Sudan University of Science and Technology

College of Graduate Studies

Immunohistochemical Detection of Neuroblastoma Rat Sarcoma in Thyroid Tumors among Sudanese Patients

الكشف النسيجي الكيميائي المناعي عن ورم ارومي عصبي ساركوما في أورام الغدة الدرقية لدى المرضى السودانيين

A dissertation submitted for partial fulfillment of the requirement of M.Sc. degree in medical laboratory sciences (histopathology and cytology)

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الآية

قال الله تعالي:

(وَإِنْ تَعُدُّوا نِعْمَةَ اللَّهِ لَا تُحْصُوهَا إِنَّ اللَّهَ لَغَفُورٌ رَحِيمٌ)

صدق الله العظيم

سورة النحل الآية (18)

Dedication

I dedicate this work to...

Who gave me the meaning of the life my parents..

My brothers..

Acknowledgment

Firstly I am grateful to Allah for giving me the knowledge, strength, patience to complete this work.

I am grateful to my Supervisor Dr. AbuElgasimAbass, for his perfect supervision, advice, encouragement and support from the early stage of this research as well as giving me opportunities of experiences throughout the project.

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Abstract

This is descriptive retrospective case study conducted in Omdurman teaching hospital during the period from January 2017 to August 2017. The study aimed to detect the expression of N-RAS in thyroid tumors among Sudanese patients. Total of 40 fixed paraffin blocks previously diagnosed as thyroid tumors, 28 (70%) were malignant samples include, 16(40%) papillary thyroid cancer, 8(20%) follicular carcinoma and 4(10%) medullary thyroid carcinoma, and 12(30%) samples were benign include, 9(22.5)% samples multinodular goiter and 3(7.5%) samples werefollicular adenoma were enrolled in this study.

One section of 3 microns was cut from each block and stained by immunohistochemicalmethod (avidinbiotin technique) for N-RAS detection. The data obtained were analyzed using SPSS computer program version 20.

The patients ages ranged between 11-78 years with mean age of 39years, most of them 32(80%) were less than 50 years and the remaining 8(20%) were above 50 years.

The sex of patient showed the majorly of them were female 28(70)%and 12(30%) were male.

N-RAS showed positive expression in 10(25%)samples. All of them were malignant, 6(15%) samples were papillary thyroid cancer, 3(7.5%) samples were follicular carcinoma and 1(2.5%) sample weremedullary thyroid carcinoma, While 30(75%) samples were negative for N-RAS divided as 18(45%) in malignant and 12(30%) in benign, with significant correlation between N-RAS expression and thyroid tumorsdiagnosis (P.value0.036).

The study concluded that N-RAS expression is associated with malignant form of thyroid tumors.

المستخلص

اجريت هذه الدراسة الوصفية التراجعيةفي مستشفى أم درمان التعليمي وذلك فيالفترةما بينيناير 2017 إلي اغسطس2017.

هدفت الدراسة للكشف عن ظهور (ان– راس) في أورام الغدة الدرقية بين السودانيين.

حيث تم جمع 40 كتلة برافين مسبقا كأورام للغدة الدرقية، منها 28(70%) عينات خبيثة ، شملت 16(40%) سرطانالغدة الدرقية الحلمي، 8(20%)سرطان المسامات(الجريبات)، 4(10%)سرطان الغدة الدرقية النخاعية،و12(30%)منها كانت عينات حميدةشملت، 9(22.5%)، تضخم الغدة الدرقية متعددةالعينات، 3 (7.5 %) عينات من الورم الحميد المسامي(الجريبي) تم إدراجهافي هذه الدراسة.

حيث تماخذجزء واحد سمك 3 ميكرون من كل كتلة تم صبغهبالطريقة النسيجية الكيميائية المناعية (تقنية البيوتين أفيدين) للكشف عن(ان–راس)،تم تحليل البيانات التي حصلعليها باستخدام برنامج الحزم الإحصائية للعلوم الاجتماعية برنامج الكمبيوترالإصدار 20.

تراوحت أعمار المرضى بين 11–78 سنة،بمتوسط عمر 39 عامًا،حيثكانمعظمهم 32 (80%) دون سن الـ 50 سنة، بينماكانت الـ 8 المتبقية (20%) فوق 50 عامًا.

أظهرتالدراسة أن معظمهم من الإناث 28 (70%)وأن 12 (30 %) من الذكور.

ظهر (ان–راس)تعبيرإيجابيا في 10 (25 %) عينة،كان جميعها عينات خبيثة، 6 (15%)كانت عينات سرطان الغدة الدرقية الحلمي ، 3 (7.5 %) عينات السرطان المسامي، 1 (2.5 %) عينة سرطان الغدة الدرقية النخاعية ، بينما30 (75 %) كانت عينات سلبية لـ (ان–راس) تم تقسيمها، 18(45%) أنهاخبيثة و 12 (30%) أنهاحميدة، مع وجود علاقة كبيرة بين(ان–راس) وتشخيص ورم الغدة الدرقية(القيمة الإحتمالية 0.036).

وخلصت الدراسة إلى أن ظهور (ان–راس)يرتبط مع الشكل الخبيث من أورام الغدة الدرقية.

Table of contents

Chapter Three

Materials and methods

Chapter Four

Results

Chapter Five

Discussion, Conclusion &Recommendation

List of tables

List of figures

List of abbreviations

PTC	Papillary Thyroid Carcinoma
FTC	Follicular Thyroid Carcinoma
ATC	Anaplastic Thyroid Carcinoma
MTC	Medullary Thyroid Carcinoma
NRAS	NeuroblastomaRat Sarcoma Virus
ICH	Immune Histochemical Staining
DAP	Diaminobenzidine
DPX	DistyrenePlasticizer Xylene
SPSS	Courses In Political And Social Statistic
HRP	Horse Reddish Peroxidase

Chapter One

Introduction

Chapter One
Introduction

1.1Introduction:

Thyroid cancer considered as the most common endocrine malignancy, it includes papillary thyroid carcinoma (PTC) (80%), follicular carcinoma (15%), poorly differentiated carcinoma (<1%) and an plastic carcinoma (<2%) (Nikiforov, *etal.*, 2009).

Thyroid cancer is the16thmost common cancer worldwide, with around 62.000 new cases diagnosed in 2015 in United State, this represented 3.8% of all new cases (Alexandar, *et al.*, 2012).

Thyroid cancer incidence rates are highest in Northern America and lowest in Western Africa, but this partly reflects varying data quality worldwide(Ferlay, *et al.*, 2013).

Thyroid cancer estimated there were 166 patientsof thyroid cancer cases diagnosed in Sudan in 2017 (Mohammed, 2017).

Risk factors of thyroid tumors includes exposure to radiation**,** environmental toxicants, geneticbackground, cigarette smoking, weight, andiodine intake (Terauchi, *et al.*, 2005).

The diagnosis of thyroid disease tumors by physical examination, blood tests, removing sample of thyroid tissue imaging tests, and genetic testing(Haugen, *etal*., 2015).

Treatmentof thyroid cancer done by surgery, thyroid hormone therapy, radioactive iodine, external radiation therapy, chemotherapy, injecting alcohol in to cancers, and supportive care(Tomoda, *et al.*, 2015).

Neuroblastoma rat sarcoma virus (N-RAS) is an enzyme that in humans is encoded by the NRAS gene. It was the third rat sarcoma virus (RAS) gene to be discovered, andwas named N-RAS, for its initial identification in human neuroblastoma cells(Marshal, *et al.*, 2016).

Previous study demonstrated that N-RAS (Q61R) IHC is a highly sensitive and specific tool that is useful for differentiating follicular-patterned thyroid tumorsand differential diagnosis of benign versus malignant thyroid lesion (Noaki, *et al.,* 2016).

1.2 Objectives:

1.3.1General objective:

To study the expression of N-RAS in thyroid tumors among Sudanese patients.

1.3.2Specific objectives:

1- TodetectN-RAS expression in thyroid tumors by usingimmunohisto-chemistry

2- To correlate between N-RAS expression andhistopathological diagnosis of thyroid tumors.

Chapter Two

Literature Review

Chapter Two

Literature Review

2.1 Scientific background:

Thyroid cancer is a malignant neoplasm that originates from follicular or parafollicular thyroid cells and is categorized as papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), an plastic thyroid carcinoma (ATC) and medullary thyroid carcinoma (MTC)(Cardis, *et al.,* 2006).

Most thyroid cancer malignant are derived from thyroid follicular cell and classified as papillary follicular or anaplastic thyroid carcinoma small percentage of thyroid carcinoma is derived from para follicular Ccell and called medullary thyroid carcinoma(Romei, 2012).

2.2 Structure of thyroid gland:

The thyroid gland is a butterfly-shaped organ that sits at the front of the neck. It is composed of two lobes, left and right, connected by a narrow isthymus, the thyroid weighs 25 grams in adults, the thyroid is supplied with arterial blood from the superior thyroid artery, a branch of the external carotid artery, and the inferiorthyroid artery, a branch of the thyrocervical trunk, and sometimes by an anatomical variant the thyroid arterywhich has a variable origin(Neffer and Frank, 2014).

2.3 Thyroid neoplasm's:

2.3.1Benign tumors of thyroid:

2.3.1.1Multinodular goiter:

Nodular goiter result from focal hyperplasia of follicular cell at one site, 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 driving force behind multi -nodular goiter growth is an intrinsically abnormal growth (Khatawkaret, *et al.,* 2015).

2.3.1.2Follicular adenoma:

Follicular adenoma is grossly described as a solitary, encapsulated nodule ; the size can be extremely variable, ranging from a few millimeters to 10-15cm.The color vary from tan to light brown with solid and fleshy appearance it can resemble multinodular goiter due to secondary changes in hemorrhage and cystic degeneration. On the basis of microscopic features, several variants have been described, including oncocytic adenoma (Hürthle cell adenoma), adenoma with clear cell change, atypical adenoma, hyalinizing trabecular adenoma, adenoma with bizarre nuclei and rare types such as adenomawith adipose (adenolipoma)cartilagenous (adenochondroma) metaplasia (Rosai, *et al.,* 1992).

2.3.2 Types and classification of thyroid cancer:

2.3.2.1 Papillary thyroid cancer:

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 radiation, accounting for about 80% of thyroid cancers. While 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(Wreesmann, *et al.,* 2004).

2.3.2.2 Follicular thyroid cancer:

It accounts for about 10% of thyroid cancers. Like papillary thyroid cancer, follicular thyroid cancer usually grows slowly. Its outlook is similar to papillary cancer, and its treatment is the same. Follicular thyroid carcinoma (FTC) is a well differentiated tumor. In fact, FTC resembles the normal microscopic pattern of the thyroid. FTC originates in follicular cells and is the second most common cancer of the thyroid, after papillary carcinoma. Follicular and papillary thyroid cancers are considered to be differentiated

thyroid cancers; together they make up 95% of thyroid cancer cases (Hezel, 2014).

2.3.2.3 Medullary thyroid cancer:

This is the only type of thyroid cancer that develops in the para follicular cells of the thyroid gland. It accounts for 3% to 10% of thyroid cancers. Medullary cancer cells usually make and release into the blood proteins called calcitonin and carcinoembryonic antigen, which can be measured and used to follow the response to treatment for the disease (Schlumberger, *et al.,* 2008).

2.3.2.4An aplastic thyroid cancer:

Anaplastic thyroid cancer (ATC) is the most aggressive thyroid tumor and one of the most aggressive cancers in humans. Anaplastic thyroid carcinoma affects more women than men, but the female-to-male ratio is of about 2–3:1, lower than that of papillary or follicular carcinoma. It arises from the follicular cells of the thyroid gland but does not retain any of the biological features of the original cells, such as uptake of iodine and synthesis of TG. The peak incidence is in the sixth–seventh decades (mean age at diagnosis 55–65 years) and the prevalence is fortunately very low (<2% of all thyroid tumors)(Cooper, *etal.,* 2009).

2.4 The epidemiology of thyroid cancer:

Thyroid cancer is the 16thmost common cancer worldwide, with around 62, 000 new cases diagnosed in 2015 in united statesthis represented 3.8% of all new cases (Alexandar, *etal.,* 2012).

Thyroid cancer incidence rates are highest in Northern America and lowest in Western Africa, but this partly reflects varying data quality worldwide(Ferlay, *etal.*, 2013).

Thyroid cancer represented 1-2% of all human malignancies. The annual incidence varies among countries and it is estimated that 1.2-2.6 men and 2.0-3.8 women/100, 000 individuals are affected worldwide.This incidence has

been increasing in the last decades, likely due to an "over –diagnosis "of small cancers that would have remained occult and that have been likely revealed because of an increased diagnostic scrutiny rather than a real increased of incidence.The annual mortality rate of thyroid cancer is 0.5/100.000 both in men and women.Thyroid cancer is 2-4 times more frequent in female than in males. The mean age at diagnosis is 40-45year for papillary tumor (PTC) and 50-55 for follicular tumors(FTC).They are very rare in children (Fadda, *etal.*, 2017).

2.5 Risk factors of thyroid tumors:

A risk factor is anything that increase chance of getting a disease like cancer includes:

2.5.1 Exposure to radiation:

Exposure to moderate levels of radiation to the head and neck may increase the risk of papillary and follicular thyroid cancer. Such sources of exposure include, radiation therapy for hodgkin lymphoma, exposure to radioactive iodine, and exposure to ionizing radiationincluding radioactive fallout from atomic weapons(Jemal, *etal.*, 2010).

2.5.2 Breast cancer:

A recent study showed that breast cancer survivors may have a higherrisk of thyroid cancer, particulary in the first 5years after diagnosed with breast cancer at a younger age (Miller, *etal.*, 2009).

2.5.3 Genetic background:

Some types of thyroid cancer are associated with genetics. Abnormal RET oncogene, which can passed from parent to child, may cause MTC. Blood tests and genetic tests can detect the gene. People with MTC are encouraged to have genetic testing to determine if a mutation of the RET proto-oncogeneis present. If so, genetic testing of parents, siblings, and children will be recommended (Villanueva, *et al.*, 2003).

2.5.4 Age:

Thyroid cancer can occur at any age, but abut 2-third of all cases are found in people between the age of 20-55(Mack, *et al.,* 2003).

2.5.5 Iodine intake:

Iodine is needed for normal thyroid faction.In the United States, iodine is added to salt to help prevent thyroid problems (Brent, *et al.,* 2007).

2.5.6 Gender:

Thyroid cancer occurs about 3 times more often in women than in men (Strieder, *et al.,* 2003*).*

2.5.7 Family history:

A family history of MTC increases person's risk. People with MEN2 syndrome are also at risk for developing other type of cancers, and family history of precancerous polys in the colon increases the risk of developing papillary thyroid cancer(Jour, *etal*., 2017).

2.6 Diagnosis of thyroid tumors:

1.6.1. Fine needle aspiration:

Fine-needle aspiration biopsy is the most sensitive, specific, and cost-effective test for thyroid cancer(Mazzaferri, *et al.,* 2004*).*

1.6.2. Ultrasound:

Ultrasound play significant role in thyroid cancer diagnosis and management by helps in detecting the presence of a small malignant lesion in the contralateral lobe and can be used as follow-up imaging for disease surveillance and early detection of tumor recurrence(Wong and Anil.2005).

1.6.3. CT/ scan:

A procedure that makes a series of detailed pictures of areas inside the body taken from different angles (Michel, *et al.,* 2006).

2.7 Treatment options thyroid cancer:

2.7.1 Surgery:

Thyroctomy and dissection of central neck compartment is initial step in treatment of thyroid cancer in majority of cases (Ferlay, *et al.*, 2013).

2.7.2 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, *etal.*, 2006).

2.7.3Post 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 <0.1 mu/L or up to 0.5 mu/L for lower-risk patients (Cooper, *etal*., 2009).

2.8 Thyroid cancer prognosis:

The argument against early diagnosis and treatment is based on the logic that many small thyroid cancers (mostly papillary) will not grow or metastasize. This viewpoint holds the overwhelming majority of thyroid cancers are over diagnosed (that is, will never cause any symptoms, illness, or death for the patient, and even if nothing is ever done about the cancer). Including these over diagnosed cases skews the statistics by lumping clinically significant cases in with apparently harmless cancers (Hofman, 2013).

2.9 N-RAS:

Gtase N-RAS or N-RAS is aguanine-nucleotide binding protein.Receptor tyrosine Kinases and G-protein coupled receptors activate RAS, which then stimulates the MAK signal pathway. Point mutations (Q61R) in RAS prevent

the GAP- mediated inhibition of the MAPK pathway in certain cancers such as lung carcinoma and melanoma.AbnormalMAPK signaling may lead to uncontrolled cell proliferation, resistance to apoptosis and cancer therapy. The frequencies of theNRASQ61R in FTAs and FTCs were significantly higher than that inNH(P=.046 and P=.001, respectively)(Noaki, *et al*., 2016).

At histology, 12 cases of follicular carcinoma, cytologically defined as follicular lesions, 1papillary cancer, 7 follicular adenoma,and4hyper plastic nodules were found. Of these, 4showed positive IHC for anti NRASQ61R antibody, the remaining cases had negativeIHC(Massi, *etal*., 2015).

The presence of NRAS codon 61 mutation,was significantly associated with the distant metastasis. The NRAS codon61 mutation status might bepotential prognostic factor in FTC patients(Kyung, *etal*., 2014).

Chapter Three

Materials and methods

Chapter Three
Materials and methods

3.1 Materials:

Archive tissue blocks obtained from samples of thyroidtumorswere used in this study.

3.2 Methods:

3.2.1 Study design:

This is a hospital based descriptive retrospective case study aimed to detectNRAS expression in thyroid tumors using immunohistochemical method.

3.2.2 Study sample:

Forty thyroid tissueblocks were obtained from tissue previously diagnosed asthyroid tumors28 of them were thyroid cancer and 12were thyroid hyperplasia at Omdurman teaching hospital during the period from January to December 2017.Patient age and sex were obtained from patients file.

3.2.3 Study area:

This study was held inOmdurman teaching hospital in 2017.

3.2.4 Immunohistochemicalstaining:

Immunehistochemical staining procedure was carried out using new indirect dextran polymer immune peroxidase technique. Sections of 3µm thickness were obtained from formalinfixedparaffinembedded tissue by using a rotary microtome. Followingdeparaffinization in xylene, slides were rehydrated through a graded series of alcohol(100%, 90%, 70%, and 50%) and were placed in water. Samples were steamed for antigen retrieval using water bath, slides were placed in coplin jar containing enough Tris-EDTA buffer (pH 9.0)to cover the sections, then was boiled at high temp for 20 minutes.Then washed in phosphate buffer saline (pH7.4) for five minutes.Then circulated by Dako pen, allowed sections to cool at RT.The sections were covered with 3%

hydrogen peroxidaseand methanol for 10 minutes, to block endogenous peroxidase activity, then slides were incubated with 50µl of primary antibody (N-RAS), (ready to use) for 30 minutes at room temperature in a humiditychamber, and then washed with PBS for 3 minutes. Thenincubated in dextran polymer – Horse Reddish peroxidase (HRP) secondary antibody for 15 minutes, 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 5 minutes. Slides were counter stained withMayer'shematoxylinthenuseddistyrene plasticizer xylene(DPX)mountmedia.

3.2.5: Result interpretation:

All quality control measures were adopted, detection of one cell with brown nucleus per one field considered as positive results.

3.2.6:Dataanalysis:

Data were analyzed using version 20.0 SPSS computer program, frequencies means and chi-square test values were calculated.

3.2.7:Ethical considerations:

Sample collected after taking ethical acceptance from Omdurman teaching hospital administration.

Chapter Four

Results

Chapter Four

Results

The study includes forty samples, 28 (70%) were malignant samples include, 16(40) papillary thyroid cancer, 8(20%) follicular carcinoma and 4(10%) medullary thyroid carcinoma, and 12(30%) samples were benign include, 9 (22.5%) samples multinodular goiter and 3(7.5%) follicular adenoma (table 4.1).

The patient age ranged between 11-78 years with mean age 39 years, most of them 32(80%) were less than 50 years and the remaining 8 (20%) were above 50years(table 4.2).

The patients sex revealed that 12(30%) patients were male and 28(70%) patients were female (table 4.3).

Malignant thyroid tumors revealed positive expression of N-RAS in10(25%)samples and negative expression of N-RAS in 18 (45%) samples while all benign thyroid tumorsshowed negative expression of N- RAS 12(30%) samples.This result showed significant statistical association (p-value 0.036), as indicated in table (4.5).

Table (4. 1) Frequency of histopathological diagnosis among study samples

Histopathological diagnosis		**Frequency**	**Percent**	
Malignant	Papillary thyroid cancer	16	40 %	70%
	Follicular thyroid cancer	8	20%	
	Medullary thyroid cancer	4	10%	
Benign	Follicular adenoma	3	7.5%	30%
	Multinodular goiter	9	22.5%	
Total		**40**	**100%**	

Table (4. 2) Distribution of age among study population

Age group (years)	**Frequency**	**Percent**
<50	32	80%
>50	8	20%
Total	**40**	**100%**

Table (4. 3) Distribution of sex among study population

Sex	**Frequency**	**Percent**
Male	12	30%
Female	28	70%
Total	**40**	**100%**

Table (4. 4) Correlation between N-RAS expression and histopathological diagnosis of thyroid tumors:

Histopathological diagnosis	**N-RAS**				**Total**		**P-value**
	Positive		Negative				
	N	%	N	%	N	%	
Malignant	10	25%	18	45%	28	70%	
Benign	0	0%	12	30%	12	30%	0.036
Total	**10**	**25%**	**30**	**75%**	**40**	**100%**	

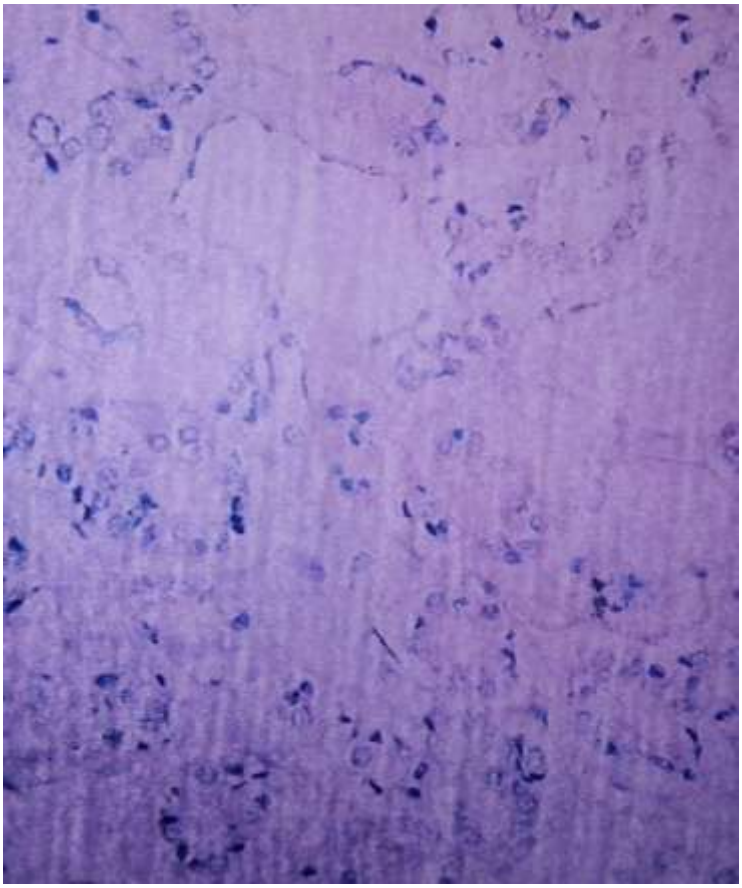

Fig (4. 1) Benign thyroid tumors showed negative expression of N-RAS

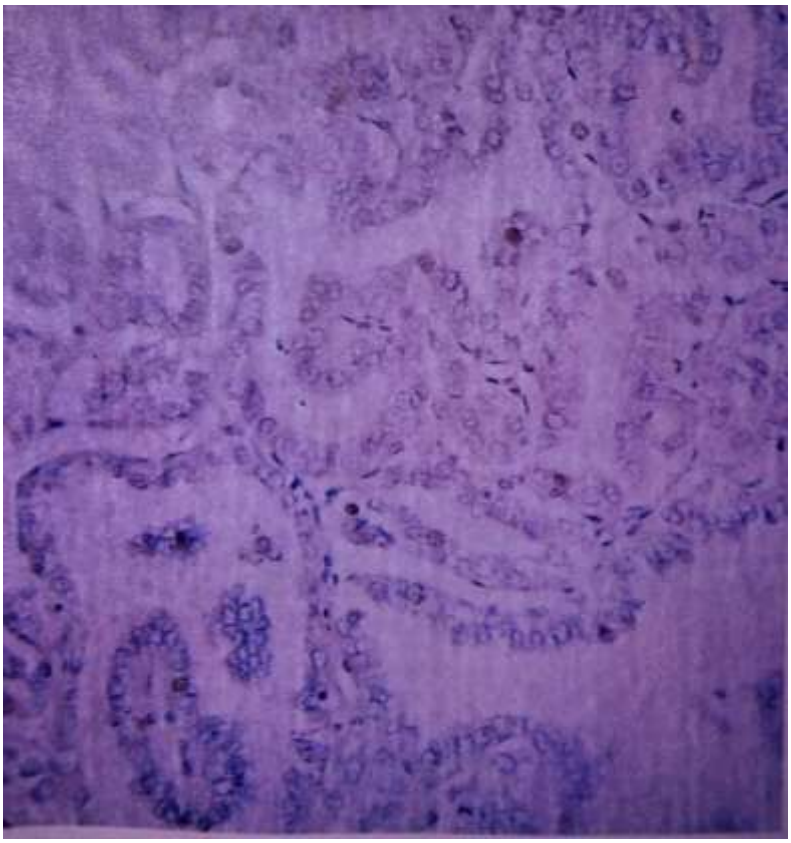

Fig (4. 2) Malignant thyroid tumors showed positive expression of N-RAS

Chapter Five

Discussion, Conclusion &Recommendation:

Chapter Five

Discussion, Conclusion &Recommendation:

5.1 Discussion:

In this study forty samples were investigated by immunehistochemical stain 28(70%) were malignant samples, and 12(30%) samples were benign.

Concerning the age of study population, most patients were aggregating in less than 50 years; the middle age was 39 years. This result compatible with (Rosai, *etal.*, 2011), who reported thatthyroid cancer more common in young and middle-aged adults,the median age at diagnosis is 40 years. It is caused by environmental, genetic and hormonal factors.

The study revealed that most of patients were female; female/male ratio was 4:1. This result agrees with (Topliss, *etal.*, 2004), who reported that female to male ratio of patients was 2.9:1, also agrees with (Strieder, *etal.*, 2003), who reported that thyroid cancer occur about 3 time in women than men.

Positive expression of NRAS in 10(25%) patients and negative expansion of NRAS in 30(75%) patient. this result showed significant statistical association (p.value 0.036).This result agrees with(Naoki, 2016), who reported that the frequencies of the NRASQ61R in thyroid carcinoma 29% (10/35) was significantly higher than that in nodular hyperplasia 2%(1/41).And disagrees with (Crescenzi, *et al.*, 2017), who reported that high reliability of IHC to identify NRAS Q61R mutated thyroid lesions.

5.2Conclusion:

From this study we conclude that:

The age of the thyroid cancer patients in our study samples is commonly less than 50 years.

Most histological type of thyroid cancer is papillary thyroid cancer.

The sex of the patients in our study is commonly female.

There is association between N-RAS and histological diagnosis of the thyroid tumors.

5.3 Recommendation:

From this study we recommend that:

Further study should be done on expression of NRAS in thyroid tumors with large sample size.

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Appendix

Appendix1

Materials and instruments used for processing and staining of the specimensinclud

Include:

- Disposable gloves.
- Rotary microtome.
- Microtome knives.
- Positively charged slides(thermo).
- Cover glasses.
- Dry oven.
- Water path (PT LINK).
- Coplin jars.
- Humidity chamber.
- Ethanol (100%, 90%, 70%, 50%).
- Xylene.
- Eosin.
- Mayer’s haematoxylin (haematoxyline, Dw, potassium or ammonium alum, sodium iodate, citric acid and chloral hydrate).
- Tris EDTA buffer (PH 9).
- Phosphate buffer saline (PH 7.4).
- Peroxidase blocker (0.3% hydrogen peroxide in methanol).
- Primary antibody (anti-human NRAS).
- Secondary antibody (dextran polymer conjugated secondary antibody horse reddish peroxidase).
- DAB (3.3 di amino Benz aldehydetetra hydrochloride) Substrate solution.
- DPX.

Appendix2

ZYTOMED SYSTEMS

Rabbit anti-N-Ras (Q61R)

Cat. No. and Size:	**515-4740**: 0.1 ml rabbit monoclonal antibody purified by protein A/G in PBS pH 7.6 with 1 % BSA and less than 0.1% sodium azide. **515-4742**: 0.5 ml ml rabbit monoclonal antibody purified by protein A/G in PBS pH 7.6 with 1 % BSA and less than 0.1% sodium azide. **515-4744**: 1 ml ml rabbit monoclonal antibody purified by protein A/G in PBS pH 7.6 with 1 % BSA and less than 0.1% sodium azide. **515-4741**: 7 ml pre-diluted rabbit monoclonal antibody purified by protein A/G in TBS pH 7.6 with 1 % BSA and less than 0.1% sodium azide.
Intended Use:	For research use only. Not for use in diagnostic procedures.
Clone:	SP174
Immunogen:	Synthetic peptide of human N-Ras protein containing N-Ras (Q61R) point mutation.
Isotype:	Rabbit IgG
Epitope:	Not determined
Molecular Weight:	21 kDa
Species Reactivity	Human (tested). Others not tested.
Description:	GTPase N-Ras or N-Ras is a guanine-nucleotide binding protein. Receptor tyrosine kinases and G-protein-coupled receptors activate Ras, which then stimulates the MAPK signal pathway. Point mutations (Q61R) in Ras prevent the GAP-mediated inhibition of the MAPK pathway in certain cancers such as lung carcinoma and melanoma. Abnormal MAPK signaling may lead to uncontrolled cell proliferation, resistance to both apoptosis and cancer therapy.
Applications:	Immunohistochemistry (IHC) on formalin-fixed, paraffin-embedded tissue sections, Western Blotting.
IHC Procedure:	**Antibody Dilution:** If using the concentrate format of this product, dilute the antibody 1:100. The dilutions are estimates; actual results may differ because of variability in methods and protocols. **Antigen Retrieval:** Boil tissue sections in EDTA buffer, pH 8.0 for 10 min followed by cooling at RT for 20 min. **Primary Antibody Incubation:** 10 minutes at RT.
IHC Positive Control:	SK-MEL-2 Cell Line
Western Blotting:	Recommended starting protocol: Dilute the antibody 1:400. Incubate for 1 hour at room temperature. The dilution is an estimate; actual results may differ because of variability in methods and protocols. Optimal dilution and procedure should be determined by the end user.
Western Blotting Positive Control:	SK-MEL-2 Cell Line
Cellular localization:	Membrane, Cytoplasm