بسم الله الرحمن الرحيم

Sudan University of Science and Technology
College of Graduate Studies

Immunohistochemical Detection of Cyclin D1 in Thyroid Tumors among Sudanese Patients

الكشف النسيجي الكيميائي المناعي عن السايكلين دي1 في أورام الغدة الدرقية لدى المرضى السودانيين

A Dissertation Submitted In Partial Fulfillment for the Requirement of M.Sc. Degree in Medical Laboratory Science (Histopathology and Cytology)

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الآية

قال تعالى :

"إِنَّ الَّذِينَ آمَنُوا وَعَمِلُوا الصَّالِحَاتِ إِنَّا لَا نُضِيعُ أَجْرَ مَنْ أَحْسَنَ عَمَلا"

صدق الله العظيم

(سورة الكهف : الآية 30)

Dedication

I dedicate my dissertation to:

My parents

Sister & brothers

My family & friends

&

Those who played a great role in my life

Acknowledgements

Thanks first and last for **Allah**, who gave me strength, wellness and health to start and complete this study.

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For all those who have support, stood by me, and helped me in this research, I send my thanks and deepest gratitude.

Abstract

This is a hospital based analytical case control study was conducted at Omdurman Teaching Hospital during the period July to September 2017. The study aimed to detect Cyclin D1 in thyroid tumors among Sudanese patients.

A total of 40 formalin fixed paraffin blocks previously diagnosed as thyroid tumors were selected. 17 (42.5%) samples were papillary thyroid carcinoma , 9 (22.5%) were follicular thyroid carcinoma, 2 (5%) were medullary thyroid carcinoma, 2 (5%) were anaplastic thyroid carcinoma and 10 (25%)samples were benign thyroid tissue (multinodular goiter), one section of 3μm thickness was cut by rotary microtome from each block and stained by immunohistochemical method (avidin-biotin immune technique) for Cyclin D1detection. The data obtained was analyzed using SPSS computer program version 16.

Patients age ranged between 11 to 65 years with mean age 37 years, most of the patients were less than 45 years.

The sex of the patients revealed that 10 (25%) were males and 30 (75%) were females.

Positive expression of Cyclin D1 was detected in (24/30) of malignant samples, while (6/30) samples showed negative expression, and (8/10) of multinodular goiter samples showed negative expression for cyclin D1, and (2/10) samples were positive. This result showed significant association between expression of cyclin D1 and type of thyroid tumors (P.value=0.001).

The study concludes that the positive expression of Cyclin D1 is associated with malignant thyroid tumors rather than benign tumors.

المستخلص

أجريت هذه الدراسة المستشفوية التحليلية الحالة الضابطة في مستشفى أم درمان التعليمي خلال الفترة من يوليو الى سبتمبر 2017 لتحديد دور سايكلين د1 في أورام الغدة الدرقية لدى المرضى السودانيين.

جمع أربعون قالب شمعي مغمورة في البرافين تم تشخيصها مسبقا اورام الغدة الدرقية. كان تشخيص كالاتي : 17 (42.5%) عينة من الورم الحليمي الخبيث للغدة الدرقية، و 9 (22.5%) عينات من سرطان الغدة الدرقية الجرابي، عينتين (5%) من سرطان الغدة الدرقية النخاعي و عينتين (5%) من سرطان الانابلاستيك، و 10 (25%) عينات تضخم الغدة الدرقية الحميد. تم قطع القوالب بسمك ثلاثة مايكرون وصبغها بطريقة الكشف النسيجي الكيميائي المناعي باستخدام تقنية البايوتين افيدين للكشف عن سايكلين د1. تم تحليل البيانات التي تم الحصول عليها باستخدام برنامج الحزم الإحصائية للعلوم الاجتماعية اصدار 16.

تراوحت أعمار المرضى بين 11 الى65 سنة مع متوسط عمر 37 سنة ومعظم اعمارالمرضى تركزت تحت 45 عاما.

كان جنس المرضى 10 رجال(25%) و 30 (75%) منهم امراة.

تم الكشف عن التعبير الإيجابي لسايكلين د1 في (24\30) من العينات الخبيثة، في حين أظهرت (6\30) عينات التعبير السلبي، و (8\10) من العينات الحميدة تم التعبير السلبي فيها للسايكلين د1 و (2\10) اطت نتيجة ايجابية. وأظهرت هذه النتيجة ارتباطا معنويا بين التعبير الإيجابي للسايكلين د1 وأورام الغدة الدرقية. . (القيمة الاحتمالية=0.001).

خلصت الدراسة الى ان ظهور سايكلين د1 يرتبط مع الاورام الخبيثة للغدة الدرقية اكثر من الاورام الحميدة.

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Chapter one

Introduction

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1.1Introduction:

Thyroid cancer is cancer originating from follicular or parafollicular thyroid cells, these cells give rise to both well-differentiated cancer (papillary thyroid cancer and follicular thyroid cancer) and poorly differentiated cancer (anaplastic thyroid cancer), the C cell or parafollicuale cell produces the hormone calcitonin and is the cell of origin for medullary thyroid cancer (Carling, *et al*. 2011).

Thyroid cancer is the most common endocrine malignancy with the increasing new patients diagnosed worldwide. Thyroid cancer diagnoses are increasing at a rate faster than any other malignancy in the United States. In 2017, there will be 56,870 new cases, accounting for 3.4% of all cancers, and 2,010 people will die of thyroid cancer.(National cancer institute. 2017)

Thyroid cancer incidence rates are three-fold higher in women than in men (Siegel, *et al*. 2017).

Thyroid cancer in the African continent are as follows (papillary: 6.7-72.1%, follicular: 4.9-68%, anaplastic: 5-21.4%, and medullary: 2.6%-13.8%) (Anthonia, *et al*. 2011).

In Sudan, Follicular carcinoma is most common type 43%, followed by papillary 27%, anaplastic 10% and 2% for medullary carcinoma (Elmakki, 2017).

The risk factors of thyroid cancer are multifactorial factors such as environmental, genetic and hormonal factors. Radiation is also a causative factor for follicular adenomas and carcinomas (Vinay, *et al*. 2004).

The clinical approach for thyroid tumors should start with proper history taking and clinical examination, morphologic assessment, blood test and immunohistochemical studies on tissue biopsies (Chan, 2000).

treatement of thyroid cancer is primarily by surgery (Kebebew, *et al*. 2000). Also suppression of thyroid function is carried out routinely by the exogenous administration of thyroid hormones (Rosai. 2004).

Cyclin D1, a member of the family of cyclins, is a 36-kD, a nuclear protein that functions as the regulatory subunit of cyclin-dependent kinases (CDK4 and CDK6) and controls the progression of the G1/S phases of the cell cycle (Yang, *et al*. 2006).

Deregulation of cyclin expression results in the loss of control of normal cell growth and oncogenesis (Wang, *et al*.1998).

Over expression of cyclin D1 has been demonstrated in thyroid carcinomas (Lee, *et al*. 2010).

Melck (2007) evaluated the expression of cell cycle regulators, including cyclin D1, in tissue microarrays of 100 benign and 105malignant thyroid lesions, he demonstrated over expression of cyclin D1 in 87.1% of malignant and 45.7% of benign thyroid lesions.

1.2 Objectives:

1.2.1 General objective:

To detect the expression of cyclin D1 in thyroid tumors among Sudanese patients and its correlation with histopathology diagnosis.

Chapter two

Literature review

Chapter two
Literature review

2. Literature review:

2.1Location and description:

The thyroid gland is a butterfly-shaped organ that sits at the front of the neck. It composed of two lobes, left and right, connected by a narrow isthmus, it weighs 25 grams in adults (Neil, *et al*. 2008). The gland is usually larger in women, and increases in size in pregnancy (Ort, *et al*. 2007).

2.2Histology:

At the microscopic level, there are three primary features of the thyroid, follicles, follicular cells, and parafollicular cells (Fawcett, *et al*. 2002).

Thyroid follicles are small spherical groupings of cells, play the main role in thyroid function (Neil, *et al.* 2008).

They consist of a rim that has a rich blood supply, nerve and lymphatic presence, that surrounds a core of colloid that consists mostly of thyroid hormone precursor proteins called thyroglobulin (Young, *et al*. 2006).

Follicular cells vary in shape from flat to cuboidal to columnar (Young, *et al*. 2006). Scattered among follicular cells and in spaces between the spherical follicles is another type of thyroid cell, parafollicular cells (Neil, *et al.* 2008). These cells secrete calcitonin and so are also called C cells (Hazard, 1977).

2.3Disorders of the thyroid gland:

2.3.1Benign disorders:

2.3.1.1Hyperthyrodism:

Hyperthyroidism is the condition that occurs due to excessive production of thyroid hormone by the thyroid gland (Bahn, *et al.* 2011). Causes

include multinodular goiter, toxic adenoma, inflammation of the thyroid, eating too much iodine, and too much synthetic thyroid hormone (Devereaux, *et al*. 2014).

2.3.1.2Hypothyrodism:

It is a common disorders of endocrine system where is the secretion of thyroid hormone is decreased (Danish. 2010).Worldwide, too little iodine in the diet is the most common cause of hypothyroidism (Chakera, *et al*. 2012). In countries with enough iodine in the diet, the most common cause of hypothyroidism is Hashimoto's thyroiditis (Ferri, *et al.* 2010).

2.3.1.3Gravis disease:

It is an autoimmunodisease characterized by the presence of immunoglobulin G directed against the thyroid stimulating hormone receptors in the thyroid cells, affecting female 4-5 times more commonly than males (Danish, 2010). About 25% to 80% of people with the condition develop eye problems. (Brent, *et al*. 2008).

2.3.1.4Diffuse nontoxic goiter and multinodular goiter:

Enlargement of the thyroid, or goiter is the most common manifestation of thyroid disease. They reflect impaired synthesis of thyroid hormone, most often caused by dietary iodine deficiency. The degree of thyroid enlargement is proportional to the level and duration of thyroid hormone deficiency (Vinay, *et al*. 2003).

2.3.1.5Thyroditis:

Thyroiditis is the inflammation of the thyroid gland due to various causes like autoimmune disorders, infections, drugs, exposure to physical agents, radiation and without any apparent predisposing factors (Shashikala, *et al*. 2016).

2.3.2Neoplasm of the thyroid gland:

2.3.2.1Adenoma:

It is encapsulated tumor of the thyroid showing evidence of follicular cell differentiation. Follicular adenoma is difficult to analyze because of the lack of

consistent criteria for distinguishing hyperplastic nodules and adenomas. The risk of progression to malignancy in males is relatively greater. Changing incidence of the occurrence of adenoma is related to changes in iodine intake, and in radiation exposure of differing populations (Bonnedbaek, *et al*. 2000).

2.3.2.2Carcinoma:

2.3.2.2.1Follicular thyroid carcinoma (FTC):

It is a malignant epithelial tumor. It is more common in women. At variance with papillary carcinoma, follicular carcinoma rarely occurs in children. The incidence of follicular carcinoma is higher in iodine-deficient areas. The frequency of follicular carcinoma has also decreased recently, due to the exclusion from this category of the follicular variant of papillary carcinoma. Both iodine deficiency and irradiation have been implicated in the development of follicular carcinoma. The cell of origin is the follicular epithelial cell (Simoes, *et al*. 2004).

2.3.2.2.2Papillary thyroid carcinoma (PTC):

It is the most common type of thyroid cancer, representing 75% to 85 % of all thyroid cancer cases (Hu, *et al*. 2008). It occurs more frequently in women and presents in the 20–55 year age group. It is also the predominant cancer type in children with thyroid cancer, and in patients with thyroid cancer who have had previous radiation to the head and neck, it is often well-differentiated, slow-growing, and localized, although it can metastasize (Dinets, *et al*. 2012).

2.3.2.2.3Medullary thyroid carcinoma (MTC):

MTC comprises 5 to 10% of all thyroid malignancies. Two forms of medullary carcinoma exist: sporadic and familial. Up to 25% of these tumors are heritable (Guiu, *et al*. 2004).

2.3.2.2.4Anaplastic thyroid carcinoma (ATC):

It is highly malignant tumor that histologically appears wholly or partially composed of undifferentiated. Occurs mainly in the elderly, with a female to male ratio of 1.5:1.The incidence is approximately one or two cases/million annually. A higher incidence has been reported in endemic goiter regions. Most cases of anaplastic carcinoma show evidence of a pre-existing differentiated or poorly differentiated thyroid carcinoma (Ordonez, *et al*. 2004).

2.4Epidemiology:

Thyroid cancer is the most common endocrine malignancy with the increasing new patients diagnosed worldwide.

Thyroid cancer diagnoses are increasing at a rate faster than any other malignancy in the United States. In 2017, there were 56,870 new cases, accounting for 3.4% of all cancers, and 2,010 people will die of thyroid cancer (National cancer institute. 2017).

The incidence of thyroid cancer in South Korean aged 50–59 in the years 2003–2007 was 120 cases per 100000 (Vaccarella, *et al*. 2016).

Thyroid cancer in the African continent are as follows (papillary: 6.7-72.1%, follicular: 4.9-68%, anaplastic: 5-21.4%, and medullary: 2.6%-13.8%) (Anthonia, *et al.* 2011).

In Sudan, Follicular carcinoma is most common type 43%, followed by papillary 27%, anaplastic 10% and 2% for medullary carcinoma (Elmakki, 2017).

Of the all 26,652 new cancer cases in Sudan registered between 2000 and 2006 in both sexes, the top five cancers were cancer of the breast 4652 (17.5%), while the thyroid were 349 (2.5%) (Elimam, *et al*. 2014).

2.5Risk factors of thyroid cancer:

2.5.1 Radiation:

Exposure to ionizing radiation is a well documented risk factor for thyroid cancer. (Gabriella, *et al*. 2013).

Medical and dental diagnostic examinations have specifically increased thyroid exposure to X-rays (Mettler, *et al*. 2000).

2.5.2TSH levels and iodine intake:

Iodine deficiency causes an increase of thyroid stimulating hormone (TSH), a major growth factor for thyroid follicular cells. There is a clear increase of thyroid cancer after prolonged iodine deficiency leading to increased TSH (Dal, *et al*. 2009).

2.5.3Thyroid nodules:

The prevalence of malignancy in single nodule has been estimated at 5% (Hegedus, 2004).

2.5.4Diet, lifestyle, and environmental pollutants:

Dietetic factors that interfere with iodine organification and thyroid hormone synthesis, such as cruciferous vegetables, could also affect thyroid cancer risk (Peterson, *et al*. 2012).

Environmental pollutants like asbestos, benzene, formaldehyde, may induce abnormal thyroid cell proliferation, favoring a precancerous state (Zhang, *et al*. 2008).

2.5.5 Reproductive factor:

It has been hypothesized that estrogen increases the levels of TSH in the body, in turn increasing thyroid growth and thyroid cancer (Schottenfeld, *et al*. 2006).

2.5.6 Family history and age:

Thyroid cancer risk has been examined in relation to family history of cancer in several epidemiologic studies (Xu, *et al*. 2012).

Age is an important prognostic features of thyroid cancer, where any patient over 60year is nearly always has a highly malignant thyroid cancer with a very poor prognosis (Crile, *et al*. 1953).

2.6Diagnosis of thyroid cancer:

2.6.1 History and physical examination:

A strong family history of thyroid cancer or prior radiation exposure to the head and neck raise the suspicion of thyroid cancer (Patel, *et al*. 2006).

On physical examination, malignant nodules are harder and fixed while a benign nodule is rubbery, soft and moves easily (Udelsman, *et al*. 1999).

2.6.2 TSH measurement:

TSH measurement early in the workup of a thyroid nodule can efficiently identify patients with a nodule and hyper- thyroidism, a solitary hyperfunctioning nodule is rarely malignant (Belfiore, *et al*. 1990).

2.6.3 Measuring serum thyroglobulin (Tg):

Measuring serum thyroglobulin (Tg) in patients with thyroid cancer can assist with the long-term follow-up of patients treated for thyroid cancer (Mazzaferri, *et al*. 2003).

2.6.4Fine needle aspiration biopsy (FNAB):

Fine needle aspiration is currently regarded as the test of choice for the diagnosis and management of thyroid cancer (Cheuk, *et al*. 2004).

FNA biopsy has resulted in improved diagnostic accuracy, a higher malignancy yield at the time of surgery, and significant cost reductions **(**Castro,*et al*. 2005).

2.6.5Immunohictochemical marker:

Immunohistochemical studies are essential to confirm a diagnosis of thyroid cancer. The claimed utility of some antibodies, such as Hector Battifora Mesothelial–1 (HBME-1) that can help to distinguish malignant thyroid neoplasm from benign thyroid tumors remain to be confirmed (Chan. 2000).

Studies have focused on identifying IHC markers that can help in differentiating benign from malignant lesions, several markers have been investigated on aspiration biopsy material and histologic specimens such as CK19, galectin-3, HBME-1, CK 903, Ret oncoprotein, CD 44, CD 57, cyclin D1 and p27 (Barroeta, *et al*. 2006).

2.7Treatment of thyroid cancer:

2.7.1Surgery:

The extent of surgery for thyroid cancer remains controversial. This is especially true for small, encapsulated, well differentiated tumors, for the majority of thyroid cancer measuring 1 cm or more a total thyroidectomy is recommended (Cooper, *et al*. 2009).

2.7.2Radioactive iodine:

Remnant ablation with radioactive iodine is the standard adjuvant treatment in selected patients with thyroid cancer (Tuttle, *et al*. 2010).

2.7.3Chemotherapy:

Chemotherapy in differentiated thyroid carcinoma has been considered for decades to be the only systemic therapy with palliative purpose, but its effectiveness in well-differentiated radioiodine refractory thyroid cancer is not well established,

although there is evidence that indicates it could have greater activity with modern drugs (Ana, *et al*. 2016).

2.8Cyclin D1 and its relation with thyroid cancer:

Cyclin D1, a member of the family of cyclins, is a 36-kD, a nuclear protein that functions as the regulatory subunit of cyclin-dependent kinases (CDK4 and CDK6) and controls the progression of the G1/S phases of the cell cycle (Yang, *et al*. 2006).

Deregulation of cyclin expression results in the loss of control of normal cell growth and oncogenesis (Wang, *et al*. 1998).

Overexpression of Cyclin D1 is associated with a number of human cancers include: breast cancer, bladder carcinoma, pancreatic carcinomas, non-small cell lung cancers, and head and neck squamous cell carcinomas (Alan, 2002).

Over expression of cyclin D1 has been demonstrated in thyroid carcinomas (Lee, *et al*. 2010).

Although little is known about the role cyclin D1 plays in the pathogenesis of thyroid carcinoma, normal thyroid follicular cells do not show immunoreactivity for cyclin D1 on immunohistochemistry, but cyclin D1 is overexpressed in up to 60% of papillary thyroid carcinomas (Basolo, *et al*. 2000), (Wang, *et al*. 1998).

In 2007 diagnostic utility and evaluated the expression of cell cycle regulators, including cyclin D1 were studied, in tissue microarrays of 100 benign and 105malignant thyroid lesions, over expression of cyclin D1 was demonstrated in most of malignant thyroid lesions (87.1%) (Melck, *et al*. 2007).

Park studied the diagnostic value of cyclin D1 in the differential diagnosis of thyroid tumors, over expression of cyclin D1 found in malignant thyroid tumors (Park, *et al*. 2007).

A heterogeneity in distribution and intensity of cyclin D1 in benign and malignant thyroid tumors was reported by Seybt, which disqualifies as a primary diagnostic marker in these tumors, however, it may be helpful in distinguishing follicular adenoma and papillary thyroid carcinoma. Its expression by thyroid tumors suggests a role in tumor development and may be an early event in thyroid neoplasia (Seybt, *et al*. 2012).

Kim, *et al* (2002) studied the expression of cyclin D1 in various lesions of thyroid, their result was expressed of cyclin D1 in the nuclei of most papillary carcinomas (79.2%). Focal nuclear immunoreactivity was noted in nodular goiters (23.5%) and follicular neoplasms (10%), they conclude that most papillary carcinomas express cyclin D1, whereas it is less common in follicular neoplasms and nodular goiter, this may be helpful in diagnostically difficult cases.

Holah compared the expression of cyclin D1 between the papillary thyroid carcinoma and multinodular goiter and conclude that the expression is associated with neoplasm aggressiveness, including advanced stage and presence of lymph node metastasis. Absence of cyclin D1 expression in normal noncancerous follicular cells and multinodular goiter cases indicates their role in early carcinogenesis of papillary thyroid carcinoma (Holah, *et al*. 2017).

Chapter three

Materials and method

Chapter three

Materials and methods

3.1 Materials:

Archived tissue blocks of thyroid tumors tissues were used in this study.

3.2 Methods:

3.2.1 Study design:

This is a hospital based analytical case control study aimed to detect cyclin D1 expression in thyroid tumors tissues using immunohistochemistry.

3.2.2 Study samples:

Tissue blocks obtained from thirty samples previously diagnosed as thyroid cancer and ten samples which previously diagnosed as benign thyroid tissues were used. Patient's data (age, histopathological diagnosis) were obtained from the patient's files.

3.2.3 Study area:

This study was held in Omdurman hospital laboratory during the period from July to September 2017.

3.2.4 Sample processing:

Sections of 3μm thickness were cut by rotary microtome, mounted in positively charged slides then dewaxed in oven for 30 minutes.

3.2.5 Immunohistochemical staining:

Immunohistochemical staining was carried out using indirect streptoavidin-biotin immune technique. Tissue sections (3μm) were deparaffinized in xylene and

rehydrated in graded alcohol (100%, 90%, 70%, 50%) and water two minutes for each. Antigen retrieval was performed by using water bath with citrate buffer (pH 6.8).Then slides were incubated for ten minutes in 0.3% hydrogen peroxide to block endogenous peroxidase activity.

The slides were treated with anti cyclin D1 primary antibody for 20 minutes and washed in phosphate buffer saline (pH 7.4), then treated with biotinylated secondary antibody for 20 minutes then incubated in strepotavidin-horseradish peroxidase for 15 minutes, washed in phosphate buffer saline (pH 7.4), incubated in 3-3 diaminobenzidine tetra hydrochloride (DAB) substrate solution for 7 minutes, washed in running tap water. Then counterstained in Mayer's haematoxylin stain for 1 minute, dehydrated, cleared and mounted in DPX mounting media (Bancroft, et *al*. 2013).

3.2.6 Data analysis:

Data analysis was done using SPSS16 computer program. Frequencies mean and Chi-square test values were calculated.

3.2.7 Result interpretation:

All quality control measures were adopted; positive and negative control slides were used during immunohistochemical staining. Detection of more than 5 cells with brown nucleus per one field considered as positive result.

3.2.8 Ethical consideration:

Hospital administration agreements were taken ethically for archived sample and patient’s data collection.

Chapter four

Result

Chapter Four

Results

4. Result:

The age of study population range between 11 and 65 years with mean age of 37 years.

Most of patients were more than 45 years (72.5%), while (27.5%) were more than 45 years old as indicated in table (4.1).

Most patients were females 30 (75%) while the males were 10 (25%). Table (4.2).

The study includes forty samples, 30 (75%) samples were malignant and 10 (25%) samples were multinodular goiter. The diagnosis of malignant samples include papillary thyroid carcinoma in 17 (42.5%) samples, follicular thyroid carcinoma in 9 (22.5%) samples, medullary thyroid carcinoma in 2 (5%) samples, and anaplastic thyroid carcinoma in 2 (5%) samples as indicated in table (4.3).

Cyclin D1 positive expression was found (24/30) in malignant samples, while (6/30) samples showed negative expression, while (8/10) of multinodular goiter were negative expression for cyclin D1, and (2/10) were positive for cyclin D1. This result showed significant association (P.value=0.001) as indicated in table (4.4).

Table (4.1): Distribution of age group among study population:

Age group (years)	Frequency	Percent
Less/equal 45 years	29	72.5%
More than 45 years	11	27.5%
Total	40	100.0%

Table (4.2): Distribution of sex among study population:

Sex	Frequency	Percent
Males	10	25%
Females	30	75%
Total	40	100%

Table (4.3): Distribution of histopathological diagnosis among study samples:

Type of study samples	Histopathological diagnosis	Frequency	Percent
Malignant	Papillary thyroid cancer	17	42.5%
	Follicular thyroid cancer	9	22..5%
	Medullary thyroid cancer	2	5%
	Anaplastic thyroid cancer	2	5%
Benign	Multinodular goiter	10	25%
Total		40	100%

Table (4.4): Relation between Cyclin D1 expression and histopathological diagnosis of thyroid tumor:

Histopathological diagnosis	Expression of Cyclin D1		Total	P.value
	Positive N (%)	Negative N (%)		
Malignant	24(60.0%)	6(15%)	30(75%)	0.001
Benign	2(5%)	8(20%)	10(25%)	
Total	26(65%)	14(35%)	40(100%)	

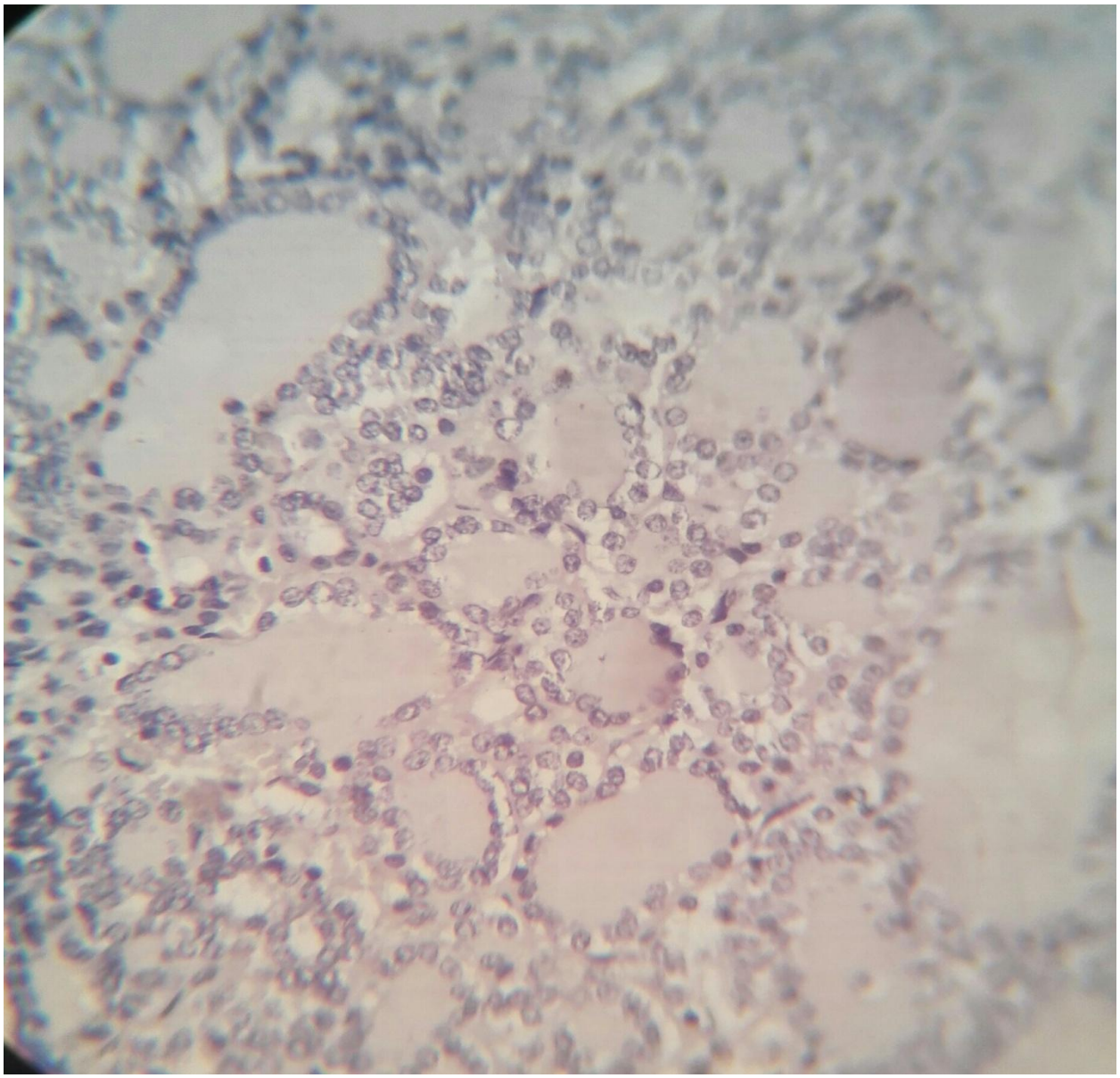

Photo (4.1): Multinodular goiter show negative nuclear expression for Cyclin D1 (40X)

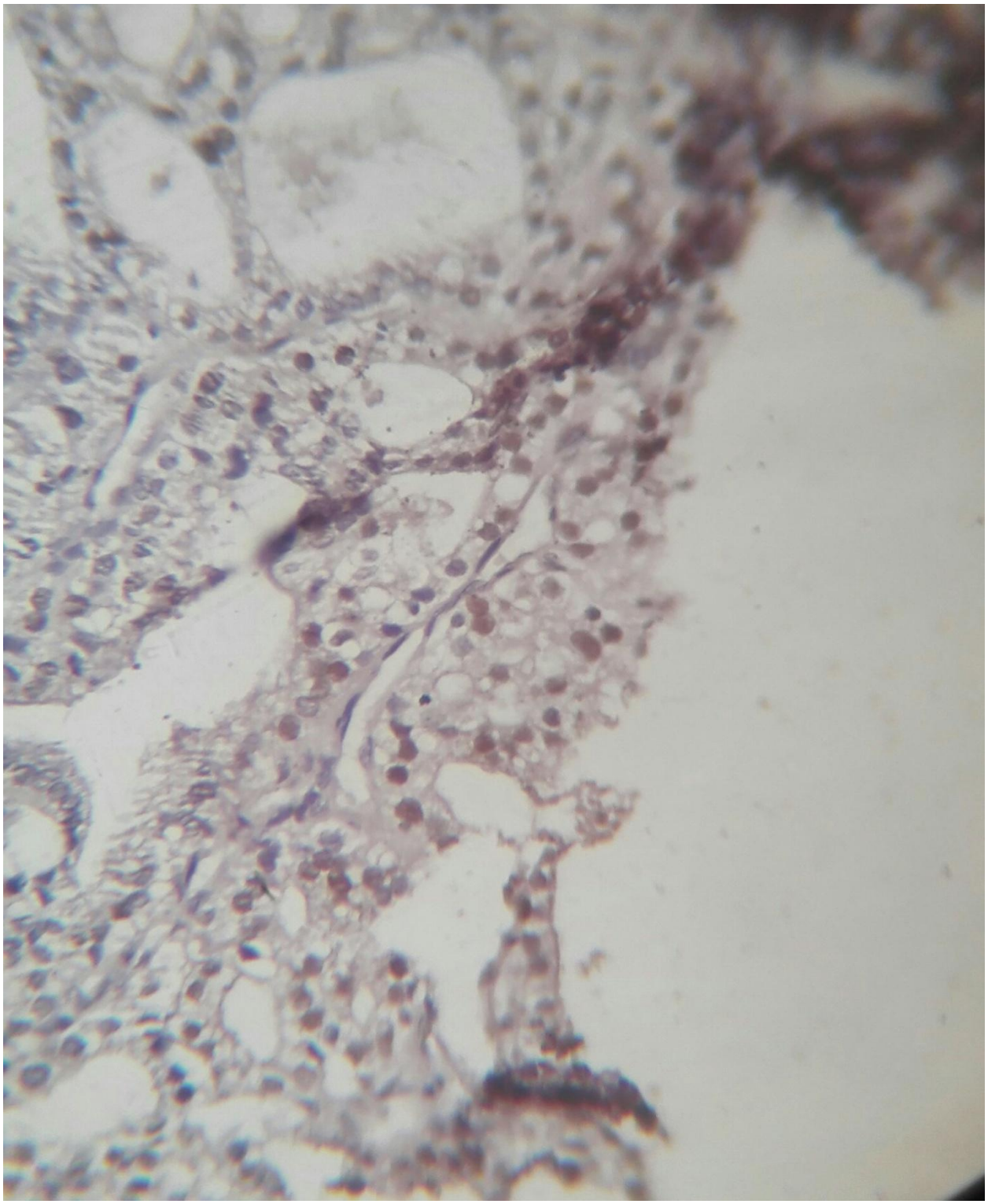

Photo (4.2): Follicular thyroid carcinoma show positive nuclear expression for Cyclin D1 (40X)

Chapter five

Discussion, conclusion and recommendations

Chapter Five

Discussion, conclusion and recommendations

5.1 Discussion:

The present study aimed at immunohistochemical staining for detection of Cyclin D1 in thyroid tumor among Sudanese patients.

Regarding the age of the patients the study revealed that most of patients were less than 45 years. This result compatible with Rosia, *et al*. (2011), who reported that the thyroid cancer more common in young and middle aged adults. The mean age at diagnosis is 37 years.

The study revealed that most of patients were females (75%). This result agrees with Girardi (2018), who reported that females were (79.6%) of most patients, also agree with Schottenfeld (2006), who hypothesized that the estrogen increases the levels of TSH in the body, in turn increasing thyroid growth and thyroid cancer.

The most histological type of thyroid cancer is papillary thyroid cancer, this result agrees with Livolsi, *et al*. (2004), who reported that papillary thyroid carcinoma (PTC), most of all thyroid carcinomas and it is the most common malignant tumor of the thyroid.

The positive expression of Cyclin D1in malignant thyroid tumors showed significant relation (P.value= 0.001). Cyclin D1 expression showed marked characteristic of nuclear staining expression within most malignant cases (papillary thyroid carcinoma, follicular thyroid carcinoma and anaplastic thyroid carcinoma). This result compatible with Park and Kwak (2007), they found over expression of this marker in malignant thyroid tumors, and compatible with Melck, *et al*. (2007), who demonstrated over expression in 87.1% of malignant thyroid tumors.

but not compatible with Khloud, *et al*. (2016), who found that there is no significant relation between Cyclin D1 expression and thyroid carcinoma from the side of histopathological type.

5.2Conclusion:

On basis of this study we conclude the follow:

- The age of the thyroid cancer among study group is commonly less than 45 years.
- Most patients of thyroid tumors were females.
- Most histological type of thyroid cancer – in the study samples – is papillary thyroid cancer.
- Cyclin D1 positive expression is associated with malignant thyroid tumor.

5.3 Recommendations:

On basis of this study we recommend the follow:

Further study should be done for expression of Cyclin D1 in thyroid cancer with large sample size.

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Appendices

Instrument and Material:

Instrument:

Disposable gloves.

Rotary microtome.

Microtome Knives.

Coplin jars.

Oven .

Staining racks.

Coated slides.

Water path.

Cover glass.

Dako pen.

Humidity chamber.

Materials:

Mayer`s haemtoxylin .

Xylene.

Ethyl alcohol (absolute, 90%, 70%, 50%).

Distilled water.

Peroxidase blocker .

Primary antibody (Cyclin D1).

Secondary antibodies (biotinylated secondary antibody).

3.3 di amino benzidine tetra hydrochloride in substrate buffer.

DPX mounting media.

Phosphate (PH 7.4) component:

Solution A (0.2 M sodium di hydrogen orthophosphate, 3.12g di sodium hydrogen orthophosphate, 100 ml DW).

Solution B (0.2 M sodium di hydrogen orthophosphate, 2.83g di sodium hydrogen orthophosphate, 100ml DW) (9.5ml from solution A +40.5ml solution B).

Citrate buffer (PH6.8) component:

Solution A (0.2M sodium di hydrogen orthophosphate, 2.83g di sodium hydrogen orthophosphate, 100ml DW).

Solution B (2.1g citric acid, 100ml DW)(27.7ml from solution A+22.8ml from solution B).

Mayer`s haematoxlin component :

Haematoxlin powder	1 gm
Potassium alum or ammonium alum	50gm
Sodium iodate	0.2gm
Citric acid	1 gm
Chloral hydrate	50gm
Distilled water	1000ml

Ammoniated water:

Concentrated ammonia	0.05ml
Tap water	99.95 ml