



Study of the Thyroid Gland's Cyclin D1 (Immune Histochemical Approach)

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Abstract

One of the most common tumors is thyroid cancer many factors contributed to this cancer one of them is cyclin dependent kinases 1 (CDKs1) which play a role in regulation of cell cycle. The study was aimed to evaluate the expression of CDK1 in thyroid carcinoma by immunohistochemical technique. There were 100 individuals enrolled in this study divided into two groups : patients with thyroid cancer 50 (20 benign thyroid cancer , 30 malignant thyroid cancer) and 50 controls as second group from October 2021 till April 2022 at Imam Hussein medical city Karbala Iraq. The thyroid tissue sample of all individual were undergone for immune histochemistry test for CDK1. The result showed significant difference (P value < 0.00001) in expression of CDK1 between benign and malignant thyroid cancer their expression in benign cases 8(40%), for malignant cases 14(46.1%) {(10 (33.33%) classical type and 4(13.33%) follicular type} only 9 (30%) while only 1% of benign cases were strong staining. All apparently healthy thyroid tissue was negative for expression of CDK1. The study was concluded that

Cyclin D1 overexpressed in Papillary thyroid carcinoma in comparison with follicular adenoma.

Introduction

Cyclin D1 and other cyclins that serve as CDK activators and CDK inhibitors, respectively, promote cell cycle advancement. It is becoming more and more clear that human cancer is caused by changes in the cyclin genes and an overexpression of different cyclins. There is much research to be done on Cyclin D1's function in the development of thyroid papillary carcinomas [1, 2, 3]. One of the characteristics of cancer is the breakdown of regulatory control over the cell cycle, which results in unchecked cell growth. Cyclins, which are cyclin-dependent kinases' (CDKs') regulatory components, regulate how quickly replicating cells move through critical cell cycle checkpoints [2, 3, 4, 5]. The cell cycle progresses from G1 to S. The cyclin achieves its maximum concentration buildup in the late stages of G1 and thereafter disappears. Cyclin D1 and growth factors are coupled while a cell is in the S phase of a cell cycle to encourage cells that are not currently active to enter the cell cycle [6, 7]. Excessive proliferation of cells with a truncated G1 phase that do not respond to normal growth stimuli results in a disproportionate buildup of cyclin D [11, 8]. Gene translocation, viral insertions in the cyclin gene area, gene duplication, and chromosomal fragmentation can all lead to overexpression. It can also be caused by cancer cells becoming more sensitive to outside stimuli. Examples include using hormones to activate the body [9, 10, 11]. Damage to the genetic system causes a shift in the levels of key components that control the cell cycle and other processes. Pathogenic factors interfering with the organ's normal function. Homeostasis issues are caused by internal control points in the cell's interior. The loss of homeostasis is a condition that occurs when the body's equilibrium is disrupted, which can lead to a variety of problems. Neoplastic transformation is a term used to describe the change of a cell into a different cell type [12, 13]. Overexpression of the cyclin D1 protein has been associated with a more aggressive behavior in a variety of cancers, phenotype of the tumor and a worse prognosis. The most frequent genetic mutation that results in CDK1 overexpression is cyclin D1 amplification, which has been shown to occur in a significant portion of breast, esophageal, lung, and other malignancies, as well as squamous carcinomas of the head and neck [14, 15]. It is unknown how CDK1 influences the development of thyroid carcinoma. On immunohistochemistry, normal thyroid cells do not show nuclear positive for cyclin D1. However, Hurthle cell carcinomas are also common, and papillary thyroid carcinomas have demonstrated evidence of CDK1 expression [16, 17, 18]. In the majority of instances, immunohistochemical examinations of normal thyroid follicular cells revealed no nuclear staining for CDK1. The expression of CDK1 is either nuclear or cytoplasmic, or both, according to certain researchers, who have shown CDK1 immunoreactivity in the cytoplasm of normal thyroid follicular cells [18]. Compared to follicular adenoma, papillary thyroid cancer had significantly higher nuclear CDK1 immunoreactivity [19]. According to certain research, thyroid oncogenesis is influenced by cyclin D1 expression. The expression of cyclin D1 is an early event in carcinogenesis, in contrast to how most authors treat these alterations [20, 21, 22].

Material And Method:

Case control study was included 100 individual enrolled in Imam Hussein Medical City in Karbala from period October 2021 till April 2022. All the consent had been taken from Karbala Health Directorate, Karbala Medical College Research Committee and Imam Hussein Medical City in addition to verbal consent was taken from the patients to use their tissue biopsies and their data for research purpose. The sample involved in this study were divided into two groups. First group patients with thyroid tumor were 50 cases with mean age 43.68 (17- 76). 20 of those were benign (15 female and 5 male), 30 malignant (22 female and 8 male) while the other group were control 50 (40 female and 10 male) their age matches the age of patients. All the cases were diagnosed by two specialist histopathologists each evaluated every case to confirm the diagnosis. The thyroid tumor was divided into follicular adenoma and papillary

carcinoma. The follicular adenoma was appeared as discrete, single tumors, encapsulated, with the non-neoplastic gland was typically compressed by adenomas. Regardless of the tumor's growth style or level of aggressiveness, PTC-nuclear alterations such nuclear clearance, nuclear grooves, expansion, and overlapping, as well as cytoplasmic pseudoinclusions, are the diagnostic criteria for papillary thyroid carcinoma malignancy. Based on the criteria outlined by LiVol [23], the follicular variant of papillary thyroid carcinoma was chosen. The malignant thyroid tumor showed modest transcapsular invasion, and the other had a vascular invasion core. The majority of papillary thyroid malignancies were encapsulated tumor [8].

Immunohistochemical Analysis

The postoperative material was embedded in paraffin after being fixed in 10% buffered formalin. One portion was chosen for the immunohistochemistry research after standard histological assessment and diagnosis. Deparaffinized sections (3 m thick) were then rehydrated using a succession of xylene and alcohol. After antigen retrieval by microwave treatment in 10mM citrate buffer, endogenous peroxidase activity was suppressed in 3% hydrogen peroxide (pH 6.0). Cyclin D1 monoclonal antibody (Clone: DCS-6, DAKO/Denmark), diluted 1:3. For detection, HRP was used with the DAKO LSAB+ System. It was done with diaminobenzidine (Liquid DAB+, DAKO/Denmark). when the sections were counterstained using the modified Mayer's hematoxylin chromogen. Dark and homogeneous or granular nucleus staining regard as positive. Sections of the cyclin D1-highly expressed mantle-cell lymphoma served as positive controls. The immunehistochemical scoring depend in CDK1 expression was (score 0: Negative, none of the cells revealed positivity for the marker; score 1: Weak or mild staining, (5-10% in 1000 tumor cell); score 2: Moderate staining, less than 25% of tumor cells are stained positive; score3: Strong staining, (25-50%) of tumor cells are stained positive; & score 4: Highly strong staining, over 50% of tumor cells are stained positive [24].

Analyses of statistics

The data were analyzed using IBM SPSS analytic software version 20. The quantitative data was analyzed by using the Chi Square test with a P-value less than 0.05.

Result

Malignant cases of thyroid cancer 14(46.1%) were showing positive expression while benign thyroid cancer only 8(40%) of the cases were positive. All the control cases had no expression of CDK1. P-value >0.00001 was showing significant difference between benign and malignant cases in CDK 1 expression as showing in table (1).

Table (1):expression of CDK1 in papillary thyroid cancers and benign (follicular adenoma) thyroid cancer.

Type of cells	negative	positive	Case No.
Benign (follicular adenoma)	12(60%)	8(40%)	20
Malignant papillary type	16(53.9%)	14(46.1%)	30
control	50 (100%)	0	50
Total	78	22	100

P-value is less than 0.00001

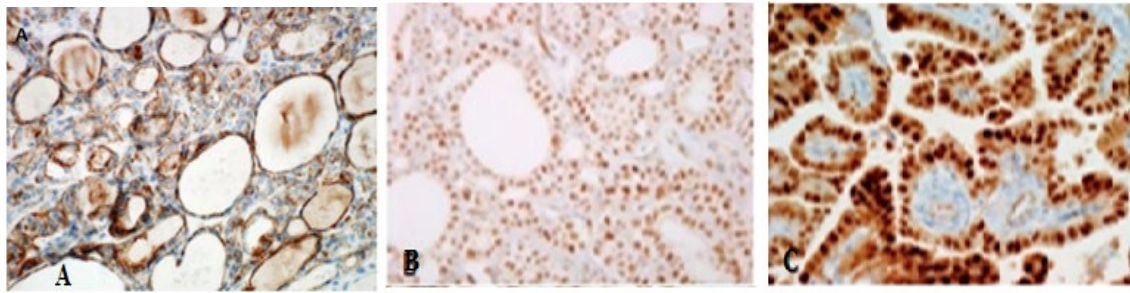


Figure 1: CDK1 expression in thyroid adenoma(a), in thyroid papillary carcinoma follicular variant (b),DK1 in classical type thyroid papillary carcinoma(c)

Table 2: Expression of Cyclin D1 with histological subtypes

Histological subtype	Negative	Positive	Case No.
Benign (follicular adenoma)	12(60%)	8(40%)	20
Classical papillary carcinoma	6(20%)	10(33.33%)	16
Papillary carcinoma follicular variant	10(33.33%)	4(13.33%)	14
Control	50 (100%)	0	50
Total	78	22	100

The p value was 0.156862 it was nonsignificant.

The CDK1 expression scoring distribution of different study categories in study sample in benign cases 4(20%) were showing low positivity ,2(10%) moderate positivity and 2(10%) strong positivity. For classical type1(3.33%) low positivity ,5(16.66%) moderate positivity and 4(13.33%) were showing strong positivity. In follicular 1(3.33%) low positivity, 1(3.33%) moderate positivity and 2(6.66%) strong positive. P value was 0.226571 as showing in table (3).

Table (3) Scoring distribution of CDK1 in different study categories

Category	Scoring				Total
	Negative	Low Positive	Moderate Positive	Strong Positive	
Benign thyroid tumor	12 (60%)	4(20%)	2(10%)	2(10%)	20
Malignant Thyroid tumor classical type	6(20%)	1(3.33%)	5(16.66%)	4 (13.33%)	16
Malignant Thyroid tumor follicular variant	10(33.34%)	1(3.33%)	1(3.33%)	2(6.66%)	14
Total	28	6	8	8	50

Discussion

Cyclin D1, a nuclear protein belonging to the cyclin family with a molecular weight of 36 kDa, controls the progression of the G1/S phase of the cell cycle by functioning as CDK4 and CDK6's regulatory component [25]. Oncogenesis and normal cell proliferation are no longer under control when cyclin expression is dysregulated. It has been shown that thyroid carcinomas, among other human malignancies, overexpress cyclin D1 [16 6 14].Cyclin D1 is immunohistochemically absent from healthy cells. Studies have been done to find out whether cyclin D1 is expressed in thyroid tumors. Muro-Cacho et al 1999 [26]. Nakashima, M., et al 2004 [17]. In this study we used immunohistochemistry to examine the expression of cyclin D1 in 100 cases, 20 benign(15 female and 5 male), 30 malignant(22female and 8 male),20 cases classical papillary cell carcinoma and 10 cases papillary thyroid carcinoma with follicular variant , and 50(40 female and 10 male) control tissues. The mean age 43.68 and ranged from 17 to years 76 patients. Liu, Haiyan, et al 2022[27].studied the

expression of cell cycle regulators, such as cyclin D1, in TMAs from 100 benign and 105 malignant thyroid tumors. They found that 45.7% of benign thyroid lesions and 87.1% of malignant thyroid lesions both overexpressed cyclin D1. 34 typical papillary cell carcinoma, 10 hardly invasive follicular thyroid 2.54 lesions compared to benign thyroid lesions, although its usefulness as a diagnostic marker is constrained by the range of staining patterns.

In conclusion, there is no overexpression of cyclin D1 in healthy thyroid tissue but a large overexpression in thyroid tumors that are more malignant than thyroid adenomas expression could contribute to the development of tumors could might have prognostic value.

Conclusion

In conclusion, Cyclin D1 was absent in normal control thyroid tissue, it is overexpressed Papillary thyroid carcinoma (classical and follicular variant) in comparison with follicular adenoma. This overexpression can help tumors grow and may also have prognostic significance.

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