Sudan University of Science and Technology

College of Graduate Studies

Immunohistochemical Detection of Epithelial Membrane Antigen in Thyroid Gland Tumors Among Sudanese Patients

الكشف النسيجي الكيميائي المناعي عن مستضد الغشاء الطلائي في أورام الغدة الدرقية لدى المرضى السودانيين

A dissertation submitted for partial fulfillment for the requirement of master degree in medical laboratory science (Histopathology and Cytology)

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الآية

بسم الله الرحمن الرحيم

قال تعالي:

﴿فَقُلْتُ اسْتَغْفِرُوا رَبَّكُمْ إِنَّهُ كَانَ غَفَّارًا (10) يُرْسِلِ السَّمَاء عَلَيْكُم مِّدْرَارًا

(11) وَيُمْدِدْكُمْ بِأَمْوَالٍ وَبَنِينَ وَيَجْعَل لَّكُمْ جَنَّاتٍ وَيَجْعَل لَّكُمْ أَنْهَارًا (12)﴾

صدق الله العظيم

سورة نوح من الآية (10-12)

Dedication

I dedicate this research to my father, my mother, my sister and my brothers.

Acknowledgment

First and foremost, I thank Allah for letting me live to see this dissertation through. I am forever indebted to Alla who support and give me power to do this dissertation.

I would like to thank my supervisor Dr. Abu ElgasimAbassAwadElkareem, for his patience, continuous guidance throughout my dissertation with his knowledge.

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Thanks to all my friends in the master program.

Finally, I am grateful to my family for their constant support and encouragement.

Abstract

This is a hospital based analytical retrospective case control study which was conducted at Omdurman teaching hospital, during the period from January to April 2017. The study was aimed to detect epithelial membrane antigen expression in thyroid tumors using immunohistochemistry.

Forty paraffin embeded blocks were collected from patients samples previously diagnosed as thyroid tumors, 30 (75%) were malignant thyroid tumors and 10 (25%) were benign thyroid tumors.

One section of 3μm from each paraffin block was cut by rotary microtome, then stained by immunohistochemical method (new indirect technique). The data obtained was analysed using SPSS program version 11.5 .

The age of patients ranged between 11 and 65 years with mean age of 36 years. Most of patients were less than 50 years representing 31 (77.5%) and the remaining 9(22.5%)were more than 50 years.

Epithelial membrane antigen was revealed positive result in 16/30 samples of malignant tumors while 14/30 samples showed negative expression, while all benign thyroid tumors samples 10/10 showed negative expression for epithelial membrane antigen, this result showed significant association between epithelial membrane antigen expression and tumors of thyroid gland (P. value =0.003).

Regarding the histopathological subtypes of thyroid malignant tumor, epithelial membrane antigen expression was found positive in 11/30 samples of papillary thyroid carcinoma, 3/30 samples of follicular thyroid carcinoma, 2/30 samples of hurthle carcinoma. This result showed no association between epithelial membrane antigen expression and subtypes of thyroid malignant tumors (P. value =0.076).

The study concluded that the expression of epithelial membrane antigen was associated with malignant tumors of thyroid gland with no association with histological subtype of malignant tumors.

المستخلص

أجريت هذه الدراسة المستشفوية الاسترجاعية الحالة والحالة الضابطة في مستشفى أم درمان التعليمي, في الفترة من يناير إلي أبريل2017, هدفت الدراسة للكشف عن ظهور مستضد الغشاء الطلائي في أورام الغدة الدرقية باستخدام كيمياء الأنسجة المناعية .

جمع أربعون قالب شمعي من عينات مرضى كانوا مشخصين مسبقا علي أنهم مصابون بأورام الغدة الدرقية, 30 (75%) منهم كانوا مشخصين بأورام الغدة الدرقية الخبيثة, و10 (25%) كانوا مشخصين بأورام الغدة الدرقية الحميدة .

قطعت المقاطع بسمك 3 مايكروميتر من كل قالب شمعي باستخدام المشراح الدوار وصبغت بواسطة طريقة كيمياء الأنسجة المناعية (الطريقة الجديدة غير المباشرة), واستخدام برنامج الحزم الإحصائية للعلوم الاجتماعية, النسخة 11.5 لتحليل البيانات.

تراوحت أعمار المرضى بين 11-65 عام بمتوسط عمر 36 سنة. أظهرت الدراسة أن معظم المرضى كانت أعمارهم اقل من 50 سنة, وكان عددهم 31 مريض بنسبة (75.5%) و 9 مرضى بنسبة (22.5%) كانت أعمارهمأكثر من 50 سنة .

أظهرت الدراسة أن مستضد الغشاء الطلائي كان موجب الظهور في16\30 عينه من أورام الغدة الدرقية الخبيثة وسالب الظهور في 14\30 المتبقية . بينما كل عينات أورام الغدة الدرقية الحميدة 10\10 كانت سالبة الظهور للمستضد الغشاء الطلائي، مع وجود علاقة ذات دلالة إحصائية بين ظهور مستضد الغشاء الطلائي وأورام الغدة الدرقية (القيمة الاحتمالية =0.003).

فيما يتعلق بالعلاقه بين مستضد الغشاء الطلائي والأنواع النسيجية للورم الخبيث, كان مستضد الغشاء الطلائي موجب الظهورفي11\30عينة سرطان الدرقية الحليمة، 3\30 عينة سرطان الدرقية الجريبية,2\30 سرطان خلية هرتل. مع عدم وجود علاقة ذات دلالة إحصائية بين ظهور مستضد الغشاء الطلائي والنوع النسيجي للورم الخبيث (القيمةالاحتمالية =0.076).

خلصت الدراسة إلي أن ظهور مستضد الغشاء الطلائي له ارتباط مع أورام الغدة الدرقية الخبيثة مع عدم وجود علاقة مع النوع النسيجي للأورام الخبيثة.

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Chapter One

Introduction

Chapter one

Introduction

1.1 Introduction:

Thyroid cancer is a cancer originating from follicular or parafollicular thyroid cells (Carling and Udelsman, 2014*).*

Thyroid cancer is the sixteenth most common worldwide, with around 230,000 new cases diagnosed in 2012 with rate of 6.10/100,000 women and 1.90/100,000

men (Carlo, *et al*. 2015).

Globally in 2012, estimated numbers of deaths from thyroid cancer were 27,000 in omen and 13,000 in men, corresponding to mortality rates of approximately 0.6/100,000 women and 0.3/100,000 men (Ross, *et al*. 2014).

In Sudan follicular thyroid carcinoma was the commonest (42%) followed by papillary thyroid carcinomas (22.3%) and anaplastic thyroid carcinomas (21.4%) (Bakheit, *et al*. 2012).

Risk factors for thyroid cancer include ionizing radiation, iodine deficiency, alcohol consumption, and dietary factor (Luigino, *et al*. 2009).

Methods of diagnosis of thyroid cancer is via fine needle aspirate (FNA), ultrasonography, immunohistochemistry, tissue biopsy, and molecular diagnosis (Shrikant and Hossein, 2016).

Treatment for thyroid cancer are including near-total thyroidectomy, administration of radioactive iodine, thyroid hormone suppression therapy, and chemotherapy (Jacqueline, *et al*. 2007).

Epithelial membrane antigen it is called MUC-1 (CD227) which is a high molecular weight protein expressed on the apical luminal membrane of glandular epithelial cells (Apostolopoulos, *et al*. 2015).

Ostrowski and Merino (1996), reported that epithelial membrane antigen had strong immunoreactivity in papillary thyroid carcinoma.

Ordonez (1991), reported that about 31.3% of anaplastic thyroid carcinoma showed positive reactivity for epithelial membrane antigen.

1.2 Objectives:

1.2.1 General objective:

To study the expression of epithelial membrane antigen in thyroid tumors among Sudanese patients.

1.2.2 Specific objectives:

1-To detect the expression of epithelial membrane antigen in thyroid tumor tissues using immunohistochemical method.

2-To correlate the epithelial membrane antigen expression with histopathological diagnosis of thyroid tumors.

Chapter Two

Literature Review

Chapter Two

Literature Review

2.1 Structure of the thyroid gland:

The thyroid is a highly vascular, brownish-red gland located anteriorly in the lower neck, extending from the level of the fifth cervical vertebra down to the first thoracic. The gland varies from an H to a U shape and is formed by 2 elongated lateral lobes with superior and inferior poles connected by a median isthmus, with an average height of 12-15 mm, overlying the second to fourth tracheal rings (Cumming, *et al*.1998).

The isthmus is encountered during routine tracheotomy and must be retracted (superiorly or inferiorly) or divided, Occasionally, the isthmus is absent, and the gland exists as 2 distinct lobes (Williams, and Bannister, 1995).

Each lobe is 50-60 mm long, with the superior poles diverging laterally at the level of the oblique lines on the laminae of the thyroid cartilage. The lower poles diverge laterally at the level of the fifth tracheal cartilage (Naido, *et al*. 2007) .

Epithelial cells are of 2 types: principal cells (follicular) and parafollicular cells (clear, light cells). Principal cells are responsible for formation of the colloid (iodothyroglobulin), whereas parafollicular cells produce the hormone calcitonin, a protein central to calcium homeostasis. Para follicular cells lie adjacent to the follicles within the basal lamina (Gravante, *et a*l. 2007).

2.2 Pathology of the thyroid gland:

2.2.1 Hyperthyroidism:

It is defined as the presence of free thyroxine and triiodothyronine levels within the reference range and a reduced serum thyroid stimulating hormone (TSH) level (Molla and Belete, 2016).

2. 2. 2 Hypothyroidism:

It is defined as failure of the thyroid gland to produce sufficient thyroid hormone to meet the metabolic demands of the body (David, *et al*. 2012).

2.2.3 Tumors of the thyroid gland:

2.2.3.1 Benign tumors:

Most of thyroid nodules are benign colloid nodules composed of irregularly enlarged follicles containing abundant colloid (Sheppard and Frankly, 1992). Causes of benign solitary nodules are thyroid adenomas, cysts, thyroiditis or it may be the largest nodule of a small, clinically unrecognized multinodular goiter (Tan, *et al*. 1995).

Multinodular goiter is a structurally and functionally heterogeneous thyroid enlargement, most often caused by iodine deficiency (endemic goiter) or by medication, malnutrition, inherited defects in thyroid hormone synthesis, and growth stimulating antibodies. With increasing age thyroid function may become more autonomous and subclinical hyperthyroidism or overt hyperthyroidism may develop (Berghout, *et al*. 1990).

2.2.3.2 Malignant tumors:

Thyroid cancer is the most common malignant disease in endocrine system and is rapidly increasing in incidence (Jemal, *et al*. 2011) .

According to World Health Organization (WHO) 2004 classification thyroid cancer classified into, Primary epithelial thyroid cancer which comprise many types e. g : Papillary carcinoma ,Follicular carcinoma, Poorly differentiated carcinoma ,Undifferentiated (Anaplastic) carcinoma .And secondary non epithelial thyroid cancer e. g Angiosarcoma , Teratoma , Smooth muscle tumors (Katoh, *et al*. 2015).

2.2.3.3 Malignant thyroid subtypes:

2.2.3.3.1 Primary epithelial thyroid cancer:

2.2.3.3.1.1 Papillary thyroid carcinoma:

It is the most common type of thyroid cancer, representing 75 percent to 85 percent of all thyroid cancer cases. 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).

2.2.3.3.1.2 Follicular thyroid carcinoma:

It is the second malignant tumor originating from the follicular cells of the thyroid, and it is accounts for 10–20 % of differentiated thyroid carcinomas (Asari, *et al*. 2009).

In iodine-deficient areas, the relative rate of FTC tends to be higher, up to 40 % of all cases of differentiated thyroid carcinomas (Avanzo, *et al*.2004).

Thyroglobulin (Tg) can be used as a tumor marker for well-differentiated follicular thyroid cancer (Belge, *et al*. 2008).

2.2.3.3.1.3 Medullary thyroid carcinoma:

It is a form of thyroid carcinoma which originates from the parafollicular cells (C cells), which secrete calcitonin and carcinoembryonic antigen (CEA), which are sensitive biomarkers for the disease (Dionigi, *et al*. 2007).

Medullary tumors is a rare form of thyroid cancer comprising approximately 4% of all thyroid cancers (Maria, *et al*. 2014).

Patients present with a thyroid nodule with or without cervical lymphadenopathy, and frequently with distant metastases to the liver, lungs, and bone (Roman, *et al*. 2006).

It is occurs in hereditary and sporadic forms, and it is aggressive behavior is associated with the clinical presentation and type of RET mutation (Stamatakos, *et al*. 2011).

2.2.3.3.1.4 Squamous cell thyroid carcinoma:

It is a rare malignant neoplasm of thyroid gland which shows tumor cells with distinct squamous differentiation. The incidence of SCTC is less than 1% out of thyroid malignancies (Syed, *et al*. 2011).

The Squamous cell thyroid carcinoma is biologically aggressive malignant neoplasm which is associated with rapid growth of neck mass followed by infiltration of thyroid-adjacent structures (Booya, *et al*. 2006).

2.2.3.3.1.5 Hürthle cell carcinoma:

It is a variant of follicular cell carcinoma of thyroid. It may present as a low-grade tumor or as a more aggressive type (Mohamed, *et al*. 2004).

The natural history of Hürthle cell carcinoma (HCC) is not well understood. It is accounts for <5% of all differentiated thyroid malignancies. Hürthle cells are characterised by eosinophilic cytoplasm with trabecular/follicular growth pattern (Baloch, *et al*. 2001).

2.2.3.3.1.6 Anaplastic thyroid cancer:

It is a rare aggressive tumor arising from the follicular cells of the thyroid gland, and it is a highly aggressive tumor that belongs to the group of killer tumors with median survival time not longer than 6-8 months (Chiacchio, *et al*. 2008).

2.2.3.3.2 Secondary non epithelial thyroid cancer:

2.2.3.3.2.1 Primary thyroid lymphoma :

It is a lymphoma involving the thyroid gland alone, accounts for only 5% of all thyroid malignancies and approximately 3% of all non-Hodgkin's lymphoma (Ansell, *et al*.1999).

It is a rare malignancy intrinsically associated with Hashimoto's thyroiditis, presenting with an enlarging goiter and associated pressure symptoms (Siun, *et al*. 2013).

2.2.3.3.2.2 Angiosarcoma:

It is a rare soft tissue tumor ,account for 1-2% of all sarcoma ,mainly occurring in the skin , head and neck superficial soft tissues (Joana, *et al*.2014).

2.3 Epidemiology of thyroid cancer:

Thyroid cancer is the most common endocrine cancer ,approximately 1.0%–1.5% of all new cancers diagnosed each year in the USA (Shahbaz, *et al*. 2015).

Thyroid cancer is the fifth most common cancer in women (Jemal, *et al*. 2010).

Thyroid cancer incidence increased, on average, 3.6% per year during 1974-2013, primarily related to increases in papillary thyroid cancer (Hyeyeun, *et al*. 2017).

The documented prevalence rates of 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 and Sonny, 2011).

The thyroid cancer accounted for 349 (2.5%) of the all 26,652 new cancer cases in Sudan (Mohammed, *et al*. 2014).

The prevalence of thyroid cancer among multinodular goiter in Sudan was 13.5% (Tadele, *et al*. 2014).

2.4 Risk factors of thyroid cancer:

2.4.1 Radiation:

The thyroid irradiated more than other tissues because of its position in the body and its ability to concentrate iodine (Mettler, *et al*. 2008).

2. 4. 2 Diet:

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

2. 4. 3 Alcohol consumption:

It has been reported to have multiple effects on the hypothalamo-pituitary-thyroid axis and the functioning of the thyroid gland, It has been reported to cause direct suppression of thyroid function by cellular toxicity (Yatan and Koushik, 2013).

2.4. 4 Iodine deficiency:

The autopsy studies of occult thyroid carcinoma showing higher microcarcinoma rates, the case control studies suggesting lower risk of thyroid cancer with higher total iodine intakes (Michael and Valeria, 2015) .

2.5 Methods of diagnosis of thyroid cancer:

2. 5. 1 Fine Needle Aspiration (FNA):

Fine-needle aspiration cytology (FNAC) of thyroid has been introduced as the most reliable and cost-effective method for diagnosing of clinically important thyroid disorders (Redman, *et al*. 2012).

It can be performed by palpation or with ultrasound guidance (Morgan, *et al*. 2003).

The use of FNA has led to a reduction in the number of patients requiring surgery and increased the diagnostic yield of cancers at thyroidectomy (Ogilvie, *et al*. 2006).

2. 5. 2 Ultrasonography:

An ultrasound examination focus on the size of the nodule, its composition, the presence of additional nodules, and any sonographic appearance suggestive of malignancy (Papini, *et al*. 2002).

2. 5. 3 Tissue biopsy:

Patients with enlarged thyroid gland sometimes require surgical biopsy for diagnosis, and there have been many case series describing the pathology found at biopsy (Rose and Thomas, 2005).

2. 5. 4 Immunohistochemistry:

The appropriate use of immunohistochemistry by applying a panel of immunomarkers and using a standardized technical and interpretational method may complement the morphologic assessment and aid in the accurate classification of difficult thyroid lesions (Haiyan and Fan, 2015).

2. 5. 5 Molecular study:

Recent molecular studies have described a number of abnormalities associated with the progression and dedifferentiation of thyroid carcinoma, these molecular events are often associated with specific stages of tumor development (Nikiforov, 2011).

2.6 Treatment of thyroid cancer:

2. 6. 1 Total thyroidectomy:

Initial total thyroidectomy can be safely performed for both benign and malignant thyroid diseases (Jisheng, *et al*. 2016).

2. 6. 2 Radioactive iodine (RAI):

Radioactive iodine (RAI) in the form of I^{131} is used in treatment of patients with differentiated papillary and follicular thyroid cancer, It is typically used after thyroidectomy (Mallick, *et al*. 2012).

2. 6. 3 Thyroid hormone replacement therapy:

It is done after thyroidectomy in thyroid cancer and it is not only required to replace endogenous TH, but it is generally thought to inhibit tumor growth indirectly by its negative feedback effects on pituitary TSH secretion (Zeina, *et al*. 2016).

2. 6. 4 Chemotherapy:

Chemotherapy involves the use of anticancer drugs to kill cancer cells. Some patients who receive chemotherapy for thyroid cancer may also need external radiation therapy (Bergese, *et al*. 2011).

2.7 Epithelial membrane antigen (EMA):

Epithelial membrane antigen (EMA) or MUC1 belongs to a heterogeneous group of heavily glycosylated proteins and is expressed in most normal and epithelial neoplastic cells. EMA is also expressed in plasma cells, anaplastic large cell lymphoma , malignant histiocytosis and erythroleukemia (Leong, *et al*. 2003).

Epithelial membrane antigen immunoreactivity is well preserved in paraffin sections of routinely processed tissues, facilitating application of this technique in diagnostic surgical pathology (Pincus and Kurtin, 1985).

EMA is best considered a broad-spectrum antibody that is reactive against many types of adenocarcinoma. Breast and skin adnexal tumors are strongly positive. A lesser degree of staining is seen in carcinomas of the endometrium, kidney, thyroid, stomach, pancreas, lung, colon, ovary, prostate and cervix , embryonal carcinomas, medullary carcinomas of thyroid, squamous carcinomas, sarcomas, lymphomas, and melanomas (Verdu, 2011).

2.8 EMA and thyroid cancer:

Yamamoto *et al*. (1992), reported that immunohistochemistry staining was demonstrated in papillary thyroid carcinoma.

Murli (2010), reported that epithelial membrane antigen is usually absent in medullary thyroid carcinoma.

Shvero *et al*. (2003), reported that anaplastic thyroid carcinomas and Hurthle cell carcinomas were positive for epithelial membrane antigen.

Wilson *et al*. (1986), reported that 90% papillary thyroid carcinoma, 70% follicular thyroid carcinoma, 20% anaplastic thyroid carcinoma and 30% medullary thyroid carcinomas were positive for epithelial membrane antigen.

Chapter Three

Materials and Methods

Chapter Three

Materials and Methods

3.1 Materials:

Archived tissue blocks obtained from thyroid gland samples previously diagnosed as thyroid carcinoma and multinodular goiter were selected for this study.

3.2 Methods:

3.2.1 Study design:

This is analytical retrospective case control study aimed to detect the expression of EMA in thyroid tumors among Sudanese patients using immunohistochemistry.

3.2.2 Study samples:

Thirty paraffin blocks previously diagnosed as thyroid carcinoma and 10 benign thyroid nodules were selected from Omdurman teaching hospital. Patient identification data (age and diagnosis), were obtained from patient's records.

3.2.3 Study area:

This study was conducted at Omdurman teaching hospital, during the period from January to April 2017.

3.2.4 Immunohistochemical staining:

Immunohistochemical staining was carried out using new indirect dextran polymer immune peroxidase technique. Tissue sections were deparaffinized in xylene and rehydrated through graded alcohol (100%, 90%, 70%, 50%) to water. The antigens were retrieved using water bath with tris EDTA buffer (pH 9.0) for 5 minutes and

then cooled down to room temperature for 20 minutes. Endogenous peroxidase activity was blocked by 3% peroxidase blocker for 10 minutes. The slide then treated with epithelial membrane antigen primary antibody for 20 minutes at room temperature in a humid chamber, then washed in phosphate buffer saline (pH 7.4) for 3 minutes. Then sections were incubated in dextran polymer-HRB (horseradish peroxidase) secondary antibody for 15 minutes, then washed in three changes of phosphate buffer saline for 3 minutes, after that incubated in 3,3 diaminobenzidine tetrahydrocholoride substrate solution for 5 minutes, then washed in runing water. then counter stained in Mayer's haematoxylin stain for one minutes. After that dehydrated, cleared and mounted in DPX mounting media (Bancroft, *et al*. 2013).

3.2.5 Result interpretation:

All quality control measures were adopted, positive and negative control sections were used during immunohistochemical staining. Detection of more than 5 cells with brown cytoplasm or membrane per one field considered as positive result.

3.2.6 Data analysis:

The obtained results and variables arranged in standard master sheet, then analyzed using statistical package for social science (SPSS) program. Frequencies, means and Chi squire tests were calculated.

3.2.7 Ethical consideration:

Specimens were taken from Omdurman teaching hospital ethically after taken ethical clearance.

Chapter Four

Results

Chapter four

4. Results

The study included forty samples, 30 (75%) samples were malignant tumors and 10 (25%) samples were benign tumors.

The age of study population ranged between 11 and 65 years with mean age of 36 years. Most patients were less than 50 years representing 31 (77.5%) and the remain 9 (22.5%) were more than 50 years as indicated in table (4.1).

The histopathological diagnosis of study samples includes 17 (42.5%) papillary thyroid carcinoma, 8 (20%) follicular thyroid carcinoma, 3 (7.5%) medulary thyroid carcinoma, 2 (5%) hurthle thyroid carcinoma and 10 (25%) benign thyroid tumor as showed in table (4.2).

Epithelial membrane antigen positive expression was found in 16/30 samples in malignant samples while 14/30 samples showed negative expression, while all benign thyroid tumors showed negative expression for epithelial membrane antigen, this result showed significant association (P . value =0.003) as indicated in table (4.3).

Types of malignant tumor showed positive expression for epithelial membrane antigen in 11 (36.6%) samples of papillary thyroid carcinoma, 3 (10%) samples of follicular thyroid carcinoma, no detected samples of medulary thyroid carcinoma, 2 (6.7%) samples of hurthle carcinoma. This result showed no association between epithelial membrane antigen expression and types of malignant tumors (P .value =0.076) as showed in table (4.4).

Table (4.1): Distribution of age groups among the study population

Age	**Frequency**	**Percent**
≤ 50 years	31	77.5%
≥ 50 years	9	22.5%
Total	40	100%

Table (4.2): Frequency of histopathological diagnosis among the study population

Histopathological diagnosis	Type	Frequency	Percent
Malignant	Papillary thyroid carcinoma	17	42.5%
	Follicular thyroid carcinoma	8	20%
	Medullary thyroid carcinoma	3	7.5%
	Hurthle carcinoma	2	5%
Benign	Multinodular goiter	10	25%
Total		40	100%

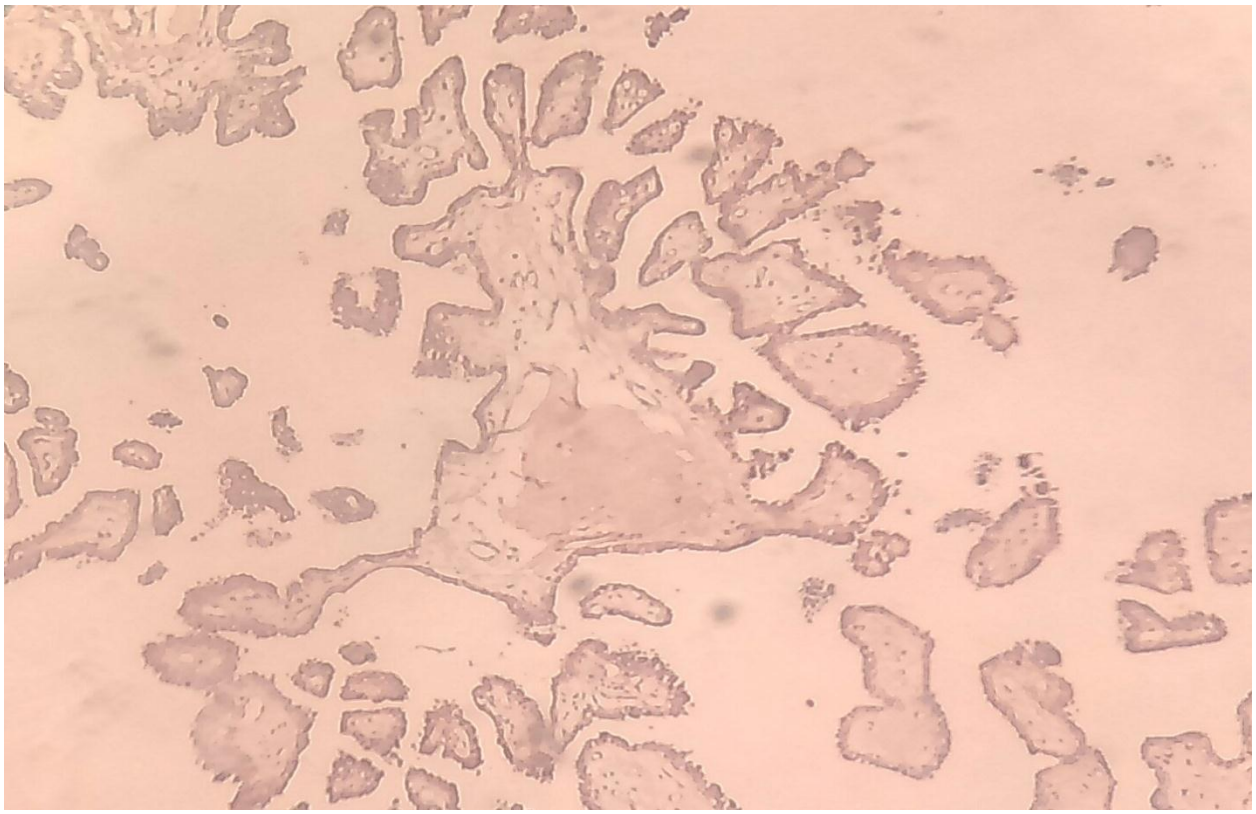
Table (4.3): Relation between the expression of EMA and histopathological diagnosis of thyroid tumors

Histopathology diagnosis	EMA expression		P. value
	Positive	Negative	
	N (%)	N (%)	
Benign	0 (0%)	10 (25%)	0.003
Malignant	16 (40%)	14 (35%)	
Total	16 (40%)	24 (60%)	

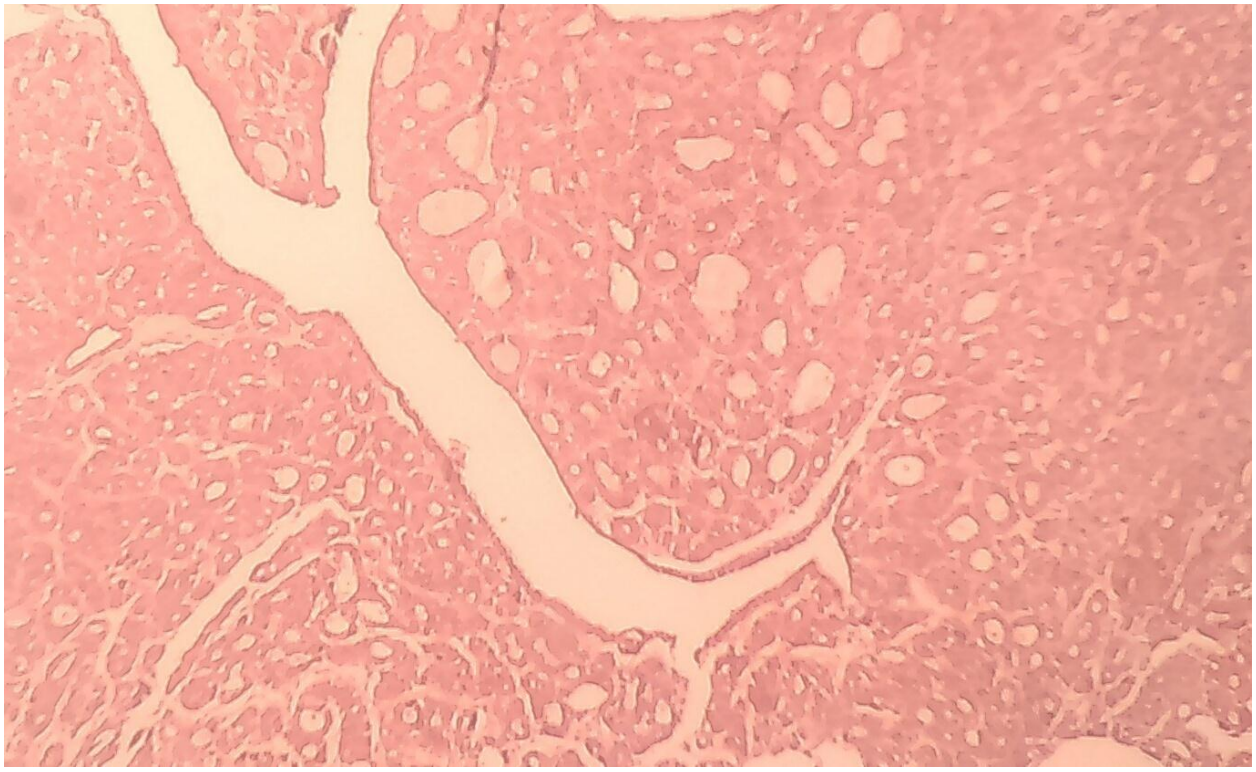
Table (4.4): Relation between epithelial membrane antigen and type of malignant tumors

EMA Expression	**Types of malignant tumor**				**Total**	**P. value**
	Papillary Thyroid Carcinoma	Follicular thyroid carcinoma	Medullary Thyroid carcinoma	Hurthle cell Thyroid carcinoma		
Positive	11(36.6%)	3 (10%)	0 (0%)	2 (6.7%)	16 (53.3%)	0.076
Negative	6 (20%)	5 (16.7%)	3 (10%)	0 (0%)	14 (46.7%)	
Total	17(56.6%)	8 (26.7%)	3 (10%)	2 (6.7%)	30 (100%)	

*EMA : epithelial membrane antigen.



Microphotograph (4.1): Papillary thyroid carcinoma showed membranous positive expression of epithelial membrane antigen (40x).



Microphotograph (4.2):Papillary thyroid carcinoma showed negative expression of epithelial membrane antigen (40x).

Chapter Five

Discussion

Chapter Five

5. Discussion

The present study included 40 samples of thyroid lesions stained by immunohistochemistry for epithelial membrane antigen. Concerning the age group of the study population, the study revealed that most patients were less than 50 years indicating that patients less than 50 years are more affected with thyroid cancer. This result is compatible with Davis and Welch, (2006), who reported that common involved age by thyroid carcinoma was the age group of 20-44 years. While disagreed with Norra, *et al*. (2015), who reported that risk of developing thyroid cancer increases with age.

The histopathological diagnosis of the study population revealed that more frequent type of thyroid cancer was papillary thyroid carcinoma. This result is compatible with Salter, *et al*. (2010), who reported that papillary thyroid carcinoma comprises most malignant thyriod neoplasms. It also agreed with Ceresini, *et al*. (2012), who reported that about 80% of malignant thyroid are papillary thyroid carcinomas.

Epithelial membrane antigen expression was detected in malignant conditions compared to benign conditions, which suggest that epithelial membrane expression is more frequent expressed in malignant conditions. This is agreed with Kilicarslan, *et al*. (2000), who reported that epithelial membrane antigen was significantly over expressed in thyroid cancer as compared with benign thyroid tumors. It also agreed with Mitselou, *et al*. (2002), who reported that expression of epithelial membrane antigen in thyroid carcinoma was higher than that in benign thyroid tumors.

The present study revealed there was no significant association between epithelial membrane antigen expression and type of malignant tumor. This result is agreed with Brasanac, *et al*. (1993), who reported that was no significant association between the expression of epithelial membrane antigen and pathological type. It also agreed with Beltrami, *et al*. (1987), who reported that expression of epithelial membrane antigen did not show significant relationship with tumor subtype.

Chapter Six

Conclusion and Recommendations

Chapter Six

Conclusion and Recommendations

6.1 Conclusion:

On the basis of this study we concluded that:

- Most thyroid cancer patients in this study appear to be less than 50 years old.
- Most histological type of thyroid cancer is papillary thyroid cancer.
- EMA expression is associated with malignant tumors of thyroid with no association with subtypes of thyroid malignant tumors.

6.2 Recommendations:

On the basis of this study we recommended that:

- Similar studies should be carried in larger sample size.

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Appendices

Appendix 1:

Materials and instruments used for processing and staining of the specimens include:-

- Disposable gloves
- Microtome knife
- Positively charged slides (thermo)
- Cover glass
- Dry oven
- Water bath
- Embedding center
- Coplin jars
- Humidity chamber
- Ethanol (100%, 90%, 70%, 50%)
- Mayer s haematoxylin
  (Haematoxylin, DW,K or ammonium alum,sodium iodate, citric acid, chororal hydrate).
- Reaction buffer
- Primary antibody (Anti-Epithelial membrane antigen)
- Secondary antibody (dextran polymer conjugated secondary antibody - HRP)
- Tris EDTA buffer(PH 9)
- Phosphate buffer saline (PH7.4)
- Peroxidase blocker
  (0.3% hydrogen peroxide in methanol)

- Bluing Reagent (0.1 M Li_2CO_3, 0.5 M Na_2CO_3)
- DAB (3,3 diaminobenzidine tetrahydrocholoride) substrate solution
- Xylene.
- DPX mounting media.

Appendix 2:

Monoclonal Mouse Anti-Human Epithelial Membrane Antigen
Clone E29
Code No. M 0613
Lot 072. Edition 08.08.02

Intended use

For in vitro diagnostic use.

DAKO Monoclonal Mouse Anti-Human Epithelial Membrane Antigen, Clone E29, is intended for use in immunocytochemistry. The antibody labels epithelial cells in a wide variety of tissues and is a useful tool for the identification of neoplastic epithelia (1). Differential identification is aided by the results from a panel of antibodies. Interpretation must be made within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Introduction

Epithelial membrane antigen (EMA) belongs to a heterogenous population of human milk fat globule (HMFG), proteins. HMFG is a complex secretory product of mammary epithelium and EMA can be recovered from the aqueous phase of skimmed milk following extraction in chloroform and methanol. Besides in milk, these proteins are present in a variety of epithelia of both normal and neoplastic types. A number of monoclonal and polyclonal antisera have been raised against these molecules and anti-EMA has been extensively studied in a large number of neoplastic conditions, most often in conjunction with other antibodies (2).

EMA is valuable as a marker in the detection of breast carcinoma metastases in histological sections of liver, lymph node, and bone marrow, and is useful for differentiating anaplastic carcinoma from malignant lymphomas, and for the recognition of spindle cell epithelial malignancies (1, 3).

Reagent provided

Monoclonal mouse antibody provided in liquid form as cell culture supernatant dialysed against 0.05 mol/L Tris/HCl, pH 7.2, and containing 15 mmol/L NaN_3.

Clone: E29 (1). Isotype: IgG2a, kappa.

Mouse IgG concentration: 240 mg/L. Total protein concentration: 8.9 g/L.

Immunogen

Human milk fat globule membrane preparation (1, 4).

Specificity

In Western blot analysis of the immunogen the antibody labels bands of 265-400 kDa (1).

Precautions

1. For in vitro diagnostic use.

2. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.

Storage

Store at 2-8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the user must verify the conditions. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact DAKO Technical Services.

Specimen preparation

Paraffin sections: The antibody can be used on paraffin-embedded tissue sections fixed in formalin, Bouin's, Zenker's and B5 solution (5). Heat-induced epitope retrieval in 10 mmol/L citrate buffer, pH 6.0, or DAKO Target Retrieval Solution, code No. S 1700, is recommended. The tissue sections should not dry out during the treatment or during the following immunocytochemical staining procedure.

Frozen sections and cell preparations: Can be used for labelling acetone-fixed frozen sections (1).

Staining procedure

Dilution: DAKO Monoclonal Mouse Anti-Human Epithelial Membrane Antigen, code No. M 0613, may be used at a dilution range of 1:50-1:100 when applied on formalin-fixed, paraffin-embedded sections of tonsil and using 20 minutes heat-induced epitope retrieval in 10 mmol/L citrate buffer, pH 6.0, and 30 minutes incubation at room temperature with the primary antibody. Optimal conditions may vary depending on specimen and preparation method, and should be determined by each individual laboratory. The recommended negative control is DAKO Mouse IgG2a, code No. X 0943, diluted to the same mouse IgG concentration as the primary antibody.

Visualization: DAKO LSAB®+/HRP kit, code No. K 0679, and DAKO EnVision™+/HRP kits, code Nos. K 4004 and K 4006, are recommended. Follow the procedure enclosed with the selected visualization kit. For frozen sections and cell preparations, the DAKO APAAP kit, code No. K 0670, is a good alternative if endogenous peroxidase staining is a concern. Follow the procedure enclosed with the selected visualization kit.

Automation: The antibody is well-suited for immunocytochemical staining using automated platforms, such as the DAKO autostainer.

Product-specific limitations

The antibody labels plasma cells, and EMA appears to be common in plasma cell neoplasms, but is also encountered occasionally among other types of lymphoma. However, it should be emphasized that such cases usually are clearly identifiable on purely morphological grounds as being lymphoid in nature (1).

Performance characteristics

In normal breast and other secretory epithelia, labelling is predominantly localized to apical luminal membranes. In neoplasms, cytoplasmic and apical luminal membrane staining are the most common patterns of immunoreactivity with peripheral membrane staining or other patterns also occuring (5).

Normal tissues: The antibody labels epithelial cells in a wide variety of tissues and mesothelial cells. Included are sweat ducts and sebaceous glands of the skin, epithelium of the gastro-intestinal tract, acini and ducts of the breast, exocrine cells in pancreas, bladder epithelium, distal tubules of the kidney, cervix, endometrium, respiratory epithelium, thyroid and bile ducts. No labelling was observed in epidermis, endocrine cells in pancreas, glomeruli and proximal tubules of the kidney, central nervous system, peripheral nervous system, connective tissue, hepatocytes, and lymphoid tissue, except for occasional plasma cells (1).

Abnormal tissues: The antibody labels a wide variety of neoplastic epithelia, and also neoplastic mesothelial cells (1). In a large study of 2081 epithelial, mesenchymal and hematopoietic neoplasms, the antibody provided a 98.6% specificity and a positive predictive value of 99.2% for neoplastic epithelial differentiation when it was used in combination with anti-leucocyte common antigen (LCA) (2). It was shown that the antibody labelled 105/354 cases of soft tissue and intracranial tumours, with most positive cases among synovial sarcomas, spindle cell malignant mesotheliomas, epithelioid sarcomas, cordomas and choroid plexus tumours, 38/169 cases of small round cell tumours and small cell sarcomas, 13/158 cases of germ cell neoplasms, 23/23 cases of spindle cell "sarcomatoid" carcinomas, 815/918 cases of other epithelial malignancies, including squamous, Merkel cell, breast, gastric adeno-, colonic adeno-, pancreatic, salivary gland, bladder, uterine, ovarian, vaginal, pulmonary, prostatic, thyroid, thymic, hepatic, renal cell, and nasopharyngeal carcinomas (2). In lymphomas, the antibody labelled 10/22 cases of null or T-cell type CD30+ ALCL of small cell, monomorphic, or pleomorphic subtypes (6), 34/35 cases of p80/ALK+, 1/6 cases of CD56/57+ T/NK-cell, 2/7 cases of EBV+ cytotoxic large T-cell, 2/8 cases of low-grade cytoxic T-cell and 6/10 cases of cytoxic Hodgkin's-like lymphomas (7). No labelling was observed in 18/18 cases of basal cell carcinoma, 12/12 cases of hepatocellular carcinoma, 43/43 cases of malignant melanoma and 69/69 cases of endocrine neoplasms, with the exception of poorly differentiated lesions in 6 cases of islet cell tumours (2).

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