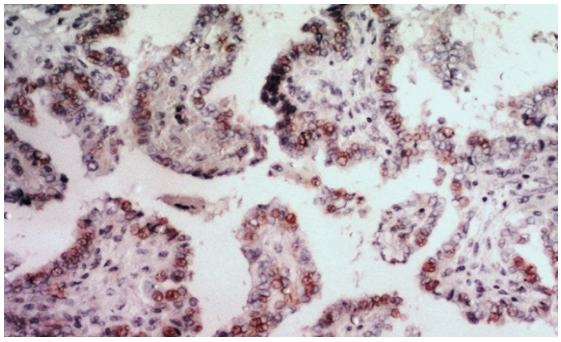

**Figure 1.** Nuclear estrogen receptors expression in the tumor cells of papillary thyroid carcinoma (IHC staining, ER x200).

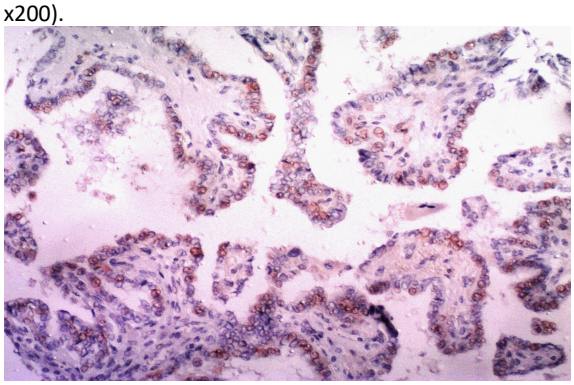

**Figure 2.** Nuclear progesterone receptors expression in the tumor cells of papillary thyroid carcinoma (IHC staining, PR x200).

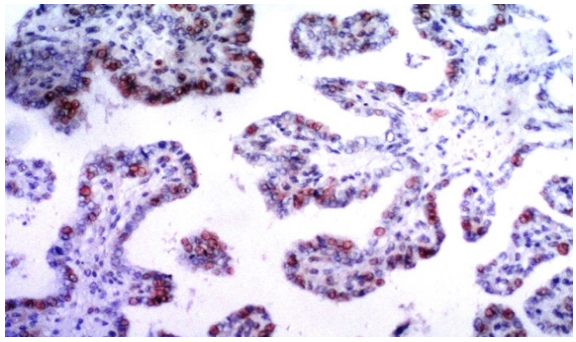

**Figure 3.** Nuclear androgen receptors expression in the tumor cells of papillary thyroid carcinoma (IHC staining, AR x200).

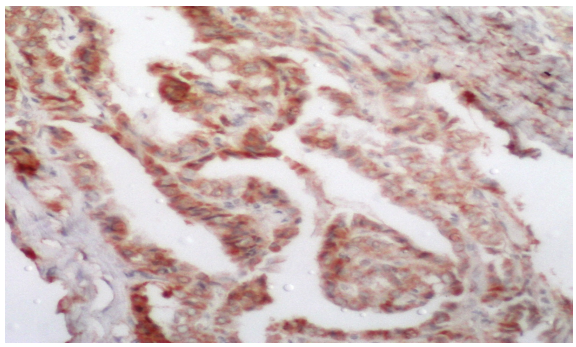

**Figure 4.** Weak HER-2/neu staining in papillary thyroid carcinoma (IHC staining, HER2 neu x200).

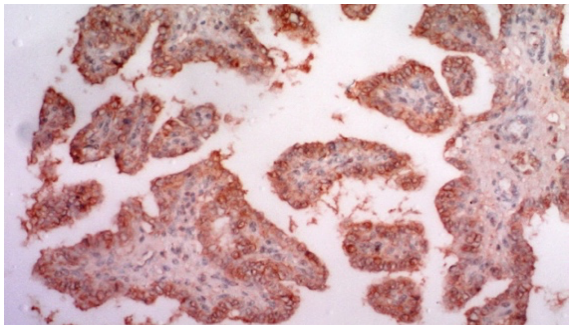

**Figure 5.** Moderate HER-2/neu staining in papillary thyroid carcinoma (IHC staining, HER2 neu x200).

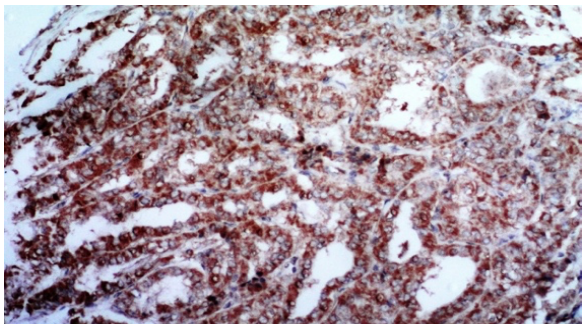

**Figure 6.** Strong HER-2/neu staining in papillary thyroid carcinoma (IHC staining, HER2 neu x200)

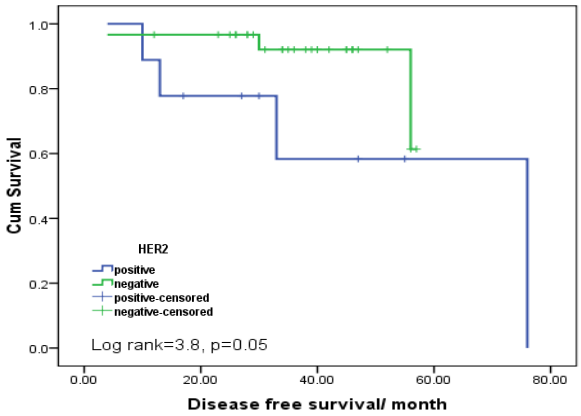


**Figure 7.** Disease free survival of HER-2/neu positive and negative cases (Kaplan-Meier curve).

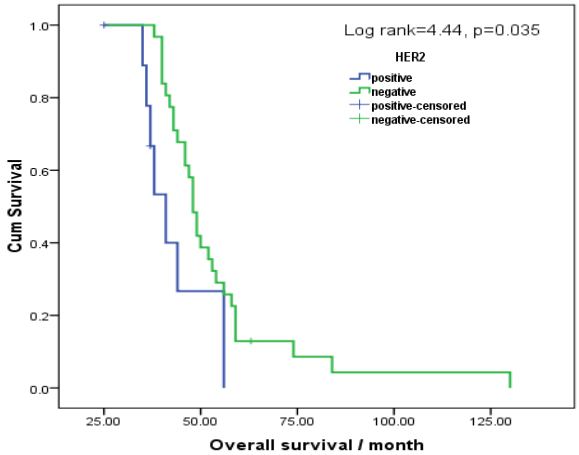


**Figure 8.** Overall survival of HER-2/neu positive and negative cases (Kaplan-Meier curve).

Regarding therapy toxicity, severe postoperative hypocalcemia was reported in 1 of our cases. Hypocalcemia is one of the well-known thyroidectomy complications (Safioleas et al., 2006). The reported mild early side effects of the used I131 agrees with the results of Fard-Eshfahani *et al*. (Fard-Esfahani et al., 2014). On follow up, nodal recurrence was encountered in 19% of our cases similar to that found by Maksimovic (Maksimovic 2018).

We reported the significant relation of older ages, tumor size and extra-thyroid extension with later nodal recurrence. This result is in harmony with that of Suh *et al*.(Suh et al., 2015) and Cho *et al*.(Cho et al., 2012). We investigated the expression of ERs, PRs, ARs and HER-2/neu receptors in PTCs aiming to discover the possible use of the available therapeutic alternatives. ERs and PRs positivity were reported in 81% and 73% of our cases, respectively. On the other hand, Sturniolo *et al*. (Sturniolo et al., 2016) reported 26% and 46%, Jalali-Nadoushan *et al*.(Jalali-Nadoushan et al., 2016) reported 47% and 5.6% and Chen *et* al.(Chen et al., 2015) reported 59% and 54% respectively. Consequently, there is a considerable expression variability among cases of PTCs in the different studies. Indeed, the value of ERs expression in PTC is a dilemma. ERs expression beside being of low intensity in the majority of our positive cases, it was of no prognostic value. Similarly, Vaiman et al. (Vaiman et al., 2010) concluded that ERs expression assessment may not be necessary. In addition, Jalali-Nadoushan (Jalali-Nadoushan et al., 2016) did not found any correlation between ERs expression and any of the clinical or pathologic parameters. On the other hand, Sturniolo et al. (Sturniolo et al., 2016) proved the good prognostic impact of ERs expression in PTCs. Chen et al. (Chen et al., 2015) and Vannucchi et al. (Vannucchi et al., 2015) found a significant correlation of ERs expression with the tumor size and Dai et al. (Dai et al., 2017) stated that concomitant ERs alpha expression and HER-2/ neu denoted aggressive behavior.

As regard PRs expression in the present study, it was significantly associated with post-operative residual tumor in the whole-body iodine scan denoting the aggressive behavior of PTCs with positive PRs expression. However, the studies of

Galali-Nadoushan et al. (Jalali-Nadoushan et al., 2016) and Sturniolo et al (Sturniolo et al., 2016) did not find significant association of PRs expression with any of the tested tumor factors. In contrast, Vannucchi et al (Vannucchi et al., 2015) found that PRs expression was significantly correlated with tumor size.

The present study recorded no association between androgen expression and any of the studied tumor factors. However, Magri *et al*. (Magri et al., 2012) reported strong association with capsular invasion. In our study, we found no significant association between HER-2/neu expression and any of hormone receptors expression similar to the result of Dai *et al*. (Dai et al., 2017)

The epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptor tyrosine kinases. Evidence suggests that the EGFR is involved in the pathogenesis of different malignancies (Sugishita et al., 2013). Our results proved the significant association between positive HER-2/neu expression, tumor size, extra-thyroid extension and nodal recurrence. Moreover, HER-2/neu positivity was among the independent prognostic factors for recurrence. There was statistically significant worse DFS and OAS of HER-2/neu positive cases in comparison to HER-2/neu negative ones. These results coincide with those of Caria et al.,(Caria et al., 2016), Ciobanu et al.,(Ciobanu Apostol et al., 2017), Elsayed et al.,(Elsayed et al., 2020), Fisher et al., (Fisher et al., 2013), Rabee et al., (Rabiee et al., 2017), and contradict that of Siraj et al. (Siraj et al., 2017).

## CONCLUSION

We concluded that expression of PR and HER-2/neu receptors was associated with tumor aggression. Despite the radicality of treatment by surgery and the ablative iodine, regular follow up is needed especially for cases with large tumor size and extra-thyroid extension as nodal recurrence is not rare. On the other hand, the value of ERs and ARs expression was not proved. Larger multicenter retrospective studies and clinical trials are needed to confirm these observations. These could define high risk group of patients, especially for nodal recurrence, and have impact on target therapy with improvement of DFS and OAS.

## FUNDING

The authors received no specific funding for this work.

## CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

## ETHICS APPROVAL

This study was approved by the Institutional Research Board (IRB) of Mansoura University (R.18.12.354) in view of the retrospective nature of the study and all the procedures being performed were part of the routine care.

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