Immunohistochmical Expression of Cyclin D1 Protein in Sudanese patients with thyroid cancer

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Abstract:

Background:- The thyroid cancer is the second most common type of malignancy in the worldwide is found in estimates 60,220 new cases approximately 3.6% in 2013 [1].

Objective:- The aim of the present study was to determine the prognostic value of cell cycle regulators cyclin D1 in the thyroid cancer. And correlate the immunohistochemical findings with prognostic parameters including patient's age, tumor size, histopathological types and gender.

Methods:- A total number of forty (40) Formalin fixed-paraffin embedded sections were collected from Sudanese patients with thyroid carcinoma; registered in who underwent surgery at many hospitals Such as (Ministry of Defense Hospital, Hospital of Al-Ribat University, and Yestbshroon Hospital) with random selection and the data were obtained from clinical charts and histopathology reports, H&E and immunohistochemistry for cyclin D1 were done for all samples of thyroid cancer.

Result:- The patient's results are the number of male=10 (P=4➤

10.0%, N=6 ⟶ 15.0% T=2 ⟶ 5.0%) while the number of female=30 (P=23⟶57.5 %, N=7 ⟶ 17.5%- T=7 ⟶ 5.0%), which thyroid cancer types such as (papillary carcinoma, follicular carcinoma, anaplastic carcinoma, medullary carcinoma). And the quantification was based on the Intensity and distribution of nuclear staining, expression levels of cyclin D1 has no correlation with gender, patient's age, tumor size and histopathological types, were statistically significant(P.V=<0.05).

Conclusion:- According to these findings we conclude that there is no correlation between the cyclin D1 expression and thyroid carcinomas from the side of histopathological type, gender and tumor size and patient's age.

Key words: Immunohistochmical Expression of Cyclin D1 Protein, thyroid cancer, Sudan

INTRODUCTION:

The thyroid cancer it is slow-growing cancer and typically develops in only one lobe or the two lobes of the thyroid gland [6]. The thyroid cancer shown the most rapid rise of incidence in worldwide estimates 60,220 new cases 3.6% in 2013 [1].And is6.8%among the male since 1998, and 6.9%among females since 2002[2].

According to the data has recently released by American National Cancer Institute (NCI), that is 6.5%per year from 1997 to 2006 [2], The age-standardized incidence rate for thyroid cancer was 14per 100,000 persons in Canada in 2012 [3] The thyroid cancer was the second most common malignancy among females in 2008[4], and in the Sudan it is 4.5% in 2015 [5]. And our studies focused on four types of cancers occur in the thyroid gland such as {papillary carcinoma, anaplastic carcinoma, follicular carcinoma and medullary carcinoma}. The cyclin D1 it is one of the activator types for the cyclin dependent kinases which is cell cycle regulator in G1 phase of cell cycle and it has relationship with the thyroid malignant progression

has not yet to be established. In our study we aimed to correlate between immunohistochemical expression of cyclin D1 in the histopathological types of thyroid carcinoma and prognostic parameters.

MATERIALS & METHODS:-

A total of tissue specimens are 40 patients, who received surgery for the thyroid cancer at the Histopathology Department, from many hospitals such as (Ministry of Defense Hospital, Hospital of alribat University and Yestbshroon Hospital) in 2015 were randomly selected.

Complete clinical follow up data and paraffin-embedded thyroid cancer specimens were available for all patients. We used in diagnosis the haematoxylineand eosin such as Mayer's for (7 min).While the eosin for (1 min)[7]. And in the advanced technique (immunohistochemistry) we used positively charged slides (Fisher brand). Then antigen retrieval (water bath for 40 min) and the slides will be placed in slide coplin jar containing enough sodium citrate buffer (pH 9.0).

While we use the thermo kits reagents that contains the blocking agent (peroxidase suppressor) for (10 min). The primary antibody (cyclin D1) for (20 min).

And the secondary antibody (dextran labeled polymer) for (20 min). While the chromogen3, 3 diaminobenzidine tetra hydrochloride (DAB) for (5 Min). And phosphate buffer saline (PBS) for (several times (3).The mayer's used as counterstain for (5 min) and after that bluing for (10 min) then left for drying and then put the slides in the xylene and finally the mounting media and cover glass[8, 9].

The Ethical approval was obtained from ethical committee in Sudan, Khartoum in Al-Neelain University.

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RESULTS:-

The clinical details of the thyroid cancer cases are summarized in30 patients were females while the males patients is 10 which effectively drawing male: female ratio 1:3 the most cases of thyroid cancer showed immunolabelling reaction for the cyclin D1.

We found there is no correlation between the immunohistochemical expression of cyclin D1 and gender, patient's age, tumor size and histopathological types, all that due to (P.Value=<0.05).

*Gender result:-
Female (T=30⟶ 75.0%, P=23 ⟶ 57.5%, N=7⟶ 17.5%), Male (T=10 ⟶ 25.0%, P=4 ⟶ 10.0%, N=6⟶ 15.0%), P. value= (4.596) which is statistically insignificant

*Patient's age result:-
<60 (T=11⟶ 27.5%, P=7⟶ 17.5%, N=4⟶ 10.0%), ≥60 (T=29 ⟶ 75.5%, P=20 ⟶50.0%,N=9⟶ 22.5%), P. value= (0.103) which is statistically insignificant.
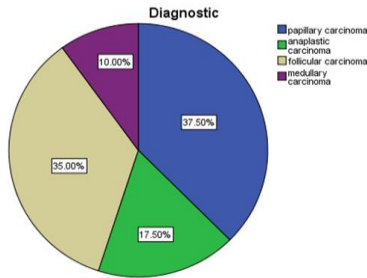
*Tumor size result:-
>5 (T=31 ⟶ 77.5%, P=19⟶ 47.5%, N=12 ⟶ 30.0%), ≤5 (T=9 ⟶ 22.5%, P=8⟶ 20.0%, N=1 ⟶ 2.5%), P. value= (2.422) which is statistically insignificant.

*Histopathological types result:-
The Papillary carcinoma (T=15 ⟶ 37.5%, P=9⟶25.5%, N=615.0%). Anaplastic carcinoma (T=7 ⟶ 17.5%, P=5 12.5%, N=2 ⟶ 5.0%), Follicular carcinoma (T=14⟶35.0%, P=1230.0%, N=2 5.0%), Medullary carcinoma (T=4

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━➤ 10.0%, P=12.5%, N=37.5%), P. value= (5.845) which is
statistically insignificant.

* (1) Descriptive pie chart proved the correlation percentages between the diagnostic parameters and reaction level:-
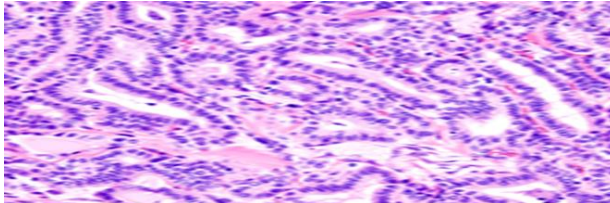

Precentages of thyroid cancer types

***Table (2) Descriptionof degree of expression of cyclin D1 in
histopathological types of thyroid cancer:-**

Reaction level	Diagnostic				Total
	papillary carcinoma	anaplastic carcinoma	follicular carcinoma	medullary carcinoma	
positive	9	5	12	1	27
% of Total	22.5%	12.5%	30.0%	2.5%	67.5%
negative	6	2	2	3	13
% of Total	15.0%	5.0%	5.0%	7.5%	32.5%
final Total	15	7	14	4	40
Final % of Total	37.5%	17.5%	35.0%	10.0%	100.0%

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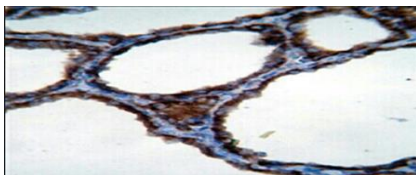with thyroid cancer**

**Table (3) Summery of Correlation of cyclin D1 expression with
histopathological characteristics:-**

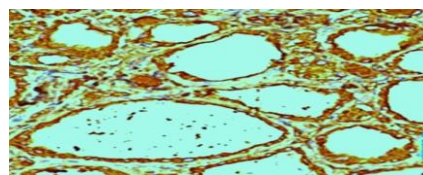| Characteris-	Case (no)	Negative	Positive	x^2 test	P-Value
Gender					
Male	10	6	4	0.032	4.596
Female	30	7	23		
Age (years)					
<60	11	4	7	0.748	0.103
≥60	29	9	20		
Tumor size (diameter)					
<5	31	12	19	0.120	2.422
≥5	9	1	8		
Histopathological types					
Papillary carcinoma	15	6	9		
Anaplastic carcinoma	7	2	5	0.119	5.845
Follicular carcinoma	14	2	12		
Medullary carcinoma	4	3	1		

*** Figures (5):- (A) Thyroid cancer tissue section with H&E, (B)
Thyroid cancer tissue sectionnegative immunoreactivity with cyclin
D1, (C) thyroid cancer tissue section positive immunoreactivity with
cyclin D1:**

(A)

(B)

(C)

STATISTICAL ANALYSIS:-

Analysis was performed using SPSS version21 for windows
exp7 and it is used for significant difference in the

immunolabelling of cyclin D1 depend on prognostic parameters such as age, sex and tumor size which the cyclin D1 expression were included in the univariate analysis to determinethe thyroid cancer univariate analysis using x^2 test, chi square, crosses tab and frequencies to determine the influence of the cyclin D1 in different prognostic parameters.

DISCUSSION:-

Although the field of thyroid oncology has much profited from a large body of information obtained by immunohistochemistry, cytogenenetics, and molecular biology, the issue of identification of the most aggressive thyroid carcinoma has not been solved [14]. Pathologists still debate about clinical and histopathological parameters and their predictive value [12], gradually turning toward new possible markers or combinations of the old and new markers.

In current study our data showed 67.5% of the thyroid carcinoma had positive cyclin D1 staining and this results are close to those of Temmim L 1 et al [15] which their study demonstrated 70% of cyclin D1 positively in thyroid carcinoma cases, but less than those of Khoo ML et al [11] which demonstrated 89% positivity.

The types of thyroid carcinoma (Medullary carcinoma, papillary carcinoma, follicular carcinoma and anaplastic carcinoma) had positive cyclin D1.

In our study cyclin D1 expression has no correlation with the histopathological types, gender and tumor size and patient's age, and is support those of Temmim L 1 et al [15] and Khoo ML et al [11]; which they found there is no correlation between the cyclin D1 and Prognostic parameters as histopathological types, gender and tumor size but not with patients age.

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But our against those ofWangs1 et al [13] and Valdi Pesutic-
Pisac 1 et al [16]; which their result indicated the cyclin D1 was
associated significantly with the prognostic parameters.
So finally we advise that only cyclin D1 is immunoreactivity
marker that could be helping full in the diagnosis which that
has no correlation with the prognostic parameters.

CONCLUSION:-

According to these findings we conclude that the
immunohistochemical expression and analysis of cyclin D1 has
no correlation with the thyroid   carcinoma andprognostic
parameters.

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