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Expression of ER/PR in Thyroid Lesions with Special Reference to Papillary Carcinoma of Thyroid

Shazieya Akhtar[1], Noor Jahan Ali[2], Subuh Parvez Khan[3]

[1]Senior Resident, Department of Haematopathology, SKIMS, Soura, Srinagar, India
Registrar, Department of Haematopathology, Sher-I-Kashmir Institute of Medical Sciences, Soura, Srinagar, India

Abstract: ***Aims and Objectives: (i)Comparison of ER/PR positivity between neoplastic thyroid lesion Shazieya Akhtar, Noor Jahan Ali, Subuh Parvez Khan. Materials and Method: Total 350 cases were taken. The specimen were examined externally and then opened as per conventional method.The tissues were processed as per standard procedure. Results: Out of 264 neoplastic cases 51 (19.3%) cases were ER+ and 74 (28%) cases were PR+. Expression of ER and PR between neoplastic and non-neoplastic groups was statistically significant.Out of 56 benign cases 7(12.5%) cases were ER + and 12 (21.4%) cases were PR +. Out of 208 malignant cases 44 (21.2%) cases were ER + and 62 (29.8%) cases were PR+. Maximum percentage of ER + (20.4%) and PR + (27.8%) cases were >4cm in size. ER expression was found in 32.1% of papillary carcinoma thyroid whereas PR expression was found in 35.2% of papillary thyroid carcinoma and 42.3% of medullary thyroid carcinoma. Conclusion: The study demonstrates that ER and PR expression occurs more frequently in neoplastic than in non-neoplastic lesions of thyroid. Papillary carcinoma and Medullary carcinoma of thyroid show significant expression of ER and PR receptors. ER/PR expression with various age groups, sex and size of tumour don't show any statistical significance.***

Keywords: Medullary carcinoma, non-neoplastic, ER / PR.

1. Introduction

Thyroid gland is one of the largest endocrine gland situated in front of a neck below the level of cricoid cartilage and weighs approximately 15 -20 gm. Many markers have been documented immunohistochemically in normal follicular cells, most of them being also expressed in well differentiated tumours of these cells. These include

a) Reactivity for thyroglobulin, thyroid peroxidase, tri-iodothyronine and thyroxine which are found in colloid and follicular cell cytoplasm.[1,2,3]
b) Thyroid transcription factor-1 (TTF-1) is an extremely useful marker for thyroid follicular cells and tumours composed of them.[4]
c) Low molecular weight keratins, CK 7, CK18 and to a lesser degree CK8 and CK19.[5]
d) Epethelial membrane antigen.
e) Vimentin.[5]
f) Estrogen and progesterone receptors.[6]
g) Blood group antigens.[7]
h) Metal binding proteins; ceruloplasmin, lactoferrin, transferrin, metalothionein.[8,9]

Malignant disease of the thyroid gland is three times more common in females than in males except for medullary carcinoma where incidence is identical. The differences in thyroid cancer incidence between females and males and the suspicion that sex hormones may be implicated as a casual factor have given rise to studies that evaluate expression of sex hormone receptors in malignant, benign and normal thyroid tissue, as well as their expression in females and males. These studies are highly variable in their methodologies including protocols for detection of ER, PR, criteria for receptor positivity and thyroid specimen type.

Aims and Objectives

- Comparison of ER/PR positivity between neoplastic thyroid lesions and non-neoplastic thyroid lesions.
- To study the expression ER/PR in thyroid carcinomas with reference to papillary carcinomas.

2. Materials and Method

The study was conducted in the department of pathology at the Sheri-i-Kashmir Institute of Medical Sciences (SKIMS) Srinagar, Kashmir and include retrospective data analysis of three and a half years and a prospective data analysis of one and a half year.

The retrospective study data of 250 cases were taken from January 2011 to June 2014 and a prospective study data of 100 cases from July 2014 to December 2015.

The specimens were examined externally and then opened as per conventional method after overnight fixational in 10% formalin.

Gross photographs of the specimen were taken.
Minimum of three sections from tumour were taken.
Appropriate section from isthmus and other lobe of thyroid were taken.
Sections from adjacent fibrofatty tissue piece were taken.
Associated lymph nodes were dissected and grossed.

The tissues was processed as per standard procedure:

Tissue Processing Techniques

1) All specimens were fixed in 10% neutral buffered formalin for atleast 48 hours.
2) The specimen was routinely embedded serially sectioned at a thickness of 5mm and is then put in micro-cassettes.

3) The specimen was then passed through a series of increasing concentration of alcohol.

Steps	Treatment	Time
1-2	80% alcohol (2 times)	1+1
3	90 % alcohol	1
4-6	100% alcohol	1+1+1

4) Cleaning: it was done by xylene

Steps	Treatment	Time
7-8	Xylene (2 times)	1+1

5) Infiltration and impregnation: it was carried out in paraffin oven for 2-4 hours at a temperature between 50-60 0c.

Steps	Treatment	Time (hours)
9-10	Paraffin (2 times)	1+1

6) Embedding: Tissue was put in leukhardts box embedded and melted paraffin wax is poured into the box. The box was kept in cold water until it hardens.
7) Section /cutting: 5 to 6 micrometer sections were cut by the knife of microtome. Four to five micometer thickness sections were cut on the microtome and stained by Haematoxylin and Eosin stain

Hematoxylin and Eosin Staining Procedures

1) Dewax sections in xylene for 5 minutes.
2) Remove xylene with absolute alcohol for one minute.
3) Gradually hydrate the sections with low grade alcohol, first treat with absolute alcohol followed by 90%, then with 70% alcohol and finally 50% alcohol.
4) Wash with water.
5) Stain in alum hematoxylin for 5 minutes.
6) Wash well in running tap water until sections are blue.
7) Dip in 1% acid alcohol for 5 -10 seconds.
8) Wash well in tap water until sections are again blue 5 minutes.
9) Stain in 1% aqueous eosin for 3 minutes.
10) Wash in running tap water for 1 minute.
11) Dehydrate in absolute alcohol.
12) Clear in xylene and mount in DPX.

3. Observations and Results

The present study was conducted on thyroidectomy specimens in the Department of Pathology in Sheri Kashmir Institute of Medical Sciences Soura Srinagar.Our study included a total of 350 cases. A total of 350 cases comprising 86 cases of non-neoplastic lesions and 264 of neoplastic lesions of thyroid were selected.

The distribution of these lesions is shown in.

Distribution of studied cases according to various histological types		
Type of Lesion	No.	Percentage
PTC	159	45.4
MTC	26	7.4
MNG	70	20
HCA	12	3.4
AC	11	3.1
FA	44	12.6
FC	12	3.4
HT	13	3.7
GD	3	0.9
Total	350	100

Majority of the cases were females (n=299) constituting 85.4% with male:female ratio of 1:5.84.

Gender Distribution of studied cases		
Gender	No.	Percentage
Male	51	14.6
Female	299	85.4

Comparison of hormone receptor expression among non-neoplastic and neoplastic lesions showed that out of 86 non-neoplastic cases, no case was positive for either ER or PR. Out of 264 neoplastic cases, 51 (19.3%) cases were ER positive and 74 (28%) cases were PR positive. The observation was statistically significant with a p-value of <0.001.

Comparison of hormone receptor expression among non-neoplastic &neoplastic lesions

Category	ER Expression		PR Expression		Total
	Positive	Negative	Positive	Negative	
Non Neoplastic Lesions	0 (0%)	86 (100%)	0 (0%)	86 (100%)	86
Neoplastic Lesions	51 (19.3%)	213 (80.7%)	74 (28%)	190 (72%)	264
P-value	<0.001*		<0.001*		350

Distribution of ER and PR cases among different types of thyroid carcinoma. This expression of hormone receptors was found to be statistically significant.

Association of ER/PR expression with different thyroid malignancies				
Type of Malignancy	ER Expression		PR Expression	
	Positive	Negative	Positive	Negative
PTC	51 (32.1%)	108 (67.9%)	56 (35.2%)	103 (64.8%)
MTC	0 (0%)	26 (100%)	11 (42.3%)	15 (57.7%)
AC	0 (0%)	11 (100%)	0 (0%)	11 (100%)
FC	0 (0%)	12 (100%)	0 (0%)	12 (100%)
P-value	<0.001*		0.002*	

4. Discussion

The present study was conducted in the Department of Pathology, SKIMS. In the present study a total of 350 cases comprising of non-neoplastic, benign and malignant lesions of thyroid were selected.The present study was conducted with the aim to evaluate ER and PR expression in 350 cases. Majority of the cases were female (n=299) constituting 85.4% of the total cases with a male:female ratio of 1:586. Immunohistochemical expression of estrogen and progesterone receptors (ER and PR) was studied in all the cases. Estrogen expression was seen in 51 cases (14.6%) and progesterone expression was seen in 74 cases (21.1%). Largest group of cases n = 246 (85.4%) were negative for both ER and PR (ER-/PR-), while 21 cases (6%) revealed ER +/PR+ pattern. Expression of ER and PR was correlated with each other and a significant number of cases (76.3%) were found to be either positive or negative for both receptors. The observation was statistically significant with a p value of < 0.001.

Out of 86 non-neoplastic lesions no case was either ER + or PR +. Out of 264 neoplastic lesions 51(19.3%) cases were ER + and 74 (28%) cases were PR+ respectively which was statistically significant with a p value of <0.001. The data indicates that there is a significant difference in the

expression of ER and PR receptors among neoplastic and non- neoplastic lesion. According to our study, it was present only in neoplastic lesions. The studies evaluating ER and PR status among neoplastic and non-neoplastic lesions show mixed results. Similar findings were observed by previous authors Molteni et al,[10] Diaz et al,[11] Hong et al,[12]Taakeichi et al,[13]Bonacci et al,[14]Colomer et al,[15]Karisson et al,[16]Lewy-Trenda ,[17]Belchet et al[18] while studies by Clark et al[19] , Hiasa et al,[20] Imai et al[21] , Metaye et al[22], Van Hoeven et al,[23]Yane et al,[24] and Tavangar et al[25] negate the findings. The possible difference can be due to variability in their sample size, thyroid tissue type, methods of detection of ER and PR including scoring system, age groups and race.

In our study ER expression was found only in Papillary thyroid carcinoma (32.1%) and PR expression was found in both papillary thyroid carcinoma (35.2%) and medullary thyroid carcinoma (42.3%). Our results are statistically significant with P value of <0.001 and 0.002 respectively. From these results we can infer that ER /PR positivity is present more in differentiated thyroid carcinomas than undifferentiated variants.Similar findings were observed by Dan Chen et al[26],Huang Y et al[27], Jin Ying et al[28], Van Hoeven et al[23], LewyTrenda[17], Kawabata et al[29] and Kansakar et al[30], Also Takeichi et al[13], Hiasa et al[20], Tavangar et al[25] and Kavanagh et al[31] observed that ER positivity decreases with increase of dedifferentiation of thyroid cancer. Diaz et al[11] noted that single case of PTC with foci of dedifferentiation lacked ER protein expression. Takeichiet al[13] evaluated ER immunoreactivity in malignant thyroid lesions of various grades from menopausal patients and found that the number of ER immunoreactive cells in a cancer significantly decreased with the degree of tumour dedifferentiation. Hiasa et al[20] also reported a trend towards decreasing ER positivity with increasing dedifferentiation and two studies by Inoue et al found that although 34 out of 124 PTC were ER positive, none of the ten poorly-differentiated thyroid cancers were ER positive. A study reported by Kavanagh et al[31] evaluating 111 thyroid tumours also found that ER α expression was predominantly found in non-anaplastic lesions and was significantly associated with well differentiated cancers. Egawaet al[32] observed that expression of ER α was slightly more in follicular and anaplastic carcinoma.

5. Conclusion

The study demonstrates that ER and PR expression occurs more frequently in neoplastic than in non-neoplastic lesions of thyroid .There is no significant difference in the expression of ER /PR between benign and malignant lesions but within various malignant lesions of thyroid, there occurs significant difference in the expression of ER and PR. Papillary carcinoma and Medullary carcinoma of thyroid show significant expression of ER and PR receptors.

References

[1] De Micco C, Kopp F, Vassko V, Grino M. In situ hybridization and immunohistochemistry study of thyroid peroxidase expression in thyroid tumours. Thyroid 2000; 10: 109-115.
[2] Lima MA, Gontijo VA, Schmitt FCL. Thyroid peroxidase and thyroglobulin expression in normal human thyroid glands.EndocrPathol 1998; 9: 333-338.
[3] Stanta G, Carcangiu ML, Rosai J.The biochemical and immunohistochemical profile of thyroid neoplasia.PatholAnnu 1988; 23(pt 1): 129-157.
[4] Katoh R, Kawaoi A, Miyagi E, Hemmi A, Komiyama A,Oyama T, Shibuya M. Thyroid transcription factor -1 in normal ,hyperplastic and neoplastic follicular thyroid cells examined by immunohistochemistry and non – radioactive in situ hybridization. Mod Pathol 2000; 13: 570-576.
[5] Fonseca E, Nesland JM, Hoie J, Sobrinho SM. Pattern of expression of intermediate cytokeratin filaments in the thyroid gland: an immunohistochemical study of simple and stratified epithelial type cytokeratins. Virchows Arch 1997; 430: 239-245.
[6] Bur M, Shiraki W, Masood S. Estrogen and progesterone receptor detection in neoplastic and non-neoplastic thyroid tissue. Mod Pathol 1993; 6: 469-472.
[7] Gonzalez-Campora R, Garcia-Santana JA, Jorda I, Heras NM et al. Blood group antigens in differentiated thyroid neoplasms. Arch Panthol Lab Med 1998; 122: 957-965.
[8] Raphael SJ, Asa SL. Immunohistochemical localization of metal binding proteins in thyroid tissues and tumours. EndocrPathol 1992; 3: 182-187.
[9] Schmid KW, Greeff M, Hittmair A, Totsch M, Ofner D, Dockhorn-Dworniczak B, Bocker W, Jasani B. Metallothione expression in normal ,hyperplastic and neoplastic thyroid follicular and para follicular c cells using monoclonal antimetallothione in antibody E9.Endocr Pathol. 1994; 5: 114-122.
[10] Molteni A, Warpeha RL, Brizio-Molteni L, Fors EM. Estradiol receptor binding protein in head and neck neoplastic and normal tissues. Arch Surg 1981; 116(2): 207-10.
[11] Diaz NM, Mazoujian G, Wick MR. Estrogen –receptor protein in thyroid neoplasms. An immunohistochemical analysis of papillary carcinoma, follicular carcinoma, and follicular adenoma. Arch Pathol Lab Med.1991 Dec; 115(12): 1203-7.
[12] Hong GS, Sng IT, Soo KC. Oestrogen receptors in well differentiated thyroid cancers. Ann Acad Med Singapore 1991; 20(6): 767-9.
[13] Takeichi N, Ito H, Haruta R, Matsuyama T, Dohi K, Tahara E. Relation between estrogen receptor and malignancy of thyroid cancer. Jpn J Cancer Res 1991; 82: 19-22.
[14] Bonacci R, Pinchera A, Fierabacci P, Giglotti A, Grasso L, Giani C. Relevance of estrogen and progesterone enzyme immunoassay in malignant, benign and surrounding normal thyroid tissue. J Endocrinol Invest 1996; 19(3): 159-64.
[15] Colomer A, Martinez-Mas JV, Matias- Guiu X, Llorens A, Cabezas R, Prat J et al. Sex- steroid hormone receptors in human medullary thyroid carcinoma. Mod Pathol 1996; 9(1): 68-72.
[16] Karlsson MG, Hardell L, HallquistA.No association between immunohistochemical expression of p53,c-erbb-2, Ki-67, estrogen and progesterone receptors in female papillary thyroid cancer and ionizing radiation. Cancer Lett 1997; 120: 173-7.

[17] Lewy-Trenda , Estrogen and progesterone receptors in neoplastic and non neoplastic thyroid lesions. Pol J Pathol 2002; 53(2): 67-72.
[18] Blechet C, Lecomte P, De Calan L, Beutter P, Guyetant S. Expression of sex steroid hormone receptors in C cell hyperplasia and medullary thyroid carcinoma. Virchows Arch 2007; 450(4): 433-9.
[19] Clark OH, Gerend PL, Davis M, Goretzki PE, Hoffman PG Jr. Estrogen and thyroid-stimulating hormone (TSH) receptors in neoplastic and non-neoplastic human thyroid tissue. J Surg Res 1985; 38: 89-96.
[20] Hiasa Y, Nishioka H, Kitahori Y, Yane K, Nakaota S, Ohshima M et al. Immunohistochemical analysis of estrogen receptors in 313 paraffin section cases of human thyroid tissue. Oncology 1993; 50: 132-6.
[21] Imai Y, Yamakawa M, Matsuda M, Kasajima T. Endogenous sex hormone and estrogen binding activity in thyroid cancer. HistolHistopathol 1989; 4(1): 39-45.
[22] Metaye T, Millet C, Kraimps JL, Aubouin B, Barbier J, Begon F. Estrogen receptors and cathepsin D in human thyroid tissue. Cancer 1993; 52(6): 1991-6.
[23] Van Hoeven KH, Menendez-Botet CJ, Strong EW, Huvos AG. Estrogen and progesterone receptor content in human thyroid disease. Am J ClinPathol 1993; 99: 175-81.
[24] Yane K , Kitahori Y, Konishi N, Okaichi K, Ohnishi T, Miyahara H et al. Expression of the estrogen receptor in human thyroid neoplasms. Cancer Lett 1994; 84(1): 59-66.
[25] Tavangar SM, Monajemzadeh M, Larijani B, Haghpanah V. Immunohistochemical study of oestrogen receptors in 351 human thyroid glands. Singapore Med J 2007; 48(8): 744-7.
[26] Dan Chen, Wenjing QI, Pengxin Zhang, Lifen Wang. Expression of the estrogen receptor α, progesterone receptor and epidermal growth factor receptor in papillary thyroid carcinoma tissues. ONCOLOGY LETTERS 2015; 10: 317-320.
[27] Huang Y, Dong W, Li J, Zhang H, Shan Z and Teng W. Differential expression patterns and clinical significance of estrogen receptor-α and β in papillary thyroid carcinoma.BMC Cancer. 2014 May 29; 14: 383.
[28] Y Jin, Y Wang, F. Li, Z-Y.Si . Expression of ER ,PR and Cerb-2 in papillary thyroid carcinoma and their clinical significances. Cancer Research and Clinic 2013; 25(5): 339-341.
[29] Kawabata W, Suzuki T, Moriya T, Fujimori K, Naganuma H, Inoue S et al. Estrogen receptors (α and β) and 17 β–hydroxysteroid dehydrogenase type 1 and 2 in thyroid disorders: possible in-situ estrogen synthesis and actions. Mod Pathol 2003; 16(5): 437-44.
[30] Kansakar E ,Chang YJ, Mehrabi M, Mittal V. Expression of estrogen receptor, progesterone receptor and vascular endotheluial growth factor-A in thyroid cancer. Am Surg 2009; 75(9): 785-9.
[31] Kavanagh DO, Mcllroy M, Myers E, Bane F, Crotty TB, Mc Dermott E et al. The role of oestrogen receptor {alpha} in human thyroid cancer: Contributions from coregulatory proteins and the tyrosine kinase receptor HER2. EndocrRelat Cancer 2010; 17: 255-64.
[32] Egawa C, Miyoshi Y, Iwao K, Shiba E, Noguchi S. Quantitative analysis of estrogen receptor-α and β messenger RNA expression in normal and malignant thyroid tissues by real time polymerase chain reaction. Oncology 2001; 62: 293-8.