Sudan University of Science and Technology

College of Graduate Studies

Immunohistochemical Detection of Cytokeratin 19 in Thyroid Tumors

الكشف النسيجي الكيمائي المناعي للسايتوكيراتين في اورام الغدة الدرقية

AdissertationSubmitted in Partial Fulfillment of M.Sc.Degree in Medical Laboratory Science (Histopathology and Cytology)

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Dedication

I dedicate this work to

my life mother and father

my loving husband Sami

to my sisters , brothers ,

to my friends .

hopefulness the successful to all persons **.**

Acknowledgment

This research took me months, by that time; I have met a great people whose contribute in many ways to come out with this project, it is a pleasure to convey my gratitude to them all in my humble acknowledgment .

Firstly all thanks to Allah for giving me the strength to complete this work.

Secondly I would like to note my gratitude to my life mother and father for his loving and supporting my sisters and brothers for encouragement me and to loving husband SamiHassan . Thirdly I would like to note my gratitude to prof.Dr.MohammedSiddig for his supervision, advice, encouragement and guidance from the very early stage of this research as well as giving me opportunities of experiences throughout the project.

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Abstract

This is retrospective study aimed to detect the expression of CK19 tumor marker in thyroid tumor using immunohistochemistry. The study was conducted in Omdurman Military Hospital and Omdurman Teaching hospital during the period from January to September2017.Forty paraffin embedded tissue blocks previously diagnosed as thyroid tumors were selected.The patients information was collected from the files of patients. The patients ages ranges between 19-82 years, with mean age40 years. Tissue sections were cut and stained using dextrane-polymer technique for immunohistochemical detection of ck19tumor marker .The obtained data were analyzed using SPSS computer program,frequency, mean and chi-square value were calculate .Out of 40 patients with thyroid tumors, 30 (75%) were females,while 10(25%)were males. Regarding the histological diagnosis the 20malignant cases were 9,7,3,1,papillary carcinoma, follicular carcinoma , medullary carcinoma and anaplastic carcinoma respectively. and the 20 benign lesions were 10goiter and 10 follicular adenoma. Ck19 showed positive result in 7,2,1,0,2,1samples in papillary carcinoma, follicular carcinoma, medullary carcinoma, anaplastic carcinoma, goiter, follicular adenoma respectively,with significant relation between ck19 expression and histopathologicaldiagnosis p.value:0.04.The study concluded that the ck19 tumor marker is highly expressive marker in thyroid carcinomas.

المستخلص

أجريت هذه الدراسة التراجعية بمستشفي أم درمان التعليمي ومستشفى أم درمان العسكري في الفترة مابين يناير وحتي سبتمبر 2017 . هدفت الدراسة للكشف عن واسمة الاورام سيتوكيرتين 19 عند مرضي أورام الغدة الدرقية.

تم إختيار 40 قالب مدعم بشمع البرافين من مرضي مشخصون مسبقا باورام الغدة الدرقية تراوحت اعمارهم بين 19-82 سنة, متوسط اعمارهم 40 سنة. تم صبغ المقاطع النسيجيةبطريقةالانسجة المناعية الكيميائية باستخدام تقنية الدكستران بوليمر,للكشف عن واسمة الاورام سيتوكيراتين19.ثم تحليل البيانات باستخدام البرنامج الاحصائي المحوسب .SPSS وتم حساب التردد والمتوسط ومربع كاي.

من مجموع 40 مريضآ بإورام الغدة الدرقية 30 (75%) منهم كانوا اناثآ بينما 10 (25%) كانوا ذكورآ, وكانت نتيجة تشخيص الانسجة المريضةالاورام الخبيثة كانت موزعة كالأتي1,3,7,9 سرطان غدة حلمي وسرطان غدة جريبي ,سرطان غدةنخاعي, سرطان غدة كشمي, علي التوالي، وكانت نتائج الاورام الحميدة (20) موزعة بين 10تضخم الغدة و10الورم الحميد الجريبي . اعطي سيتوكيراتين 19 نتائج ايجابية في 1,3,0,1,2,7 سرطان الغدة الحلمي والجريبي واالنخاعي,الكشمي وتضخم الغدة الدرقية والورم الغدى المسامى على التوالي مع وجود علاقة بين إفراز ck19 والتشخيص النسيجي القيمة الاحتمالية0.04.

خلصت الدراسة الي ان واسمة الاورام سيتوكيريتين 19عالية الإفراز في سرطان الغدة الدرقية.

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CHAPTRE ONE

INTRODUTION

CHAPTRE ONE

1.1INTRODUTION:

Thyroid cancer is the most common malignancy of the endocrine system , representing 3.8% of all new cancer cases in united states and is the ninth most common cancer overall , The American cancer society estimate that 62,450 people in the united states diagnosed with thyroid cancer in 2015 and 1950 deaths result from the disease mortality, survival, incidence data were collected by the national cancer institute (Surveillance, Epidemiology, Results [SEER] Program), the centers for disease Control and Prevention (National Program of Cancer Registries), and the north American association of central cancer registries , mortality data were collected by the national center for health statistics(Copeland, *et al*.2015).. In 2016, 1,685,210 new cancer cases and 595,690 cancer deaths are projected to occur in the united states (Murphy *et al*.2016). The diagnosis of thyroid tumor include physical examation, thyroid function tests that measure blood levels thyroxin ,triiodothyronine and thyroid-stimulating hormone , ultrasonography,which provide best information about shape and structure of nodules , fine needle aspiration biopsy helps to distinguish between benign and malignancy , also thyroid scan help to evaluate thyroid nodules. (Haugen *et al* . 2015) . Cytokeratin 19 is the lowest molecular weight keratin (40 KDa) and is widely present in simple epithelial , CK19 is expressed differentially in various types of thyroid lesions in which a malignant transformation is generally paralleled by increased level of CK19 expression (Camby*et al* .2001).

1.2 Objectives:

1.2.1General objective:

To study the expression of Ck19 in thyroid tumors among Sudanese patients using immunohistochemistry.

1.2.2 Specific objectives:

1. TodetectCk19 expression in thyroid tumors by using immunohistochemistry.
2. To Correlate between Ck19 expression and histopathological diagnosis.
3. To correlate between Ck19 expression and thyroid malignant subtype.

CHAPTER Two

Literture review

CHAPTER Two

2-Literture review

2.1 Anatomy and physiology of thyroid gland:

The thyroid gland is a butterfly-shaped organ that sits at the front of the neck. It iscomposed of two lobes, left and right, connected by a narrow isthmus. The thyroid weighs 25 grams in adults, with each lobe being about 5 cm long, 3 cm wide and 2 cm thick, and the isthmus about 1.25 cm in height and width. The gland is usually larger in women, and increases in size in pregnancy. the thyroid sits near the front of the neck, lying against and around the front of the larynx and trachea. Thethyroid cartilage and cricoid cartilage lie just above the gland, below the Adam's apple. The thyroid gland is covered by a thin fibrous capsule, which has an inner and an outer layer. The outer layer is continuous with the pretracheal fascia, attaching the gland to the cricoid and thyroid cartilages, via a thickening of the fascia to form the posteriorsuspensory ligament of thyroid gland. This causes the thyroid to move up and down with swallowing the inner layer extrudes into the gland and forms theseptae that divides the thyroid tissue into microscopic lobule (Netter*et al*.2014).

The thyroid gland secretesthyroid hormones, which primarily influence themetabolic rate and protein synthesis. The hormones also have many other effects including those on development. The thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) are createdfromiodine and tyrosine. The thyroid also produces the hormonecalcitonin, which plays a role in calcium homeostasis.

Hormonal output from the thyroid is regulated by thyroid-stimulating hormone (TSH) secreted from theanterior pituitary gland, which itself is regulated by thyrotrophic releasing hormone (TRH) produced by the hypothalamus(Boron *et al* .2012).

2.2 Histology of thyroid gland:

The thyroid gland weighs 15–25 g and is composed of two lobes joined by the isthmus, with approximately 40 % of people having a pyramidal lobe. The thyroid is composed of lobules, each of which is composed of 20–40 follicles. Each follicle is surrounded by a basement membrane and lined by follicular cells. Colloid is present in the lumen of the follicles. C cells are calcitonin-producing cells in the thyroid. They are in interfollicular areas but are difficult to identify in normal thyroid tissue with hematoxylin and eosin alone. C cells migrate from the neural crest to the ultimobranchial bodies, which are derived from branchial pouch complexes IV and V and develop in the first five to seven fetal weeks. C cells have clear cytoplasm and oval to round nuclei; they are positive for calcitonin, chromogranin, synaptophysin, calcitonin, calcitonin gene-related peptide, somatostatin, and bombesin. Thyroid follicular cells and tumors are immunopositive for thyroglobulin, TTF1, and keratin. Interesting histologic features occasionally may be recognized in thyroid tissue, including solid cell nests, fatty metaplasia, radiation changes, drugs such as minocycline and amiodarone, and palpation thyroiditis. Thyroid tissue also may occur ectopically, and tumors may develop in this tissue(Coyne*et al.*2010).

2.3 Pathology of thyroid cancer:

2.3.1 Benign tumors of thyroid:

2.3.1.1Multinodular goiter:

Nodular goiters result from focal hyperplasia of follicular cells at one site or, most often at multiple sites within the thyroid gland. The basic process in goitrogenesis is the generation of new follicular cells, which are used either to form new follicles or to enlarge the size of newly formed follicles. The sprouting of a capillary network embedded in stromal cells is a necessary secondary event .The driving force behind multi-nodular goiter growth is an intrinsically abnormal growth.

Extra thyroidal factors such as TSH, may act upon this basic process and thereby accelerate goiter growth (Khatawkar*et al*.2015).

2.3.1.2 Follicular adenoma:

Follicular adenoma is a benign encapsulated tumor of the thyroid gland. It is a firm or rubbery, homogeneous, round or oval tumor that is surrounded by a thin fibrous capsule. A follicular adenoma is a common neoplasm of the thyroid gland. Most patients with a follicular adenoma are clinically and biochemically euthyroid. Approximately 1% of follicular adenomas are “toxic adenomas,” which are a cause of symptomatic hyperthyroidism. Hyperthyroidism usually does not occur until a functioning follicular adenoma is ≥3 cm in size (Cabanillas*et al.*2010).

2.3.2 Malignant tumors of thyroid:

2.3.2.1 Papillary carcinoma:

Papillary carcinoma (PTC) is the most common form of well-differentiated thyroid cancer, and the most common form of thyroid cancer to result from exposure to nodule in a normal thyroid parenchymaWhile papillary thyroid cancer typically occurs in only one lobe of the thyroid gland, it may arise in both lobes in up to 10% to 20% of cases. Papillary carcinoma appears as an irregular solid or cystic mass or nodule in a normal thyroid parenchyma .papillary thyroid cancer is most common in women of childbearing age(Wreesman, *et al.*2004).

2.3.2.2 Follicular carcinoma:

Follicular carcinoma is a malignant epithelial tumor showing evidence of follicular differentiation and not belonging to any of the other distinctive types of thyroid malignancy. Besides the point on follicular cell differentiation that does not usually raise any major difficulty, the two crucial issues of both definitions reside in the need to demonstrate malignancy and to exclude the presence of nuclear features typical of PTC. Malignancy is equivalent to invasiveness (capsular and/or vascular penetration), which may be difficult to disclose with certainty(Pleasance*et al* 2010).

2.3.2.3 Medullary carcinoma:

Medullary carcinoma of the thyroid (MTC) is a distinct thyroid carcinoma that originates in the Para follicular C cells of the thyroid gland. These C cells produce calcitonin. Sporadic, or isolated, MTC accounts for 75% of cases, and inherited MTC constitutes the rest. Inherited MTC occurs in association with multiple

endocrine neoplastic (MEN) type 2A and 2B syndromes, but non-MEN familial MTC also occur. Medullary thyroid cancer (MTC) is usually diagnosed on physical examination as a solitary neck nodule, and early spread to regional lymph nodes is common. Distant metastases occur in the liver, lung, bone, and brain.Sporadic MTC usually is unilateral. In association with multiple endocrine neoplasia (MEN) syndromes, it is always bilateral and multicentric, with presentation earlier in life. MTC typically is the first abnormality observed in both MEN 2A and 2B syndromes(Haugen*et al* .2016).

2.3.2.4 Anaplastic carcinoma:

Anaplastic carcinoma of the thyroid (ATC) is the most aggressive thyroid gland malignancy. Although ATC accounts for less than 2% of all thyroid cancers, it causes up to 40% of deaths from thyroid cancer. The aggressive nature of ATC makes treatment studies difficult to perform. Anaplastic carcinoma of the thyroid (ATC) generally occurs in people in iodine-deficient areas and in a setting of previous thyroid pathology (e.g., preexisting goiter, follicular thyroid cancer, papillary thyroid cancer). Local invasion of adjacent structures (e.g., trachea, esophagus) commonly occurs (Wagle*et al*.2014).

2.4 Risk factors of thyroid cancer:

2.4.1 Ionizing radiation:

Exposure to ionizing radiation, particularly during childhood, is the best established risk factor for TC(Verdecchia*et al*.2007).

2.4.2 Family history:

There is a strong association with history of benign nodules/adenoma or goiter(Verdecchia*et al*.2007).

2.4.3Iodine deficiency:Induce an increasing incidence of benign thyroid conditions, but very high iodine intake also affects thyroid function and, possibly, TC risk(Verdecchia*et al*.2007).

2.4.4 Dietary factors:

High intake of cruciferous vegetables shows a weak inverse association with TC. Among other food groups, vegetables other than cruciferous are the only food group showing a favorable effect on TC, with an approximate 20% reduction in risk for subjects with the highest consumption. No effect on TC risk of alcohol, coffee, or other food-groups/nutrients emerged(Verdecchia*et al*.2007).

2.4.5 Height and weight:

Moderate positive association with TC risk (Verdecchia*et al* .2007).

2.5 Diagnosis of thyroid cancer:

2.5.1 Thyroid hormones:

serum thyroid-stimulating hormone (TSH) level.5 The TSH is released from the anterior pituitary and signals the thyroid gland to make thyroid hormone as appropriate. When thyroid hormone levels are low, the TSH rises responsively and and nonfunctional nodules. This is an important characteristic, because hyper functioning nodules are rarely malignant. However, if a TSH is subnormal, indicating a hyperactive gland, a nuclear medicine imaging study (thyroid uptake and scan) should be performed (Nguyen *et al.*2015).

2.5.2 Fine needle aspiration:

Nonfunctioning nodules will require the use of fine-needle aspiration (FNA) for cytologic evaluation. However, if the TSH is normal or elevated, even within the upper limits of normal, a FNA is recommended(Nguyen *et al.*2015).

2.5.3 Ultrasound :

No single ultrasound feature and no combination of ultrasound features is sensitive enough or specific enough to identify malignancy by themselves. Some ultrasound features have greater correlation with certain types of cancer, such as microcalcifications with papillary thyroid cancer and its absence in follicular thyroid cancer(Nguyen *et al.*2015).

2.5.4 Sonographic features:

Certain sonographic features are highly predictive of benign nodules, such as purely cystic nodules and nodules with >50% spongiform appearance (aggregation of multiple microcystic components(Nguyen*et al*.2015).

2.6 Treatment of thyroid cancer:

2.6.1 Surgery:

Thyroidectomy and dissection of central neck compariment is initial step in treatment of thyroid cancer in majority of cases (prak*et al*.2013).

2.6.2 Levothyroxine therapy:

Thyroid hormone suppression therapy is an important part of the treatment of thyroid cancer.Immediately after surgery thyroid hormone therapy is initiated with

dual aim:to replace thyroid hormone and to suppress the potential growth stimulus of TSH on tumor cells(pacini*et al* .2006).

2.6.3 Post treatment management:

TSH suppression therapy is recommended after surgery and after therapy because differentiated thyroid cancers express TSH receptors that respond to TSH stimulation the cells respond by increasing sodium iodide symporters and thus increasing cell growth.TSH suppression can be achieved by using supraphysiologic doses of levothyroxine to suppress the TSH to <0.1mU/L or up to 0.5mU/L for lower-risk patients(Doherry*et al* .2009).

2.7 Tumor marker:

A tumor markeris abiomarker found in blood, urine, or body tissues that can be elevated by the presence of one or more types of cancer. There are many different tumor markers, each indicative of a particular disease process, and they are used in oncology to help detect the presence of cancer. An elevated level of a tumor marker can indicate cancer.Tumor markers can be produced directly by the tumor or by non-tumor cells as a response to the presence of a tumor. The diagnosis is mostly confirmed by biopsy (Benesch *et al*.2010).

2.7.1Ck19: Keratin, type I cytoskeletal 19 also known as cytokeratin-19 (CK-19) or keratin-19 (K19) is a 40 kDaprotein that in humans is encoded by the KRT19 gene. Keratin 19 is a type Ikeratin. Keratin 19 is a member of the keratin family**.** proteins The keratins are intermediatefilament responsible for thestructural integrity of epithelial cells and are subdivided into cytokeratins and hair keratins.Keratin 19 is atype I keratin. The type I cytokeratins consist of acidic proteins which are arranged in pairs of heterotypic keratin chains. Unlike its related

family members, this smallest known acidic cytokeratin is not paired with a basic cytokeratin in epithelialcells. It is specifically found in the periderm, the transiently superficial layer that envelops the developing epidermis. The type I cytokeratins are clustered in a region of chromosome 17q12-q21.KRT19 may result in the detection of either or both of these pseudogenesKRT19 is also known as Cyfra 21-1.

Due to its high sensitivity, KRT19 is the most used marker for the RT-PCR-mediated detection of tumor cells disseminated in lymphnodes, peripheral blood, and bone marrow of breast cancer patients. Depending on the assays, KRT19 has been shown to be both a specific and a non-specific marker. False positivity in such KRT19 RT-PCR studies include: illegitimate transcription (expression of small amounts of KRT19 mRNA by tissues in which it has no real physiological role), haematological disorders (KRT19 induction in peripheral blood cells by cytokines and growth factors, which circulate at higher concentrations in inflammatory conditions and neutropenia), the presence of pseudogenes (two KRT19 pseudogenes, KRT19a and KRT19b, have been identified, which have significant sequence homology to KRT19 mRNA. Subsequently, attempts to detect the expression of the authentic**),** sample contamination (introduction of contaminating epithelial cells during peripheral blood sampling for subsequent RT-PCR analysis). Moreover, Ck-19 is widely applied as post-operative diagnostic marker of papillary thyroid carcinoma**.**Keratin 19 is often used together with keratin 8 and keratin 18 to differentiate cells of epithelial origin from hematopoietic cells in tests that enumerate circulating tumor cells in blood(Dinets*et al* .2015).

Kaliszewskiet al 2016 his study to evaluate expression of cytokeration-19 in the classical subtype of papillary thyroid carcinoma . positive cytgoasmic staining was found a higher expression of ck19 was observed.

Erkilic et al 2002 his study to evaluate the use of ck19 which as shown to be effective in discriminating papillary carcinoma from follicular carcinoma of the thyroid and also to evaluated the immunorectivity of ck19 in follicularadenomas.diffusc and intense ck19 positivity was found in the cells of all pupillary carcinoma.in the multi nodular goiter group 20 of 25 cases showed no staining while the remaining of 5 were focally reactive with ck19,3 of thought the 5 were thought to be false positive owing to hemorrhage.weak and focal ck19 staining was seen in some follicular adenoma

Erdoganet al 2016 in this study 47 papillary (26 Follicular variant ,21 classic type) and 26 benign thyroids lesion (15 nodular by purplasia,10 follicular adenoma,hurtle all adenoma)were analyzed retrospectively HBME -1,ck19,and cD56 antibodies were performed with immune his to chemical methods, concluded that cD56 is helpful antibody for the differential diagnosis of benign and malignant lesions and may increase the diagnostic accuracy when used with HBME-1 and ck19.

Song et al 2011 this study, they conculated that the diagnostic efficiency of ck19for PTC was slightly better .the utilization of this makers combined with morphologic evaluation may be helpful in the differential diagnosis of papillary thyroid carcinoma in the northeastern region of china.

Cheung et al 2001 this study to examine immunohistochemical diagnosis of papillary thyroid carcinoma, ,focal ck19 staining maybe found in benign lesions ,but diffuse positivity is characteristic of pc.

Chapter Three

Materials and Methods

Chapter Three

3-Materials and Methods

3-1Studydesign*:*

This is a retrospective study aimed to detect the expression of cytokeratin19 markers in thyroid tumor using immunohistochemical technique.

3-2 Materials:

Formalin fixed paraffin wax embedded tissue blocks were selected from tissues of patients samples with thyroid tumors were used in this study.

3-3 Study population:

Forty paraffin wax embedded tissue blocks convience selected from patients their specimens were referred to histopathology laboratory in Omdurman military hospital and Omdurman teaching hospital during the period from January to September 2017.A clinical data were obtained from each patient's file.

3-4 Sample processing:

Sections of 3microns thickness were obtained from formalin fixed paraffin wax embedded tissues blocks of each patient using rotary microtome and de waxed in a hot oven .

3-5 Sample staining:

Sections (3µm) from formalin-fixed- paraffin-embedded blocks was cut and mounted onto salinized slides (thermo). Following deparaffinization in xylene,

slides were rehydrated through a graded series of alcohol and was placed in running water. Samples were steamed for antigen retrieval for CK 19using waterbath. Briefly, slides were placed in coplin jars containing enough sodium citrate buffer (pH 9.0) to cover the sections, then were boiled at 97c for 20minutes then allowed sections to cool at room temperture. Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase and methanol for 10 min, then slides were incubated with 100µ l of primary antibodies for 20 min at room temperature in a moisture chamber,. The primary antibody for was ready to use (Dako, Carpintera). and then was rinsed in phosphate buffer saline. After washing with PBS for 3 min, binding of antibody detected by incubating for20 minutes with dextrene polymer finally, the sections were washed in three changes of PBS, followed by adding 3, 3 diaminobenzidine tetra hydrochloride (DAB)as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. Slides were counterstained with mayerhaematoxylin ,dehydrate in alcohol,cleared in xylene and mounted non-aqeous-permanent mounting media(Kwaspen*et al*.1997).

3-6 Results interpretation:

All quality control measures were adopted during sample collection and processing for the assessment of immunohistochemical results positive staining for CK19 appeared as memberance cytoplasmic brown color in reactive region.

3-7 Statistical analysis:

The data was analyzed using **SPSS**computer program. Frequencies, means, and chi-square values were calculated.

3-8 Ethical consideration:

Sample were collected after taking ethical approval from each hospital to use the tissues blocks for research purposes.

Chapter Four

Results

Chapter Four:

4-Results

A total of40 blocks selected from samples of patient previously diagnosed as thyroid tumor, and stained with immunohistochemical stain to study expression of CK19 tumor marker, they were grouped in two categories20benign,20malignant the malignant was classified into four categories papillary carcinoma9(22.5%) ,3 were medullary carcinoma(7.5%) ,1 was anaplastic carcinoma(2.5%) ,7 were follicular carcinoma(17.5%)and20 were benign lesion 10 goiter ,10 follicular adenoma as control as shown in table (1) . Their age classified to two group less than or equal 40 years15 (37.5%) and greater than40 years 25 (62.5%) as shown in table(3), the majority of study population was female30(75%) while males were10(25%)as shownin table(2).

Ck19 show positive result in4 benign and negative result i16, while in malignant positive result in10 and negative result in10 with significant relation between Ck19 result and histological diagnosis.(p.v=0.05)as shown in table(5).

Table(6):Ck19 expression in subtype positive result in 7papillary,1 medullary,0 anaplastic,2 follicular carcinoma ,and negative result in 2 papillary,2 medullary,1 anaplastic,5follicular with insignificant relation between Ck19 result and malignant subtype p.value:0.145.

Table3-1 : Frequency of histopathological diagnosis :

Histpathological diagnosis		Frequency	Percent
Benign	Goiter	10	25.0
	Follicular adenoma	10	25.0
Malignant	Papillary carcinoma	9	22.5
	Medullary carcinoma	3	7.5
	Anaplastic carcinoma	1	2.5
	Follicullar carcinoma	7	17.5
Total		**40**	**100**

Table 3-2 Frequency of a sex among study population :

Sex	Frequency	Percent
Male	10	25.0
Female	30	75.0
Total	40	100.0

Table 3-3 Frequency of age group among study population :

Age	Frequency	Percent
≤40	15	37.5
>40	25	62.5
Total	40	100.0

Table 3-4 Frequency of CK 19 immunohistochemical result:

CK 19	Frequency	Percent
+Ve	14	35.0
-Ve	26	65.0
Total	40	100.0

Table 3-5 Relation between histopathological diagnosis and CK 19 results :

Histopathological diagnosis	Ck19		Total
	+ve	-ve	
Benign	4	16	20
malignant	10	10	20
Total	14	26	40

P.V: 0.04

Table 3-6 Relation between ck19 and malignant subtype :

Malignant subtype	Ck19 result	
	+ve	-ve
Papillary carcinoma	7	2
Medullary carcinoma	1	2
Anaplastic carcinoma	0	1
Follicular carcinoma	2	5
Total	10	10

P.v =.145

Chapter five

Discussion

Chapter five

5.Discussion

In this study 40 samples were collected from patients previously diagnosed as thyroid carcinoma of whom 20 were benign and 20 were malignant,Their age ranged 19 to 82 years classified to two groups less than or equal 40 and great than 40, this result were agree withMazzaferi*et al* (1999) and Preston martin *et al* (1987), that found most of papillary carcinoma were diagnosed in the third to fifth decades. And Sahoo*et al*(2001) showed higher mean age of 46years with an age range of 33-78 years. And disagree with Debdas*et al* (2012) who found the range of age 0f patient is 53 years.

In the present most study of patients are female(75%) this result agreed with Negri*et al* (1999), who found PTC was about four -times more common in females than in males and.Mazzaferri*et al* (1999), also found the majority of patient cancer are female.

And disagree with Debdas*et al* (2012), who found the majority of populations are male ,andSahoo*et al* (2001), have shown a male:female ratio of 1:9 in their 10 cases of FVPTC. The majority of malignancy in this study are papillary carcinoma this result were agreed with kaliszewski*etal* (2016),who found that papillary carcinoma more give positive for ck19 and dunderovic*et al* (2015), who found that ck19 more expressive in malignant lesions ,also Debdas*et al*(2012),who found strong expression of ck19 in thyroid malignancy .Song *et al* (2011), who found that ck19 has diagnostic significance for papillary carcinoma .ErKilic*et al* (2002),who found ck19 use to evaluate papillary carcinoma .

And disagreed with Asa*et al* (2005) ,who found ck19 cannot differentiate thyroid carcinoma and express in benign lesions and also Michel *et al* (2006),who found that CK19 more expressive in benign lesion .

Chapter six

Conclusion and Recommendation

Chapter six

6. Conclusion and recommendation:

6.1 Conclusion:

On the base of this study we conclude that:.

1. Most patients were more than 40years and majority of population are female.

2.There is significant relationship between ck19 and thyroid carcinoma with insignificant relationship with malignant subtype.

6.2 Recommendations:

On the base of this study we recommend that:

Use of ck19 as differational marker in thyroid tumor .Further study with large sample size and involving all part of the Sudan including all type of thyroid cancer should be done.

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Appendices

Appendices:

Appendix 1:

Materials and instruments

- **Disposable gloves**
- **Ethanol (90% 70% 50%)Absolute.**
- **Xylene**
- **Prarffin wax**
- **Mould**
- **Cassettes**
- **Pencil**
- **Coated slides**
- **Cover glasses**
- **Rotary microtome**
- **Microtome knives**
- **Water bath**
- **Dry oven**
- **Coupling jars**
- **Humadity chamber**
- **Mayer's haematoxylin**
- **Citrate buffer (pH6.8)**
- **Phosphate buffer (pH7.4)**
- **Peroxidase blocking solution**
- **Primary antibody (CK19)**

- **Subsrate-chromogen**

Preparationof solution

Mayer's haematoxylin

Heamatoxylinlg

Distilled water	**1000ml**
Potassium Alum	**50g**
Sodium iodate	**0.2g**
Citric acid	**1g**
Chloral hydrate	**50g**

Appendix 2:

Kit Leaflet:

Intended use :

for in vitro diagnostic use FLEX Monoclonal Mouse Anti-Human Cytokeratin19 Clone RCK108 ,Ready – to – use (DakoAutostainer /Autostainer – plus) ,is intended for use in immunohistochemistry together with DakoAutostainer /Autostainer –plus Instruments. This antibody labels epithelial cells expressing the cytokeratin 19 protien ,and is useful for the identification of epithelial tumors ,and may be useful in identifying cholangiocellular carcinomas (1).the clinical interpretation of any staining or its absence should be complemented by moephological studies using proper controls and should be evaluated within the context of the patient´s clinical history and other dignostic tests by a qualified pathologist.

Summary and explanation:

Cytokeratin 19(CK19) belongs to the intermediate filaments,which create cytoskeleton in almost all cells . in contrast other intermediate filaments. Cytokeratin (CK_2) are made up of highly complex mulitgene family of polypeptides with molecular masses ranging from 40 to 68 kDa. Twenty distinct CK polypeptides (2,3) have been revealed in various human epithelial cells (4) . they can be divided into an acidedc (type 1) and a neutrel-basic (type ii)subfamily.CK19 belongs to the acidic type of cytokeratins ,and is a low molecular mass cytokeratins (40kDa) typically expressed in simple epithelia not normally in expressed in stratified squamous epithelia ,but may be persent in modified squamous epithelia invaded by iymphocytes as well as in besal cells in non-keratinizing stratified squamous epithelium (2,4,5) .

Refer to Dako´s General instructions for immunohistochemical Staining or the detection system instructions of IHC procedures for ;1) princpile of procedures. 2) Materials Required . not Supplied ,3) Storage ,4) Specimen Perparation ,5) statning procedure,6) Quality control,7) Troubleshooting ,8) interpretation of staining ,9) General limitations .

Reagent provided:

Ready-to-use monoclonal mouse antibodyprovided in Uquid from in a buffer contioning stabilizing protein and 0.015 mol/l-sodium azide clone RCK108 Isotypr:IgG1,kappa.

Immunogen:

Cytoskeletal precaration from human bladder cance cell line T24(1).

Specificity:

in Westrn blotting of cytoskeleton preparations from the T24and R24 cell lines as well as the human squamous carcinoma cell line HaCaT,the antibody labels a single band corresponding to CK19(1).

In immunohistochemistry on diferent human tissuse , the antibody labels only epithelia ,whereas cells known not to contain CK19 , e.ghebatocytes , are not labeled(1)

Precautions:

1- for professional users .

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 .

3- As with any product derived from biological sources ,proper handing procedures should be used .

4- Wear appropriate personal protective Equipment to avoid contact with eyes and skin .

5- Unused solution should be disposed of according to locam , state and federal regulations .

Storage :

store at 2 -8c^{o} ,Don't use after expiration date stamped on vial ,if raegents are stored under any conditions other than those specified , the conditions mustd verified by the user , there are not obivous signs to indicate instability of this product .Therefore,positive and negative controls should be run simultaneously with patient spesimens . if unexpected staining is observed which cannot be explained by variations in laboratory procedures and aproblem with the antiboey is suspected contact Dako Technical Support .

Specimen preparation including materials required but not supplied :

The antibody can be used for labeling formalin – fixed ,paraffin – embedded tissue sections ,Tissue Specimens should be cut into sections of 4 μm pre-treatment with heat – induced epitope retrieval is required . Optimal results are optained by pretreating tissues using EnVision^{TM} FLEX Target Retrieval Solution , High PH (10X) ,(DakoAutostainer / Autostainer plus) (Code K8010/k8014) .

Deparaffinized sections : pre-treatment of deparaffinized formalin – fixed , paraffin – embedded tissue sections is recommended using Dako PT link(CodePT 100/PT 101) for details .

Follow the pre-treatment procedure outlined in the package insert for EnVision ™ FLEX Target Retrieval Solution ,High PH(10x) , ,(DakoAutostainer / Autostainer plus) (Code k8010 /K8014) The following parameters should be used for PT link: pre-heat temperature EnVision™ FLEX Wash Buffer (10x) ,(DakoAutostainer / Autostainer plus) (Code K8010) The following parameters should be used for PT link:pre- heat temperature : 65 cº epitope retrieval temperature and time :97 cº for 20(+-1) minutes : cool down to 65 cº. Rinse sections with diluted room temperature EnVision™ FLEX Wash Buffer (10x) (DakoAutostainer / Autostainer plus) (Code K8010) .

Paraffin embedded sections:As alternative specimen preparation both deparaffinization and epitope retrieval can be performed in the Ptlink using amodified procedure .see the PT link user Guide for instructions .after the statining procedure has been completed ,the sections most be dehydrated .cleared and mounted using permanent mounting mediaum .

The tissue sections should not dry out during the treatment or during the following immunohistochemical staining precedure .for greater adherence of tiusse sections to glass slides ,the use of DakoSilanized slides (Code S3003) is recommmended .

Staining procedure inculding materials required but not supplied:

The recommened visualization system is EnVision™ FLEX High PH,(DakoAutostainer / Autostainer plus) (Code K8010) .the staining steps and incubation time are pre-programmed into the soft ware of ,(DakoAutostainer /

Autostainer plus) instruments ,using the following protocol :Template protocol:FLEXTU2(200µl dispense volume)or FLEXRTU3(300µl dispense volume).

Autoprogram :CK19(without counter staining)or CK19H(with counter staining).

The Auxiliary step should be set to 'rinse buffer 'in statining run with ≤10 slides .for statining run with >10 slides in Auxiliary step should be set to 'none 'This ascertionscomperable wash times.

All incubation steps should be performed at room temperture .for detials .

Optimal conditions may very deoending on specimen and preperation methods ,and should be determined by each individual laboratory .if the evaluating pathologist should desire a different staining intensity , aDako Application Specialist /Technical service specialist can be contacted for information on re-programming of the protocol .Verity that the perforamance of the adjusted protocol is still valid by evaluating that the staining pattern is identical to the staining pattern .

Counterstainung in hematoxylin is recommened using EnVision™ FLEX hematoxylin (DakoAutostainer / Autostainer plus) (Code K8018.Non-aqueous permanent mounting medium is recommeded .

Positive and negative controls should be run simultaneousty using the same protocol as the paitent specimens .the positive control tissuse should inculde liver and tonsil and the cells/structures should display reaction patterns as described for sthesetiussesin'performancescharasteristics' in all positive specimens the

recommended negative control reagent is FLEXNegative control ,Mouse (DakoAutostainer / Autostainer plus) (Code IS750).

Staining interpretation :

cells labeled by the antibody display cytoplasmic staining .