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IMMUNOHISTOCHEMICAL EXPRESSION OF BETA CATENIN IN THYROID TUMORS – A PROSPECTIVE STUDY

Pathology

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ABSTRACT

Aim and objectives: To evaluate the expression of beta-catenin in well differentiated and poorly / undifferentiated thyroid tumors. Also to identify tumors which may not have favourable outcome.

Methods : In this study , paraffin blocks from 30 thyroidectomy specimens are stained with monoclonal antibodies reactive with beta-catenin. These include 22 cases of papillary carcinoma of thyroid (PTC) (16 cases of conventional PTC, 3 cases of Follicular variant, 2 cases of cribriform morular variant , 1 case of hobnail variant), 4 cases of Follicular carcinoma(FC), 3 cases of Medullary carcinoma (MC) and 1 case of Poorly differentiated carcinoma (PDC)

Results : Papillary carcinoma conventional type, other variants of PTC and FC express residual membranous positivity and diffuse cytoplasmic positivity with occasional nuclear positivity. PTC,FVPC and hobnail variant show few nuclear inclusion positivity. PDC shows diffuse cytoplasmic positivity with severe loss of membrane positivity

Conclusion: Loss of membrane positivity of beta catenin with cytoplasmic and nuclear expression correlates well with aggressive behaviour i.e increased invasiveness and metastasis.

KEYWORDS

PTC- Papillary carcinoma of thyroid, FVTC- Follicular variant of papillary carcinoma of thyroid, FC- follicular carcinoma, MC-medullary carcinoma, PDC- Poorly differentiated carcinoma, Beta-catenin

INTRODUCTION

Aim of this study is to determine the role of beta-catenin in well differentiated and poorly differentiated/ undifferentiated carcinomas.

BETA-CATENIN

It is a part of membrane bound cell growth signaling complex that plays a role in cell adhesion as well as promotion of growth through activation of wnt signaling pathways. Oncogenic signaling may result in release of this beta- catenin to accumulate in cytoplasm and translocate into the nucleus there by promoting the transcription genes (bcl-1 and c-myc) to induce cell proliferation.

Hence abnormal expression of this molecule in various thyroid tumors may be helpful in predicting the outcome of the disease.

β –catenin is a protein in humans encoded by the CTNNB1. Normal thyroid follicular cell shows strong immunoreactivity for beta-catenin. Thyroid tumors may show residual/weak membranous immunoreactivity and diffuse cytoplasmic and nuclear positivity of variable intensity.

AIM :

To study the immunohistochemical expression of beta catenin in thyroid tumors and thereby comparing its expression in well differentiated and poorly differentiated / undifferentiated carcinomas.

Objectives :

1. To study the expression of beta catenin in different thyroid tumors.
2. To study the value of beta catenin in differentiating well differentiated and poorly differentiated / undifferentiated carcinomas.
3. To identify tumors which may not have favorable outcome.

Role Of Immunohistochemistry In Thyroid Tumors

For most of the thyroid tumors, diagnosis can be reached by morphology alone. However, in tumors with morphological overlap as seen in follicular adenoma, follicular carcinoma and follicular variant of papillary carcinoma, diagnosis may be quite difficult. Nuclear features characteristic of papillary carcinoma can also be seen in hyalinising trabecular adenoma and multinodular goitre with papillary hyperplasia. Certain tumors have overlapping features.. Even follicular patterned lesions are identified based on the nuclear features like nuclear grooving, intranuclear pseudoinclusions and nuclear overlapping and interobserver variations are not uncommon. Thus it may lead to inappropriate diagnosis/nomenclature. Hence in order to overcome this difficulty in addition to the histopathological study of the tissue sections, many immunohistochemical markers are evaluated. These may really be helpful in distinguishing papillary thyroid carcinoma from other follicular patterned lesions.

MATERIALS AND METHODS:

Prospective study conducted from June 2016 to May 2017 at Coimbatore Medical College Hospital, Coimbatore. Total number of cases-30

Inclusion criteria:

1. All thyroidectomy specimens which include Total thyroidectomy, neartotal thyroidectomy, Subtotoal thyroidectomy, and Hemithyroidectomy.
2. Both male and female patients
3. All age are included

Exclusion criteria

Benign lesions of thyroid

METHODS

All those 30 cases reported as carcinomas were evaluated further with immunohistochemical analysis. Initially after receiving the specimens they were fixed in 10% formalin after processing they were embedded in paraffin block and stained with eosin and hematoxylin. After staining the slides were reported by the pathologists and categorized as the following

1. Papillary carcinoma
2. Follicular variant of Papillary carcinoma of thyroid
3. Cribriform morular variant of papillary carcinoma of thyroid
4. Hobnail variant of papillary carcinoma of thyroid
5. Follicular carcinoma
6. Medullary carcinoma
7. Poorly differentiated carcinoma

IMMUNOHISTOCHEMISTRY –PROCEDURE

From the selected cases blocks were taken.They were cut and mounted on the poly l lysine coated slides. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol. Next antigen retrieval was done by heating in microwave oven by using Tris –EDTA buffer at pH 7.5. The study was done using beta –catenin, a monoclonal antibody.

Table 1

INTERPRETATION: In the present study Interpretation of cytoplasmic staining of Beta –catenin are done as below

S.NO	GRADING	PERCENTAGE OF CELLS EXPRESSING BETA CATENIN
1.	0 (Negative)	No positively staining tumor cells
2.	1+ (focally positive)	Less than 25 % of cells shows positivity
3.	2+ (moderate positivity)	25 – 50 % of cells shows positivity
4.	3+(diffusely positive)	Greater than 50% of cells shows positivity

Table 2

In the present study Membranous Staining of beta-catenin was graded as follows

Proportion of staining	Grading	Corresponds to membrane staining
No staining	zero	negative
<25 %	1+	Severe loss of membrane staining
25% - 50 %	2+	Moderate loss of membrane staining
50% - 75 %	3+	Mild loss of membrane staining
75% -100%	4+	Membrane staining of Normal thyroid follicular cells. No loss of membrane staining

OBSERVATION AND RESULTS

A total of 30 cases of thyroidectomy specimens for different thyroid tumors, received over the period of June 2016 to May 2017 were studied. After obtaining the Ethical clearance from the Ethical committee of Coimbatore Medical College and Hospital, Coimbatore, the study was conducted. We evaluated 30 thyroid tumors which include papillary carcinoma conventional type and few other variants of papillary carcinoma, follicular carcinoma, poorly differentiated carcinoma and medullary carcinoma. In these thyroid tumors the histomorphological pattern and immunohistological pattern of expression of beta- catenin were studied, analysed and compared with the literature.

Table 3: Distribution of different thyroid tumors

Types of Carcinoma	Number of cases	Percent (%)
Papillary Carcinoma	22	73.4
Follicular Carcinoma	4	13.3
Medullary Carcinoma of Thyroid	3	10.0
Poorly Differentiated Carcinoma	1	3.3
Total	30	100.0

Table 4

Staining of Beta Catenin	Papillary Carcinoma n=22	Follicular Carcinoma n=4	Poorly Differentiated Carcinoma n=1	Medullary carcinoma thyroid n=3
0	0(0.0%)	0(0.0%)	0(0.0%)	3(100.0%)
1	1(4.6%)	1(25.0%)	0(0.0%)	0(0.0%)
2	7(31.8%)	3(75.0%)	0(0.0%)	0(0.0%)
3	14(63.6%)	0(0.0%)	1(100.0%)	0(0.0%)

Intensity of Cytoplasmic Staining of BetaCateninin Thyroid Tumors

BETA –CATENIN IN THYROID TUMORS

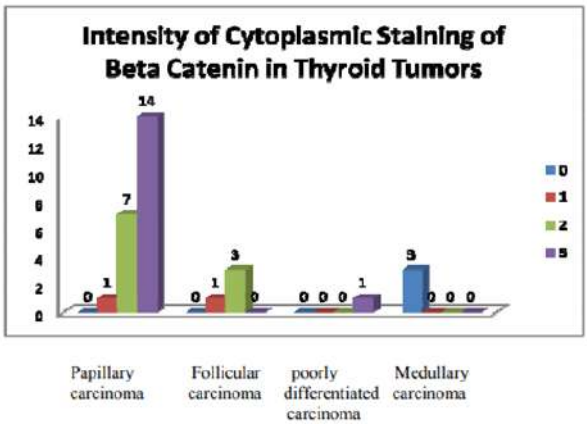

Chart 1

In the present study beta-catenin in papillary carcinoma of thyroid shows 3+ membranous positivity in 54.6% of cases (12 out of 22 cases) and 2+membranous positivity in 45.45% of cases (10 out 22 cases). Follicular carcinoma shows membrane positivity of 1+ in50% of cases (2 out of 4 cases) and membrane positivity of 2+ in remaining 50% of cases (2 out of 4 cases). In the present study, Poorly differentiated carcinoma show 1+ membranous staining and medullary carcinoma shows negative membranous staining in all the cases studied .

In overall, Papillary carcinoma show strong/moderate cytoplasmic beta catenin positivity and strong/moderate membrane positivity. In follicular carcinoma both cytoplasmic and membrane positivity are moderate or less. Poorly differentiated carcinoma show strong/diffuse cytoplasmic positivity and very minimal membane positivity.

Table 5 Statistical analysis of cytoplasmic beta-catenin expression in papillary carcinoma thyroid with and without metastasis

Papillary carcinoma	Cytoplasmic staining 3+	Cytoplasmic staining 2+	P value
With metastasis	11	0	.005*
Without metastasis	3	7	

*-Statistically significant (P<0.05)

Sensitivity=78%

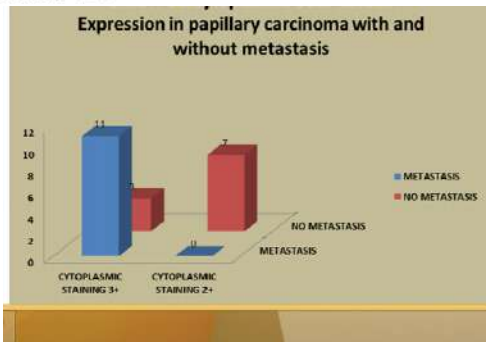

Chart 2

Table 6- statistical analysis of membranous expression of beta-catenin in papillary carcinoma of thyroid with and without metastsis

Papillary carcinoma	Membranous staining 2+	Membranous staining 3+	P value
With metastasis	9	2	0.004*
Without metastasis	1	10	

*-Statistically significant (P<0.05)

Sensitivity -90%

Specificity- 83%

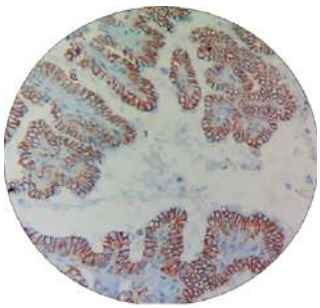

Figure: 1 IHC- papillary carcinoma conventional type shows residual membranous and cytoplasmic staining of beta-catenin. occasional nuclear inclusions show positivity Low power view(40x).

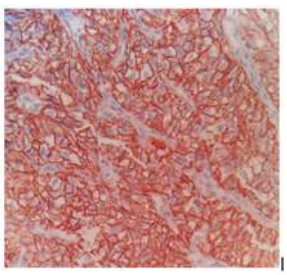

Figure: 2 IHC- papillary carcinoma conventional types shows strong cytoplasmic and residual membranous positivity of beta – catenin .High power view (40x).

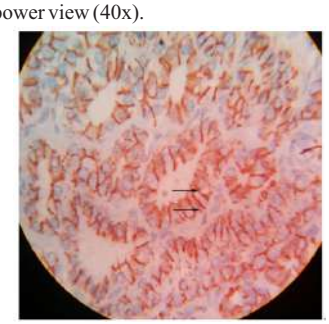

Figure 3: IHC- papillary carcinoma conventional type diffuse cytoplasmic and residual membrane positivity shown in oil immersion

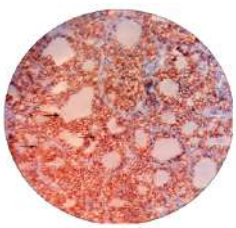

Figure 4: Follicular variant of papillary carcinoma of thyroid showing strong cytoplasmic and residual membranous staining of beta-catenin. Focal nuclear positivity also seen (arrow). Low power view (40 x).

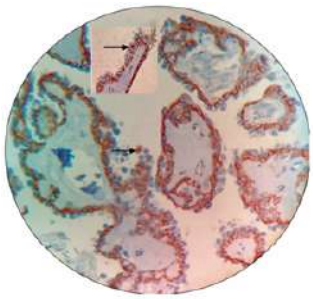

Figure 5: IHC- Cribriform morular variant of papillary carcinoma shows loss of membrane positivity (red arrow) with focal areas of strong cytoplasmic positivity(black arrow) High power view (40x).

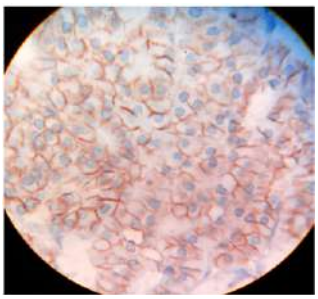

Figure 6: IHC- follicular carcinoma of thyroid shows moderate cytoplasmic positivity with focal areas of residual membranous positivity .oil immersion view(100x).

Figure 7: IHC- Diffuse cytoplasmic staining of beta-catenin in poorly differentiated carcinoma of thyroid. High power view (40x).

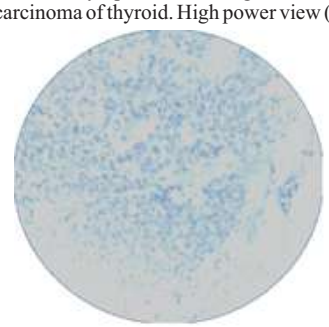

Figure 8: IHC- Medullary carcinoma of thyroid shows negative staining for beta-catenin. Low power view (10x)

DISCUSSION:

Thyroid lesions are most common endocrine tumors comprising 6 to 10%. They are more common in females and in the age group between 35 to 65 years. Every year about 3.8% of new cases of thyroid cancer occur. Prognosis and followup vary in various thyroid tumors and confirming the diagnosis is essential. Diagnosis of thyroid tumors by the usual hematoxylin and eosin stained sections may be challenging because of the overlapping histological features. Interpretation of thyroid tumors particularly follicular pattern may be difficult. Similarly the characteristic nuclear features seen in papillary carcinoma of thyroid may not be present in all cases. Encapsulated tumors with follicular pattern and some of the follicular lesions with incomplete vascular or equivocal capsular invasion may be difficult to differentiate. In these shortcomings, immunohistochemistry may be useful in distinguishing these lesions.. In this study, the usefulness of beta-catenin in differentiating the thyroid tumors and prognostic significance were analysed and it is correlated with age, sex and

histomorphology. The observation and results of this study were compared with the previously conducted studies. As per the study conducted by Farahati J et al in 1997 thyroid tumors most commonly occurred in the age group between 30 to 60 years. In our study we also observed that thyroid Tumors were common in the age group 30 to 50 years .This is in accordance with the above study Chen A Y et al in 2009, observed that thyroid tumors were more common in females when compared to males .In our study, we also observed that incidence of thyroid tumors were more in females than males, with female to male ratio of 4:1 as shown in table 2. According to the statistics released by National cancer institute in 2018 papillary carcinoma is the predominant malignancy constituting 70- 80 % of all thyroid cancers followed by follicular carcinoma constituting 10-15%. In the present study we obtained similar results with papillary carcinoma constituting 73.4%, followed by follicular carcinoma 13.3%. In 2013, Fabio Muradas Girardi et al, did a retrospective study of prevalence of variants of papillary carcinoma of thyroid in a single centre. They found out that classic or conventional type of papillary carcinoma to be the common type constituting 31.7 %, followed by follicular variant and oncocytic variant constituting 16% and 1.9% respectively. Other variants of about 0.1 to 0.7 % only. In our study, classic or conventional papillary carcinoma of thyroid was found to be the most common type constituiting 72.7% followed by follicular and cribriform variant comprising 13.6% and 9 % respectively. Hence, this correlates with the previous study which also shows predominance of conventional type of papillary carcinoma of thyroid. S.Rezk et al, studied the diagnostic utility of beta-catenin in thyroid lesions and observed that papillary carcinoma, follicular carcinoma and follicular variant of papillary carcinoma showed strong cytoplasmic / membranous staining and minimal residual membranous staining in 87%,80% and 71% respectively. They also observed that the intranuclear inclusions were distinctly positive in 83% of papillary carcinoma of thyroid and 20% of follicular variant of papillary carcinoma.

Ishigaki et al, in their study observed cytoplasmic /membrane immunoreactivity for beta –catenin in 67 % of papillary carcinoma and 25% in follicular carcinoma. In this present study papillary carcinoma showed 2+ and 3 + membranous staining and cytoplasmic positivity varying from 1+(one case only) , 2+ (in some cases) and 3+(most of the cases). Follicular carcinoma show 1+ and 2 + membranous positivity and 1+ and 2+ cytoplasmic staining. Poorly differentiated carcinoma show 1+ membranous positivity and 3+ cytoplasmic positivity.

Sethi K et al in 2011, in their study have analysed beta-catenin and few other markers as a diagnostic and prognostic marker in thyroid carcinoma. They observed that the diagnostic accuracy of beta –catenin was 96% and 94% for the diagnosis of papillary thyroid carcinoma and follicular carcinoma respectively. They also observed that they were strongly expressed in invasive papillary carcinoma and follicular carcinoma. In our study, among the 22 cases of papillary carcinoma 11 cases had metastasis and other 11 did not have metastasis. On comparing the membranous and cytoplasmic staining between those with metastasis and those without metastasis, the following observation was found. Betacatenin membrane staining in papillary carcinoma with metastasis show 2+ (corresponding to more loss of membrane staining) in 9 out of 11 cases and 3+ (corresponding to less loss of membrane staining) in 2 out 11 cases with metastasis. The cytoplasmic staining in all the 11 cases with metastasis show 3+(strong/diffuse) cytoplasmic positivity. Among the 11 cases without metastasis 10 cases showed 3+ membranous staining (corresponding to less loss of membrane staining) and 1 case showed 2+ membrane staining(corresponding to more loss of membrane staining). Cytoplasmic staining was variable with 1+ in one case , 2+ in 7 cases and 3+ in 3 cases. The 3 cases without metastasis but with 3+(diffuse/strong) cytoplasmic positivity may behave aggressively and may need more close follow up. Junk C K et al in 2009, in their study, IHC of beta-catenin found to be showing strong cytoplasmic and nuclear staining in cribriform morular variant of PTC, but in our study we observed strong cytoplasmic with occasional nuclear positivity only.

Rossai D et al in 2013, observed that 80% poorly differentiated carcinoma showed immunoreactivity for beta-catenin, which seems to be related to a worse prognostic behaviour. They concluded that poorly differentiated carcinoma represents an intermediate step in the biological process of dedifferentiation towards anaplastic thyroid carcinoma. This shift was underlined by the beta-catenin expression and hence worse clinical behaviour

Hence the loss of beta- catenin membrane reactivity and its cytoplasmic / nuclear localisation has been found to correlate with loss of differentiation and poor prognosis. In our study papillary carcinoma with metastasis show strong cytoplasmic positivity and moderate residual membrane positivity(i.e translocation of beta-catenin from membrane to cytoplasm) when compared with those without metastasis. These findings are consistent with invasive and metastatic properties of thyroid tumor. So, in our study pattern of staining in different thyroid lesions is consistent with wnt signaling pathway via translocation of beta-catenin from the cell membrane to the cytoplasm/nucleus in malignant lesions. The specificity of beta-catenin as a prognostic marker with Chi square test of these variables showed significant p value

SUMMARY AND CONCLUSION

In this study, paraffin blocks from 30 thyroidectomy specimens were stained with monoclonal antibodies for beta-catenin. Tissues from normal thyroid specimens served as control.

Papillary carcinoma conventional type, other variants of papillary thyroid carcinoma and follicular carcinoma expressed diffuse cytoplasmic and focal nuclear positivity with moderate to strong residual membranous staining. Papillary thyroid carcinoma, follicular variant of papillary thyroid carcinoma and hobnail variant show few nuclear inclusions staining positive for beta-catenin. Poorly differentiated carcinoma show strong/diffuse cytoplasmic positivity for beta-catenin and minimal with membrane positivity. Hence the loss of beta- catenin membrane reactivity and its cytoplasmic / nuclear localisation has been found to correlate with loss of differentiation and poor prognosis. Papillary carcinoma with metastasis show strong cytoplasmic betacatenin positivity and moderate membranous positivity in most of the cases when compared with the cases without metastasis . This reduction of membranous positivity along with more of cytoplasmic positivity is consistent with the progressive loss of intracellular adhesions that could be related to the invasive and hence the metastatic properties of thyroid cancer cells.

The specificity of beta-catenin as a prognostic marker with Chi square test of these variables showed significant p value

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