Benghazi University

Faculty of Medicine

Evaluation of Ki-67 And P53 Expression In Papillary Thyroid Carcinoma By Immunohistochemistry And Studying Its Correlation With Clinicopathological Features And Prognosis

Thesis Submitted For Partial Fulfillment Of Master Degree In Histopathology

By

Zainab Muftah El-amami

Supervisor

Prof. Omran AL-fituri

Benghazi University, Faculty of Medicine

LIBYA

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Dedication

I’m pleased to dedicate this work to my son Mohammed .

Also, I am happy to express my gratitude to my mother

for their patience and endless love and support.

Acknowledgment

First of all, thanks to "ALLAH"

I would like to thank my supervisor Prof. Omran AL-Fituri for their support, encouragement, and patience during my work on this thesis.

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ABBREVIATIONS

AD1	activation domain 1
AD2	activation domain 2
ATC	Anaplastic thyroid carcinoma
BCL-2	B-cell lymphoma /leukaemia-2
BCR	Benghazi cancer registry
BRAF	B-type Raf kinase
CDK	cyclin dependent kinase
CSS	cause-specific survival
DBD	DNA binding core domain
DFS	Disease free survival
DTC	Differentiated thyroid cancer
FMTC	Familial Medullary Thyroid Cancer
FNA	Fine needle Aspiration
FNAB	Fine-needle aspiration biopsy
FNAC	Fine -needle aspiration cytology
FTC	follicular thyroid cancer
HMGA	high mobility group A factors

HT Hashimoto's thyroiditis

IDD iodine deficiency disorder

IHC Immunohistochemistry

Ki -67 Proliferation factor

MAPK Mitogen-activated protein kinase

MDM2 Murine double minute-2

MEN Multiple Endocrine Neoplasia

MEN2 Multiple endocrine neoplasia type 2

NG Nodular goiter

NMTC Non medullary thyroid cancer

OD Oligomerisation domain

P53 tumor suppressor gene/protein P53

PCNA proliferating cell nuclear antigen

PDC poorly differentiated carcinoma

PET Positron Emission Tomography

PPAR γ Peroxisome proliferator-activated receptor γ

PTC Papillary Thyroid Cancer

RET Rearanged during Transfection

RAI Radioactive iodine

RLN Regional lymph node

SEER	Surveillance, Epidemiology and End Results
TAb	thyroid auto antibodies
TAD	transcription – activation domain
TBG	Thyroxin - binding globulin
Tg	Thyroglobulin
TgAb	Anti-thyroglobulin Antibodies
TNM	Tumor Node Metastases classification
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
VEGF	Vascular endothelial growth facto
UICC	International Union Against Cancer
WHO	World Health Organization

Chapter I

Introduction
and
aims of the study

1.1ABSTRACT

Background and aims: Despite the generally indolent behavior of papillary thyroid cancer, we need to identify patients who experience an unfavorable outcome. Currently, several risk classification methods have been established to predict the prognosis of individual patient, TNM classification being the most widely used prognostic scoring system. To reduce the number of thyroid cancer recurrences, our aims were to find a marker or a set of markers (p53&Ki67) to predict the outcome of an individual patient better than does TNM classification alone.

Patients and methods: paraffin blocks of 32 Libyan cases of papillary thyroid carcinoma were retrieved. Immunohistochemistry was performed. Nuclear staining were evaluated. Ki67&P53 immunoexpression were categorized for statistical analysis. Statistical tests were used to determine the association of Ki67&P53 with clinicopathological characteristics; age, gender, localization , tumor size ,extrathyroid extension ,distant metastasis, histological type, lymph node involvement, lymphovascular invasion, blood Vessel invasion, pre existence hashimiotothyroiditis , multifocality.

Results: The tumor arising from left lobe show proliferation index more than the right lobe ($p < 0.028$), moreover decreased P53 expression was significantly associated with tumor localization, tumor arising in both lobes show low expression of p53, also tumor arising in left lobe or in right lobe express low P53 ($p < 0,06$). However tumor express P53 associated with lymphatic invasion and show border line significant ($p < 0.08$). No statistically significant correlation was found between Ki67& P53 expression in tumors and other clinicopathological parameters (such as age, gender, histopathological type, tumor size, extrathyroid extension, multifocality, pre-existence hashimiotothyroiditis).

Conclusion: Although the sample size of our study was small, immunohistochemical evaluation of Ki-67and p53 expression in patients with PTC may be useful for determining prognosis, in PTC patients.

Key words: papillary thyroid cancer, Ki67& P53 expression, IHC, prognosis.

1.2.Introduction

Thyroid cancers are quite rare, accounting for only 1.5% of all cancers in adults and 3% of all cancers in children (American cancer society, 2009), thyroid cancer is commonly diagnosed at a younger age than most other adult cancers. Nearly 2 out of 3 cases are found in people younger than 55 years of age. About 2% of thyroid cancers occur in children and teens (American cancer society, 2013), female are more likely to have thyroid cancer at ratio 3:1, thyroid cancer is the fastest growing cancer diagnosis in the US (Hayat et al., 2007; Jemal et al., 2007) with a total of 44,670 new cases and 1,690 deaths expected in 2010 .

Thyroid cancers encompass a variety of lesions that range from benign adenoma to malignant tumors. They also span the spectrum from well-differentiated, poorly differentiated or undifferentiated (anplastic). More than 95% of thyroid cancers are derived from thyroid follicular cells, while 2-3% of thyroid tumors (medullary thyroid cancers) are derived from the calcitonin producing C-cells .

Papillary thyroid cancer (PTC) is the most common form of thyroid cancer, representing approximately 80% of all thyroid malignancies. PTC is usually indolent and curable but this cancer can spread early to local lymph nodes and disease persistence and/or recurrence are common and associated with increased mortality (Sherman et al., 1998; Xing et al., 2007).

Follicular thyroid cancer (FTC) is the second most common thyroid cancer, representing approximately 15% of all thyroid malignancies, this cancer can develop from a pre-existing benign follicular adenoma or directly and it is characterized by hematogenous spread. Poorly differentiated (PDC) and anplastic (ATC) thyroid cancers are rare representing 2-5% of all thyroid cancers (Kondo et al., 2006; Nikiforova et al., 2009). These are very aggressive tumors that can develop de novo or from the progression of pre-existing papillary or follicular carcinomas.

Thyroid cancer is usually very treatable and is often cured with surgery. Even when thyroid cancer is more advanced, effective and well-tolerated treatment is available for the most common forms of thyroid cancer.

There are several factors, genetic and environmental, implicated in the pathogenesis of thyroid cancers. The most common genetic abnormalities found in PTC are the point mutations of BRAF and RAS genes as well as RET/PTC rearrangements. These mutations are found in more than 70% of PTCs and tend to be mutually exclusive. The more common genetic alterations in FTCs are RAS point mutations or PAX8/PPARγ rearrangements, respectively which are also usually mutually exclusive. RAS and PAX8/PPARγ rearrangements are found in ~80% of FTC. RAS and to a lesser extent PAX8/PPARγ translocations are also associated with follicular adenoma with frequencies of 20-40% and of 2-10%, respectively (Kondo et al., 2006; Nikiforova et al., 2009).

From nuclear disasters such as Chernobyl in 1986, it is clear that radiation exposure is a significant risk factor for thyroid cancer (Detours et al., 2005) but the majority of thyroid cancers appear to be sporadic in nature, most series have shown a statistical correlation between iodine deficiency and an increased incidence of thyroid carcinoma of both follicular and undifferentiated types, the tumors tend to be more advanced at the time of diagnosis than in nonendemic areas. Papillary carcinoma is the predominant type in areas without iodine deficiency, and its frequency is said to be increased in regions with high iodine uptake (Ackerman's, 2011).

For PTC several prognostic factors have been identified. These factors can be divided into four categories, backgrounds of patients, factors based on preoperative, and intraoperative and postoperative evaluations. Backgrounds of patients include age, gender, and family history. Preoperative evaluation is mainly performed by imaging studies Of these, ultrasonography is the most useful tool to detect and evaluate primary lesions (Ito et al., 2007), and regional lymph node metastasis, size, location, and multiplicity of primary lesions can be evaluated on ultrasonography lymph node metastasis can also be diagnosed based on ultrasonography criteria. Intraoperative evaluation is based on findings during surgery, including extra thyroid extension and extra nodal, tumor extension to adjacent organs. The degree of extension that is to where and how the tumor extends significantly Postoperative evaluation includes findings based on pathological and molecular examinations. There are various histological types in PTC, most of which are diagnosed on pathological examination. Molecular examination includes evaluation of cell proliferating activity, there are some

markers used one of these proliferating marker is Ki-67. Ki-67 is a protein that in human is encoded by the MKi- 67 gene (antigen identified by monoclonal antibody Ki-67) (Schonk et al., 1989). The absence of Ki-67 in quiescent cells and its universal expression in proliferating tissues created great interest on its potential role as a marker of cell proliferation (Van Dierendonck et al., 1989). Ki-67 protein is present during all active phases of the cell cycle (G1, s, G2, and mitosis) but is absent from resting cells(G0) .

Over expression is frequently seen in a variety of malignant tissues and is associated with worse survival of individuals with bladder (kilicli – camur et al., 2002) brain (Tohannessen et al., 2006) breast (Stuart – Harris et al., 2008 ; Viale et al., 2008).

Some studies on Ki-67 reported, the mean values of Ki-67 LI increased progressively from multinodular goiter to follicular adenoma, papillary carcinoma, follicular carcinoma, and medullary carcinoma, and were the highest in undifferentiated carcinoma (pujani et al., 2010). These findings are in close agreement with those of Erickson et al. They observed the highest values for Ki-6 LI in anaplastic carcinoma followed by follicular and papillary carcinoma.

Oncogenes gain of function is the most frequent molecular alteration described in thyroid cancer it mainly includes the aberrant activation of the RAS/RAF/MEK/ERK pathway (Kroll , 2004; Hunt, 2005). Loss of function of tumor suppressor proteins may also occur in thyroid cancer and includes PAX-8/PPARɣ rearrangement, PTEN down-regulation, b-catenin, and p53 mutations (Kroll 2004, Hunt 2005). While inactivating mutations of the p53 gene is very frequent in human cancers (50% of all human malignancies), they have been found in only 10% of thyroid carcinomas (Olivier et al., 2002) and mainly in poorly differentiated and aggressive histotypes.

The malignance of papillary thyroid carcinoma with p53 mutation is higher (Puglisi et al., 2000). Other research indicated that p53 mutation is possibly the fundamental causes of thyroid gland canceration caused by ionizing radiation (Park et al., 1998). So it could be concluded that p53 mutation has an effect on infiltration, lymphatic metastasis and prognosis of thyroid carcinoma.

1.3.Aims of study

1. To evaluate the expression of Ki67 and P53 in Libyan papillary thyroid cancer patients.

2. To observe the relationship between traditional prognostic parameters in correlation with Ki-67 and P53 expression in papillary thyroid cancer.

Chapter II

Review of the literature

2. REVIEW OF LITERATURE

2.1.Embryology and anatomy of thyroid gland

Embryology

In the fetus, at 3–4 weeks of gestation, the thyroid gland appears as an epithelial proliferation in the floor of the pharynx at the base of the tongue between the tuberculum impar and the copula linguae at a point later indicated by the foramen cecum. The thyroid then descends in front of the pharyngeal gut as a bilobed diverticulum through the thyroglossal duct. Over the next few weeks, it migrates to the base of the neck, passing anterior to the hyoid bone. During migration, the thyroid remains connected to the tongue by a narrow canal, the thyroglossal duct. Thyrotropin-releasing hormone (TRH) and thyroid-stimulating hormone (TSH) start being secreted from the fetal hypothalamus and pituitary at 18-20 weeks of gestation, and fetal production of thyroxine (T_4) reach a clinically significant level at 18–20 weeks. Fetal triiodothyronine (T_3) remains low (less than 15 ng/dL) until 30 weeks of gestation, and increases to 50 ng/dL at term. Fetal self-sufficiency of thyroid hormones protects the fetus against e.g. brain development abnormalities caused by maternal hypothyroidism (Zoeller, 2003). However, preterm births can suffer neurodevelopmental disorders due to lack of maternal thyroid hormones due their own thyroid being insufficiently developed to meet their postnatal needs (Berbel et al., 2010). The portion of the thyroid containing the parafollicular C cells, those responsible for the production of calcitonin, are derived from the neural crest. This is first seen as the ultimobranchial body, which joins the primordial thyroid gland during its descent to its final location in the anterior neck.

Gross anatomy

The normal adult thyroid has a shape reminiscent of butterfly. With two bulky lateral lobes connected by a thin isthmus. Each lateral lobe is 2 to 2.5 cm wide 5 to 6 cm long and 2 cm deep.The thyroid gland is located in the mid portion of the neck, where it is attached to the anterior trachea by loose connective tissue. The two lateral lobes surround the ventral and lateral aspects of the larynx and trachea, reaching the lower halves of the thyroid cartilage and covering the second, third and fourth tracheal rings.

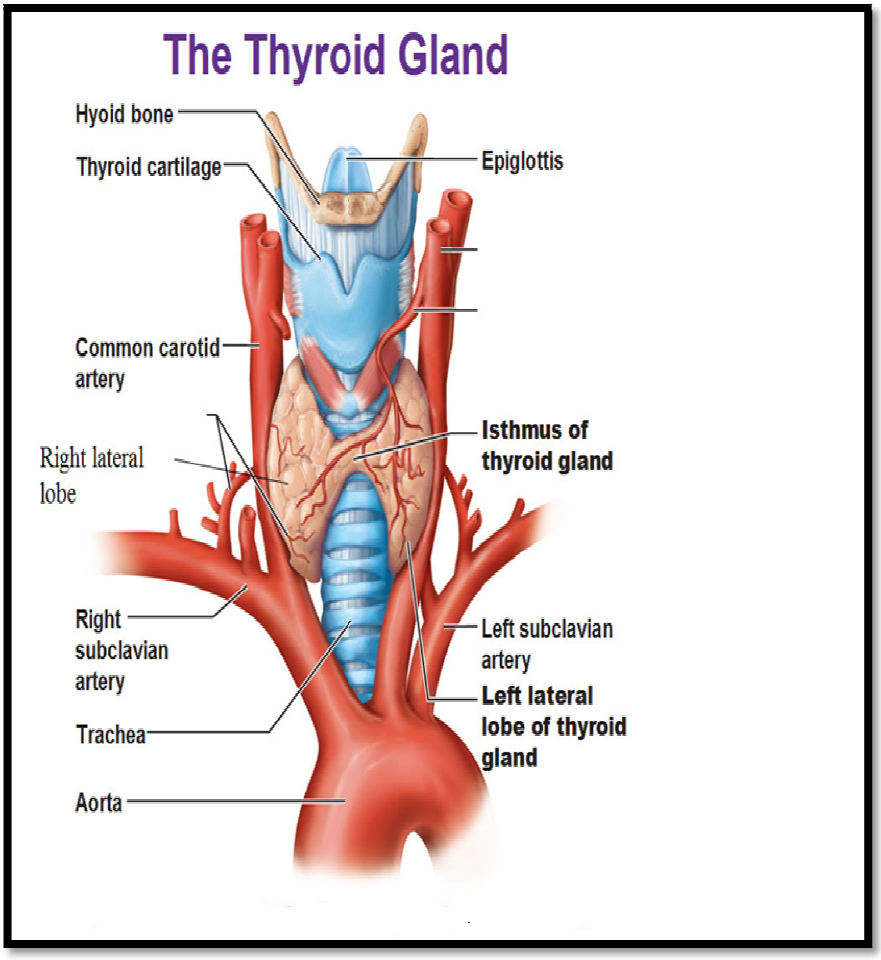


Figure2.1. Gross anatomy of thyroid gland (http://www antranik.org)

The normal weight of the adult thyroid is 15 to 25g in non goitrous areas. The color of the normal thyroid is red-brown, the blood supply of the thyroid gland derives primarily from the inferior thyroid artery (which originates from the thyrocervical trunk of the subclavian artery) and the superior thyroid artery (which arises from the external carotid). A thyroideaima artery also may be present.

The superior and medial thyroid veins and the inferior vein drain into the internal jugular and the brachiocephalic vein. The lymph vessels draining the superior portion of the thyroid lobes and isthmus collect into the internal jugular lymph nodes, whereas

those draining the inferior portion of the gland collect into the pre-and paratracheal and prelaryngeal lymph nodes (Hoyes et al., 1997).

2.2.Histology of thyroid gland

The fundamental unit of the thyroid is the follicle, around to slightly oval structure lined by a single layer of epithelial cells resting on abasement membrane. The lumen of the follicle contains colloid, a viscous material that is mostly composed by proteins secreted by the follicular cells including thyroxin-binding globulin (TBG). The follicles, which are separated from each other by a loose fibro connective tissue, have an average diameter of 200 μm.

Follicular cell

Light microscopy- The cells lining the follicles show variations in their shape and size according to the functional status of the gland. Three major types are described: flattened, cuboidal and columnar (cylindrical). Flattened cells are relatively inactive, cuboidal cells (their height equaling their width) are the most numerous and their major function is to secrete colloid. The rarer columnar cells resorb the TBG-containing colloid, liberate the active hormones, and excrete these hormones into blood vessels.

Size and position of the nucleus and some components of the cytoplasm may vary considerably. In the resting thyroid, the nucleus is round or oval is located toward the center of the cell and usually contains one nucleolus that is eccentrically located. Its chromatin may be finely granular or clumped. In actively secreting cells, the nucleus is enlarged because of the mostly apical enlargement of the cytoplasm, it acquires a basal position.

Electron microscopy

Ultra structurally, the follicular cells are arranged in a single layer around the colloid and rest on abasement membrane, approximately 35 to 40 nm in thickness, that separate them from the interstitial stroma. Microvilli emanate from the surface of the cells, their number being increased and their length greater in actively functioning cells the

cytoplasm contains variable amounts of endoplasmic reticulum, mitochondria of usually small size, and lysosomes (Hoyes et al., 1997).

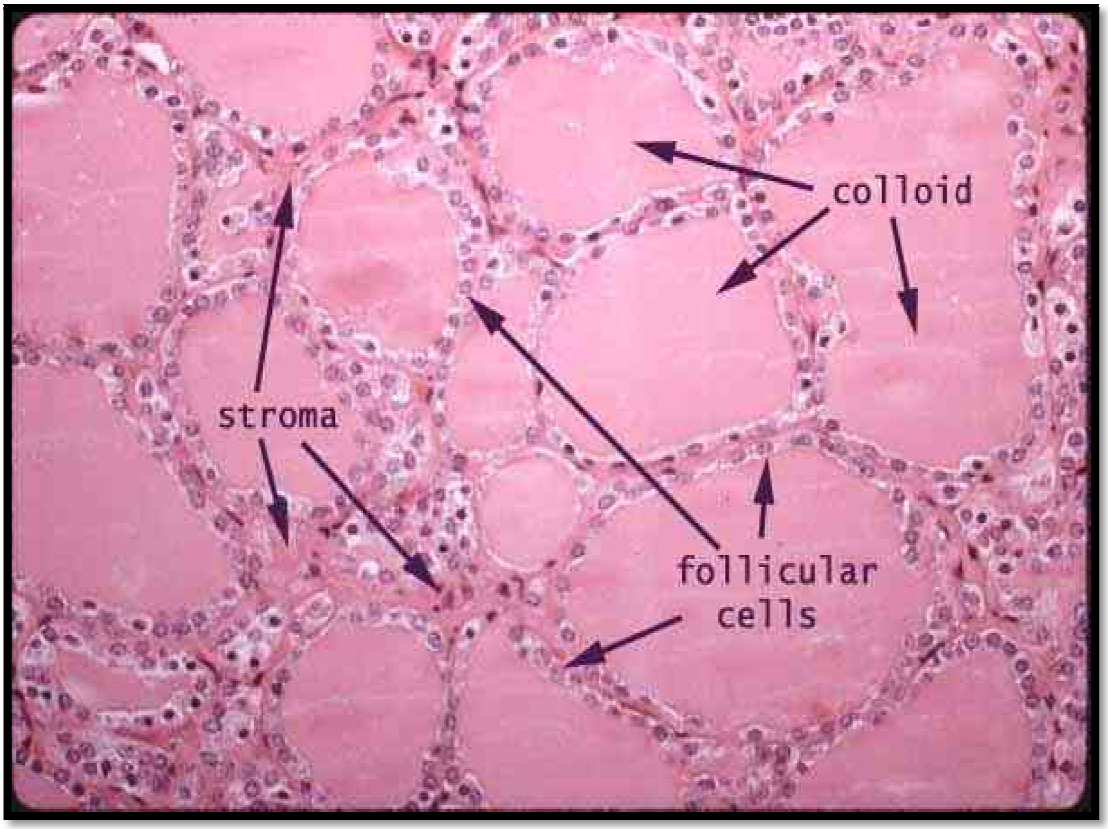


Figure2.2. Thyroid follicles and colloid the simple cuboidal epithelium lining the follicles (http://www anatomy forme.blogspot.com)

2.3.Physiology of thyroid gland

The primary function of the thyroid is production of the hormones triiodothyronine (T_3), thyroxine (T_4), and calcitonin. Up to 80% of the T_4 is converted to T_3 by peripheral organs such as the liver, kidney and spleen. T_3 is several times more powerful than T_4, which is largely a prohormone, perhaps four or even ten times more active.

Synthesis of the thyroid hormones, as seen on an individual thyroid follicular cell (Boron & Boulpaep, 2003): Thyroglobulin (Tg) is synthesized in the rough endoplasmic reticulum and follows the secretory pathway to enter the colloid in the lumen of the thyroid follicle by exocytosis, a sodium-iodide (Na/I) symporter pumps iodide (I^-) actively into the cell, which previously has crossed the endothelium by largely unknown mechanisms, this iodide enters the follicular lumen from the cytoplasm by the transporter pendrin, in a purportedly passive manner, in the colloid, iodide (I^-) is oxidized to iodine (I^0) by an enzyme called thyroid peroxidase, iodine (I^0) is very reactive and iodinates the thyroglobulin at tyrosyl residues in its protein chain (in total containing approximately 120 tyrosyl residues), in conjugation, adjacent tyrosyl residues are paired together, the entire complex re-enters the follicular cell by endocytosis.

T_4 is synthesized by the follicular cells from free tyrosine and on the tyrosine residues of the Tg. Iodine is captured with the "iodine trap" by the hydrogen peroxide generated by the enzyme thyroid peroxidase (TPO) (Ekholm &Bjorkman, 1997). And linked to the 3' and 5' sites of the benzene ring of the tyrosine residues on Tg, and on free tyrosine. Upon stimulation by the TSH, the follicular cells reabsorb Tg and cleave the iodinated tyrosines from Tg in lysosomes, forming T_4 and T_3 (in T_3, one iodine atom is absent compared to T_4), and releasing them into the blood. Deiodinase enzymes convert T_4 to T_3 (Bianco et al., 2002) Thyroid hormone secreted from the gland is about 80-90% T_4 and about 10-20% T_3.

Cells of the developing brain are a major target for the thyroid hormones T_3 and T_4. Thyroid hormones play a particularly crucial role in brain maturation during fetal development (Kester et al., 2004). A transport protein that seems to be important for T_4 transport across the blood–brain barrier (OATP1C1) has been identified. A second transport protein (MCT8) is important for T_3 transport across brain cell membranes (Jansen et al., 2005).

In the blood, T_4 and T_3 are partially bound to TBG, transthyretin, and albumin. Only a very small fraction of the circulating hormone is free (unbound) - T_4 0.03% and T_3 0.3%. Only the free fraction has hormonal activity.

The production of thyroxine and triiodothyronine is regulated by thyroid-stimulating hormone (TSH), released by the anterior pituitary. The thyroid and thyrotropes form a negative feedback loop. TSH production is suppressed when the T_4 levels are high (Johannes, 2002) The TSH production itself is modulated by Thyrotropin-releasing hormone (TRH), which is produced by the hypothalamus and secreted at an increased rate in situations such as cold exposure (to stimulate thermogenesis). TSH production is blunted by somatostatin (SRIH), rising levels of glucocorticoids and sex hormones (estrogen and testosterone), and excessively high blood iodide concentration.

An additional hormone produced by the thyroid contributes to the regulation of blood calcium levels. Parafollicular cells produce calcitonin in response to hypercalcemia. Calcitonin stimulates movement of calcium into bone, in opposition to the effects of parathyroid hormone (PTH). However, calcitonin seems far less essential than PTH, as calcium metabolism remains clinically normal after removal of the thyroid (thyroidectomy), but not the parathyroid.

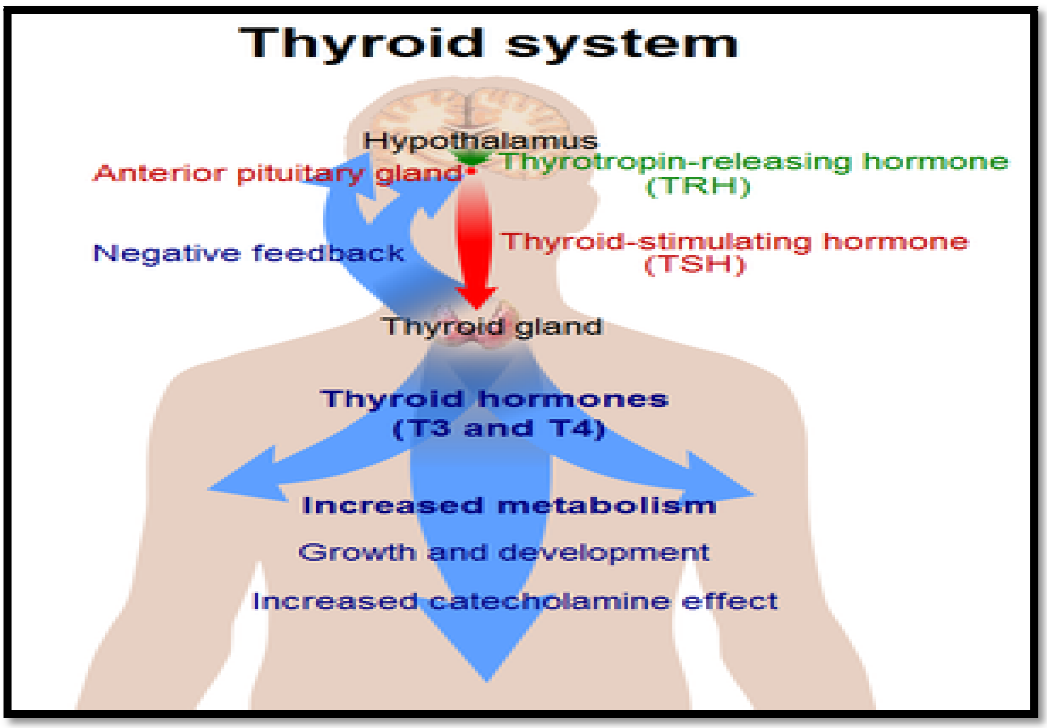


Figure2.3. Thyroid system (http://www.en.wikipedia.org)

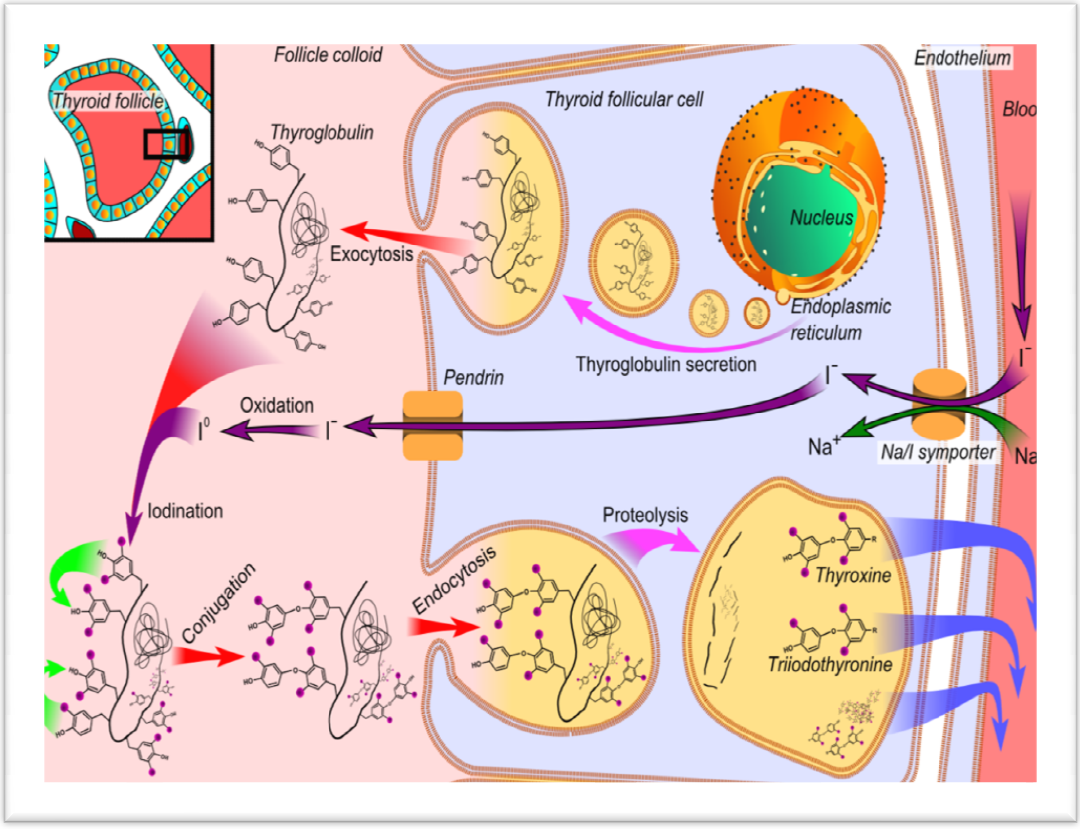


Figure2.4. Hormones of thyroid gland (http://www.howmed.net

2.4.Epidemiology

2.4.1.Incidence and mortality

In 2008, the worldwide estimated age-standardised incidence rates for thyroid cancer incidence were 4.7 and 1.5 per 100,000 women and men, respectively. Thyroid cancer's overall contribution to the worldwide cancer burden is relatively small, but incidence rates have increased over the last three decades throughout the world. This trend has been hypothesized to reflect a combination of technological advances enabling increased detection, but also changes in environmental factors, including population exposure to ionizing radiation from fallout, diagnostic tests and treatment for benign and malignant conditions (Schonfeld et al., 2011).

The American Cancer Society's estimates for thyroid cancer in the United States for 2013 are, about 60,220 new cases of thyroid cancer (45,310 in women, and 14,910 in men) and about 1,850 deaths from thyroid cancer (1,040 women and 810 men) (American Cancer Society, 2013).

In 2008 there were an estimated 33,600 new cases of thyroid cancer diagnosed in the European Union (EU-27). The highest incidence rate was estimated to be in France, where the female rate was five times higher than the rate of the lowest ranking country, Greece (18.6 versus 3.3 per 100,000 females) (Cancer Research UK, 2011).

Thyroid cancer is within the top twenty most common cancers for UK females (number 18) (cancer research UK, 2011). The highest rates for thyroid cancer in the world occur in Northern America, where the female age-standardised rate is 15.1 per 100,000 females, compared with 1.2 per 100,000 females in Middle Africa. Incidence is low in all parts of Africa. Worldwide more cases occur in females aged 15-44 than in any other age group (Ferlay et al., 2010).

The prevalence of differentiated thyroid cancer (DTC) is increasing worldwide. Iodine deficiency is a risk factor for follicular thyroid cancer (FTC). FTC is still common in developing countries, whereas papillary thyroid cancer (PTC) is the predominant subtype in developed countries. Efforts to treat iodine deficiency may improve outcomes by changing to a less aggressive subtype (Woodruff et al., 2010).

Thyroid cancer is rare in children, while in adults the incidence rates rise steadily with age. Rates peak in 35 to 39 year olds and again in the over 70s. There is a substantial number of cases at younger adult age. Almost half (48%) of all cases occur in people aged less than 50 years (Cancer Research UK, 2011). The death rate from thyroid cancer has been fairly stable for many years, and remains very low compared with most other cancers (American Cancer Society, 2013).

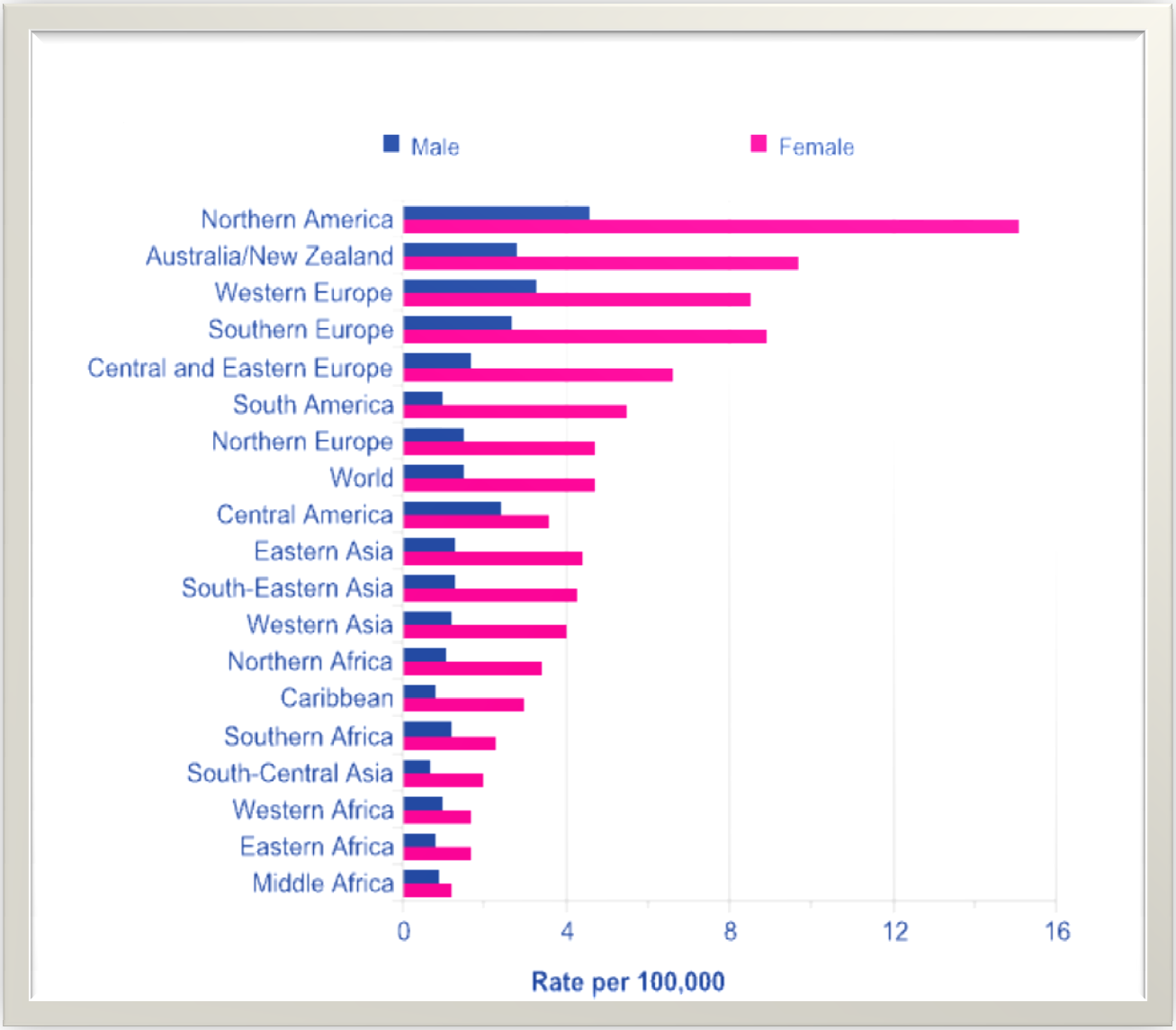


Figure 2.5. Thyroid cancer World Age-Standardized Incidence Rates, World Regions, 2008 Estimates. Source: GLOBOCAN 2008 (Ferlay et al., 2010).

In Arab world in almost all Gulf countries, thyroid is the second most common cancer site in females, preceded in all cases by breast cancer. Only in Bahrain, does thyroid cancer rank third among all cancers diagnosed in females. Outside the Gulf region, Syria is the only country in which thyroid cancer ranks among the top five cancer sites in females. While in Yemen, the bulk of thyroid cancer is of the papillary type, in other countries like Algeria and Sudan, follicular cancer is the predominant form. In both the latter countries, the number of patients presenting with advanced stages of the disease is high (Ghzi omar et al., 2010).

In Western Libya, according to Sebrata Cancer Registry during 2006, thyroid cancers constitute 1.2% of all cancers reported from collective data. M:F ratio is 1:1 and the median age at presentation is 48 year. Histopathologically, majority of cancers were papillary carcinoma. Compared with eastern Libya, the incidence in eastern Libya was higher than the incidence in Western Libya.where thyroid cancers constitute 2.4% of all cancers were registers by Benghazi Cancer Registry. A total of 27 cancers of thyroid with a huge predominance in women (22 cases, 4% of all cancers in females) over men (5 cases). The large majority of cases are microscopically verified (89%), and the most common histological type is the papillary carcinoma (El Mistiri et al., 2007).

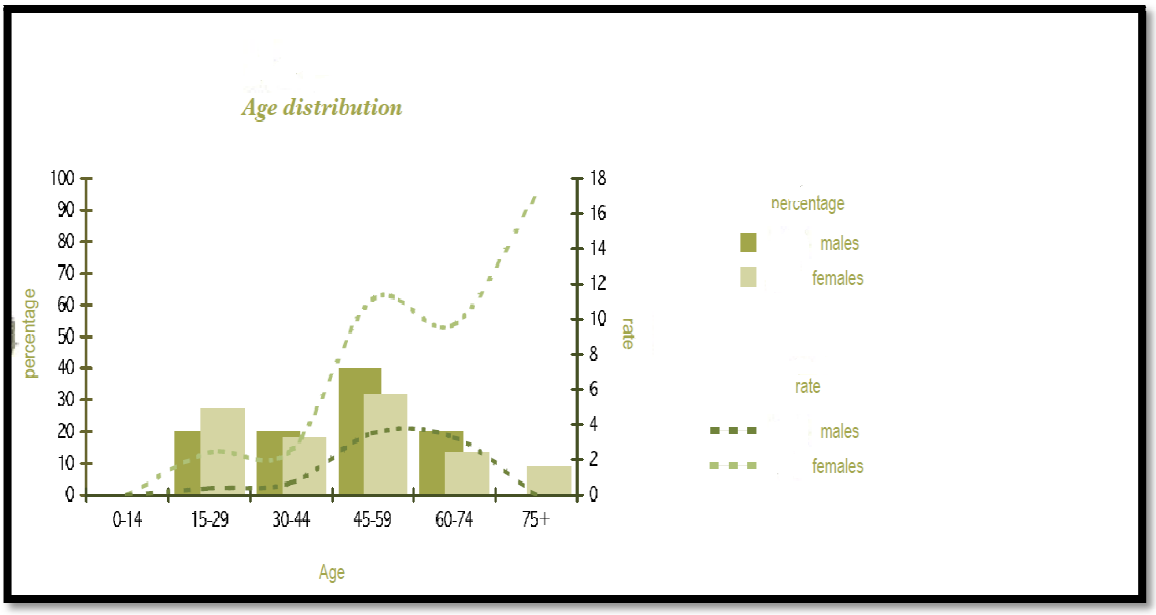


Figure2.6. Age distribution of thyroid cancer patients in Benghazi cancer registries (El Mistiri et al, 2007).

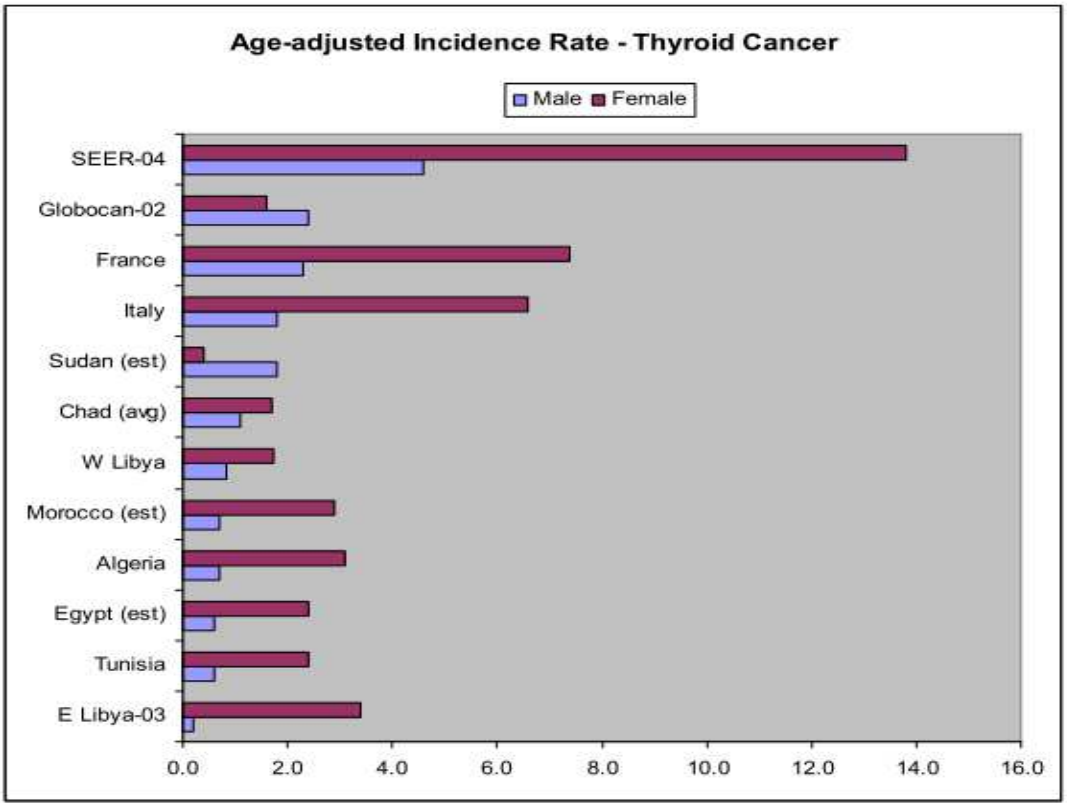


Figure2.7.Age-adjusted Incidence Rate- Thyroid (Sabratha cancer registry, 2006**)**

The incidence of thyroid cancer increased from 3.6 per 100,000 in 1973 to 8.7 per 100,000 in 2002-2004 fold increase. Virtually the entire increase is attributable to an increase in the incidence of papillary thyroid cancer, which increased from 2.7 to 7.7 per 100,000 – a 2.9 fold increase. Between 1988 and 2002, 49% of the increase consisted of cancers measuring 1cm or smaller. These trends, combined with the known existence of a substantial reservoir of subclinical cancer and stable overall mortality, suggest that increasing incidence reflects increased detection of subclinical disease, not an increase in the true occurrence of thyroid cancer (Adeniran et al., 2006).

2.4.2.Risk factors

2.4.2.1.Age

Thyroid cancer is commonly diagnosed at a younger age than most other adult cancers. Nearly 2 out of 3 cases are found in people younger than 55 years of age. About 2% of thyroid cancers occur in children and teens (American cancer society, 2013). With a mean age of 49 years and an age range of 15-84 years. In the younger population, PTC tends to occur more frequently than follicular carcinoma, with a peak in patients aged 30-50 years (American cancer society, 2009)**.**

2.4.2.2.Race

This cancer occurs more frequently in whites than in blacks. The 5-year relative survival rates by race increased from 1975 to 2003, as follows: whites: Increase from 93% to 97%, African American: Increase from 91% to 94%, all races: Increase from 93% to 97%.

2.4.2.3.Sex

The female-to-male ratio is near 3:1 and is related to the patient's age. In patients younger than 19 years, the female-to-male ratio is 3.2:1, in patients aged 20-45 years, the female-to-male ratio is 3.6:1, in patients older than 45 years, the female-to-male ratio is 2.8:1 (American cancer society, 2009).

2.4.2.4. Inherited predisposition

Familial non medullary thyroid cancer (NMTC) represents 3%–7% of all thyroid tumors and is associated with some of the highest familial risks among all cancers, with a risk of developing this type of neoplasia for first-degree relatives of 5–10-fold compared to the general population (Elena Bonora et al., 2010), Patients with familial NMTC may have more aggressive tumors with increased rates of extrathyroid extension, lymph node metastases, and larger tumors in younger patients (Nose, 2008).

Several syndromes are associated with NMTC such as Gardner's syndrome (familial adenomatous polyposis, FAP), Cowden disease (multiple hamartoma), Carney complex, and Werner syndrome (Richards, 2009).

Approximately 25% of medullary thyroid cancer is genetic in nature, caused by a mutation in the RET proto-oncogene. This form is classified as familial MTC. When MTC occurs by itself it is termed sporadic MTC. When it coexists with tumors of the parathyroid gland and medullary component of the adrenal glands (pheochromocytoma) it is called multiple endocrine neoplasia type 2 (MEN2) (Dionigi et al., 2007).

2.4.2.5.enviromental factors

2.4.2.5.1.Diet

Follicular thyroid cancers are more common in areas of the world where people's diet are low in iodine. In the United States, dietary iodine is plentiful because iodine is added to table salt and other foods (American cancer society, 2009). PTC is the predominant type in areas without iodine deficiency, and its frequency is said to be increased in regions with high iodine uptake (Ackerman's, 2011).

According to the World Health Organization(WHO) criteria, iodine deficiency is identified when the median urinary iodine (UI) level is below 10 μg/dL or goiter prevalence is greater than 5% in school children. Based on these criteria, the Arab countries have a high proportion of the total World goiter prevalence (TGP); in 1993, a prevalence rate of 22.9% for goiter was recorded for the Region. This value has increased to 37.3% in 2003 portraying a 62.9% increase as compared to 1993 (de Benoist et al., 2003). In 2003, the WHO estimated that the proportion of school-age children (6–12 years) and the proportion of the general population with insufficient iodine intake based on UI levels in the region was 55.4% and 54.1% respectively, placing it as the second most affected Region after Europe (59.9% and 56.9% respectively) (de Benoist et al, 2003). The prevalence of iodine deficiency disorder (IDD) is considered mild in seven countries of the Region including Jordan, Lebanon, Libya, Oman, Syria, the United Arab Emirates and Yemen, whereas it is found to be moderate in four countries that include Egypt, Morocco, Saudi Arabia and Sudan (Mclaren et al., 2001).

The IDD in the Region ranged from mild to moderate, with the exception of Iraq, where the IDD status is severe most likely due to inadequate intake of dietary iodine, ingestion of goitrogens (food contain chemical inhibit iodine absorption) and habitation

in regions where the soil lacks iodine (Mclaren et al., 2001). Lack or inadequate intake of fish may contributed to iodize-deficiency. In Morocco, fish consumption was negatively associated with goiter among school children in Atlas Mountains of Morocco. It was concluded that the effective programme to control the prevalence of goiter should include the following activities: encourage fish consumption, salt iodization, nutrition education (Oldham et al., 1993). However, several studies showed that the altitudes may play an important factor. In Saudi Arabia, the overall prevalence of goiter among school children 6–18 years was 24%. The prevalence was significantly higher ($P < 0.000$) in high altitude (27%, 95% CI, 24–30%) than in low altitude areas (13%, 95%, CI 8–18%) (Abu-Eshy et al., 2001). In Yemen, the prevalence of IDD among school children 6–12 years in mountain areas was 31% compared to 16.8% in lowland/coastal areas (Zein et al., 2000). Universal salt iodization (USI) and iodine supplementation are highly effective strategies for preventing and controlling iodine deficiency. USI is now implemented in nearly all countries worldwide, and two-thirds of the world's population is covered by iodized salt. The number of countries with iodine deficiency as a national public health problem has decreased from 110 in 1993 to 47 in 2007. Still one-third of households lack access to adequately iodized salt. Iodine deficiency remains a major threat to the health and development of populations around the world, particularly in children and pregnant women in low-income countries. Data on iodine status are available from 130 countries and approximately one-third of the global population is estimated to have a low iodine intake based on urinary iodine (UI) concentrations. The challenges ahead lie in ensuring higher coverage of adequately iodized salt, strengthening regular monitoring of salt iodization and iodine status in the population, together with targeted interventions for vulnerable population groups (Abdurrahman et al., 2011).

2.4.2.5.2.Radiation exposure

Patients with a history of radiation administered in infancy and childhood for benign conditions of the head and neck, such as enlarged thymus, acne, or tonsillar or adenoidal enlargement, have an increased risk of cancer as well as other abnormalities of the thyroid gland (Detours et al., 2005). The most common of them are of a benign nature and consist of nodular hyperplasia, lymphocytic thyroiditis, and fibrosis. There is also

increase in the incidence of carcinoma in this population, the large majority of the tumors being of the papillary type (Ackerman's, 2011), In this group of patients, malignancies of the thyroid gland first appear beginning as early as 5 years following radiation and may appear 20 or more years later (Carling et al., 2011). From nuclear disasters such as Chernobyl in 1986, it is clear that radiation exposure is a significant risk factor for thyroid cancer (Detours et al., 2005), especially in children (Tronko et al., 2006). Radiation given through a vein (through an IV) during medical test and treatment dose not increase the risk of developing thyroid cancer (Larsen et al., 2003).

2.4.2.5.3.Smoking

Tobacco smoking seems to be associated with a decreased risk of thyroid cancer, but, obviously, it poses more health hazards than benefits (Mack et al., 2003).

Many other conditions have been considered as predisposing to PTC (oral contraceptive use, benign thyroid nodules, late menarche, late age at first birth) (Negri et al.,1999; Franceshi et al., 1999).

2.4.2.5.4.Body Mass Index

Several case-control studies have shown an increased risk of TC in patients with high body mass index (BMI). The risk would be increased by 5-fold in obese men and 2 times in obese women (>97 percentile), compared to the risk observed in patients with weight <3rd percentile. In women (especially in postmenopausal age) a weight gain >14% appears to positively correlate with the onset of TC (Dal Maso et al., 2000; Suzuki et al., 2008).

2.4.2.6.Pre existence Hashimoto's thyroiditis

Hashimoto's thyroiditis (HT) is frequently diagnosed especially in females and is the most common cause of hypothyroidism in iodine-sufficient areas of the world, with an increasing prevalence in older patients (Hollowell et al., 2002). An association has been suggested between HT and PTC in many studies (Dailey et al., 1955; Hirabayashi & Lindsay ,1965; JBaker, 1995; Okayasu, 1997; Singh et al., 1999), even if other studies yielded conflicting results (Holm et al., 1985; Anil et al., 2010). Concurrent HT lymphocytic infiltration and PTC was associated with the female gender, smaller tumor

size, a less frequent extracapsular extension and a lower grade of TNM staging. BRAF V600E was more frequent in PTC with concomitant lymphocytic infiltration (Marotta et al., 2013). Also the link between HT and PTC is supported by the observation that rearrangements of RET oncogene (RET/PTC) frequently detected in PTC, may also be found in the thyroids of patients affected by HT (Mechler et al., 2001). By strict criteria, HT is a histological diagnosis characterized by widespread lymphocytic infiltration of the thyroid. On the other hand, PTC is often associated with a significant lymphocytic infiltration in the absence of the typical signs of autoimmune thyroiditis (Okayasu et al., 1995; Fiore et al., 2009b), and may represent a response to tumor antigens released through disruption of normal follicles by neoplastic invasion. On clinical grounds, the diagnosis of HT is based on the presence of serum thyroid auto antibodies (TAb) and of spontaneous hypothyroidism that may be present at the initial evaluation or may develop during follow-up (Vanderpump et al., 1995; Walsh et al., 2010). However, patients with nodular goiter (NG) may also have circulating TAb as expression of focal thyroiditis not evolving toward hypothyroidism, thus making the diagnosis of HT bf. The progressive reduction of thyroid function as a consequence of the autoimmune process leads to a progressive increase in serum TSH. Recently, it has been reported that in patients with nodular thyroid diseases, the risk of thyroid malignancy increases with serum TSH concentrations (Boelaert et al., 2006; Haymart et al., 2008a,b; Jonklaas et al., 2008; Polyzos et al., 2008; Fiore et al., 2009a; Jin et al., 2010). Therefore, it is possible to hypothesize that increased TSH levels may play a role in the development of PTC also in patients with nodular-HT. The results reported in the study of Fiore et al. showed that PTC is more frequent in patients with nodular-HT compared with patients with non-autoimmune NG and that the increased TSH levels, a consequence of the destruction of functioning tissue by the autoimmune process, are strictly related to the increased frequency of PTC. In agreement with this conclusion, treatment with L -thyroxine (L -T 4) reduces TSH levels and decreases the frequency of PTC in nodular-HT (Fiore et al., 2011).

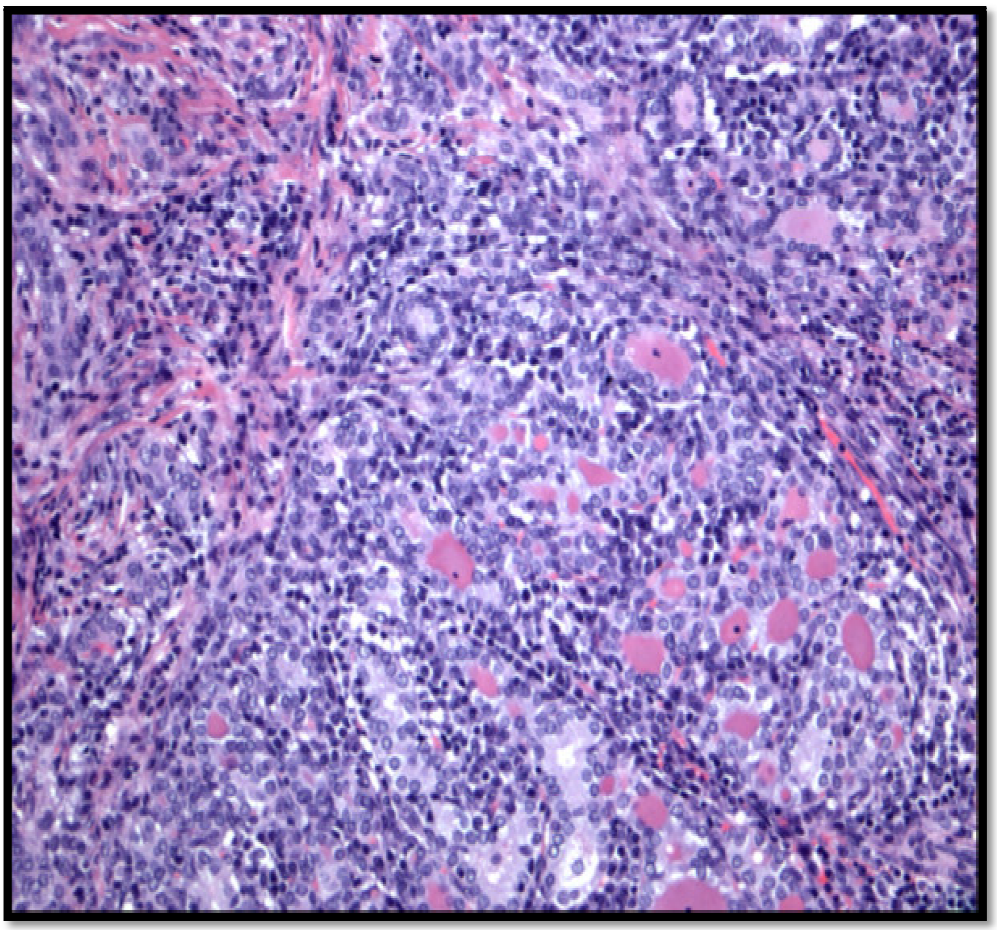


Figure 2.8. Papillary carcinoma in the setting of Hashimoto thyroiditis (El Demellawy et al. , 2008)

2.5.Molecular pathogenesis of thyroid cancer

A number of genetic alterations have been shown to be involved in the development of follicular cell-derived cancers. These point mutations and translocations occur in genes for several important signaling pathways, in particular the mitogen-activated protein kinase (MAPK) pathway, and are required for transformation of well-differentiated follicular cell-derived thyroid cancers, i.e. PTC and FTC (Kondo et al., 2006; Nikiforova et al., 2009), as described in Figure 2.9.

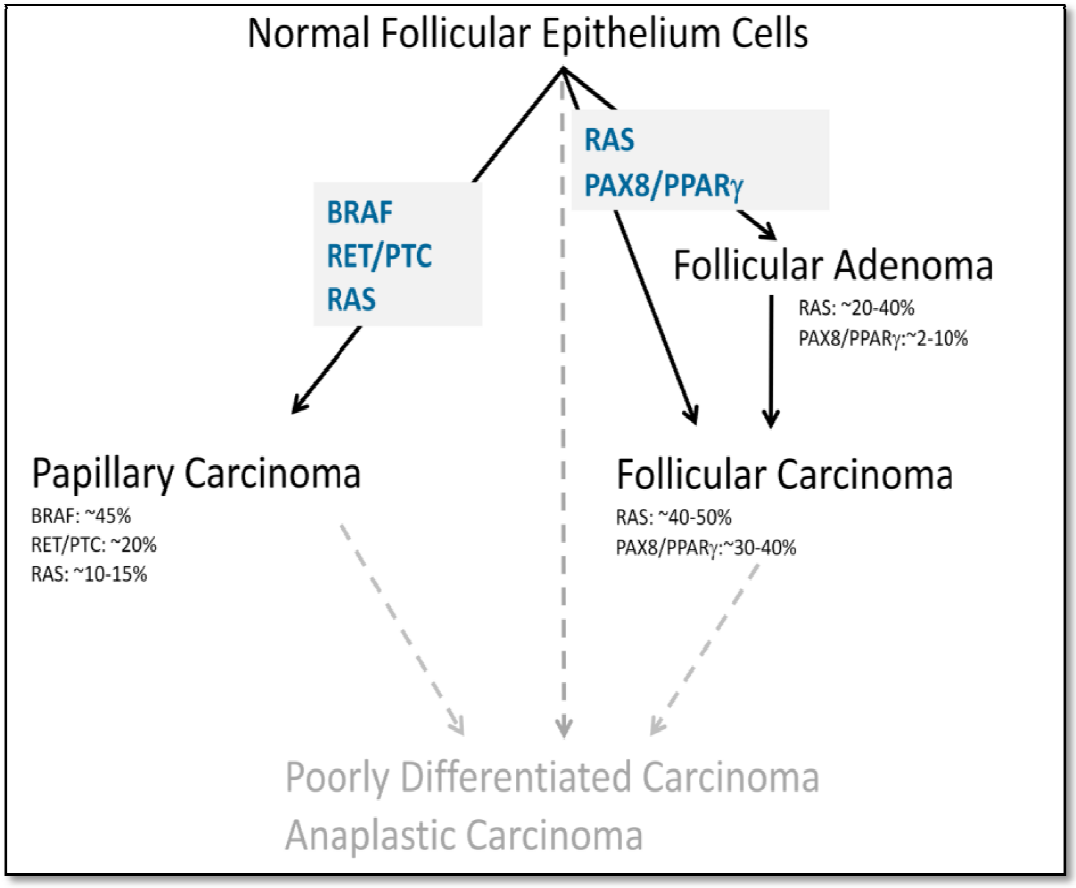


Figure2.9 Mutation and translocations involved in the pathogenesis of papillary and follicular thyroid carcinoma respectively (Asuragen Int, 2011).

Four mutation types, that is, BRAF and RAS point mutations and RET/PTC and PAX8/peroxisome proliferator-activated receptor g ($PPAR_{\gamma}$) rearrangements, constitute the majority of mutations known to occur in thyroid cancer.

2.5.1.BRAF

The BRAF mutation (V600E) is the most common mutation in PTC, occurring with a prevalence of ~45% (range 27%-87%) (Lee et al., 2007; Xing, 2007). It is mostly found in conventional PTC and the tall cell variant of PTC and less frequently in the follicular variant of PTC. BRAF point mutation is not found in follicular thyroid cancer and benign thyroid nodules. BRAF V600E mutation leads to the constitutive activation of the BRAF protein kinase of the MAPK pathway. Recent study have established the BRAF V600E mutation is the only independent predictor of central compartment lymph node metastasis in PTC and can be utilized to guide the extent of initial surgery (Howell et al., 2013). And other studies have established the BRAF V600E as a marker of disease aggressiveness, disease recurrence, and poor prognosis (Elisei et al., 2005; Xing, 2007; Placzkowski et al., 2008), although these findings have not been confirmed in some other studies (Trovisco et al., 2005; Fugazzola et al., 2006). Other BRAF activating point mutations have been described at positions 598, 599, and 601, but these mutations are very rare compared to the activating mutation at position 600. BRAF V600E is the target for research of therapies (Santoro et al., 2006; Woyach et al., 2009).

2.5.2RET/PTC rearrangements

Rearrangements of the RET gene, called RET/PTC rearrangements, are the second most common genetic alteration described in PTC. They occur in ~20% of sporadic PTC, although their prevalence has been shown variable among studies, mostly due to variations in the geographical distribution, the different methodologies used for its detection and tumor heterogeneity (Zhu et al., 2006). These rearrangements are specific for PTC and PTC variants, such as the oncocytic (Hurthle cell) variant, and are usually not found in benign tumors. RET/PTC rearrangements are more prevalent in radiation-induced PTC. At least 11 different RET/PTC rearrangements have been described, the two most common in sporadic (i.e. non-radiation induced) PTC being RET/PTC1 (60-70% of positive cases) and RET/PTC3 (20-30% of positive cases).

2.5.3.RAS

Point mutations within RAS genes involve codons 12, 13, and 61 of NRAS, HRAS and KRAS, with mutations of NRAS and HRAS at codon 61 and of KRAS at codon 12/13 being the most common. Mutant RAS proteins constitutively activate the MAPK and PI3K/AKT pathways.

In contrast to the other markers, RAS mutations are not restricted to a particular histological subtype of thyroid tumor. RAS mutations are found in ~ 10-15% PTCs (higher in follicular variant of PTC) but are more prevalent in FTC, where they are associated with 40%-50% of the cancers. RAS mutations are also found in ~35% of poorly differentiated and ~50% of anaplastic thyroid cancers, where the presence of RAS mutations seems to correlate with more aggressive tumor behavior and poor prognosis (Kondo et al., 2006; Nikiforova et al. ,2008; Ruggeri et al., 2008). RAS mutations are also found in 20%-40% of follicular adenoma.

2.5.4.PAX8/PPARγ rearrangements

PAX8/PPARγ rearrangements are found in 30-40% of conventional FTC and in ~5% of oncocytic carcinomas (Nikiforova et al., 2003; Placzko wski et al., 2008). Tumors associated with PAX8/PPARγ usually carry a favorable prognosis. Tumors with PAX8/PPARγ rearrangement do not usually carry any RAS mutation, suggesting that the development of FTC involves two independent pathways associated with either PAX8/PPARγ translocation or RAS mutation (Nikiforova et al., 2003). PAX8/PPARγ rearrangements are also found in 2-10% of follicular adenomas, and in the follicular variant of PTC (Nikiforova et al., 2003; Castro et al., 2006). PAX8/PPARγ translocations have been reported in a very low percentage (0%-1%) of PTC (Marques et al., 2002).

2.6.Histopathologic types of thyroid neoplasm

The histological classification recommended below is modified from the WHO published recommendations (Delellis et al., 2004). This protocol applies only to carcinomas and does not apply to lymphomas, sarcomas or metastatic tumors to the thyroid gland. Given the fact that the classification of papillary carcinoma is predicated on the combination of architectural and cytomorphologic findings.

I. Papillary carcinoma Variants (in alphabetical order)
   - Classical (usual)
   - Clear cell variant
   - Columnar cell variant
   - Cribriform-morular variant
   - Diffuse sclerosing variant
   - Follicular variant
   - Macrofollicular variant
   - Microcarcinoma (occult, latent, small, papillary microtumor)
   - Oncocytic or oxyphilic variant (follicular variant, non-follicular variant)
   - Solid variant
   - Tall cell variant
   - Warthin-like variant

II. Follicular carcinoma
   - Variants:
     - Clear cell variant
     - Oncocytic (Hürthle cell) variant

III. Poorly differentiated thyroid carcinomas including insular carcinoma

IV. Medullary carcinoma

V. Undifferentiated (anaplastic) carcinoma

VI. Carcinoma, type cannot be determined

Another classification for thyroid tumor (I) Benign Tumors the most common benign thyroid neoplasm is follicular adenoma and rare benign tumors include dermoid cysts, lipoma, hemanigoma, and teratoma (seen mainly in infants).

(II) Malignant tumors.

The major subtype of thyroid carcinoma and their relative frequencies include the following

- Papillary carcinoma (75% to 85% of cases)
- Follicular carcinoma (10% to 20% of cases)
- Medullary carcinoma (5% of cases)
- Anaplastic carcinoma (5% of cases)
- Other malignant tumor

  Malignant lymphoma

  Sarcomas of various microscopic types have been reported in the thyroid (Robbins and Cotran, 2007).

2.6.1.Follicular adenoma

Is defined as a benign encapsulated tumor that shows evidence of follicular cell differentiation. It is the most common thyroid neoplasm, most patients are euthyroid adults who initially have a thyroid lump, which on scan is usually cold sometimes cool or warm, many patients with thyroid adenomas have elevated circulating levels of thyroglobulin but few of the tumors are associated with clinical hyperthyroidism (so called toxic adenomas). It has been suggested that these autonomously functioning tumors are more common in regions with iodine deficiency.

Adenomas may exhibit a variety of patterns, singly or in combination: normofollicular (simple), Macrofollicular (colloid), microfollicular (fetal), and trabecular/solid (embryonal). Mitoses are rare or absent in the follicular adenomas. Secondary degenerative changes such as hemorrhage, edema, fibrosis, calcification, bone formation, and cystic degeneration are common.

Enzyme histochemical and immunohistochemical profile of adenomas mirrors that of the normal follicle. There is reactivity for low-Molecular Weight keratin and

thyroglobulin in the cytoplasm and for laminin and other basement membrane components around the follicles. Several variants of follicular adenoma have been described. Hurthle cell adenoma, Hyalinizing trabecular adenoma and Adenoma with bizarre nuclei (Ackerman's, 2011).

2.6.2.Papillary carcinoma

is the most common type of thyroid cancer (Hu et al., 2008) representing 75% to 85% of all thyroid cancer cases (Robbins & Cotran, 2007). It occurs more frequently in women and presents in the 30-40 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 (Dinets et al., 2012). Recurrence, metastases, and cancer death may occur in a few patients and are more commonly associated with more aggressive tumors, such as tall cell, columnar cell, or diffuse sclerosing variants of the PTC (Asioli et al., 2010).

Gross features

The size of the primary tumor ranges from microscopic to huge. A very high proportion of thyroid cancers measuring less than 1cm in diameter are of papillary type .Grossly most cases are solid, whitish, firm , and clearly invasive, fewer than 10% are surrounded by complete capsule marked cystic changes are seen in about 10% of cases. Sometimes papillary formations are evident to the naked eye.

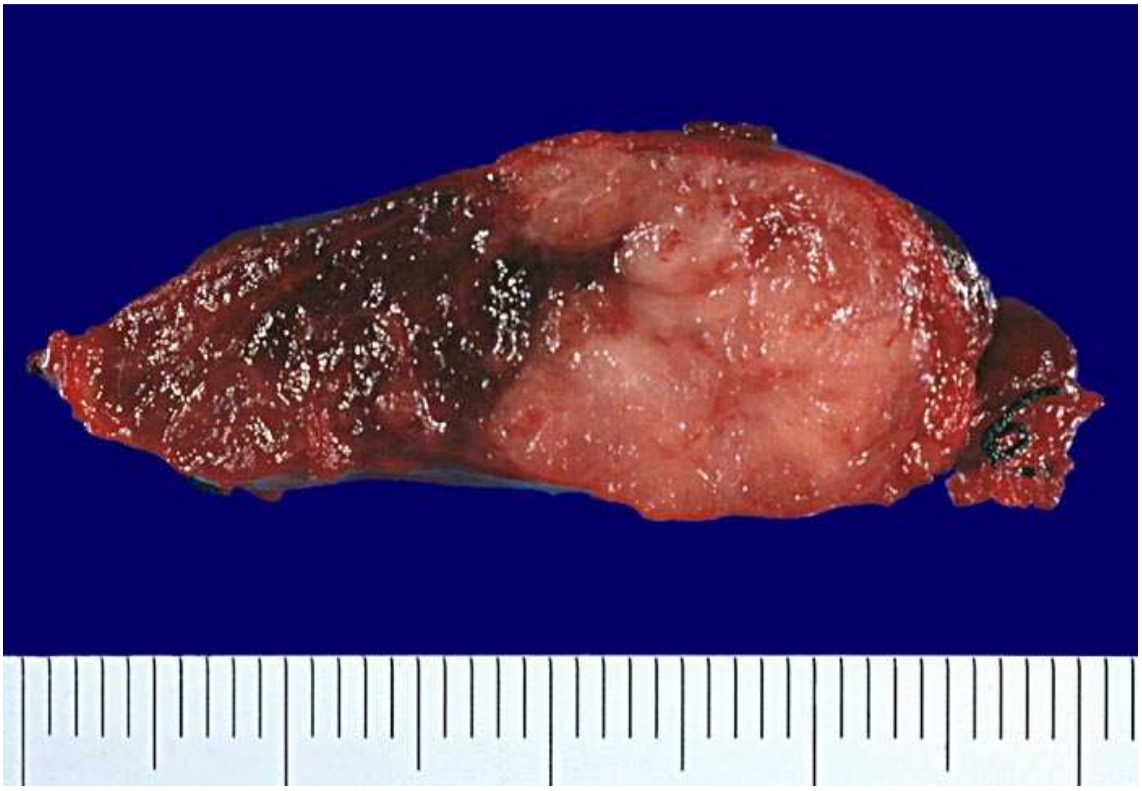


Figure2.10. Gross appearance of papillary thyroid carcinoma (www.psapath.com)

Microscopic features

Microscopically, the diagnosis of papillary carcinoma depends on the presence of certain architectural features (mainly in the form of true papillae) and /or characteristic nuclear changes. The papillae are usually complex, branching, and randomly oriented, with a central fibrovascular core and a single or stratified lining of cuboidal cell. The stroma of the papillae may be edematous or hyaline, and it contain lymphocytes foamy macrophages, hemosiderin, these papillae are nearly always associated with the formation of follicles. The follicles tend to be irregularly shaped, often tubular and branching.

The nuclear features of papillary carcinoma, which are as important diagnostically as the presence of papillae, consist of

1. Ground glass (optically clear) nuclei, which often have a large size and an overlapping quality. The nucleolus is usually inconspicuous and pushed against the nuclear membrane which appears thickened. This change is

present in sections obtained from paraffin-embedded material but is less apparent or absent in frozen sections or cytology material.

2. Nuclear pseudo inclusions - these represent invaginations of the cytoplasm and appear as sharply outlined acidophilic formations. In contrast to the ground glass feature, the pseudo inclusions are also readily apparent in specimens from frozen section and aspirations.
3. Nuclear grooves - these tend to occur in oval or spindle nuclei, are usually attached along the longest nuclear axis and represent- like the pseudo inclusions- the morphologic expression of infoldings of a redundant nuclear membrane.
4. Nuclear microfilaments - A few cases have been described in which the nuclear clearing is due to the accumulation of fine thread like fibrils.

Mitoses are very scanty or absent. Over half of the cases show extensive fibrosis, the appearance of this fibrosis range from sclera hyaline to highly cellular psammoma bodies are seen in approximately half of cases. They may be located in the papillary stalk, in fibrous stroma, or between tumor cells in solid foci. Their presence strongly suggests the diagnosis of PTC. They represent a very important clue to the diagnosis not only in paraffin sections, but also in frozen sections, cytology preparations. Theses laminated basophilic structures stain for mucin, calcium, and iron and appear to arise from necrosis of individual tumor cells.

Areas with a solid/ trabecular pattern of growth are present in 20% of the cases and foci of squamous metaplasia in a similar number; the prominence of trabecular formations places the tumor in a poorly differential category.

Lymphocytic infiltration of stroma is seen in a fourth of cases, blood vessel invasion is found in only 5% of cases.

Ultra structural features

The most distinctive feature of the cells of papillary carcinoma is the highly indented nuclear membrane with formation of pseudo-inclusions and multilobation. The cytoplasm is rich in mitochondria, lysosomes and intermediate filaments. The apical surface exhibits microvillus differentiation (Ackerman's, 2011).

Variants

1-Classic PTC

The classic PTC shows a papillary architecture with branching (DeLellis et al., 2004; Khan & Nose, 2010). The papillae are covered by cells with eosinophilic cytoplasm and enlarged nuclei. The polarity of the cells may be abnormal or lost in some tumors. Squamous metaplasia may be present. Psammoma bodies with concentric lamellae composed partly of thyroglobulin are more common in some variants of PTC. Some tumors may also contain multinucleated giant cells (Lloyd et al., 2011).

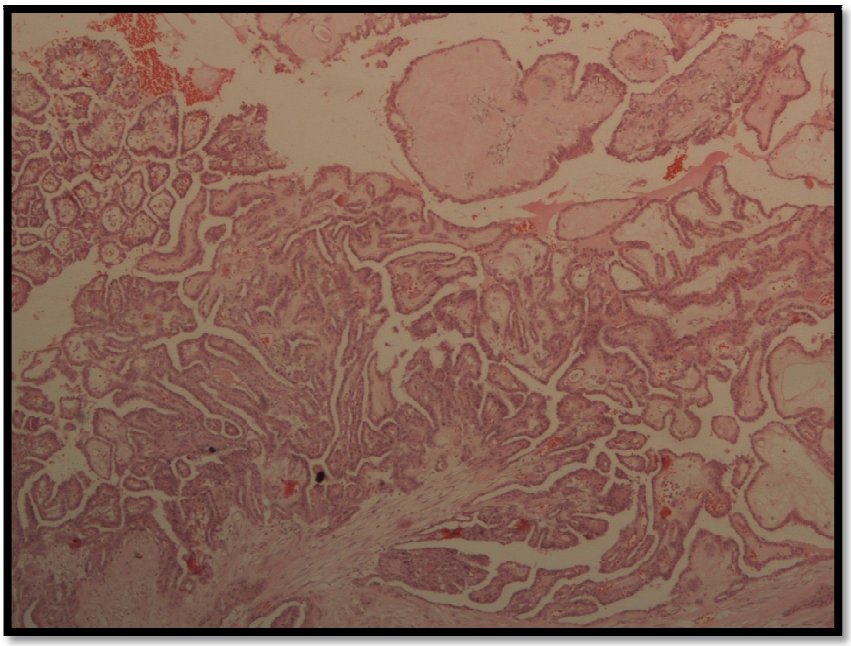


Figure 2.11 Classic papillary thyroid carcinoma (H&EX10) (Department of Pathology, Faculty of Medicine, Benghazi University).

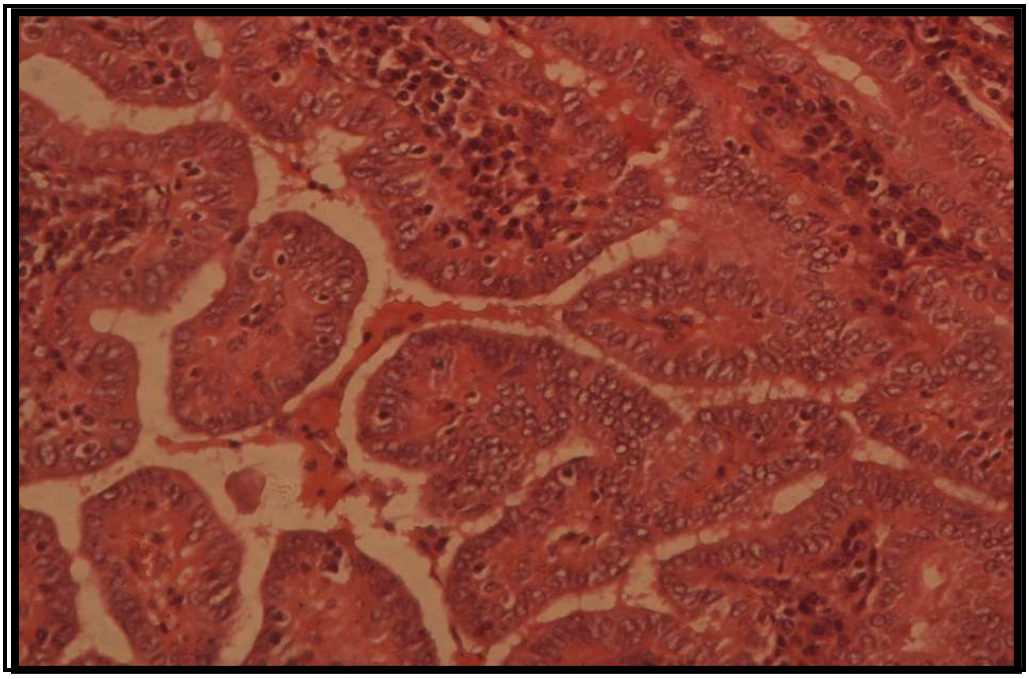


Figure 2.12 Papillary carcinoma showing the spectrum of cytological features, the papillae are covered by cells with pale nuclei, the nuclei are comparatively chromatin rich. They are ovoid, with grooving and distinct nucleoli. (H&EX40) (Department of Pathology, Faculty of Medicine, Benghazi University).

I. Papillary microcarcinoma

This is defined as a papillary carcinoma measuring 1cm or less in diameter most cases have a satellite configuration and correspond to so-called occult sclerosing carcinoma or non encapsulated sclerosing tumor, where as other show partial or near total encapsulation, with or without tumor outside the capsule. It is a common incidental finding in thyroid glands removed for other reasons it is associated with cervical node metastases in about one third of cases, but distant metastases are exceptionally rare, and the prognosis is generally excellent.

II. Encapsulated variant

This is defined as a papillary carcinoma totally surrounded by a capsule. It may still be associates with nodal metastasis, but the incidence of distant metastases or tumor death is nearly zero in contrast to papillary carcinomas, these lesions are hot on thyroid scan and are accompanied by a pale, vacuolated colloid. The follicular cells tend to be low

columnar, with basally located normochromatic or hyperchromatic nuclei (Ackerman's, 2011).

III. Follicular variant

These tumors look like follicular neoplasm when examined grossly. They are composed of follicles of variable sizes. The colloid is usually darker or hypereosinophilic compared to the colloid in adjacent non-neoplastic thyroid and may show scalloping "bubble gum" appearance. Occasional multinucleated giant cells are present within the follicles. The cytological features of PTC are important to establish the diagnosis in these tumors (DeLellis et al., 2004; Khan & Nose, 2010). Supportive features for the diagnosis are an invasive pattern of growth, fibrous trabeculation (Akerman,s,2011). The diagnosis of follicular variant of PTC can be quite difficult and controversial (Lloyd et al.,2004; Wallander et al.,2010). The prognosis of these tumors is similar to the typical PTC. An exception is the diffuse (multinodular) follicular variant, which has a more aggressive clinical course (DeLellis et al., 2004). The prognosis of follicular variant of PTC also depends on whether they are completely encapsulated or invasive (Khan & Nose, 2010; Rivera et al., 2010).

Follicular variant of PTC can look like a follicular neoplasm except for the cytological features. Because these tumors can be easily confused with follicular adenomas and follicular carcinomas, the use of immunohistochemical and molecular markers can be very useful in confirming the diagnosis in difficult cases. The follicular variant of papillary carcinoma can be viewed as the balanced result of two opposing biologic properties of the tumor cell differentiation in the form of secretory activity (There by making colloid-filled follicles) and proliferation. When one of these forces predominates over the other, two further variant emerge

A. Solid variant

This tumor, particularly common in children, results when proliferation predominates over secretion. It is characterized by solid nests of generally round shape that can be viewed as filled- up follicles; it is distinguished from other poorly differentiated carcinoma because the nuclear features remain those of papillary carcinoma.

B. Macro follicular variant

This variant has large dilated follicles, so that the tumor resembles not so much a follicular neoplasm as a hyperplasic nodule.

C. **Diffuse(multinodular)variant**

in this very unusual form, most of a thyroid lobe or sometimes both lobes are diffusely involved by the tumor growth, which is difficult to recognize because of its very diffuseness.

D. Encapsulated follicular variant

This tumor type, which has become the single most common Source of consultation material in thyroid pathology and the subject of considerable controversy, can be defined as a neoplasm surrounded by a capsule and having the cytoarchitectural features of papillary carcinoma, to make a diagnosis of this variant, the nuclear alterations should be widespread and well developed, and supportive features (Such as intratumoral sharply defined fibrohyaline bands, elongated and branching follicles, abortive follicles, and dense eosinophilic colloid) should be present.

V . Diffuse sclerosing variant

This variant is characterized by diffuse involvement of one or both thyroid lobes, dense sclerosis, abundant psammoma bodies, extensive solid foci, squamous metaplasia, and heavy lymphocytic infiltration. Clinically it may be misdiagnosed as Hashimoto's thyroiditis. Nodal metastases are nearly always present, lung metastases are common.

VI . Oncocytic (oxyphilic variant)

In this variant, the nuclear features remain those of papillary carcinoma but the cytoplasm is abundant and has a granular oxyphilic quality.

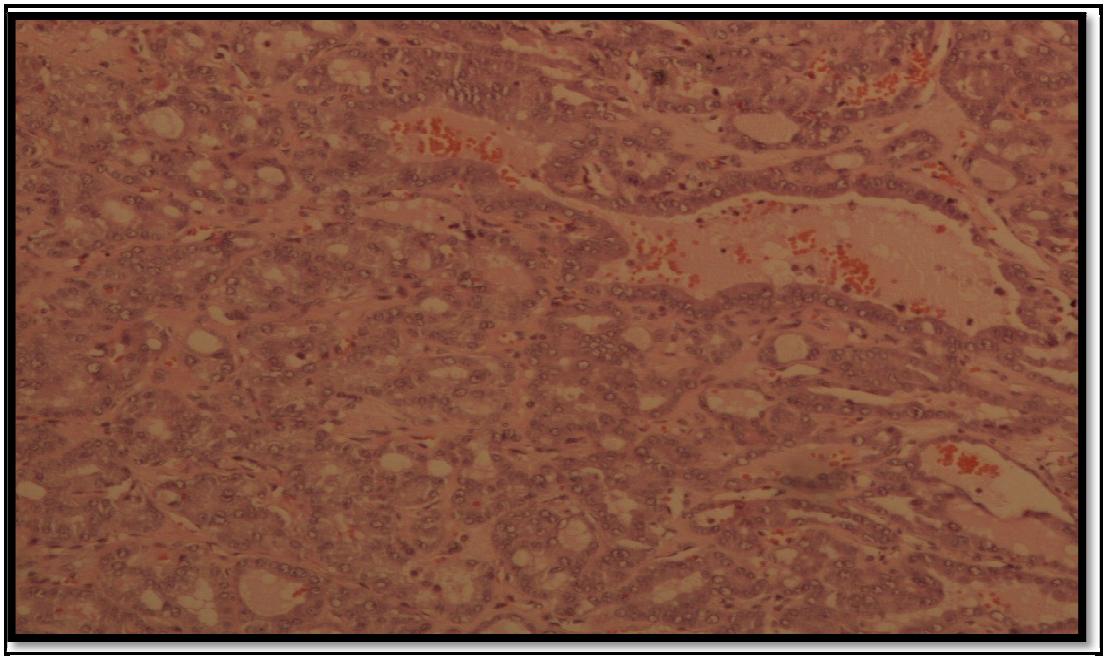


Figure 2.13. Follicular variant papillary carcinoma (H&EX20) (Department of Pathology, Faculty of Medicine, Benghazi University)

VII. Tall cell and columnar variants

The tall cell variant is a type of papillary carcinoma characterized by papillae lined by a single layer of 'tall' cells and an abundant acidophilic cytoplasm. These features should be present in at least half of the tumor for it to be placed into this category. This variant tends to affect older patients and clinical course is more aggressive. In the columnar variant, there is prominent stratification, and the cytoplasm is clear. The prognosis is very poor.

VIII. Cribriform-morular variant:

As the name indicates, this variants is characterized by the presence of a cribriform pattern of growth and morular formations some of the cases are sporadic, where as others are seen as part of a genetically determined syndrome that includes colonic adenomatous polyposis.

IX. Papillary carcinoma with exuberant nodular fasciitis like stroma

In this variant, the prominence of the stromal reaction of the tumor may obscure the neoplastic epithelial component (Akerman's, 2011).

X. Papillary thyroid carcinoma with prominent Hobnail features:

it is new aggressive variant of moderately differentiated papillary carcinoma, multifocal has variable size complex papillary structures lined by cells with increased nuclear / cytoplasmic ratios and apically placed nuclei that produced a surface bulge (hobnail appearance) (Asioli et al., 2010).

PTC can undergo dedifferentiation or transformation to anaplastic carcinoma(Kleihues et al.,2004; Khan & Nose , 2010). In a study of 109 anaplastic carcinomas, Albores Saavedra et al. found that 46.8% PTC coexisted with anaplastic carcinoma (34 conventional type 14 tall cell variant and 3 follicular variant) supporting the concept of dedifferenation of PTC leading to anaplastic carcinoma (Albores Saavedra et al., 2007).

2.6.3.Follicular carcinoma

Follicular carcinoma become a relatively rare neoplasm whose identification largely depends on the presence of invasion of the capsule, blood vessels, or adjacent thyroid it shares with papillary carcinoma the same predilection for females, but it occurs on the average, in patients who are a decade older. Its microscopic appearance is extremely variable, ranging from well formed follicles to a predominantly solid growth pattern. Poorly formed follicles, cribriform areas, or trabecular formation may be present, mitotic activity and nuclear atypia are usually seen but may be entirely lacking. Psammoma bodies are absent, and squamous metaplasia is exceptionally rare. Immunohistochemically follicular carcinomas are reactive for Tg, low-molecular-weight keratin, EMA and basement membrane components such as laminin and type IV collagen.

Depending on its degree of invasiveness follicular carcinoma has been subdivided into a minimally invasive and a widely invasive form.

Minimally invasive follicular carcinoma

Is a grossly encapsulated tumor, often with a solid and fleshy cut surface/ the pattern of growth usually resembles that of an adenoma of embryonal, fetal or atypical type since the diagnosis of malignancy depends entirely on the demonstration of blood vessel and / or capsular invasion microscopically. The vessels should be of venous caliber be located in or immediately outside the capsule and contain one or more clusters of tumor cells attached to the wall and protruding into the lumen. Interruption of the capsule must be full thickness for the process to qualify as capsular invasion.

Widely invasive follicular carcinoma

It shows widespread infiltration of blood vessels and / or adjacent thyroid tissue. It often lacks encapsulation altogether in contrast to papillary carcinomas; follicular carcinomas of either subtype are almost always solitary and practically never occult. Metastases are usually blood borne rather than to regional nodes (Ackerman's, 2011).

2.6.4.Anaplastic carcinoma

Other name undifferentiated carcinoma usually in elderly patients as a rapidly growing mass associated with hoarseness, dysphagia, and dyspnea. Extra–thyroidal extension is encountered at the time of initial presentation in most of the cases. Grossly, a highly necrotic and hemorrhagic solid tumor mass is seen replacing large portions of the organ.

Microscopically, the term undifferentiated or anaplastic carcinoma is used in the thyroid gland in connection with two major categories that sometimes coexist, the first is undifferentiated in the sense that it does not make follicles, papillae, or even trabecular or nests, the second category is actually composed of two patterns which are often seen together and which are sometimes grouped under the qualifier of sarcomatoid: spindle cell and giant cell. They may exhibit fascicular or storiform pattern of growth, heavy neutrophilic infiltration, prominent vascularization, and cartilaginous/osseous metaplasia most if not all undifferentiated thyroid carcinomas arise as a result of anaplastic transformation of a per –existing well-differentiated tumor, usually papillary carcinoma but also follicular carcinoma, Hurthle cell carcinoma. The mortality rate is over 95%, the mean survival is less than 6 months, and the immediate cause of death is usually involvement of vital structures in the neck (Ackerman's, 2011).

2.6.5.Medullary carcinoma

Is the original and still the preferred term for a distinctive type of thyroid malignancy composed of C (parafollicular) cells, grossly, the typical tumor is solid, firm, and nonencapsulated but relatively well circumscribed and has a gray to yellowish cut surface most tumors are located in the midportion or upper half of the gland, corresponding to a greater concentration of cells in this region.

Microscopically, the classic presentation is represented by a solid proliferation of round to polygonal cells of granular amphophilic cytoplasm and medium-sized nucleus, separated by a highly vascular stroma, hyalinized collagen, and amyloid coarse calcification is common and can be prominent enough to be detected radiographically.

In fine needle aspiration preparations, medullary carcinoma is characterized by eccentric nuclei, “neuroendcrine-type” chromatin, inconspicuous nucleoli, binucleated and multinucleated cells, ill-defined cell borders, and clean back ground. Sometimes the amyloid material can be identified.

Immunohistochemically, the tumor cells are reactive for epithelial markers such as keratin, general thyroid marker such as TTF-1, pan-endocrine markers such as NSE, chnomogranin A,B,C and most important – the specific product of (cells i.e calcitonin. They are also consistently positive CEA and generally negative for Tg .The amyloid of medullary carcinoma reacts with the generic stains for this substance and has atypical microfibrillary appearance ultra structurally. It also shows reactivity for calcitonin, suggesting that its production may be related to the secretion or degradation of this hormone (Ackerman’s, 2011).

2.7. Diagnosis of Thyroid cancer

2.7.1.History and Physical examination

The most common presentation of thyroid cancer is an asymptomatic thyroid mass or a nodule that can be felt in the neck. For any patient with a thyroid lump that has developed recently, obtain a history regarding every prior exposure to ionizing radiation and the lifetime duration of the radiation exposure, consider a family history of thyroid cancer, some patients have persistent cough, difficulty breathing, or difficulty swallowing, Pain is seldom an early warning sign of thyroid cancer. Other symptoms (e.g pain, stridor, vocal cord paralysis, hemoptysis, rapid enlargement) are rare. These symptoms can be caused by less serious problems. At the time of diagnosis, 10-15% of patients have distant metastases to the bones and lungs and, initially, are evaluated for pulmonary or osteoarticular symptoms (e.g pathologic fracture, spontaneous fracture).

Palpate the patient's neck to evaluate the size and firmness of the thyroid and to check for any thyroid nodules. The principal sign of thyroid carcinoma is a palpable, firm, and nontender nodule in the thyroid area. This mass is painless. Some patients have a tight or full feeling in the neck, hoarseness, or signs of tracheal or esophageal compression. Usually, signs of hyperthyroidism or hypothyroidism are not observed (American Cancer Society's, 2009) .

2.7.2.Laboratory tests and Imaging studies

TSH, T3 and T4 measured to determine thyroid gland state (hypothyroidism or hyperthyroid) but it is important to remember that thyroid function tests are not indicators of thyroid cancer and most people with thyroid cancer have normal thyroid function. If patient has a family history of MTC ,the doctor will test blood calcitonin and calcium levels .An elevated calcitonin level can indicate cancer (Lai et al., 2008).

Thyroid scan

A thyroid scan or nuclear medicine scan, test the gland's function. After a radioactive tracer (dye – iodine or technetium) is injected, a special camera captures images of the

thyroid gland and measures the amount of dye the gland(nodules) absorbs. Normal and abnormal test results are reported as functioning **(normal)**, cold **(underactive)** or hot **(over active)**. suspicious cold nodules can be further evaluated by a procedure called fine needle aspiration hot nodules do not generally require biopsy.

Results from imaging studies may assist doctor in confirming thyroid cancer diagnosis. Different types of imaging studies include x – ray. computed tomography **(CT scan)** magnetic resonance imaging **(MRI)** and positron emission tomography **(PET scan)** (Lai et al., 2008).

2.7.3.Fine needle Aspiration (FNA)

depending on the size of the nodule, the doctor may perform F**NA** in his office, An anesthetic numbs the area, although is usually not painful small tumors **(less than half an inch)** may require biopsy using ultrasound to guide needle placement fine needle Aspiration usually involves taking several samples that are microscopically examined by a pathologist. Some times FNA results are not conclusive. If the doctors has reason to think the nodule may be cancerous ,the doctor may recommend a biopsy using a larger needle ,open biopsy or removal of the thyroid gland **(**lobectomy). These procedures are performed under general anesthesia in an operating room (Lai et al., 2008).

FNA cytology is currently the most reliable diagnostic test for thyroid nodules and establishes the definitive diagnosis of a benign or malignant lesion in the majority of cases, whereas 10 – 40% of all FNA samples are diagnosed as indeterminate for malignancy (Gharib et al., 2007).

The general category of indeterminate cytology encompasses several sub categories, that is follicular lesion of indeterminate significance **(FLUS)**, follicular neoplasm /Hurthle cell neoplasm, and suspicious for malignancy, which correlate with the estimated risk of malignancy of 5-10%, 20 - 30% ,and 50 - 75%, respectively (Baloch et al., 2008).

Owing to the lack of definitive diagnosis ,most patients with indeterminate cytology undergo surgery ,although only 8 - 17% of surgically removed thyroid nodules are malignant (Mazza Frri et al., 1993; Baloch et al., 2002). Patient with indeterminate

FNA cytology and malignant tumors are not adequately treated as well as most of them initially undergo thyroid lobectomy and later have another surgery to complete thyroidectomy.

Molecular testing of FNA samples may significantly improve the accuracy of cytological diagnosis of thyroid nodules .most experience is BRAF mutations. The results of **BRAF** testing in 2766 **FNA** samples have been reported in 18prospective and retrospective studies (Hayashida et al., 2004; Chung , 2006; Jin et al., 2006; Kumagai et al. ,2007; Kim et al.,2008; Nikiforov et al., 2009).

Among 581 **BRAF** – positive nodules tested in **FNA** samples in these studies ,580 were papillary carcinomas on pathological examination of the resected nodules ,whereas one was diagnosed as a benign nodule ,resulting in the false – positive rate of 0.2%. this reportedly benign nodule had histopathological diagnosis of atypical nodular hyperplasia**,** importantly 15– 40%of **BRAF**–positive **FNA** samples are indeterminate or non diagnostic by cytology (Coheny , 2004; Salvatore et al., 2004; Pizzolanti et al., 2007; Kim et al., 2008; Joys et al., 2009; Nikiforov et al., 2009). Indicating that testing for **BRAF** is helpful in establishing the definitive diagnosis of cancer in nodules with indeterminate cytology.

Detection of **RAS** mutation which was the second most common mutation after **BRAF**, also appeared to be of high diagnostic value in **FNA** samples, as it conferred an 87 – 100% probability of malignancy. The biggest diagnostic impact can be achieved by testing **FNA** samples for a panel of mutations rather then for a single mutation (Nikiforov et al., 2009; Ohori et al., 2010). Gupta et al. evaluated patients undergoing FNA prospectively with a panel of molecular markers. Results were sixty-eight aspirates from 66 patients were positive for RAS mutations including 63 cytologically indeterminate (93%), 3 malignant (4%), and 2 benign (3%) specimens. Their conclusion were most RAS-positive thyroid cancers have indeterminate cytology, lack suspicious ultrasound features, and are histologically low-grade follicular variant histology PTC. Lymph node and distant metastases are uncommon but bilateral disease is frequent. Total thyroidectomy should be considered for initial surgical management of most patients with RAS-positive FNA results (Gupta et al., 2013).

Many studies have explored the diagnostic utility of molecular testing for a panel of mutations consisting of **BRAF, RAS, RET/PTC**, and **PAX8/PPAR$_\gamma$** (Nikiforov et al., 2009; Ohori et al., 2010). the guide – lines recommend the use of molecular markers, such as **BRAF, RAS, RET/PTC, and PAX$_\gamma$ / PPAR$_\gamma$**, for indeterminate F**NA** cytology to help guide patient management (Yuri Nikiforov, 2011).

Over helps of molecular testing of fine-needle aspiration (FNA) in diagnose thyroid cancer, the molecular testing of cytologically indeterminate FNA results is cost saving predominantly because of reduction in two-stage thyroidectomy. Appropriate use of emerging molecular testing techniques may thus help optimize patient care, improve resource use, and avoid unnecessary operation (Yip et al., 2012).

Table 2.1. Improved diagnosis from molecular testing of thyroid FNA biopsies (Nikiforova, 2009; Xing et al., 2009; Cantara et al., 2010).

Positive Result	Probability of malignancy
Cytology	44- 60%
Cytology plus Molecular testing	80- 90%
BRAF Testing	99.8%

2.7.4.Grading

Histological grading of thyroid tumors is not commonly performed and is not included in RCPath dataset. However, grading may provide useful additional prognostic information (Hay et al., 1987; Akslen et al., 2000). It is therefore recommended that, where possible, a grade be assigned to the primary tumor as follows

G1 Well differentiated

G2 Moderately well differentiated

G3 Poorly differentiated

G4 Undifferentiated

GX Grade cannot be assessed

For papillary tumors, a simple grading system based on a combination of marked nuclear atypia, tumor necrosis and vascular invasion has been proposed (Hay et al., 1987). Grade 1 tumors have none of these feature, Grade 2 one or more. For follicular tumors, the presence of an insular, solid or other less well differentiated component in a predominantly follicular lesion would warrant Grade 2. Predominance of the dedifferentiated component would place the tumor in Grade 3 (Geoffrey et al., 2006).

2.7.5.Staging

The American Joint Committee on Cancer (AJCC) has designated staging by TNM classification to define thyroid cancer (Edge et al., 2010).

Table 2.2. American Joint Committee on Cancer (AJCC) clinical TNM classification of thyroid cancer (Edge et al., 2010).

Primary Tumor (T)

TX	Primary tumor cannot be assessed.
T0	No evidence of primary tumor.
T1	Tumor ≤2 cm in greatest dimension limited to the thyroid.
T1a	Tumor ≤1 cm, limited to the thyroid.
T1b	Tumor >1 cm but ≤2 cm in greatest dimension, limited to the thyroid.
T2	Tumor >2 cm but ≤4 cm in greatest dimension, limited to the thyroid.
T3	Tumor >4 cm in greatest dimension limited to the thyroid or any tumor with minimal extrathyroid extension (e.g., extension to sternothyroid muscle or perithyroid soft tissues).
T4a	Moderately advanced disease.
	Tumor of any size extending beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, esophagus, or recurrent laryngeal nerve.
T4b	Very advanced disease.
	Tumor invades prevertebral fascia or encases carotid artery or mediastinal vessels.
[c]T4a	Intrathyroidal anaplastic carcinoma.
[c]T4b	Anaplastic carcinoma with gross extrathyroid extension.

[a]Reprinted with permission from AJCC

[b]All categories may be subdivided: (s) solitary tumor and (m) multifocal tumor (the largest determines the classification).

[c]All anaplastic carcinomas are considered T4 tumors.

Regional Lymph Nodes (N)[a,b]

NX	Regional lymph nodes cannot be assessed.
N0	No regional lymph node metastasis.
N1	Regional lymph node metastasis.
N1a	Metastases to Level VI (pretracheal, paratracheal, and prelaryngeal/Delphian lymph nodes).
N1b	Metastases to unilateral, bilateral, or contralateral cervical (Levels I, II, III, IV, or V) or retropharyngeal or superior mediastinal lymph nodes (Level VII).

Distant Metastasis (M)[a]

M0	No distant metastasis.
M1	Distant metastasis.

Table 2.3. American Joint Committee on Cancer (AJCC) stage grouping (Anatomic Stage/Prognostic Groups[a,b]) (Edge et al, 2010)

Stage	**T**	**N**	**M**
Papillary or follicular (differentiated)			
YOUNGER THAN 45 YEARS			
I	Any T	Any N	M0
II	Any T	Any N	M1
45 YEARS AND OLDER			
I	T1	N0	M0
II	T2	N0	M0
III	T3	N0	M0
	T1	N1a	M0
	T2	N1a	M0
	T3	N1a	M0
IVA	T4a	N0	M0
	T4a	N1a	M0
	T1	N1b	M0
	T2	N1b	M0
	T3	N1b	M0
	T4a	N1b	M0
IVB	T4b	Any N	M0
Stage IVC	Any T	Any N	M1
Medullary carcinoma (all age groups)			
I	T1	N0	M0
II	T2	N0	M0
	T3	N0	M0
III	T1	N1a	M0
	T1	N1a	M0
	T2	N1a	M0

Stage	**T**	**N**	**M**
	T3	N1a	M0
IVA	T4a	N0	M0
	T4a	N1a	M0
	T1	N1b	M0
	T2	N1b	M0
	T3	N1b	M0
	T4a	N1b	M0
	Stage IVB	T4b	Any N
IVB	T4b	Any N	M0
IVC	Any T	Any N	M1
Anaplastic carcinoma [c]			
IVA	T4a	Any N	M0
IVB	T4b	Any N	M0
IVC	Any T	Any N	M1

Stage I papillary thyroid cancer

Stage I PTC is localized to the thyroid gland. In as many as 50% of cases, there are multifocal sites of papillary adenocarcinomas throughout the gland. Most papillary cancers have some follicular elements, and these may sometimes be more numerous than the papillary formations, but this does not change the prognosis. The 10-year survival rate is slightly better for patients younger than 45 years than for patients older than 45 years.

Stage II papillary thyroid cancer

Stage II PTC is defined as either: (1) tumor that has spread distantly in patients younger than 45 years, or (2) tumor that is larger than 2 cm but 4 cm or smaller and is limited to the thyroid gland in patients older than 45 years. In as many as 50% to 80% of cases, there are multifocal sites of papillary adenocarcinomas throughout the gland. Most papillary cancers have some follicular elements, and these may sometimes be more numerous than the papillary formations, but this does not appear to change the prognosis.

Stage III papillary thyroid cancer

Stage III is PTC in patients older than 45 years that is larger than 4 cm and is limited to the thyroid or with minimal extrathyroid extension, or positive lymph nodes limited to the pretracheal, paratracheal, or prelaryngeal/Delphian nodes. Papillary carcinoma that has invaded adjacent cervical tissue has a worse prognosis than tumors confined to the thyroid.

Stage IV papillary thyroid cancer

Stage IV is PTC in patients older than 45 years with extension beyond the thyroid capsule to the soft tissues of the neck, cervical lymph node metastases, or distant metastases. The lungs and bone are the most frequent distant sites of spread, though such distant spread is rare in this type of thyroid cancer. PTC more frequently metastasizes to regional lymph nodes than to distant sites. The prognosis for patients with distant metastases is poor (Edge et al., 2010).

2.8.Screening and prevention

No screening is indicated for the general population. Risk-directed screening should be considered when the primary care physician identifies patients with: Familial thyroid cancer, including medullary thyroid cancer (MTC), history of neck irradiation in childhood , family history of multiple endocrine neoplasia type 2 (MEN2). The following carry a statistically increased risk of thyroid malignancy but no screening is recommended: Endemic goiter, hashimiotothyroiditis (risk of lymphoma), family or personal history of thyroid adenoma , Cowden's syndrome (macrocephaly, mild learning difficulties, carpet-pile tongue, with benign or malignant breast disease), familial adenomatous polyposis (Geoffrey et al., 2006).

Prevention

There are several scattered activities are carried out in the Arab countries to prevent and control of nutritional problems, one of them are Control programmes for iodine deficiency disorders (IDD). Control programmes for IDD are not usually targeted to specific age or sex groups, e.g., women or children, but rather to whole populations. Successful IDD control programmes would result in the promotion of iodine status in, along with other groups, women, adolescent girls and children. Consequently, improvements in their physical and mental health will occur. The Region has been very active in this area over the past two decades, with support from the WHO, the United Nations Children's Fund and the International Council for the Control of iodine Deficiency Disorders. Not all countries in the Region, however, have national control programmes. In Tunisia, IDD has been officially declared by the WHO to be under control, and in Jordan, Lebanon, the Syrian Arab Republic and Yemen it is said to be almost under control. Seventeen of the remaining countries have ongoing programmes for universal salt iodization and 16 have appropriate legislation for this. In the Region as a whole, about 51% of households currently consume iodized salt (Djazayery, 2004). Previous head or neck irradiation in childhood is a possible cause of thyroid cancer in adults. Exposure to radiation should be limited whenever possible. In cases of populations or individuals being contaminated with radioactive iodine, the thyroid can be protected by administering potassium iodide (Kutkov et al., 2011).

2.9.Treatment

The treatment of patients with PTC consists of four main components: adequate surgical extirpation of disease, adjunctive RAI ablation in selected cases, TSH suppression, and surveillance. The overall management strategy for any individual patient depends on preoperative and Intraoperative findings as well as the final TNM classification and postoperative evaluation. The surgical options for resection of the primary cancer include thyroid lobectomy versus total or near-total thyroidectomy. One of the ongoing debates in the treatment of patients with PTC over the past several decades has been the extent of thyroidectomy, particularly for small, intrathyroidal, low-risk, well-differentiated PTCs. Because of the low incidence of thyroid cancer and its overall good prognosis, no controlled, prospective studies have compared the surgical treatment options, so these debates are thus likely to continue. Those supporting thyroid lobectomy as the treatment of choice argue that some studies have shown no survival benefit to more extensive thyroidectomy (Shaha et al., 1997; Sanders et al., 1998; Haigh et al., 2005), and that lobectomy is associated with lower rates of complications, such as recurrent laryngeal nerve injury and permanent hypoparathyroidism. Arguments supporting total thyroidectomy include reports that have shown that more extensive thyroidectomy is associated with lower recurrence rates and a survival benefit compared with lobectomy (Hay et al., 1998; Bilimoria et al., 2007). Also, in the hands of experienced endocrine surgeons, the complication rates are comparable for total thyroidectomy and lobectomy (Sosa et al., 1998; Hundahl et al., 2000) PTC is multifocal in up to 80% of cases and bilateral in up to 60% (Katoh et al., 1992), and removal of the entire thyroid gland facilitates the use of postoperative RAI to treat residual microscopic or metastatic disease and allows for the use of serum thyroglobulin (Tg) postoperatively as a sensitive marker for recurrent disease. Consensus guidelines recommend total or near-total thyroidectomy as the preferred initial procedure for patients with PTC, with absolute indications including a history of radiation exposure, familial thyroid cancer, tumor size greater than 4 cm, known extra thyroidal extension, cervical lymph node or distant metastasis, or an aggressive histological variant of PTC (Cooper et al., 2006; Sherman et al., 2007). Another area of controversy in the treatment of patients with PTC is the extent of lymphadenectomy. Lymph node metastases from PTC are very common, with contemporary series of prophylactic neck dissections

demonstrating a prevalence of 33% to 63% for central neck (pre- or paratracheal [level VI]) nodal metastases, and a prevalence of 57% to 64% for lateral neck (internal jugular vein levels II, III, and IV) nodal metastases not detected by preoperative ultrasonography (Pereira et al., 2005; Shindo et al., 2006; Ito et al., 2006; Ito et al., 2007) because some studies have shown that lymph node metastases have no impact on overall survival, particularly in patients younger than age 45 years (Sanders et al., 1998; Greene et al., 2002; Toniato et al., 2008). Therapeutic compartment oriented lymph node dissection is indicated for patients with known cervical nodal metastases. Selective node removal, or "berry picking" should not be done because patients with PTC may develop recurrent disease within a previously dissected compartment, possibly necessitating high-risk revisional lymphadenectomy. Lymph node metastases are usually identified preoperatively by palpation or by ultrasonography and confirmed by FNA biopsy. The central compartment of the neck is less completely evaluated by preoperative ultrasonography (Kouvaraki et al., 2003) and should also be evaluated at the time of thyroidectomy, with a central neck dissection performed if a suspicious appearing lymph node is confirmed to contain metastatic PTC on frozen section analysis. The role of prophylactic lymphadenectomy in the treatment of patients with PTC is controversial. Prophylactic lateral neck dissection is not done in Europe and the United States. Prophylactic central neck dissection, however, remains an area of controversy. Proponents of routine prophylactic central neck dissection argue that central neck nodal metastases are common (Pereira et al., 2005; Ito et al., 2006; Shindo et al., 2006). Some studies have shown that regional lymph node metastases are associated with higher rates of recurrent disease (Simon et al., 1996; Mazzaferri et al., 2001), and patients who develop recurrent disease have higher rates of cancer-specific mortality (Mazzaferri et al., 1994; Mazzaferri et al., 2001). Opponents of routine prophylactic central neck dissection argue that other studies have shown that lymph node metastases have no impact on overall survival, and that this procedure is associated with a higher risk of complications, with reported rates of transient vocal cord paralysis of 2% to 7%, rates of transient hypoparathyroidism of 14% to 60%, and rates of permanent hypoparathyroidism of 2% to 5%. Major consensus guidelines disagree with respect to prophylactic central neck dissection, the ATA guide-lines recommend considering routine central compartment neck dissection for patients with PTC but state that near total or total thyroidectomy without central neck dissection may

be an alternative approach when followed by RAI (Cooper et al., 2006). On the other hand, the NCCN guidelines do not advocate routine central neck dissection and only recommend it if lymph nodes are palpable or biopsy-proven positive for metastatic disease (Sherman et al., 2008). Locally advanced disease may be overt or found incidentally at the time of thyroidectomy. If aerodigestive tract invasion is seen on preoperative imaging or endoscopy, the patient's performance status and extent and progression of metastatic disease should help guide the decision to perform an extensive laryngotracheal or full-thickness esophageal resection versus palliation with intraluminal laser tumor debulking or stenting. Internal jugular vein invasion should be managed by unilateral resection; the rare circumstance of bilateral internal jugular vein invasion may be managed with bilateral resection, but these two procedures should be staged at least 6 weeks apart. External-beam radiotherapy should be considered for positive margins. The second component of the global treatment strategy for patients with PTC is RAI ablation. It is usually administered 4 to 12 weeks after surgery, and its purpose is to destroy any remnant thyroid tissue after thyroidectomy and to treat occult or known metastatic disease. A controversy in the use of RAI is that although some studies show that it reduces rates of recurrence and cancer-specific mortality (DeGroot et al., 1990; Samaan et al., 1992; Mazzaferri et al., 1994; Sawka et al., 2004), other studies have shown no benefit (Hundahl et al., 1998; Sanders & Cady, 1998; Hay et al., 2002; Sawka et al., 2004), particularly for patients classified as having low-risk PTC. Both the ATA and NCCN consensus guidelines recommend RAI ablation for all patients with PTC except select patients with stage I disease who are at very low risk for recurrence (e.g., those with well-differentiated, unifocal tumors smaller than 1 cm, no extrathyroidal extension or vascular invasion, and no lymph node or distant metastases) (Cooper et al., 2006; Sherman et al., 2008). In addition, the NCCN guidelines recommend omitting RAI ablation in patients who have a negative whole-body RAI scan with Tg levels below 1 ng/mL and negative anti-Tg antibodies under conditions of adequate TSH stimulation (Sherman et al., 2008). The isotope used for ablation is iodine 131 (I 131), which is administered in oral form as sodium iodide and has a half-life of 8 days. Thyroid cells are unique among all cells of the human body as they have the ability to absorb iodine. Thyroid cells will absorb and concentrate also radioactive iodine, and the radioactivity destroys the cell. Because papillary and follicular thyroid cancer cells retain this ability to absorb iodine, radioiodine thus destroys any remaining

normal thyroid tissue and occult microscopic carcinoma. This means that even metastatic disease in most cases will be treatable (Schlumberger, 1998; Sherman et al. 2005). Contraindications to the use of RAI include pregnancy and lactation. Women of childbearing age should have a negative pregnancy test result before treatment and are advised to not become pregnant for at least 6 to 12 months after treatment. Preparation for treatment includes following a low-iodine diet for 1 to2 weeks and discontinuing all iodide-containing products (including multivitamins), thyroid hormones, and amiodarone to allow the TSH to increase to above 30 mIU/mL and stimulate maximal uptake of the I 131 (Meier et al., 2002). Some physicians treat patients after administration of recombinant human TSH (rhTSH) rather than after withdrawal of thyroid hormone. Based on a prospective, international, multicenter trial, the US Food and Drug Administration approved the use of rhTSH to facilitate RAI ablation of thyroid tissue remnants in patients who have undergone near-total or total thyroidectomy for well differentiated thyroid cancer in the absence of metastatic disease (Pacini et al., 2006). In recent study Sherman demonstrated the addition of recombinant human thyrotropin to diagnostic testing replaced the requirement for thyroid hormone withdrawal and symptomatic hypothyroidism that had been necessary to generate sufficient endogenous thyrotropin for radioiodine scanning and thyroglobulin testing, so recombinant human thyrotropin-stimulated testing continues to be a valuable component of follow-up testing in the first year after initial treatment of differentiated thyroid cancer (Sherman, 2013).

The third component in the global treatment strategy for patients with PTC is administration of supraphysiologic doses of thyroid hormone in the form of levothyroxine (LT4). The rationale behind this therapy is to suppress TSH, which is a known stimulator of thyroid cell proliferation. Large retrospective and prospective studies, as well as a meta-analysis, have demonstrated that patients treated with TSH suppressive doses of LT4 have a decreased risk of major clinical adverse events, particularly in patients with PTC who fall into a high-risk group (Mazzaferri et al., 1994; Cooper et al., 1998; McGriff et al., 2002). The ATA consensus guidelines recommend TSH suppression to below 0.1 mIU/mL for high-risk patients and to between 0.1 and 0.5 mIU/mL for those in the low-risk group (Cooper et al., 2006). The last component in the global treatment strategy for patients with PTC is surveillance.

Tumor burden should be monitored periodically by experienced physicians. The measurement of serum TSH, Tg, and anti-Tg levels; cervical ultrasonography; and RAI scanning are all sensitive for the presence of residual or recurrent disease. Anti-Tg antibodies, which are present in about 25% of patients with thyroid cancer and can falsely lower serum Tg levels (Spencer et al., 1999), should always be measured concurrently with TSH and Tg. Serial anti-Tg antibody levels may be followed as a surrogate marker for Tg (Cooper et al., 2006). The strategies used for PTC surveillance are highly variable and institution specific. What follows is a basic strategy based on consensus guide-lines. Initial follow-up should occur at 6 and 12 months after RAI remnant ablation and should consist of measurement of TSH, Tg, anti-Tg antibodies, and cervical ultrasonography. If the unstimulated Tg level is undetectable (<1 ng/mL) and the cervical ultrasonography results are negative, Tg should be remeasured under conditions of LT4 withdrawal or rhTSH stimulation (Cooper et al., 2006; Sherman et al., 2008). If the patient is disease free (Tg <2 ng/mL), retesting should be done on an annual basis. If the stimulated Tg is detectable (>2–5 ng/mL), however, then the patient should undergo a whole-body RAI scan (WBS). If the WBS results are positive, further management depends on the site(s) of recurrent disease. If the WBS results are negative, FDG-PET scanning should be considered for further evaluation (Sherman et al., 2008). Patients with a locoregional recurrence should have it confirmed by FNA biopsy and then undergo surgery if the recurrence is resectable. These locoregional recurrences are usually lymph node metastases that survived the initial RAI ablation, and appropriate treatment consists of a formal lymph node dissection of the involved lateral or central compartment. If the recurrence is in a previously dissected compartment, then excision of the solitary recurrence is appropriate, although other surrounding lymph nodes are often found to be positive at the time of surgery. This is recommended even in the presence of distant metastatic disease to palliate symptoms or to prevent subsequent airway or esophageal obstruction. Resection of locoregional recurrences should be followed by another treatment dose of RAI if the tumor is iodine avid. Cytotoxic chemotherapy has not traditionally been found to be effective in the treatment of patients with PTC (Leaf et al., 2000). It is not indicated in the adjuvant treatment of patients with resected PTC. For advanced, RAI-resistant differentiated thyroid carcinoma, the agent that has historically been implemented is doxorubicin. Response

rates of up to 40% have been reported, although the duration is short lived (Gottlieb et al., 1992).

Molecular targeted therapy

Novel therapies for the treatment of patients with advanced or metastatic PTC include redifferentiation agents, which are agents that target the RAS pathway, the BRAF pathway, vascular endothelial growth factor and its receptors, the epidermal growth factor receptor pathway, and other angiogenic pathways with agents such as thalidomide and proteasomes (Braga-Basaria et al., 2003). Some agents, such as sunitinib and sorafenib, inhibit multiple receptor tyrosine kinases and thus target multiple pathways involved in tumor growth. A recent phase II trial implementing sorafenib for patients with metastatic, iodine-refractory thyroid carcinoma demonstrated an overall clinical benefit rate (partial response or stable disease) of 77% (Gupta-Abramson et al., 2008). The data are preliminary, and further trials are needed to validate the drug's long-term efficacy. Enrollment in clinical trials for patients with advanced thyroid cancer is strongly encouraged.

Pregnancy and thyroid cancer

Thyroid cancer, is often detected in young female patients. Therefore, pregnancy following thyroid cancer is not infrequent, and about 10% of thyroid cancers occurring during the reproductive years are diagnosed during pregnancy or in the early post-partum period. Differentiated thyroid cancer (DTC) in young people generally has an excellent prognosis, and disease-free survival among women with DTC diagnosed during pregnancy may not differ from that in age-matched non-pregnant women with similar disease (Gibelli et al., 2011).

The management of thyroid cancer diagnosed during pregnancy requires careful consideration of risks to mother and fetus. Surgery is indicated, but evidence regarding the optimum timing is unclear. Thyroidectomy in the first trimester of pregnancy carries a high risk of abortion, but may be performed safely in the 2 nd trimester. Alternatively surgery can be deferred until after delivery, provided that the tumor is monitored regularly (e.g by ultrasound) and found to be reasonably stable. In cases of advanced or aggressive disease delays in treatment would be undesirable, and termination of

pregnancy may (rarely) need to be considered. 131 I ablation or therapy must be avoided in pregnancy. Suppressive thyroxine therapy is safe during pregnancy. A thyroid nodule presenting during pregnancy should be investigated by FNAC. Radioiodine scans are contraindicated in pregnancy and breast-feeding .

Pregnancy in the treated patient

In accordance with ARSAC, it is recommended that women should defer conception for a minimum of 6 months and men for a period of 4 months following 131 I ablation or therapy (ARSAC, 2006). A small risk of spontaneous abortion may persist for up to 1 year after high dose 131 I ablation or therapy (Casara et al,. 1993; Dottorini et al., 1995; Schlumberger et al., 1996; Schlumberger et al., 1997; Ayala et al., 1998). There is no risk of previous 131 I ablation or therapy to the fetus, provided the recommendations are followed (Casara et al.,1993; Dottorini et al., 1995).

I. Suppressive levothyroxine therapy should continue during pregnancy and to achieve this, the dose should be increased as soon as pregnancy is confirmed by approximately 25% (Mandel et al., 1990) and further adjusted if necessary according to monitoring of thyroid function tests.
II. The thyroid status should be checked by measurements of serum TSH and free thyroxine during each trimester to ensure that TSH remains suppressed, as levothyroxine requirements may increase during pregnancy (UK guidelines for the use of thyroid function tests, 2006).
III. For men there should be a minimum period of 4 months from 131 I ablation or therapy before unprotected intercourse takes place (Administration of Radioactive Substances Advisory Committee, 2006).

Thyroid cancer in childhood

Differentiated thyroid cancer is rare in children. Children at particular risk are those previously exposed to radiotherapy to the head or neck. Thyroid nodules are more likely to be malignant in children than in adults so surgical excision may be appropriate even if findings from FNAC suggest benign disease. Thyroid cancer in children aged 10

years or less is more aggressive than in adults and risk of recurrence is higher (Schlumberger et al., 1987; Jarzab et al., 2000). The general principles of management are similar to those in adults, however the managing team must include a paediatric endocrinologist, paediatric oncologist, total thyroidectomy followed by TSH suppression is recommended for most patients, selective neck dissection is recommended for children with clinically positive neck nodes (Thompson et al., 2004) , 131 I ablation is recommended for all children particularly those aged under 10 years, but the decision about 131 I ablation should be individually determined (La Quaglia et al., 2000; Jarzab et al., 2000; Thompson et al., 2004), follow-up with serial serum Tg measurements should be life-long (Laundau et al., 2000).

2.10.Prognostic factors

several prognostic factors have been identified for PTC. Traditional factors can be divided into 4 categories, backgrounds of patients, factors based on preoperative, and Intraoperative and postoperative evaluations.

2.10.1.Traditional prognostic factors

2.10.1.1. Backgrounds of patients include

1. Age

Patient age is an important background factor for predicting prognoses. Several classification systems have adopted age as a prominent factor in deciding whether carcinoma should be considered high risk. For example

●**AGES** – age**,** Grade, Extent of disease, size .

●**AMES –** age, Metastasis, Extent of disease, size.

●**MACIS**– metastasis ,Age at presentation ,completeness of surgical resection ,Invasion **(extra thyroidal)**, size (New York Thyroid Center, 2007) (this is a modification of the AGES system), It is probably the most reliable staging method available.

MAICS

The MAICS system of estimating the prognosis of PTC was developed by the mayo clinic and was based on careful evaluation of a large group of patients .It is probably the most reliable staging method available (New York Thyroid Center, 2010). It assigns scores to the main factors involved, and uses the sum of this scores to calculate the prognosis.

Table 2.4. The MAICS system for prognosis of PTC (New York Thyroid Center, 2010)

factors	score
Distant metastasis: spread of the cancer to areas out side the neck	Yes 3 No 0
Age at the time the tumor was discovered .	Less than 39 yrs 3.1 over 40 yrs 0.08× age
Invasion into surrounding areas of the neck as seen by the naked eye	Yes 1 No 0
Completeness of surgical resection (or removal) of the tumor	In complete 1 Complete 0
Size of the tumor	0.3×size in cm

Sum of MAICS score	20 yr survival
< 6.0	99%
6.0- 6.99	89%
7.0- 7.99	56%
> 8.0	24%

Most patients fall in the low risk category (MACI S score less than 6.0) and are cured of the cancer at the time of surgery (New York Thyroid Center, 2010). Children with multiple lung metastases and /or a miliary aspect still have an excellent long-term prognosis if given adequate treatment (Vermeer-Mens et al., 2006). By over all cancer staging into stages I to IV, papillary thyroid cancer has a 5-year survival rate of 100% for stages I and II, 93% for stage III and 51% for stage IV (American Cancer Society's, 2009).

2-Gender

females are said to have a better prognosis than males ,although in some series the difference has not been significant (Ackerman's, 2011).

3-Family History

PTC and FTC are generally considered sporadic with the exception that these are lesions also associated with rare inherited diseases such as familial adenomatous polyposis, Gardner syndrome, and Cowden disease (Fagin, 1997; Sturgeon and Clark, 2005).

2.10.1.2.Prognostic Factors Predominantly Based on Preoperative Evaluation

1. Tumor Size

Tumor size was adopted as a factor discriminating high risk patients from others in various classification systems such as UICC classification (Sobin et al., 2002), MACIS scoring system (Hay et al., 1993), and AMES (Cady et al., 1988). In UICC TNM classification, there are two cutoffs, 2 cm and 4 cm (T1 for 2 cm or less, T2 for 2.1–4 cm, and T3 for larger than 4 cm) (Sobin et al., 2002). In AMES, 5 cm is a cutoff between high-risk and low-risk patients (Cady et al., 1988).

2. Multiplicity of Primary Lesions.

PTC is frequently multiple. There is study showed that lateral node metastasis is more frequently detected in multiple microcarcinomas than in solitary microcarcinomas (Ito et al., 2004). Also Lee et al. said the multifocal tumors in patients with papillary thyroid carcinoma are associated with increased risk of bilateral central compartment and lateral cervical lymph node metastasis (Lee et al., 2009). It is, therefore, suggested that multiplicity reflects the aggressive behavior of PTC to some extent. However, in another study said multiplicity was not an independent prognostic factor on multivariate analysis (Ito et al., 2010).

3- Clinical Lymph Node Metastasis (N).

Lymph node metastasis is a very common event and recognized as one of the most important prognostic factors. However, prominent classification systems such as AMES (Cady et al., 1988) and MACIS (Hay et al., 1993) do not adopt lymph-node metastasis as a prognostic factor. This may be possibly because these systems were based on a series of patients who underwent surgery before the establishment of routine ultrasonography as a preoperative imaging study for accurate evaluation of lymph-node metastasis. At present, lymph-node metastasis can be preoperatively evaluated on imaging studies, ultrasonography is the most useful tool for this purpose. Node metastasis detected on preoperative imaging studies is called clinical lymph node metastasis (N). Evaluation of clinical node metastasis is very important, and it was divided into two categories in UICC TNM classification (Sobin et al., 2002): N1a, central node metastasis and N1b, metastasis to the lateral or mediastinal compartment. In this classification, N1b is upgraded compared to N1a and N1b patients are further upstaged if they are aged 45 years or older. It is currently considered that the prognostic impact of clinical node metastasis can be divided into three categories: (1) clinical node metastasis measuring 3cm or larger or showing Extranodal tumor extension on Intraoperative findings (high risk), (2) clinical node metastasis smaller than 3cm without extra nodal tumor extension(intermediate risk), and (3) no clinical node metastasis (low risk).

4- Distant Metastasis at Surgery (M1).

Although rarer than FTC, PTC can metastasize not only to regional lymph nodes, but also to distant organs such as the lung, bone, and brain. Distant metastasis at surgery can be detected on imaging studies such as CT scan and PET- CT and also on postoperative radioactive iodine (RAI) ablation or whole body scan. There are no doubts that distant metastasis at surgery is one of the most important prognostic factors for CSS of patients (Ito et al., 2010). However, prognosis of M1 patients differ according to other clinicopathological features of the patient. Many previous studies analyzed M1 patients and patients showing distant recurrence during postoperative follow up as a single group and/or analyzed PTC and FTC as DTC in a single group (Pacini et al., 1994; Shoup et al., 2003; Haq & Harmer, 2005; Orita et al., 2012). Some

study has consistently shown M1 is directly linked to other clinicopathological features such as gender, tumor size, extrathyroid extension, and N factor, indicating that distant metastasis at diagnosis will more likely be found in PTC showing aggressive behavior (Ito et al., 2010). Tumor larger than 4 cm, aged 55 years or older (at the time of initial surgery) and extrathyroid extension were independent prognostic factors for CSS of M1 PTC patients.

2.10.1.3. Prognostic factors predominantly based on Intraoperative findings

1. Extrathyroid Extension.

Extrathyroid extension has been adopted in various classification systems (Cady et al., 1988; Hay et al., 1993). In the UICC TNM classification system, there are two grades of extrathyroid extension (Sobin et al., 2002). Extension to perithyroid tissue and sternothyroid muscle was graded as T3 (minimal extension), and extension to other adjacent organs such as the recurrent laryngeal nerve, esophagus, trachea, sternohyoid muscle, and jugular vein was graded as T4 (massive or significant extension). However, this classification has some limitations. This classification system is established for preoperative evaluation. However, it is significantly difficult to accurately evaluate extrathyroid extension based on preoperative evaluation unless recurrent laryngeal nerve paralysis due to carcinoma invasion and apparent intratracheal extension on CT scan or MRI can be detected. Most extrathyroid extensions are found on Intraoperative findings there is study showed that the significance of extrathyroid extension is not uniform but rather size-dependent. Prognostic significance of extrathyroid extension was less than clinical lateral node metastasis (N1b) for PTC measuring 3 cm or less, it was reversed in PTC larger than 3 cm (Fukushima et al., 2010). In the Intraoperative staging system that study established by revising the UICC TNM staging system, extrathyroid extension of tumor larger than 2 cm was regarded as a sign of high risk and that of a tumor 2 cm or smaller was a sign of intermediate risk (Ito et al., 2010). Result of this study was extrathyroid extension should be evaluated on Intraoperative findings and that minimal extension to perithyroid tissue and the sternothyroid muscle should not be considered significant. Significant extension on Intraoperative evaluation is an important factor predicting a worse prognosis for patients with PTC, especially those with a large tumor (Ito& Miyauchi, 2012).

2. Extranodal Tumor Extension.

Prognostic significance of Extranodal tumor extension has been investigated by several groups (Spires et al., 1989; Yamashita et al., 1997; Asanuma et al., 2001; Ito et al., 2007; Ito et al., 2009; Ito et al., 2010). Yamashita et al. showed that patients with pathological Extranodal tumor extension were more likely to show distant recurrence (Yamashita et al., 1997). Others said (Ito& Miyauchi), extranodal tumor extension requiring resection of adjacent organs showed a worse prognosis, especially for CSS as indicated above (Ito et al., 2007; Ito et al., 2009; Ito et al., 2010). It is strongly suggested that PTC with Extranodal tumor extension is high risk and has a high potential to show a dire prognosis (Ito& Miyauchi, 2012).

2.10.1.4. Prognostic Factors Based on Postoperative Findings

1. Pathological Lymph-Node Metastasis. PTC frequently metastasizes to the regional lymph nodes (Macdonald et al., 1957; Noguchi et al., 1987; Ahuja et al., 1991). clinical lymph-node metastasis detected on preoperative imaging studies is a significant prognostic factor, and especially, large metastatic node has a very strong prognostic impact on both DFS and CSS of PTC patients. Pathological and latent node metastases increase the rate of carcinoma recurrence to some extent but do not affect CSS of patients (Ito et al., 2004; Ito et al., 2009). In conclusion, lymph-node metastasis that can be diagnosed only on pathological examination is a moderate factor only for PTC recurrence (Ito& Miyauchi, 2012).

2. Histological Variants. Many histological variants of PTC have been adopted in the WHO classification (Sobrinho-Simoes et al., 2004). Follicular variant was the most common variant, which accounted for 7%. Follicular variant was reported to show aggressive behavior (Chang et al., 2006; Liu et al., 2006; Hagag et al., 2006). Tall cell variant is a typical variant showing an aggressive behavior (Johnson et al., 1988; Egea et al., 1993; Michels et al., 2007). Interestingly, the incidence of clinicopathological features reflecting poor prognosis such as gender, clinical lymph-node metastasis, and extrathyroid extension did not differ between tall cell variant and others although the average age of patients with tall cell variant was slightly higher than that of other patients. However, this histology independently affected DFS and CSS of PTC patients on multivariate analysis (Ito et al., 2008). Oncocytic variant accounted for 2% of PTC

and most of them were diagnosed as having Warthin-like tumor showing abundant chronic inflammatory cells that are associated with chronic thyroiditis. Previous studies showed that this variant generally had a mild character (Baloch et al., 2000; Urano et al., 2001; Ludv´ıkov´a et al., 2001). There are some more important variants of which prevalence is lower than those indicated above. Columnar cell variant is classified as an independent entity as columnar cell carcinoma (Sobrinho-Simoes et al., 2004). This carcinoma accounted only for 0.4%, but as much as 60% of patients showed carcinoma recurrence, indicating that this histological type is a sign of significantly aggressive behavior (Ito et al., 2008). Regarding the biological behavior and prognosis of the diffuse sclerosing variant, previous studies showed discrepant findings (Chow et al., 2003; Lam et al., 2006; Falvo et al., 2006). Another study said the diffuse sclerosing variant frequently showed multiple clinical node metastases and was more likely to show PTC recurrence, but the CSS of patients did not differ from that of conventional PTC (Fukushima et al., 2009). Macrofollicular variant could be diagnosed as multinodular goiter in the past in high incidences (Albores-Saavedra et al., 1991;Lugli et al., 2004). Cribriform morular variant is mostly a hereditary disease caused by the APC gene mutations associated with colonic polyposis or colon carcinoma (Dalal et al., 2006). This variant is multicentric and total thyroidectomy is mandatory regardless of carcinoma size and lymph-node status, but the prognosis of patients is generally excellent (Tomoda et al., 2004). Although not adopted in the WHO classification, encapsulated PTC generally shows a better prognosis than conventional PTC. This type is encapsulated and there is no extrathyroid extension, which may be the reason for excellent prognosis. The incidence of lymph-node metastasis is also lower than that in conventional PTC (Ito et al., 2008; Baloch et al., 2010).

3. Involvement of PDC Components, three criteria for PDC have been proposed. There are three growth patterns of PDC, solid, trabecular, and insular growth patterns, which are designated as PDC components. In order to diagnose PDC using the WHO classification, PDC components should occupy in the majority of the tumor (Sobrinho-Simoes et al., 2004). However, in the JSTS criteria (The Japanese Society of Thyroid Surgery, 2005), carcinoma with only a small portion of PDC components is diagnosed as PDC and discriminated from PTC or FTC. In the criteria for PDC in the Turin proposal (Volante et al., 2007), the absence of nuclear features of PTC and the

presence of convoluted nuclei, mitotic activity (3 × 10 HPF), or tumor necrosis were adopted in addition to the presence of a PDC component.

2.10.2 Biological prognostic markers

2.10.2.1. Oncogenes and tumor suppressor genes

The primary characteristics of tumor suppressor genes are that they encode normal cellular products involved in growth control, and both alleles must be inactivated for loss of function (i.e loss of tumor suppression) to occur. The most well known are retinoblastoma (Rb) protein, p53, p27, p21, and p16. The two best characterized are the Rb and p53 genes. Both are thought to be involved in growth control through the regulation of transcription.

1.Retinoblastoma gene (Rb)

The Rb gene is located on chromosome 13q14 and is dysfunctional in a number of types of cancer. Its normal function is to prevent the replication of damaged DNA; it does so by preventing cell replication by binding and inhibiting the transcription factor E2F (Chatterjee et al., 2004; Korenjak & Brehm, 2005). pRb is activated when it is dephosphorylated and inactivated when it is phosphorylated. Alterations in this gene have been described in many human tumors, including retinoblastoma, osteosarcoma, other sarcomas, leukemias, lymphomas, and certain carcinomas, including breast, lung, prostate, bladder, kidney, and testicular carcinoma (Reissmann et al., 1989; Cordon-Cardo et al., 1992; Hawes et al., 2000). Gene alterations are associated with advanced tumor grade and stage in a variety of tumors (Cordon-Cardo et al., 1992; Chatterjee et al., 2004). Alterations in the Rb gene correlate with loss of expression of pRb as determined by IHC (Xu H-J et al., 1991). Assessment of Rb gene loss by IHC is based on the loss of detectable nuclear staining for pRb. There is growing evidence that gene alterations may identify tumors that have a higher risk of developing metastases (Cote et al., 1998). Loss of heterozygosity, mutations, or deletions of the Rb gene usually result in the loss of pRb expression, which has been regarded as an indicator of loss of pRb function in human tumors. Ito Y et al.

examined the expression of cell proliferating markers, Ki-67, cyclin D1, p27, and retinoblastoma gene product (pRb) in papillary microcarcinomas. Cases of clinically apparent metastasis showed increased cyclin D1 expression together with decreased p27 expression and higher levels of pRb and Ki-67 expression. These findings suggest that cases of clinically apparent metastasis show significantly higher growth based on cell proliferating activity, apoptosis, and expression of metastatic suppressor than those demonstrating no or occult metastases (Ito Y et al., 2005).

2.P53

P53**(also Known protein 53 or tumor protein 53)**, is a tumor suppresser protein that in human is encoded by the *TP53* gene (Matlashewskig etal., 1984; Mcbride et al., 1986 ; Kem et al., 1991) P53 is crucial in multicellular organisms, where it regulates the cell cycle and thus functions as a tumor suppressor that is involved in preventing cancer .As such, P53 has been described as **"the guardian of the genome"** because of its role in conserving stability by preventing genome mutation (Read and Strachan, 1999) The name P53 is in reference to its apparent molecular mass. It runs as a 53 – kilodalton**(KD_a)** protein on **SDS – PAGE** .but ,based on calculation from its amino acid residues ,P53 mass is actually only **43.7 KD_a** ,this difference is due to the high number of proline residues in the protein ,which slow its migration on **SDS – PAGE** , thus making it appear heavier than it actually is (Ziemer et al., 1982).

Nomenclature

P53is also known as: cellular tumor antigen P53(Uniprot name), antigen NY – Co –13, Phospho protein P53, trans formation – related protein 53(TR p53), Tumor suppressor P53.

Gene

The P53 gene is located on chromosome 17 P13.1 ,and it is the most common target for genetic alteration in human tumors (Robbins & cotran, 2007) a little over 50% of human tumors contain mutation in this gene. While inactivating mutations of the P53 gene is found in 10% of thyroid carcinomas and mainly in poorly differentiated and aggressive histotypes (Olivier et al., 2002). Homozygous loss of P53 gene activity can occur in virtually every type of cancer, including carcinomas of the lung, colon, and breast. In

most cases, the inactivating mutations affect both P53 alleles **(**Robbins and cotran, 2007).

Structure

the P53 protein is a **DNA** – binding protein localized to the nucleus, it is 393 aminoacids long and has seven domains, an acidic **N**- terminus transcription – activation domain**(TAD)**, also Known as activation domain 1**(AD1)**, which activates transcription factors(venot etal ,1998), activation domain 2**(AD2)** important for apoptotic activity, proline rich domain important for the apoptotic activity, central **DNA** binding core domain**(DBD)**, nuclear localization signaling domain, homo – oligomerisation domain **(OD)** – is essential for the activity of P53 in vivo, **C** – terminal involved in down regulation of **DNA** binding of the central domain.

Mutations that de activate P53 in cancer usually occur in the **DBD**, most of these mutations destroy the ability of the protein to bind to its target **DNA** sequences, and thus prevents transcriptional activation of these genes. As such ,mutations in the **DBD** are recessive loss – of function mutations .molecules of P53 with mutations in the **OD** dimerise with wild – type P53 and prevent them from activating transcription. There fore OD mutation have a dominant negative effect on the function of P53. Approximately 80% of the P53 point mutation present in human cancers are located in the **DNA** – binding domain of the protein.

Function

The fact that P53 mutation are common in a variety of human tumors suggests that the P53 protein functions as a critical gate keeper against the formation of cancer Indeed, it is evident that P53acts as a **"molecular policeman"** that prevents the propagation of genetically damaged cells.The major functional activities of the p53 protein are cell-cycle arrest and initiation of apoptosis in response to DNA damage. P53 is called in to apply emergency brakes when DNA is damaged by irradiation, UV light, or mutagenic chemicals and chemicals and also in response to changes in cellular redox potential ,hypoxia, senescence ,and other stress conditions that may not directly damage DNA (Liu and Gellmann, 2002). Following DNA damage, there is a rapid increase in P53 levels .At the same time, kinase such as DNA – dependent protein kinase and ATM

(ataxia – telangectasia mutated) are activated in response to DNA damage. These enzymes phosphorylated P53, and the protein then unfolds, is able to bind to DNA, and becomes an active transcription factor. P53 stimulates transcription of several genes that mediate cell-cycle arrest and apoptosis.P53 induced cell – cycle arrest occurs late in the G1 phase and is caused by the P53 – dependent transcription of the CDK inhibitor P21. Such a pause in cell cycling is welcome because it allows the cells enough time to repair the DNA damage inflicted by the mutagenic agent. P53 also helps in the repair process directly by inducing the transcription of $GADD_{45}$ (growth arrest and DNA damage) which encodes a protein involved in DNA repair. If the DNA damage is repaired successfully, quite ingeniously. P53 activates MDM_2, whose product binds to and degrades P53, thus relieving the cell cycle block. If during the pause in cell division the DNA damage can not be successfully repaired normal P53, perhaps as a last – ditch effort ,sends the cell to the graveyard by inducing the activation of apoptosis inducing genes, such as BAX. BAX binds to and antagonizes the apoptosis – inhibiting protein BCL–2, thus BAX promotes cell death.

In view of these activities ,P53 has been right fully called a "guardian of the genome" with homozygous loss of P53, DNA damage goes unrepaired, mutations become fixed in dividing cells, and the cell turns onto a one – way street leading to malignant transformation. The ability of P53 to control apoptosis in response to DNA damage has important practical therapeutic implications. Radiation and chemotherapy, the two common modalities of cancer treatment, mediate their effects by inducing DNA damage and subsequent apoptosis. tumors that retain normal P53 are more likely to respond to such therapy than tumors that carry mutant alleles of the gene. such is the case with testicular teratocarcinomas (Chresta et al., 1996) and childhood a cute lymphoblastic leukemia's. By contrast, tumors such as lung cancers and colorectal cancers, which frequently carry P53 mutations, are relatively resistant to chemotherapy and radiotherapy, also there are several reports indicate that wild-type P53 gene delivery into anaplastic thyroid cancer cells induces a partial differention,with the re-expression of thyroid specific-genes, and makes cells more vulnerable to the effect of chemotherapy (Fagin et al., 1996; Moretti et al., 1997; Blagosklonny et al., 1998). This effect may be increased by the concomitant use of histone deacetylase inhibitors, which stimulate P53 acetylation and functional activation (Imanishi et al., 2002). Various

therapeutic strategies aimed at increasing normal P53 activity in tumor cells defective in P53 function are being investigated. One type of strategy relies mostly on the modulation of MDM_2 activity ;second uses modified adenoviruses that lyse cancer cells that lack P53 function (Robins and cotran, 2007).

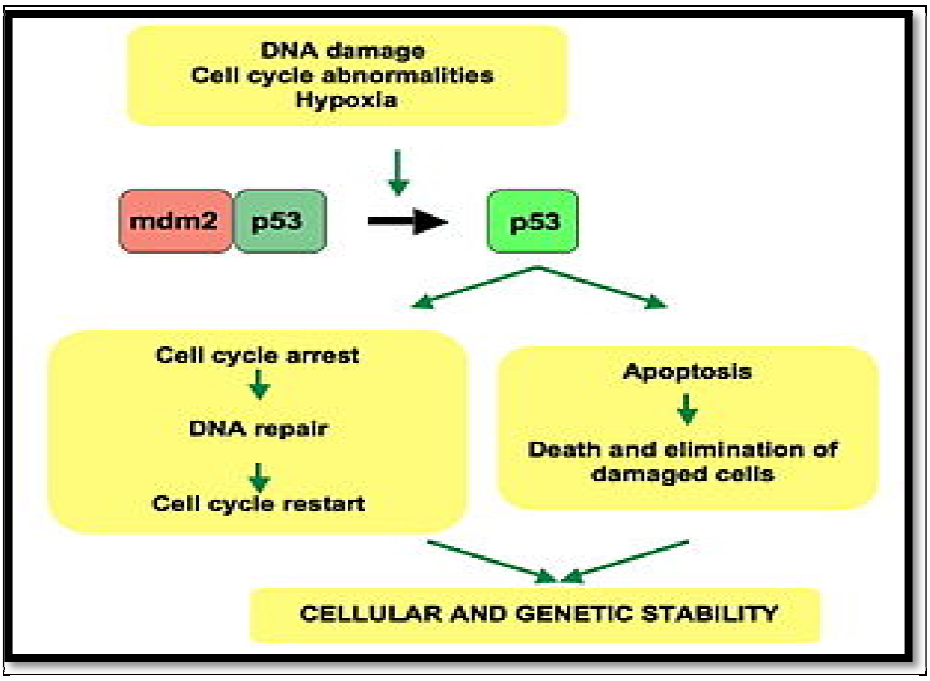


Figure2.14 p53 pathway: In a normal cell p53 is inactivated by its negative regulator, mdm2. Upon DNA damage or other stresses, various pathways will lead to the dissociation of the p53 and mdm2 complex. Once activated, p53 will induce a cell cycle arrest to allow either repair and survival of the cell or apoptosis to discard the damaged cell.

cell cycle

The cell cycle, is the series of events that take place in a cell leading to its division and duplication (replication). In cells without a nucleus (prokaryotic), the cell cycle occurs via a process termed binary fission. In cells with a nucleus (eukaryotes), the cell cycle can be divided in two periods: interphase—during which the cell grows, accumulating nutrients needed for mitosis and duplicating its DNA—and the mitotic (M) phase, during which the cell splits itself into two distinct cells, often called "daughter cells" and the final phase, cytokinesis, where the new cell is completely divided. The cell-division cycle is a vital process by which a single-celled fertilized egg develops into a mature organism, as well as the process by which hair, skin, blood cells, and some internal organs are renewed. The cell cycle consists of four distinct phases: G_1 phase, S phase (synthesis), G_2 phase (collectively known as interphase) and M phase (mitosis). M phase is itself composed of two tightly coupled processes: mitosis, in which the cell's chromosomes are divided between the two sister cells, and cytokinesis, in which the cell's cytoplasm divides in half forming distinct cells. Activation of each phase is dependent on the proper progression and completion of the previous one. Cells that have temporarily or reversibly stopped dividing are said to have entered a state of quiescence called G_0 phase (Cooper, 2000).

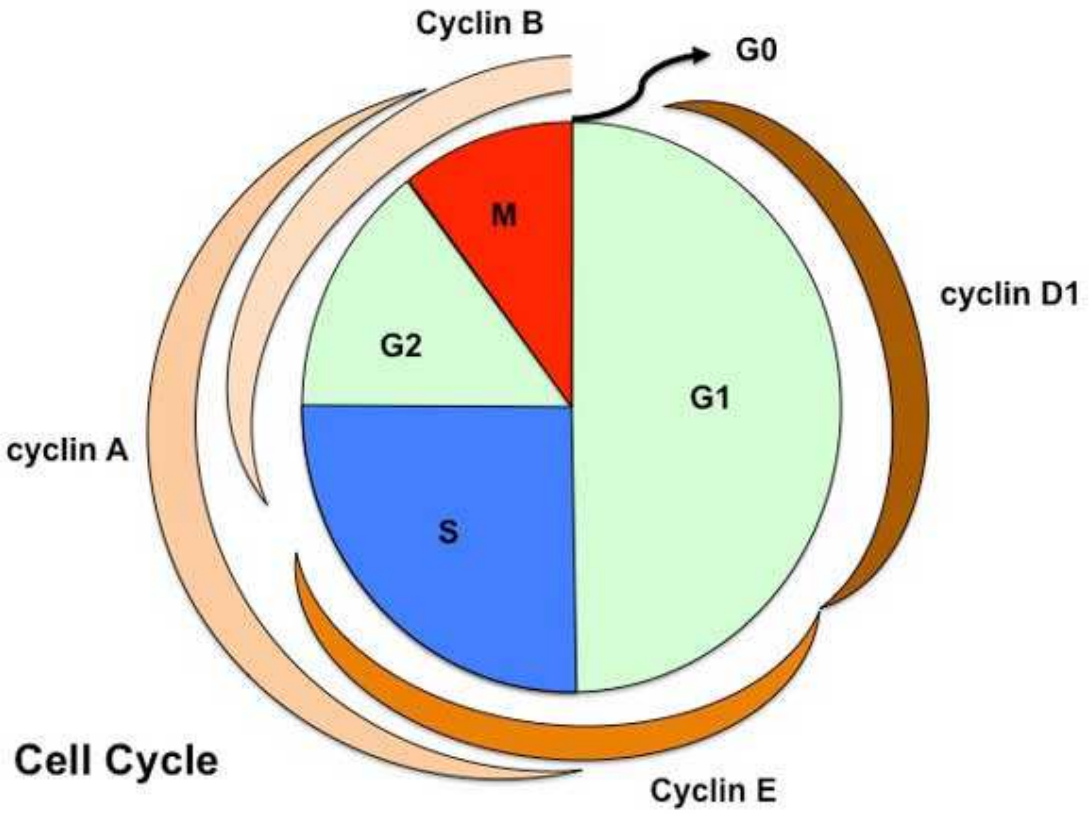


Figure.2.15 Cartoon of Cell Cycle Showing phases and cyclin levels(Cell cycle1.jpg)

G_0 phase

The term "post-mitotic" is sometimes used to refer to both quiescent and senescent cells. Nonproliferative cells generally enter the quiescent G_0 state from G_1 and may remain quiescent for long periods of time, possibly indefinitely (as is often the case for neurons). This is very common for cells that are fully differentiated. Cellular senescence occurs in response to DNA damage or degradation that would make a cell's progeny nonviable; it is often a biochemical reaction; division of such a cell could, for example, become cancerous.

Interphase

Before a cell can enter cell division, it needs to take in nutrients. All of the preparations are done during the interphase. Interphase proceeds in three stages, G_1, S, and G_2. Cell division operates in a cycle. Therefore, interphase is preceded by the previous cycle of mitosis and cytokinesis. In this stage nucleus and cytosol division does not occur. The cell prepares for division.

G_1 phase

The first phase within interphase, from the end of the previous M phase until the beginning of DNA synthesis is called G_1 (G indicating *gap*). It is also called the growth phase. During this phase the biosynthetic activities of the cell, which had been considerably slowed down during M phase, resume at a high rate. This phase is marked by the use of 20 amino acids to form millions of proteins and later on enzymes that are required in S phase, mainly those needed for DNA replication. Duration of G_1 is highly variable, even among different cells of the same species. It is under the control of the p53 gene.

S phase

The ensuring S phase starts when DNA replication commences; when it is complete, all of the chromosomes have been replicated, i.e each chromosome has two (sister) chromatids. Thus, during this phase, the amount of DNA in the cell has effectively doubled, though the ploidy of the cell remains the same (King and Roger, 2006).

G_2 phase

During the gap between DNA synthesis and mitosis, the cell will continue to grow. The G2 checkpoint control mechanism ensures that everything is ready to enter the M (mitosis) phase and divides.

Mitosis (M phase, mitotic phase)

Mitosis is the process by which a eukaryotic cell separates the chromosomes in its cell nucleus into two identical sets in two nuclei (Rubenstein et al., 2008). It is generally

followed immediately by cytokinesis, which divides the nuclei, cytoplasm, organelles and cell membrane into two cells containing roughly equal shares of these cellular components. Mitosis and cytokinesis together define the mitotic (M) phase of the cell cycle . The process of mitosis is complex and highly regulated. Errors in mitosis can either kill a cell through apoptosis or cause mutations that may lead to cancer.

Regulation of the cell cycle involves processes crucial to the survival of a cell, including the detection and repair of genetic damage as well as the prevention of uncontrolled cell division. Two key classes of regulatory molecules, cyclins and cyclin-dependent kinases (CDKs), cyclins bind and activate members of the cyclin-dependent kinase (Cdk) family to effect cell cycle progression. Cell cycle progression is controlled by the relative levels of individual cyclin family members. Progression through the G1-S-G2-M cycle follows successive oscillations in the levels of cyclins, D, E, A and B. Cyclins are grouped into classes that relate to the phase of the cell cycle they regulate. Cyclin D family members are G1 phase cyclins that regulate the entry of cells into G1 from Go. Cyclin D is upregulated by growth factor and external signals through the Ras GTPase signaling pathway. Cyclin D couples with Cdk4 and Cdk6. The cyclin-D-dependent kinases enforce commitment to enter S-phase. Cyclin D-Cdk4 hypophosphorylates retinoblastoma protein (pRB) and facilitates the expression of cyclin E. Cyclin E and Cyclin A are able to bind Cdk2 and promote the cell cycle progression through G1/S transition. Cyclin E-Cdk2 and Cyclin A-Cdk2 hyperphosphorylate and inactivate pRb. The inactivation of pRb leads to activation of E2F transcription factors. Cyclin E stimulates replication complex assembly through interaction with Cdc6. Cyclin A activates DNA synthesis by the replication complex already assembled and inhibits assembly of new replication complex. Cyclin E reinitiates the replication complex that is blocked by cyclin A. Cyclins B1 and B2 are M-phase cyclins. Cyclin B1 and cyclin B2 and their catalytic partner, Cdk1 (cdc2, p34 kinase), are components of the M phase/maturation promoting (MPF) factor that regulates processes that lead to assembly of the mitotic spindle and sister-chromatid pair alignment on the spindle (Leone et al., 1998; Mateyak et al., 1999; Coverley et al., 2002; Keenan et al., 2004).

Two families of genes, the cip/kip family (CDK interacting protein/Kinase inhibitory protein) and the INK4a/ARF (Inhibitor of Kinase 4/Alternative Reading Frame) prevent

the progression of the cell cycle. Because these genes are instrumental in prevention of tumor formation, they are known as tumor suppressors.

The cip/kip family includes the genes p21, p27 and p57. They halt cell cycle in G_1 phase, by binding to, and inactivating, cyclin-CDK complexes. p21 is activated by p53 (which, in turn, is triggered by DNA damage e.g. due to radiation). p27 is activated by Transforming Growth Factor of β (TGF β), a growth inhibitor.

The INK4a/ARF family includes p16INK4a, which binds to CDK4 and arrests the cell cycle in G_1 phase, and p19ARF which prevents p53 degradation. Synthetic inhibitors of Cdc25 could also be useful for the arrest of cell cycle and therefore be useful as antineoplastic and anticancer agents.

Cell cycle checkpoints are used by the cell to monitor and regulate the progress of the cell cycle (Stephen, 1996) Checkpoints prevent cell cycle progression at specific points, allowing verification of necessary phase processes and repair of DNA damage. The cell cannot proceed to the next phase until checkpoint requirements have been met. Several checkpoints are designed to ensure that damaged or incomplete DNA is not passed on to daughter cells. Two main checkpoints exist: the G_1/S checkpoint and the G_2/M checkpoint. G_1/S transition is a rate-limiting step in the cell cycle and is also known as restriction point.An alternative model of the cell cycle response to DNA damage has also been proposed, known as the postreplication checkpoint. p53 plays an important role in triggering the control mechanisms at both G_1/S and G_2/M checkpoints (Robbins and Cotran, 2007).

Role of p53 in Cancer

P53 in prostate cancer

Mutant p53 expression is a late event in localized prostate cancer (Hall et al., 1995; Mottaz et al., 1997), usually present in higher-grade cancer(Fan et al., 1994) and elevated in untreated metastatic cancer (Heidenberg et al., 1995; Moul et al., 1996), hormone refractory cancer (Hall et al., 1995; Heidenberg et al., 1995), and recurrent cancer (Moul et al., 1996). Inactivation of p53 is associated with late progression of prostate cancer and may be a marker of survival in stage T2-3N1-3M0 (Qian

et al., 2002). Protein expression of p53, Ki-67, and bcl2 were evaluated in archival paraffin-embedded radical prostatectomy specimens from 162 patients of clinically localized cancer by Moul et al. to determine the clinical use of p53, Ki-67, and bcl2 immunohistochemical protein expression in the primary tumor as combined predictors of disease progression. The study concluded that p53, Ki-67, and bcl2 have potential as biomarkers to predict recurrence in patients with clinically localized prostate cancer after radical prostatectomy. All three markers were clearly correlated with recurrence estimates at 6 years (Moul et al., 1996). The same conclusion was obtained by (Bauer et al., 1996).

P53 in lung cancer

The prognostic value of p53 status in non-small cell lung cancer has been investigated in 148 patients with clinical stage I-IIIB disease. Patients with mutations in p53 had a significantly higher risk for lung cancer-related death and for death from all causes than those with wild-type p53.

These results indicate that mutations in defined structural and functional domains of p53 may be useful molecular biological markers for prognosis and treatment strategy in non-small cell lung cancer patients (Skaug et al., 2000).

P53 in colon cancer

There is no doubt about the role of p53 mutations in the progression of colorectal tumours. Claudia Valentina et al. investigate the expression of PCNA, Ki-67 and p53 antibodies in colorectal carcinomas(CRC) and to establish the relationship between these markers and some particular histological findings of colorectal carcinomas, they concluded that the p53 overexpression was associated with the histological grade of the colorectal adenocarcinomas and with the nonmucinoscarcinomas and tended to be more frequent in the colorectal carcinomas with a high proliferative activity (Claudia Valentina et al., 2007). P53 are more frequent in advanced CRC and are associated with worse prognosis in this stage of disease (Iacopetta et al., 2006), it is believed that P53 mutations play a role in the adenoma-carcinoma transition of tumors during pathological process (López et al., 2012).

Several studies reported an elevated expression of p53 in tumors. In breast tumors, 24 of 30 cases showed an increased expression of p53, but low or undetectable levels in normal breast tissue (Bourdon et al., 2005). An increase of p53α mRNA was also found in renal cell carcinoma (Song et al., 2009).

P53 in Thyroid cancer

Among thyroid tumors, p53 mutations are generally restricted to PDTC and ATC (Donghi et al., 1993; Segev et al., 2003). Point mutations of p53 occur in approximately 60% of ATC and in 25% of PDTC (Dobashi et al., 1994; Segev et al., 2003). Moreover, in tumors with both well-differentiated and anaplastic components, p53 mutations were present only in the anaplastic component (Ito et al., 1993; Matias-Guiu et al., 1996; Takeuchi et al., 1999). These findings are consistent with the hypothesis that p53 inactivation likely serves as a second hit, triggering tumor dedifferentiation and progression to PDTC and ATC.

p53 expression has been found to be significant independent prognostic factors for thyroid cancer (Hay et al., 2002; Mallick, 2010). Also recent study indicate that p53 expression is predictor of prognosis in PTC, and their use as diagnostic tools in the clinical setting is therefore warranted (Lee YM and Lee JB, 2013).

Naomi Morita et al. examined the immunohistochemical expression of P53 protein in PTC to investigate the relations between its expression and the clinicopathological features. Overexpression of P53 protein in the primary tumor was observed in 43% of cases. Statistical analysis revealed significant correlation between P53 protein expression in the primary tumor and large tumor size, the presence of lymph node metastasis, and the mean number of lymph node metastases. The results of this study suggest that Immunohistochemistry for P53 in the primary tumor could be useful in the clinical evaluation of patients with PTC. Moreover, P53 protein overexpression in lymph node metastasis may be useful as a treatment guide or target for lymph node recurrences (Naomi Morita et al., 2008).

The relationship between exposure to radiation and p53 mutation is less clear. Studies performed in the Belarus population after the Chernobyl accident did not prove a definitive correlation between radiation exposure and p53 mutations in thyroid cancer

(Nikiforov et al., 1996; Smida et al., 1997; Suchy et al., 1998; Pisarchik et al., 2000). Some evidences suggest that p53 mutations in thyroid cancer may be favored by the genomic instability occurring during the tumor progression process (Shahedian et al., 2001). Other studies on p53 protein expression in a large series of thyroid tumor specimens suggest that, although not mutated, p53 activity may be inhibited in thyroid cancer by other mechanisms. Indeed, increased p53 protein levels were observed by Immunohistochemistry not only in anaplastic and poorly differentiated thyroid cancer, where p53 mutations are frequent, but also in well-differentiated cancers, in the absence of any p53 mutation (Soares et al., 1994; Pollina et al., 1996; Park et al., 1998). Nonfunctioning p53 can not induce Mdm2, its major degrading protein, and consequently accumulates in the cell nucleus. A strong p53 staining in paraffin embedded specimens, therefore, is considered indirect evidence of nonfunctioning p53. Nuclear accumulation of wild-type p53 protein and reduced p53 tumor suppressor function in some differentiated thyroid cancers is also suggested by the observation of a correlation between elevated p53 protein content and poor clinical outcome (Dobashi et al., 1993; Gerasimov et al., 1995; Nishida et al., 1996; Ruter et al., 1996; Hosal et al., 1997; Godballe et al., 1998; Chen et al., 1999). Wild-type p53 inactivation is also suggested by invitro studies in thyroid cancer cells: H-RAS-transformed rat thyrocytes display wild-type p53 protein accumulation (Burns et al., 1992) and isolated rat thyroid cancer cells in culture display both wild-type p53 over expression and a defect in G1 arrest in response to DNA damage (Wyllie et al., 1995). Several independent mechanisms may be hypothesized to explain the wild-type p53 inactivation in thyroid carcinomas, including p53 cytoplasmic retention (Zedenius et al., 1996) and Mdm2 over expression (Jennings et al., 1995; Zou et al., 1995). Indeed, in a large series of differentiated thyroid cancer specimens, IHC indicated that Mdm2 is over expressed and its expression level directly correlates with a poor clinical outcome (Jennings et al., 1995; Zou et al., 1995; Czyz et al., 2001; Horie et al., 2001).

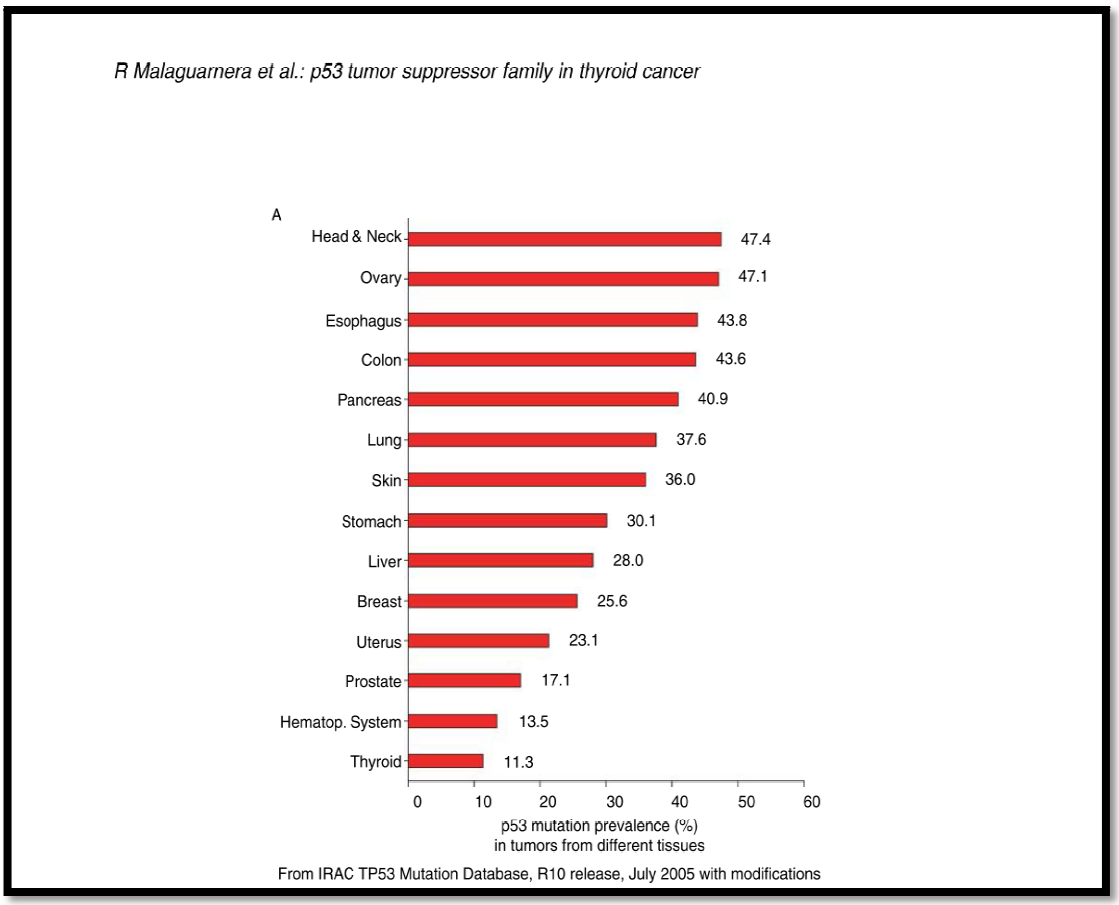


Figure 2.16. P53 mutation prevalence(%) in tumors from different tissues (Malaguarnera R et al., 2007)

Other members in p53 family: p63 and p73 Two novel genes, named p63 and p73, have been discovered as members of the p53 tumor suppressor family because of their remarkable similarity with p53 structure and functional domains(Lohrum & Vousden, 2000). Structural homology between these three proteins suggests that they also share similar tumor suppressor functions. Like p53, p63 and p73 also contain three major domains: the TAD, the DBD, and the (OD) (Moll et al., 2001). TAp63 and TAp73, containing the TA domain, TAp63 and TAp73 are very rarely mutated in cancer cells and often over expressed. These observations are in concert with a possible role of these proteins in tumor genesis (Ikawa et al., 1999;Yang et al., 2000; Moll, 2003; Flores et al., 2005). A possible role of p63 in the malignant transformation of thyroid follicular cells is supported by two lines of evidence: (1) p63 is involved in epithelial cell differentiation (Yang et al., 1998;

Reis-Filho et al., 2003; Reis-Filho & Schmitt, 2002) and (2) a number of human epithelial malignancies express a high levels of p63 isoforms (Hibi et al., 2000; Marin & Kaelin , 2000; Moll et al., 2001; Massion et al., 2003; Moll & Slade, 2004). About P73, p73 expression is a marker of thyroid cell malignant transformation, and the interactions of the various p73 isoforms within the molecular network of p53 family members are complex and await additional investigation (Malaguarnera et al., 2007).

All p53 family members are inhibited by HMGA1 up-regulation in thyroid cancer cells The high mobility group A factors (HMGA1a, HMGA1b, and HMGA2) are non-histone proteins, with several different functions, including gene transcription, malignant transformation, promotion, and metastatic progression (Reeves, 2001; Sgarra et al., 2004).

In thyroid cancer cells, all the three p53 family members are present with an expression pattern that is complex and different in different tumors (Ruter et al., 1996; Frasca et al., 2003; Vella et al., 2003; Malaguar-nera et al., 2005). In general, the tumor suppressor activity of these proteins is kept latent by several mechanisms, including interaction with p53 mutants, dominant negative isoforms over expression, and impaired activation mechanisms. Since HMGA over-expression is very common in thyroid cancer, the possibility of an interference of HMGA proteins on p53 family oncosuppressor function was investigated. In several thyroid cancer cells of different histotypes, HMGA1 gene silencing indicated that HMGA1 protein has an inhibitory effect on both ectopic and endogenous p53 family member activity. HMGA1 overexpression is another mechanism by which p53 family member function is kept latent in thyroid cancer cells and that HMGA1 directly interacts with the oligomerisation domain of p53 family transcription factors, there by preventing proper oligomerisation, DNA binding, and, as a consequence, transcriptional and tumor suppressor activity (Frasca et al., 2006). Since HMGA1 over - expression is very common in thyroid cancer and occurs also in well-differentiated histotypes, it is reasonable to suppose that the p53-blunted function due to over expressed HMGA1 is also a very common mechanism and may explain the positive. correlation between HMGA1 expression and poor prognosis in some thyroid carcinomas. These observations also suggest that p53 network inactivation may be an important

prerequisite for oncogene-driven thyroid cancer progression (Malaguarnera et al., 2007).

3-P21

p21 / WAF1 also known as cyclin-dependent kinase inhibitor 1 or CDK-interacting protein 1 is a protein that in humans is encoded by the CDKN1A gene located on chromosome 6 (6p21.2). p21 is a potent cyclin-dependent kinase inhibitor (CKI)(el-Deiry et al., 1993). The p21 (CIP1/WAF1) protein binds to and inhibits the activity of cyclin-CDK2 or -CDK1 complexes, and thus functions as a regulator of cell cycle progression at G_1. The expression of this gene is tightly controlled by the tumor suppressor protein p53, through which this protein mediates the p53-dependent cell cycle G_1 phase arrest in response to a variety of stress stimuli (Dolezalova et al., 2012). The expression of p21 is controlled by the tumor suppressor protein p53. Sometimes, it is expressed without being induced by p53. This kind of induction plays a big role in p53 independent differentiation which is promoted by p21. Expression of p21 is mainly dependent on two factors 1) stimulus provided 2) type of the cell. Growth arrest by p21 can promote cellular differentiation. p21 therefore prevents cell proliferation. The p21 protein also is important in the stress response. p21 is a transcriptional target of the tumor suppressor gene, p53; despite this, loss-of-function mutations in p21 (unlike p53) do not accumulate in cancer nor do they predispose to cancer incidence (Rodriguez&Meuth, 2006). On other hand, p21 may facilitate tumor invasion through p21-activated kinase-1(PAK1). p21-activated kinases (PAKs) are a family of serine/threonine kinases that regulate cytoskeletal dynamics and cell motility. PAKs are subdivided into group I (PAKs 1-3) and group II (PAKs 4-6) on the basis of structural and functional characteristics. Based on prior gene expression data that predicted enhanced PAK signaling in the invasive fronts of aggressive papillary thyroid cancers (PTCs). McCarty SK et al. examined PAK isoform expression in human thyroid cancer ,result was PAK isoforms are expressed in human thyroid tissues and PAK1 regulates thyroid cancer cell motility, and PAK1 and pPAK levels are increased in PTC invasive fronts. These data implicate PAKs as regulators of thyroid cancer invasion (McCarty et al., 2010).

4.p27

The p27 inhibitor is involved in the regulation of the cell cycle at the G 1 -S transition, ultimately through the inhibition of pRb phosphorylation (Cordon-Cardo, 1995). Mutations in the human p27 gene appear to be rare (Ferrando et al., 1996). Loss of p27 expression is associated with colon, breast, prostate, and gastric cancer progression (Loda et al., 1997; Porter et al., 1997; Cote et al., 1998; So et al., 2000). Erickson et al. studied expression of p27 to distinguishes papillary hyperplasia in Graves' disease from PTC and result of study was p27 protein expression is significantly higher in papillary hyperplasia of Graves' disease compared to papillary carcinoma, which may be diagnostically useful in difficult cases (Erickson et al., 2000).

2.10.2.2.Marker of cell proliferation

1-ki-67

Antigen Ki- 67 also known as Ki -67 or MKi67 is a protein that in human is encoded by the MKi 67 gene (antigen identified by monoclonal antibody Ki- 67) (Schonk et al., 1989).

The Ki-67 gene is on the long arm of human chromosome 10(10 q 25) (Fonatsch et al., 1991). Ki-67 was identified by Gerdes et al. as a nuclear non histone protein ,shortly after the corresponding antibody was described by the same group (Gerdes et al., 1983) in city of Kiel (hence "ki") after immunization of mice with the Hodgkin's lymphoma cell line L428 (67 refers to the clone number on the 96 – well plate in which it was found). The absence of ki-67 in quiescent cells and its universal expression in proliferating tissues created great interest on its potential role as a marker of cell proliferation (Van Dierendonck et al., 1989). ki-67 protein is present during all active phases of the cell cycle (G1, S, G2, and mitosis) but is absent from resting cells(G0). The cellular appearance and location of the ki-67protein throughout the cell cycle is not homogenous. During early G1, it is found as generally weakly staining discrete foci throughout karyoplasms (Kill, 1996). it progressively condense during late G1 in larger perinucleolar granules. During S and G2 phases, it is mainly found associated with the nucleolar region in larger foci as well as with some heterochromatin regions. when the nuclear membrane disrupts during early mitosis, ki-67 shows an intense expression

associated with the surface of condensed chromosomes in the cytoplasm .This intensity rapidly disappears in anaphase – telophase (Braun et al., 1988; Starborg et al., 1996). The original ki-67 monoclonal antibody, when used for immunostaing was initially reported to stain proliferating cells in unfixed tissues but not formalin – fixed paraffin embedded samples in 1992 ,cattoretti et al. reported better success in staining ki-67 in paraffin – embedded samples after development of the new antibodies MIB -1and MIB-3. These antibodies seem to produce results equivalent to the original ki-67 antibody targeting the same epitope (contained in the ki-67 motif), as demonstrated by identical bands in western immunoblots (key et al., 1993). Staining by MIB – 1 and–3 of formalin–fixed paraffin–embedded samples is greatly enhanced by antigen retrieval (most frequently by microwave heating). A though several antibodies are now commercially available to stain ki-67 in fresh and paraffin–embedded tissue, ki-67 expression is usually estimated as the percentage of tumor cells positively stained by the antibody, with nuclear staining being the most common criterion of positivity, brown granular nuclear reactivity was positive (Shi et al., 1991). The function of ki-67 antigen is associated with and may be necessary for cellular proliferation further more it is associated with ribosomal RNA transcription (Bull winker et al., 2006). Inactivation of antigen Ki-67 lead to inhibition of ribosomal RNA synthesis (Rahman zadeh, 2007).

Role of Ki-67 in cancer

The prognostic value of Ki-67 in different malignancies has been reviewed (Brown and Gatter, 2002). Over expression of Ki-67 is frequently seen in a variety of malignant tissues and is associated with worse survival of individuals with bladder (kilicli–camur et al., 2002) brain (Tohannessen et al., 2006) breast (Stuart – Harris et al., 2008 ; Viale et al., 2008) kidney (knkuri et al., 2006) lung (Shib et al. , 2000) ovary (Munstedt et al., 2004) prostate (Pollack et al., 2004), or thyroid (Tisell et al., 2004) cancer. In addition, ki-67 reactivity is included among other parameters in the World Health Organization's recommended grading system for neuroendcrine tumors of the pancreas and gastrointestinal tract (Kloppel et al., 2004) Ki-67 over expression is associated with worse survival of individuals with neuroendcrine tumor (Kloppel et al., 2004 ; Jamali et al., 2008).

Ki-67 in breast cancer

The proliferation biomarker Ki-67 is considered to be a prognostic factor for breast cancer has been investigated in several studies (Urruticoechea et al., 2005; Yerushalmi et al., 2010). The association between a high Ki-67 labelling index, poor differentiation of tumors and large tumor size in breast carcinoma were demonstrated in many studies (Railo et al., 1993; Yerushalmi et al., 2010). Various studies have shown correlations between Ki-67 and overall survival and disease-free survival, with an increased risk of recurrence in patients with a high Ki-67 (Goodson et al., 2000; Brown et al., 2002). On other hand low expression of Ki-67 was more common with favorable prognostic variables. This finding confirmed by some studies (Wenger et al., 1993; Brown et al., 1996; Urruticoechea et al., 2005; Yerushalmi et al., 2010; Nishimura et al., 2010; Boder et al., 2011).

Ki-67 in gastrointestinal tumors

Belev et al. investigated the prognostic value of Ki-67, in gastrointestinal stromal tumors (GISTs). Result was Ki-67 presents a significant prognostic factor for GIST recurrence which could be of great importance in evaluating malignant potential of disease (Belev et al., 2013).

In colorectal cancer, data have, to some extent, been contradictory. Several studies have reported no prognostic value of Ki-67 expression. One study reported an association between a low tumor cell proliferation rate at the invasive margin and poor prognosis in colorectal cancer TNM stage II (Palmqvist et al., 1999), whereas others reported an adverse prognostic value of a high Ki-67 after curative resection for colorectal cancer (Kimura et al., 2000). Another study showed that the prognostic markers in colon cancer stage II and III, treated with surgery with or without adjuvant 5-FU and leucovorin (calcium folinate) therapy, showed an improved outcome in patients with a high percentage of Ki-67-positive tumor cells (Allegra et al., 2003). As a possible explanation, more rapidly proliferating tumor cells may be more vulnerable to chemotherapy-induced tumor cell death in colon cancer stage III, a high Ki-67 immunostaining in >40% of the tumor cells are both associated with an improved RFS in colon cancer stages II and III, but not so in

rectal cancer. In addition, in colon cancer stage III, a high Ki-67 immunostaining in >40% of the tumor cells seems to be a predictive marker to the effect of adjuvant chemotherapy (fluge et al., 2009).

Ki-67 in prostate cancer

The Ki-67 labeling index of prostatic carcinoma has been said to predict tumor-specific mortality both in cases of limited disease and in cases associated with lymph node metastases (Masuda et al., 1998). The combined determination of Gleason score and proliferation index constitutes a particularly powerful prognostic tool (Chiusa et al., 1997). In a 6-year study involving 808 patients diagnosed with prostate cancer, an immunohistochemical assessment of Ki-67 expression was evaluated for its relationship to the specificity of the cancer and overall survival. Compared to information from the Gleason score and PSA, Ki-67 provided additional prognostic information (Khatami et al., 2009; Berney et al., 2009). In another study of a group of men treated with radiotherapy and androgen deprivation for prostate cancer, Ki-67 expression levels in conjunction with MDM2 were found to be correlated to distant metastasis and survivability (Khor et al., 2009). Nevertheless, further studies will be needed to validate these results and explore the possibility of combining Ki-67 with existing prognostic tools as a powerful biomarker for localized prostate cancer (Jhavar et al., 2009).

Role of Ki-67 in Thyroid cancer

It is know that the Ki-67 LI in PTC_s is lower than that in breast, lung, stomach and colon adenocarcinomas (Katoch et al., 1995) . Most authors have demonstrated low Ki-67 expression in differentiated thyroid carcinomas, but higher expression in those undifferentiated (Wallin et al., 1992; Katoh et al., 1995; Basolo et al., 1997; Okayasu et al., 1998; Tallini et al., 1999; Yoshida et al., 1999; Saiz et al., 2002).

As expected ,the mean ki-67 LI was low, but was significantly higher in the BRAF – positive patients than in the BRAF – negative patients these finding suggest that the BRAF mutation may activate the MAP kinase pathway, resulting in activated tumor cell proliferation. Activation of the MAP kinase pathway by the BRAF (V600E) mutation

may play an important role in thyroid carcinogenesis and tumor progression.Therapeutic agents targeting this pathway are considered to be a promising novel treatment modality for thyroid carcinomas, and a specific inhibitor targeting the Rafkinase has been reported (Bollag et al., 2003; Karasaides et al., 2004) it is hoped that this inhibitor will be therapeutically for those who have incurable disease.

ItoY et al. investigated Ki-67 LI in PTC and compared result with various clinicopathological features, including patient prognosis. Ki-67 LI was associated with patient age, massive extrathyroid extension, and distant metastasis at surgery, and the disease-free survival (DFS) of patients with Ki-67 LI >1% was significantly worse than that of those with Ki-67 LI <1% ($p < 0.0001$). On multivariate analysis, Ki-67 LI was recognized as an independent prognostic factor for the DFS of patients (Ito et al., 2010).

Pujani et al. demonstrated the increased Ki-67 LI progressively from multinodular goiter to follicular adenoma, papillary carcinoma, follicular carcinoma, and medullary carcinoma, and were the highest in undifferentiated carcinoma (Pujani et al., 2010). These findings are in close agreement with those of Erickson et al. They observed the highest values for Ki-67 LI in anaplastic carcinoma followed by follicular and papillary carcinoma (Erickson et al., 1998). Ziad et al. studied immunoexpression of thyroid transcription factor-1 (TTF-1) and Ki-67 in a coexistent ATC and FTC and found a significantly higher Ki-67 LI in anaplastic areas in comparison with the follicular areas. They suggested that in thyroid cancers, TTF-1 and Ki-67 could provide useful information on the differentiation activities of thyroid tumor cells and may be helpful to distinguish well-differentiated and undifferentiated areas in a mixed thyroid cancer (Ziad et al., 2008).

2-Proliferating Cell Nuclear Antigen(PCNA)

commonly known as **PCNA**, is a protein that acts as a processivity factor for DNA polymerase δ in eukaryotic cells. It achieves this processivity by encircling the DNA, thus creating a topological link to the genome. It is an example of a DNA clamp. The protein encoded by this gene is found in the nucleus and is a cofactor of DNA polymerase delta. The encoded protein acts as a homotrimer and helps increase the

processivity of leading strand synthesis during DNA replication. In response to DNA damage, this protein is ubiquitinated and is involved in the RAD6-dependent DNA repair pathway. Two transcript variants encoding the same protein have been found for this gene. Pseudo genes of this gene have been described on chromosome 4 and on the X chromosome. PCNA was originally identified as an antigen that is expressed in the nuclei of cells during the DNA synthesis phase of the cell cycle (Leonardi et al., 1992).

2.10.2.3.Growth factor

Vascular endothelial growth factor (VEGF)

Angiogenesis is a process of new blood vessel development from preexisting vasculature. The vascular endothelial growth factor (VEGF) is one of the most potent endothelial cell mitogens and plays a crucial role in both angiogenesis and lymphogenesis (Carmeliet & Jain, 2000). The microvascular density is increased in the thyroid malignancy compared with normal thyroid tissue and benign thyroid tumors (Segal et al., 1996; Akslen & Livolsi, 2000). Growing evidence from invitro and invivo experiments have shown that increased VEGF expression promotes thyroid cancer cell growth, subsequent lymph node metastasis, local invasion, and distant metastasis, where as the inhibition of VEGF signaling results in suppression of the tumor growth (Lin&Chao, 2005). also Lennard et al. found that VEGF expression correlates with tumor aggressiveness and metastatic potential. Diffuse, intense immunostaining for vascular endothelial growth factor in patients with papillary cancer has been associated with a high rate of local recurrence and distant metastases (Lennard et al., 2001).

2.10.2.4.Serum markers

1-serum thyroglobulin (Tg)

Thyroglobulin (Tg) is a protein produced by and used entirely within the thyroid gland. Tg bound to T3 and/or T4 is sometimes called colloid. Tg is used by the thyroid gland to produce the thyroid hormones T4 and T3 (Venturi et al., 2000) Thyroglobulin levels in the blood can be used as a tumor marker for certain kinds of thyroid cancer (particularly papillary or follicular thyroid cancer) (American Cancer Society, 2009). Tg is secreted by both normal and cancerous thyroid cells. In patients who have not had a

total thyroidectomy and ^{131} I ablation, the interpretation of serum Tg measurements is limited by the inability to differentiate between tumor and thyroid remnant (Spencer et al., 2005). Detectable serum Tg is highly suggestive of thyroid remnant, residual or recurrent tumor. The cut-off serum Tg concentration beyond which recurrent / persistent disease is implied depends on several variables including the assay employed by each laboratory. Individual laboratories should advise clinicians on the significance of detectable serum Tg at low concentrations .A serum Tg which is rising with time while on suppressive thyroxin therapy is highly suggestive of tumor recurrence or progression (Spencer et al., 2005) also van Herle et al. and Ruiz-Garcia et al. found that an elevated serum thyroglobulin level correlates strongly with recurrent tumor when found in patients with differentiated thyroid cancer during postoperative evaluations. Serum thyroglobulin levels are most sensitive when patients are hypothyroid and have elevated serum thyroid-stimulating hormone levels (Duren et al., 1999), samples should not be collected sooner than 6 weeks post-thyroidectomy, or ^{131} I ablation / therapy (Ozata et al., 1994; Hocevar et al., 1997; Spencer et al., 2005; UK guidelines, 2006), there is normally no need to measure serum Tg more frequently than 3 monthly during routine follow-up; for patients in remission an annual check of serum Tg should be measured while on suppressive levothyroxine treatment, since Tg release is TSH-dependent, serum TSH concentration should be determined concurrently to aid interpretation. The requesting clinician should indicate on the form whether the patient is on thyroid hormone therapy and the TSH result should be available to the laboratory performing the Tg assay, there is no need for TSH stimulation if the basal serum Tg is already detectable, Patients in whom the basal Tg remains persistently detectable (i.e while on suppressive levothyroxine therapy), or rises with subsequent assessments, require further evaluation (Spencer et al., 2005). Some people's immune systems make antibodies against thyroglobulin, which can affect test results. Because of this, levels of anti-thyroglobulin antibodies are often measured at the same time (American Cancer Society, 2012).

2-Thyroid-stimulating hormone receptor mRNAs'

Thyroid cancer cells in the circulation can be detected by measuring the mRNA of thyroid-specific genes. Among these, thyroid-stimulating hormone receptor mRNAs'

provide high diagnostic sensitivity and specificity for thyroid cancer detection. This marker can be used in synergy with current diagnostic modalities, i.e. fine-needle aspiration and ultrasound, for preoperative diagnosis and serum thyroglobulin measurement for monitoring. Gupta and Chia studies have demonstrated the high sensitivity and specificity of thyroid-stimulating hormone receptor mRNA in detecting recurrent/residual disease even in the presence of thyroglobulin antibodies. Fine-needle aspiration biopsy is currently the sole method for evaluating thyroid nodules. Indeterminate fine-needle aspiration cytology is found in approximately 15-30% of specimens. Thyroid-stimulating hormone receptor mRNA measurement in patients with indeterminate fine-needle aspiration may enhance cancer detection and save unnecessary surgeries (Gupta & Chia , 2007).

2.9.2.5.Hormone receptors

Because of thyroid cancer is three times more prevalent in females but the role of sex hormones in its pathogenesis is unknown Kansakar et al. determined the expression of estrogen receptor (ER), progesterone receptor (PR) in thyroid neoplasm's to see if expression correlated with age and sex, histological type, cancer stage, and clinical outcome. ER and PR expression is higher in tumor thyroid tissue. The over expression of sex hormone receptors in thyroid tumor suggests their role in thyroid cancer pathogenesis and needs further investigation (Kansakar et al., 2009).

2.10.2.6.Marker of cell adhesion

1.E – cadherin

E–cadherin is a glycoprotein which is the most important molecule in cell-cell adhesion in epithelial tissues; it is localized on the surfaces of epithelia cells in region of cell-cell contact known as adherence junction (Gumbiner, 1996). As a member of a large family of genes coding for calcium-dependent cell adhesion molecules (CAMS), it is essential for the formation and maintenance of epithelia.

Besides its role in normal cells, this highly conserved gene can play a major role in malignant cell transformation, and especially in tumor development and progression. Loss of E-cadherin function or expression has been implicated in cancer progression and metastasis. E–cadherin down regulation decreases the strength of

cellular adhesion within a tissue, resulting in an increase in cellular motility. This in turn may allow cancer cell to cross the basement membrane and invade surrounding tissues (Huntsman et al., 1999).

Several prognostic factors have been reported to be significant in thyroid carcinomas such as age at presentation and local tumors size, histology and distant metastasis. (Larsen et al., 2003)

The loss of E-cadherin correlates to tumor metastasis and explanation for aggressive nature of tumor and important indicator for clinical diagnosis and divergent results have been reported for thyroid carcinoma some studies confirmed that, E-cadherin staining is an independent prognostic indicator for differentiated thyroid carcinomas. It may help to uncover the small group of patients with differentiated thyroid carcinomas carrying a high risk of suffering an unfavorable clinical out come (Reinhard von et al., 1997). And other said E-cadherin expression seems to be associated with the dedifferentiation, progression, and metastatic spread of thyroid carcinomas and may be a useful marker for the prognosis of these tumors (scheumman GF et al., 1995).

2. Galectin-3 (Gal-3)

Galectin-3 (Gal-3), represents the most well-studied molecular candidate for thyroid cancer diagnosis. Gal-3 is a protein that binds to beta-galactosidase residues on cell surface glycoprotein and has also been identified in the cytoplasmic and nuclear compartment. This marker has been implicated in regulation of normal cellular proliferation and apoptosis, as well as malignant transformation and the metastasis of cancer cells, the majority of immunohistochemical studies found that Gal-3 was differentially expressed in thyroid carcinoma compared with benign and normal thyroid specimens, suggesting that Gal-3 is a good diagnostic marker for thyroid cancer (Chiu et al., 2010). In the thyroid, several reports have shown that Gal-3 is over expressed in malignant tumors (Fernandez et al., 1997; Kawachi et al., 2000; Herrmann et al., 2002; Kovacs et al., 2003; Papotti et al., 2005; Prasad et al., 2005; Cvejic et al., 2005). Gal-3 shows strong diffuse cytoplasmic staining in most cases of PTC, including the classical and follicular variant (Fernandez et al., 1997;Bartolazzi et al., 2001; Herrmann et al., 2002; Kovacs etal., 2003; Weber et al., 2004; Prasad et al., 2005; Cvejic et al., 2005). Hyperplastic nodules, nodular goiters, and normal follicular epithelium usually show

absence of Gal-3 (Fernandez et al., 1997; Bartolazzi et al., 2000; Herrmann et al., 2002). On the other hand, Gal-3 genomic expression studies have shown inconsistent results for diagnostic utility and are not recommended. Overall, the development of Gal-3 as a diagnostic marker for thyroid cancer represents a promising avenue for future study, and its clinical application could significantly reduce the number of diagnostic thyroid operations performed for cases of indeterminant fine needle aspiration biopsy cytology, and thus positively impact the current management of thyroid nodular disease (Chiu et al., 2010).

2.10.2.7.Molecular Markers

BRAF mutation (V600E) has proven to be relatively restricted to PTC and anaplastic thyroid carcinomas, and is very useful in the differential diagnosis difficult thyroid tumors. Unfortunately the follicular variant of papillary thyroid carcinomas has a BRAF mutation in only 5–20% of the time while conventional PTC shows a BRAF mutation more often (35–70%). BRAF mutations have been reported to be predictive of several factors including tumor behavior and response to radioactive iodine , but more studies are needed in this area.RET/PTC rearrangement is found mainly in PTC with a highly variable frequency (5–80%) in different geographic regions and in different studies . There are different fusions of the tyrosine kinase domain of RET. RET/PTC1 is the most common followed by RET/PTC3. In children with PTC, which developed after exposure to radiation, RET/PTC3 was the dominant rearrangement. Chromosomal rearrangements involving the TRK gene are found in about 10% of PTC. This results from the fusion of tyrosine kinase domain of TRK on chromosome 1q22 to the tropomyosin gene . Activating point mutations of RAS proto-oncogene occur in a small percentage of PTC. The N-RAS codon is the most common. Follicular variant of PTC has a higher frequency of RAS mutations than in other PTC. RAS mutations are also present in follicular carcinomas and adenomas (Nikiforova et al., 2008).

HMGA2 and insulin-like growth factor II mRNA binding protein 3 (IMP3) are highly expressed during fetal development and then tissue levels are very low to absent in adult tissues. Recent studies have shown that in many malignant neoplasms HMGA2 and IMP3 have increased levels of expression. These studies have shown that normal thyroid tissues and follicular adenomas express low levels of HMGA2 and IMP3

mRNA compared to papillary and follicular carcinomas. The levels of expression in the carcinomas range from 1.5 to greater that ten -fold higher than in adenomas or normal thyroid tissues, so measurements of the levels of expression by quantitative RT–PCR helps to separate benign from low grade malignant thyroid neoplasms in most cases (Lappinga et al., 2010; Jin et al., 2010). These observations have been examined in thyroid tissues and molecular assays are being developed to distinguish follicular adenomas from papillary and follicular carcinomas in cytologic specimens as well as in tissues using formalin fixed paraffin embedded tissues. These approaches should allow the pathologist to use molecular approaches in solving difficult diagnostic problems in thyroid pathology (Ricardo et al., 2011).

2.10.2.8.Cellular structure markers

1.Cytokeratin 19

CK19 (Keratin 19) is a member of the keratin family. The keratins are intermediate filament proteins responsible for the structural integrity of epithelial cells. Different subtypes of keratin filaments are grouped according to molecular weight. High-molecular-weight CKs (CK1, CK4, CK10, and CK13) are detected in stratified squamous epithelium. Simple or glandular epithelium expresses CK7, CK8, CK18, and CK19. The thyroid gland has been extensively studied with various antibodies to CKs in an attempt to identify differential expression patterns in normal parenchyma, benign nodules, and malignant tumors. Papillary carcinomas have been shown to express strong and diffuse immunoreactivity for CK7, CK18, and CK19 in 80% to 100% of cases(Cheung et al., 2001; Lam et al., 2001). Because CK19 detection in follicular adenomas and follicular carcinomas is often less intense and more focal than in PTC, this keratin has become one of the most commonly used to investigate thyroid lesions (Baloch et al., 1999; Cheung et al., 2001; Sahoo et al., 2001; Cerilli et al., 2002). Several authors emphasize the importance of the distribution and intensity of CK19 staining as the most critical aspects of accurate interpretation (Cheung et al., 2001; Sahoo et al., 2001; Asa &Cheung, 2001; Asa, 2004). Normal thyroid follicular epithelium is often negative, focal staining for CK19 does not rule out a diagnosis of PTC, particularly in nodules with nuclear features of PTC that are seen focally (Sahoo et al., 2001). CK19 has also been considered by many investigators to be a useful

ancillary tool for the diagnosis of papillary carcinoma in FNAC (Nasser et al., 2000; Khurana et al., 2003; Saggiorato et al., 2005). The reported sensitivity and specificity using CK19 as a single marker is as high as 92% and 97%, respectively (Nasser et al., 2000). A panel of markers including CK19 and galectin-3 was reported as reaching 100% of both specificity and sensitivity in the management of thyroid lesions with a cytologic diagnosis of follicular oncocytic tumors (Saggiorato et al., 2005).

2.HBME-1

HBME-1 is a monoclonal antibody that recognizes an unknown antigen in the microvilli of mesothelioma cells, normal tracheal epithelium, and adenocarcinoma of the lung, pancreas, and breast (mase et al., 2003; Nikiforova et al., 2003). HBME-1 has also been reported by several investigators to be a useful marker of malignancy in thyroid nodules (Mase et al., 2003; Erickson et al., 2004; Rezk et al., 2005; Saggiorato et al., 2005; Paptti et al., 2005). Overall in the thyroid, HBME-1 stains mostly follicular-derived malignant tumors, including well-differentiated and poorly differentiated carcinomas, with a variable sensitivity and specificity in different series. Most PTC show diffuse positive staining for HBME-1 (55%-100%; mean, 88%) (Cheung et al., 2001; mase et al., 2003; Saggiorato et al., 2005). One study has reported a sensitivity of 70% and 45% for the classical and follicular variant of PTC, respectively (Cheung et al., 2001). Many of the reports in which HBME-1 was also studied in normal and hyperplastic thyroid demonstrated absence of this marker (Cheung et al., 2001; Prasad et al., 2005). However these finding given the high specificity of this marker for malignancy in many series.

2.11. Immunohistochemistry as adjunct tool in Biomedical Research

Immunohistochemistry (IHC) is a technique that is regularly used in cell biology, biological research and diagnostic pathology. It relies upon the interaction between antibodies and antigens, allowing for substances to be identified within tissue samples. An antigen is a substance that is recognized by the immune system as foreign, prompting the production of antibodies and causing an immune response within the body. Antigens are usually large, complex proteins or polysaccharide molecules. An antibody is a glycoprotein that is produced by B lymphocytes in response to the presence of an antigen (Hayat, 2002). IHC is a technique used to locate antigens or

proteins in tissue sections. It utilizes this antigen-antibody interaction by labelling antibodies which will react with specific antigens. This interaction is visualized by a marker which may be a fluorescent dye, enzymes, radioactive elements or even colloidal gold (Ramos-Vara, 2005).

2.11.1. History

In 1941 - Albert H. Coons first introduces immunofluorescence as initial attempts to label antibodies were unsuccessful as the labels were not visible enough under the microscope. Using specific antibodies, Coons labeled them with fluorescent dyes in order to localize substances in tissues. This allowed for the detection of antibodies, antigens and antigenic proteins in tissues. In 1942 Coons, Creech, Jones and Berliner succeeded in tagging antibodies. These antibodies were used to detect foreign antigens in tissues. This involved using a single antipneumococcal antibody to find pneumococcal antigens in mice injected with large numbers of pneumococci.

In 1959 Singer first used an electron-dense protein in order to achieve ultra structural localization. The protein ferritin was used to tag an antibody. Electron microscopy could be used in Immunohistochemistry as a result of this as the presence of iron in the protein makes it electron-dense (Bozzola et al., 1999). In 1965 Uranium was used to develop the first electron-opaque heavy metal technique (WonTaek Lee, 2010). In 1966 Graham and Karnovsky localized the enzyme peroxidase using cytochemical methods leading to the development of the enzyme tagging method. In 1967 Nakane and Pierce developed the enzyme-labeled antibody technique by labeling an antibody with an enzyme (Bozzola et al., 1999). In 1970 Building upon the work of Graham, Karnovsky, Nakane and Pierce, Sternberger developed the peroxidase-antiperoxidase (PAP) method in an attempt to improve the enzyme-labelled method. The PAP method was an unlabelled antibody method. In 1971 Another electron-opaque heavy metal technique was developed by Faulk and Taylor using colloidal gold. This is a popular technique and can also be called the colloidal gold technique (Won Taek Lee, 2010). In 1974 The Avidin-antibiotin complex (ABC) method was developed. Similar to the PAP method, it is also an unlabelled antibody method (Won Taek Lee, 2010). In1990's - Antigen Retrieval was discovered, that the retrieval of nonreactive antigens in formalin-fixed, paraffin-embedded tissues was possible by heating sections in buffer solutions. This

increased the detection of antigens and sensitivity of methods (Ramos-Vara et al. ,1999).

2.11.2. The Process

IHC has a general process which differs slightly depending on which method is utilized. These are the steps involved: a tissue sample is collected from an animal or the patient. It can be from almost any organ in the body, the sample must be frozen or preserved quickly to prevent deterioration of the tissues. Fresh samples must be used as soon as possible. This is known as the fixation process, frozen samples are sliced to one-cell thickness and mounted, antibodies are added to the sample which bind with the antigens present in the tissue. Antibodies may be monoclonal or polyclonal, a protein solution is added to prevent the antibodies binding to non-specific proteins in a process called blocking, the sample is then incubated and washed to remove excess primary antibodies, a secondary antibody is added to the sample and similarly to previous steps, it is incubated and washed to remove any excess secondary antibodies, after mounting, these antibodies are fluorescently tagged and are visualized with a microscope.

General method adapted from IHC world (IHC World, 2007) and "Microscopy, Immunohistochemistry, and Antigen Retrieval Methods (Hayat, 2002). This method including; Direct and Indirect Method, Peroxidase-Antiperoxidase (PAP) Method, Avidin-antibiotin complex (ABC) Method, Labelled StreptAvidin Biotin (LSAB) Method, Polymeric Method and Catalysed Signal Amplification (CSA) Methods.

Controls

Use of Positive and Negative controls in IHC. Like most experiments, a control needs to be run so that the procedure can be verified and to check that the antibody being used is the correct one. The controls should be handled and processed in the same way the tissues being tested are, to ensure consistency and accurate results. There are two types of control in Immunohistochemistry; Positive control and negative control.

Positive control

Positive control is used to verify the procedure. Any negative results or steps that do not work efficiently will be eliminated or completed over again until the correct procedure and staining is established. Generally it is most appropriate to use the tissue which will be used in the experiment.

Negative control

Negative staining is used to verify the specificity of the antibody being used. The antibody must be from the same species as the primary antibody and a stain must not occur when the primary antibody is removed or replaced with serum. This type of negative control is often used in IHC as it is easy. A second type of negative staining is used, in which the staining is inhibited by a purified antigen absorbing the primary antibody. This technique is the most useful and appropriate, however isolating a purified antigen is time consuming and hard, consequently making this stain rarely used in IHC controls (IHC World, 2007).

Blocking

The process called blocking, is used to reduce or limit the amount of background staining which occurs during various IHC methods. Background staining is when cells that are not being stained for pick up some of the stain or fluorescence, leading to false positives. This is a common outcome when fixation is not completed quickly or adequately enough and also in the middle of large pieces of tissue. Background staining may also occur if the antibodies being used have been contaminated by other antibodies, through the use of impure antigen during immunization.

2.11.3. Methods

Direct Method

Direct fluorescent methods is the oldest and simplest method. It utilises one labelled primary antibody which reacts directly with an antigen within a tissue sample. A sample

is prepared and is exposed to a primary antibody. The antibodies react with the antigens resulting in an antigen-antibody interaction. The sample is washed to remove excess antibodies and is mounted and visualised under a microscope (IHC World, 2007).

Advantages

Procedure is short and quick, it can be used for quick diagnostic testing.

Disadvantages

Procedure is insensitive, only one tagged antibody binds with each antigen. If antigen concentration is low then the concentration of tagged antibodies is low and may not be enough for detection under the microscope.

Applications

Since the introduction of the more accurate and sensitive indirect method, the direct method is rarely used.

Indirect Method

The indirect method is used far more commonly than the direct method. It involves using both primary and secondary antibodies. Similarly to the direct method, the sample is exposed to primary antibodies and the antigens in the sample react with the antibodies resulting in an antigen-antibody interaction. The sample is washed to remove the excess antibodies and is then exposed to the labelled secondary antibodies which are directed against the primary antibodies causing them to bind together. The sample is mounted and can be visualised through a microscope.

The secondary antibodies may be labelled with various substances. If they are labelled with fluorescent dyes such as Texas Red, rhodamine or FITC the method becomes known as Indirect Immunofluorescence Method. Alternatively, they may be labelled with enzymes such as peroxidase or glucose oxidase. This is known as Indirect Immunoenzyme Method (IHC World, 2007).

Advantages

Only the secondary antibodies, which tend to be cheaper, need to be labelled, thus preventing wastage of primary antibodies, sensitivity is much greater than that of direct method.

Disadvantages

Secondary antibodies must be from a different animal species, procedure is laborious. The indirect method acts as a precursor to more complex methods such as the PAP method and ABC method. It is rarely used since the introduction of the more commonly used complex and sensitive successors.

2.11.4. Limitations of IHC

There are various limitations associated with IHC

- There are some tumors for which there are no antibodies available which can be accepted to be specific to that type of tumors. This means that there is no specific test for this tumor and it requires a multimodal approach in order to reach a diagnosis. An example of this is malignant mesothelioma which is a malignant epithelial neoplasm which originates from the serosal surfaces of body cavities (Suster and Moran, 2006).

- The specificity of antibodies needs to be tested using controls to avoid false-positive results which are often a result of non-specific binding (Fritschy, 2008)

- There is a limited amount of antibodies that can sourced from a single animal and specificity is reduced when the protein used for immunization and the contaminants are co-purified. This is overcome by using monoclonal antibodies, though monoclonal antibodies are more expensive (Faggiano et al., 2007).

- Immunohistochemistry has numerous methods that can be used in the lab, with some having substantial advantages over others. One such property, sensitivity is often viewed as a limitation as many methods will not produce the required results (IHC World, 2007).

2.11.5. Advantage of IHC

In diagnosis of disease IHC is an excellent detection technique and has the tremendous advantage of being able to show exactly where a given protein is located within the tissue examined. It is also an effective way to examine the tissues. The technique is even more widely used in diagnostic surgical pathology for typing tumors (e.g. immunostaining for e-cadherin to differentiate between DCIS (ductal carcinoma in situ: stains positive) and LCIS (lobular carcinoma in situ: does not stain positive) (O'Malley & Pinder, 2006).

- Carcinoembryonic antigen (CEA): used for identification of adenocarcinomas. Not specific for site.
- Cytokeratins: used for identification of carcinomas but may also be expressed in some sarcomas (Leader et al., 1986).
- CD15 and CD30 : used for Hodgkin's disease.
- Alpha fetoprotein: for yolk sac tumors and hepatocellular carcinoma.
- CD117 (KIT): for gastrointestinal stromal tumors (GIST).
- CD10 (CALLA): for renal cell carcinoma and acute lymphoblastic leukemia.
- Prostate specific antigen (PSA): for prostate cancer.
- estrogens and progesterone staining for tumor identification.
- Identification of B-cell lymphomas using CD20. Identification of T-cell lymphomas using CD3.

In cancer therapy IHC can be used to assess which tumors are likely to respond to therapy, by detecting the presence or elevated levels of the molecular target. Tumor biology allows for a number of potential intracellular targets. Many tumors are hormone dependent. The presence of hormone receptors can be used to determine if a tumor is potentially responsive to antihormonal therapy. One of the first therapies was the antiestrogen, tamoxifen, used to treat breast cancer. Such hormone receptors can be detected by IHC (Jørgensen et al., 2007). Imatinib, an intracellualar tyrosine kinase inhibitor, was developed to treat chronic myelogenous leukemia, a disease characterized by the formation of a specific abnormal tyrosine kinase. Imitanib has proven effective in

tumors, that express other tyrosine kinases, most notably KIT. Most gastrointestinal stromal tumors express KIT, which can be detected by IHC (Gold and Dematteo, 2006).

Problems of Immunohistochemistry without automation

Three critical points in IHC that depend directly on the human intervention can be delineated as follows: Appropriate handling of the specimen, its suitable fixation, its later inclusion in paraffin, as occurs with most of the samples, and the preparation of the sections are all vital in the final result of the stains. Another critical step includes the preparation of reagents, antibodies and application of solutions, times of incubation, suitable dried washing and crystals. These activities are done manually and repetitively. To present, these activities have been object of automation . The interpretation of the results is always the responsibility of an expert pathologist who is familiar with each one of the antibodies, its diagnostic possibilities and limitations. Automation simplifies the 16-step manual method down to another maximum five-step method, which more or less reduces the time per slide, ideally in less than 1 hour it must be possible to dye more than 40 slides. The different instruments must be able to function without great time spent by technical personnel. The cost per test with an automatic system, including the cost of the antibodies and reagents, would have to be same as that for the manual method. The use of closed systems would favor biosecurity in every laboratory. Examples of automated immunostainers Techmate, Horizon, Nexes, Ventana ES, Leica, and Optimax Cadenza (Grogan et al., 1993).

Chapter III

Patients and methods

3.Patients and Methods

Clinicopathological features and follow up data

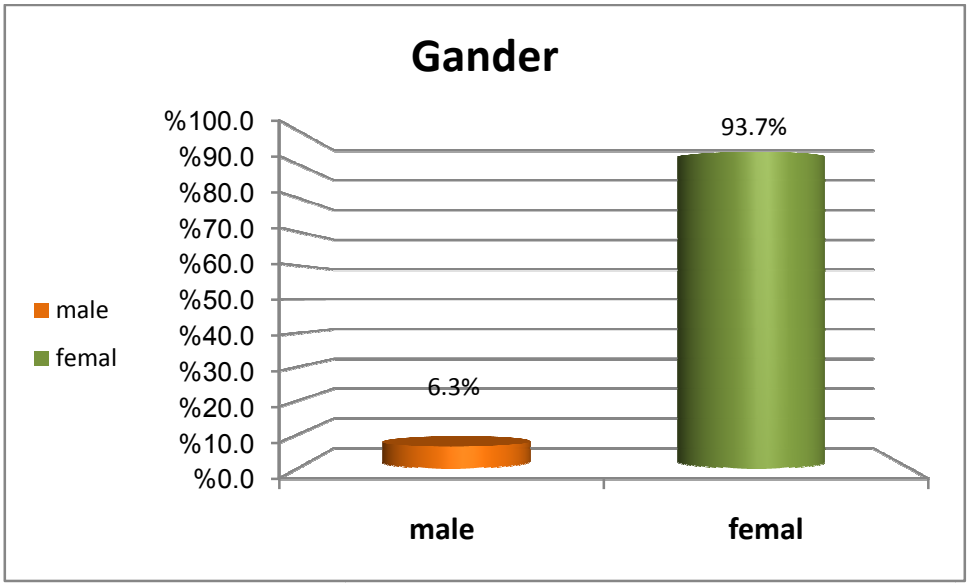
Archival samples of 32 papillary thyroid carcinoma was examined in the present study: All the tumor samples were collected from Pathology Department, Faculty of Medicine, Benghazi University between January 2004 to December 2012 based on availability of representative paraffin blocks.

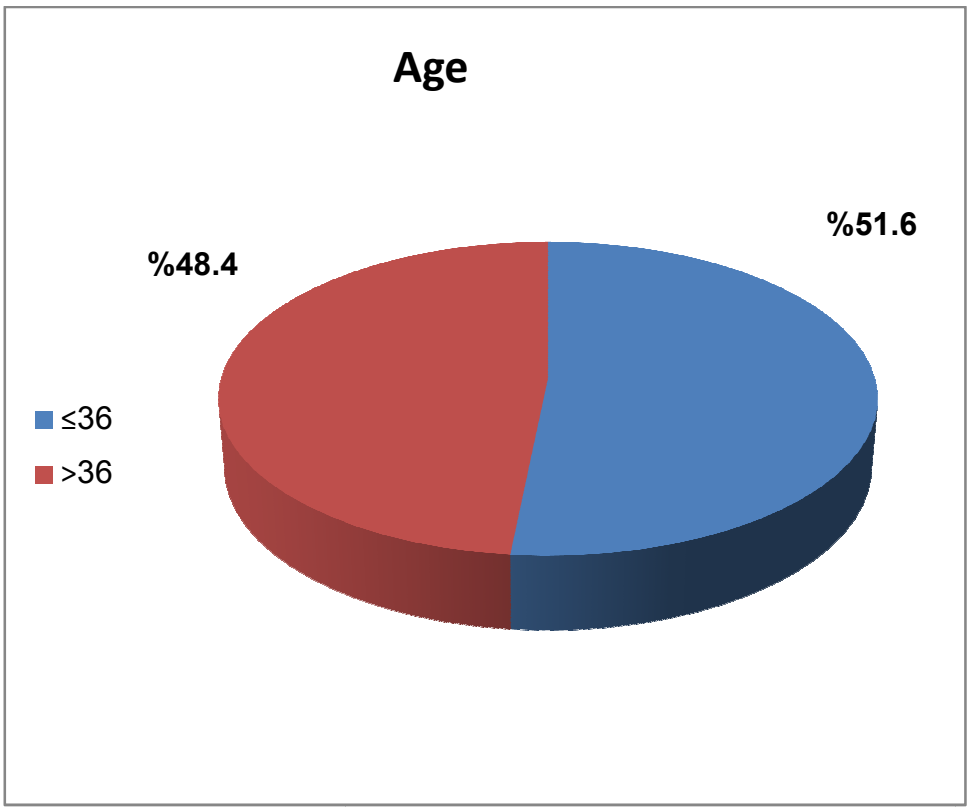
The patient's report were retrieved from the files of the Pathology Department archive in order to collect the appropriate clinical and surgical information for current study. For each patient, we obtained the following information: age, gender, localization, tumor size, extrathyroid extension, distant metastasis, type of surgery.

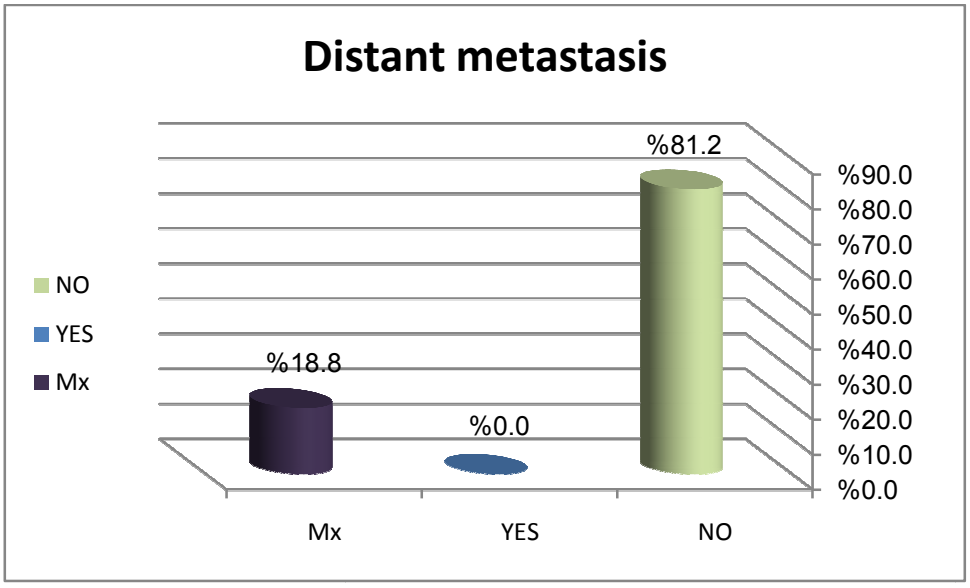
An experienced pathologist confirmed all diagnosis, and the following histopathological features were recorded included; histological type, lymph node involvement, lymphovascular invasion, blood Vessel invasion, pre existence hashimiotothyroiditis, multifocality. All tumors were classified using the histopathological criteria of WHO classification. The key clinicopathological data of patients are summarized in Table 3.1.

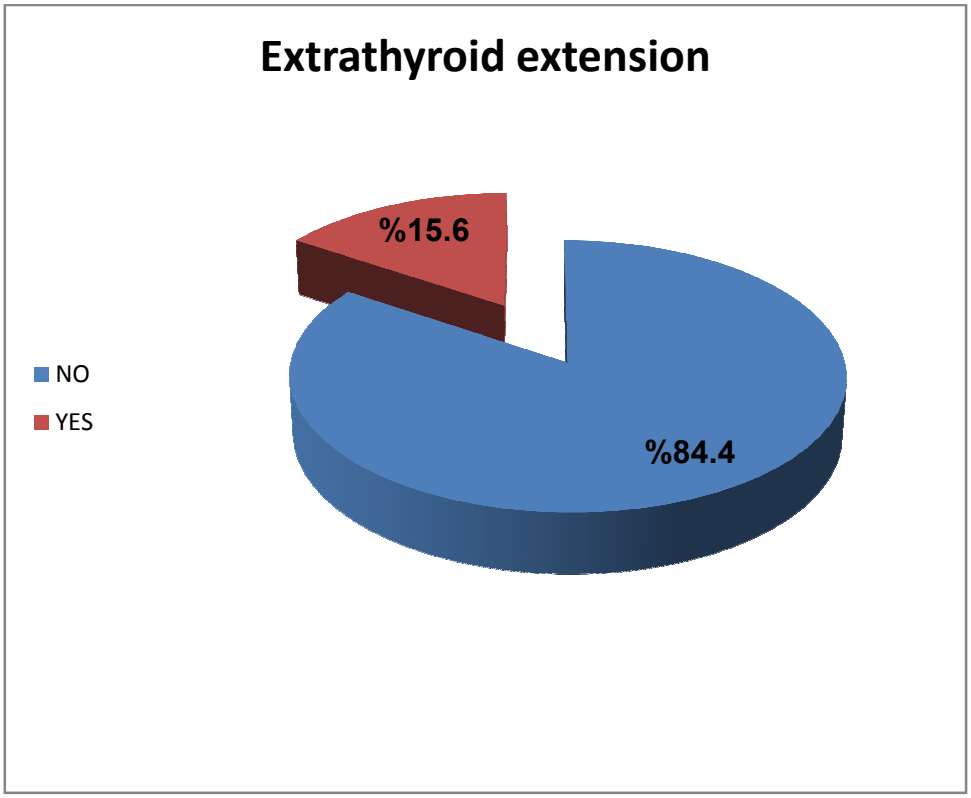
Table 3.1 Clinicopathological characteristics of the patients with PTC

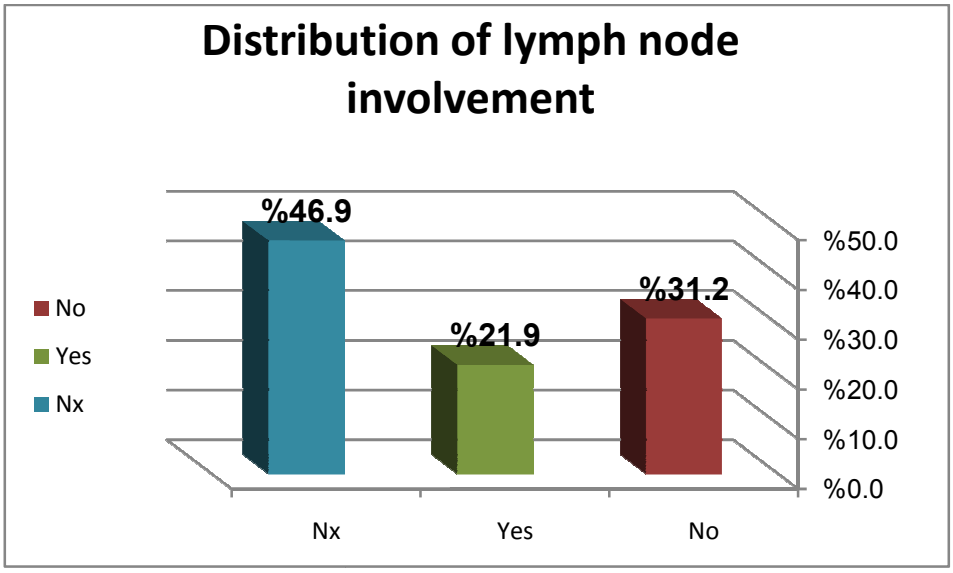
Characteristic	No of patients(%)
Gender	
Male	2 (6.3%)
Female	30 (93.7%)
Age(yrs)	
≤ 36	16 (51.6%)
> 36	15 (48.4%)
Histopathological type	
Classic variant	21 (65.6%)
Follicular variant	10 (31.3%)
Tall cell variant	1 (3.1%)
LN involvement	
No	10 (31.2%)
Yes	7 (21.9%)
Nx	15 (46.9%)
Distant metastasis	
No	26 (81.2%)
Yes	0 (0%)
Mx	6 (18.8%)
Extrathyroid extension	
No	27 (84.4%)
Yes	5 (15.6%)
Multifocal	
No	20 (62.5%)
Yes	12 (37.5%)
Localization	
Lt lobe	9 (31.1%)
Rt lobe	13(44.8%)
Both	7 (24.1%)
Lymph vessel invasion	
No	29 (90.6%)
Yes	3 (9.4%)
Blood vessel invasion	
No	32 (100%)
Yes	0 (0%)
Pre-existence hashimiotothyroiditis	
Yes	13 (40.6%)
No	19 (59.4%)

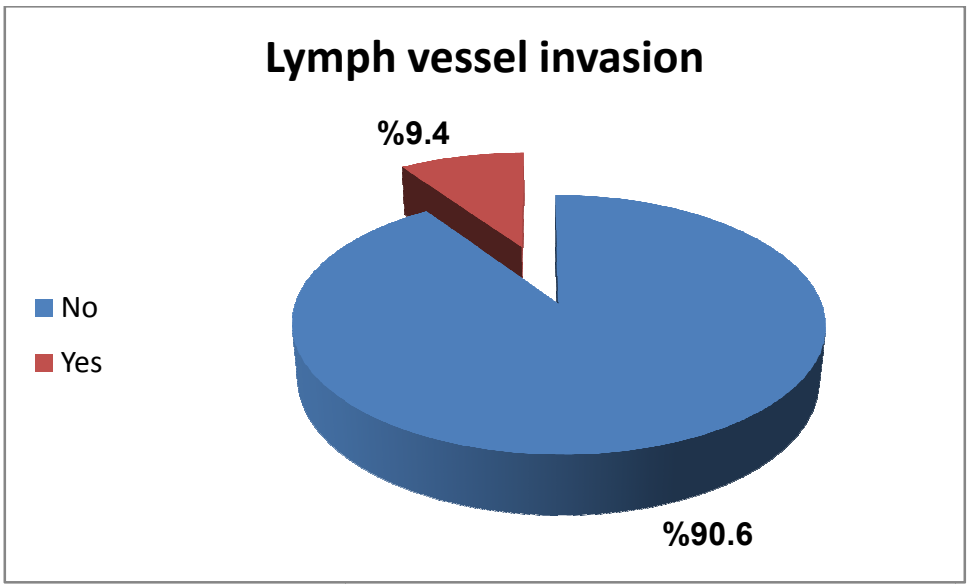

Gander
%100.0
%90.0
%80.0
%70.0
%60.0
%50.0
%40.0
%30.0
%20.0
%10.0
%0.0
93.7%
6.3%
male
femal
male
femal

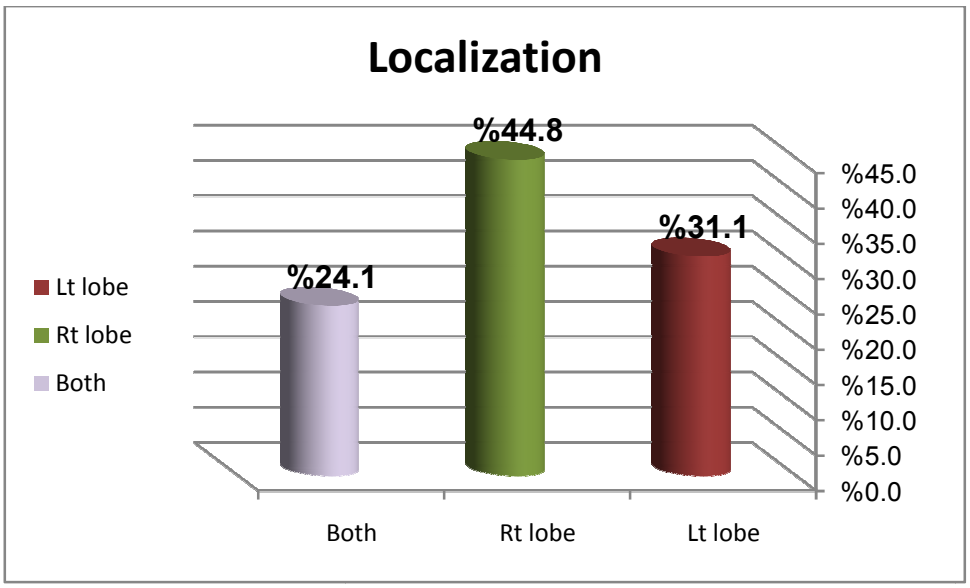

Age
%51.6
%48.4
≤36
>36

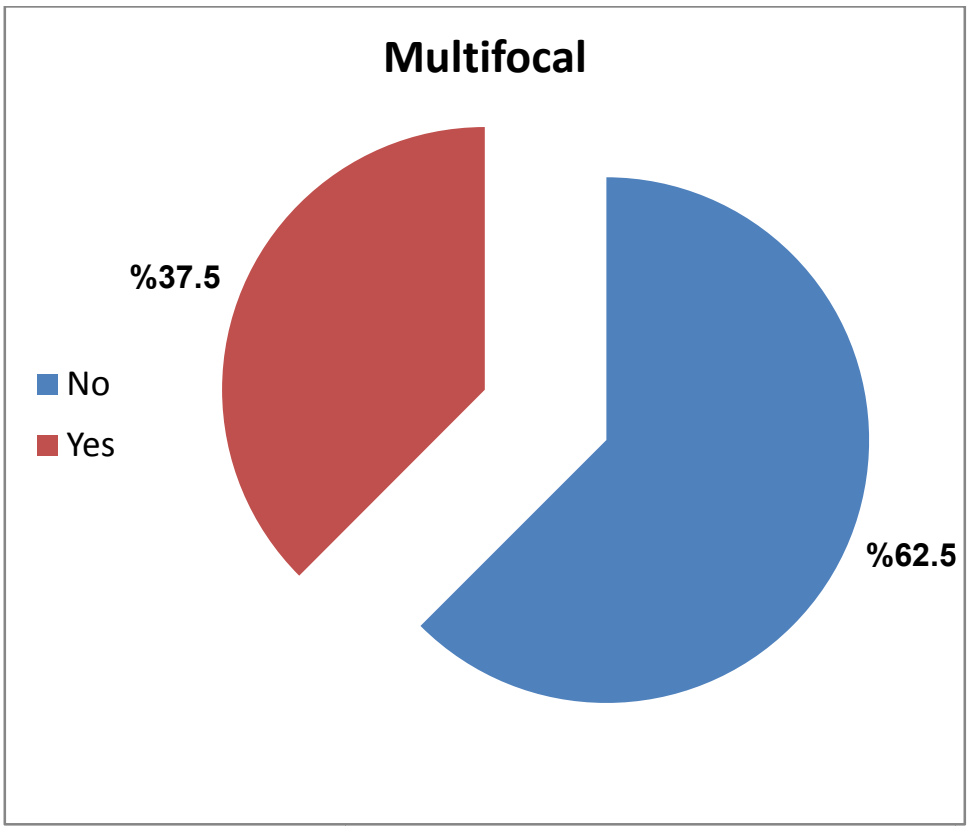

Distant metastasis
%81.2
%18.8
%0.0
%90.0
%80.0
%70.0
%60.0
%50.0
%40.0
%30.0
%20.0
%10.0
%0.0
NO
YES
Mx
Mx
YES
NO

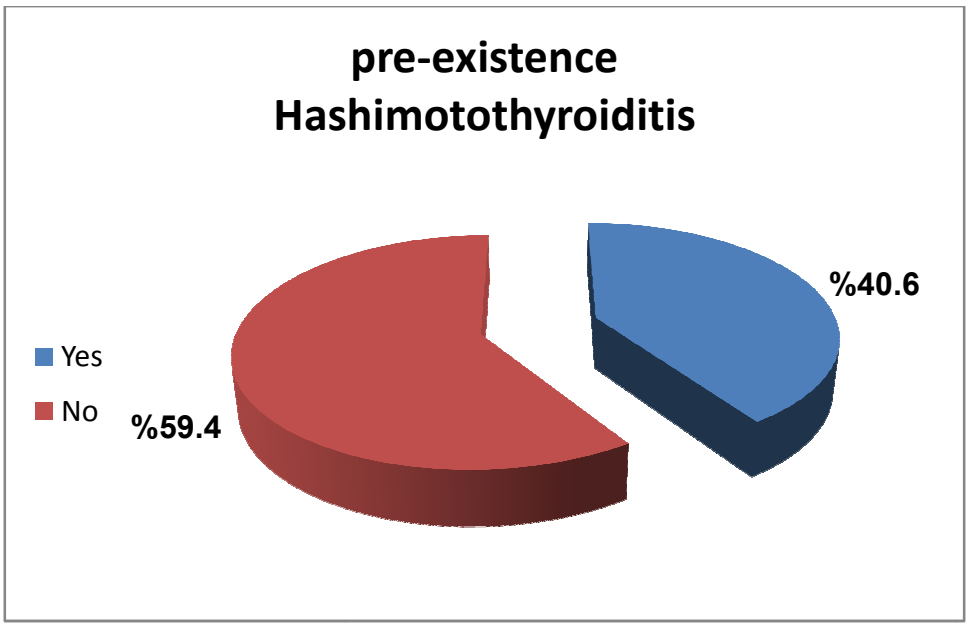

Extrathyroid extension
%15.6
%84.4
NO
YES

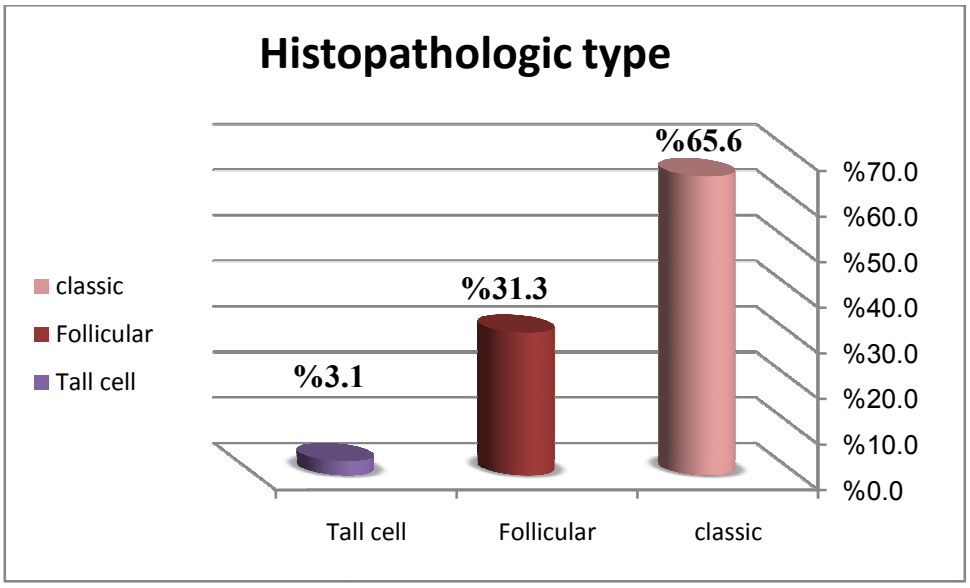

Distribution of lymph node involvement
%46.9
%21.9
%31.2
%50.0
%40.0
%30.0
%20.0
%10.0
%0.0
No
Yes
Nx
Nx
Yes
No


Lymph vessel invasion
%9.4
%90.6
No
Yes


Localization
%44.8
%31.1
%24.1
%45.0
%40.0
%35.0
%30.0
%25.0
%20.0
%15.0
%10.0
%5.0
%0.0
Lt lobe
Rt lobe
Both
Both
Rt lobe
Lt lobe


Multifocal
%37.5
%62.5
No
Yes


pre-existence
Hashimotothyroiditis
Yes
No
%40.6
%59.4


Histopathologic type
classic
Follicular
Tall cell
%65.6
%31.3
%3.1
%70.0
%60.0
%50.0
%40.0
%30.0
%20.0
%10.0
%0.0
Tall cell
Follicular
classic

Ki67&P53 Immunostaining

Formalin-fixed, paraffin-embedded PTC tissue was obtained from 32 patients. Sections were cut serially at 5µm for immunohistochemical (IHC) analysis by using microtome. IHC analysis was done using the automatic system (BenchMark XT, Ventana Medical System, Inc. Tucson, Arizona, USA). This fully automated processing of code-labeled slides included baking of the slides, solvent free deparaffinization, antigen retrieval in a cell conditioning buffer CCI (Mild: 36 minutes conditioning, and standard: 60 minutes conditioning), Slides were then incubated with the Rabbit monoclonal antiP53antibodies (clone: SP5, Catalog No: M3054, 6920 Koll Center Parkway, CA 94566, USA),and with MIB-1 monoclonal antibodies(clone:sp6, Catalog No:N3064 ,6920 Koll Center Parkway, CA 94566, USA) Dilution 1: 50 for 32 min, at 37ºC. Application of I-View™ DAB Detection Kit (Lot no. B05860AZ), which, includes: I-View DAB HRP, I-View DAB Inhibitor, I-View DAB Biotin, I-View DAB H2O2, and I-View DAB Copper. Counterstaining with haematoxylin II (C00758) was done for 4 minutes, and post-counterstaining with blueing reagent (B11129) was done for 4 minutes as well. After staining, the sections was dehydrated in ethanol, cleared in xylene, and covered with Mountex and cover slips.

Evaluation of immunohistochemical staining

Ki67and P53 staining were evaluated using regular light microscope at the magnification of x40. Nuclear staining was graded into four categories: 3+++ No blue to be seen through brown staining, nuclei appear darker than cytoplasm, 2++ Blue scarcely to be seen through brown staining, nuclei appear darker than cytoplasm, 1+ blue clearly to be seen through brown staining, 0 only blue staining.

The nuclear index (NI) was calculated with both the intensity of staining and fraction of positively-stained cells taken into account using the following formula:

$$I = 0 \times f0 + 1 \times f1 + 2 \times f2 + 3 \times f3$$

Where I; is the staining index, f0-f3 are the fractions of the cells showing a defined level of staining intensity (from 0 to 3). Theoretically, the index could vary between 0 and 3 (Lipponen & Collan, 1992; Buhmeida et al., 2008 ; Fatma Emaetig et al., 2013).

Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics (IBM Company, NY, USA) software packages (IBM PASW Statistics for Windows, version 19). Frequency tables were analyzed using the Chi-square test, with likelihood ratio (LR) or Fischer's exact test being used to assess the significance of the correlation between the categorical variables. Odds Ratios and their 95% Confidence Intervals (95%CI) were calculated where appropriate, using the exact method. Difference in the means of continuous variables was analysed using non-parametric tests (Mamm-Whitney or Kruskal-Wallis) and multiple independent samples, respectively. Analysis of variance (ANOVA) was only used deriving the mean values (and their 95%CI) of each individual stratum. In all tests, the values $p<0.05$ were regarded statistically significant.

Chapter IV

Results

4-Result

expression patterns of Ki-67 expression

The intracellular localization of ki-67 in tumor cells was predominantly nuclear in tumor area. The expression of ki-67 in PTC lesions are illustrated in the following figures respectively (Figure 4.1, 4.2). The mean value of staining ki-67 indices (NI) was (0.3), 62% of tumors express Ki-67 were below the mean(20/32), and 37.5% above the mean (12/32) the frequency of Ki-67 in PTC are illustrated in figure 4.3.

Correlation of Ki-67 expression with clinicopathological characteristics

The distribution of Ki-67 expression in tumor samples in relation to clinicopathological characteristics is presented in (Table 4.1, 4.2).

Using different cut-off points (mean, median, and 2-teir score (0 Vs 1,2,3) and (0,1, Vs 2,3). An interesting finding in our immunohistochemical study, The tumor arising from left lobe(7/9) show proliferation index more than the right lobe (3/13) ($p < 0.028$).

No statistically significant correlation was found between Ki-67 expression in tumors and other clinicopathological parameters (such as age, gender, histopathological type, tumor size, extrathyroid extension, multifocality, Pre-existence Hashimiotothyroiditis).

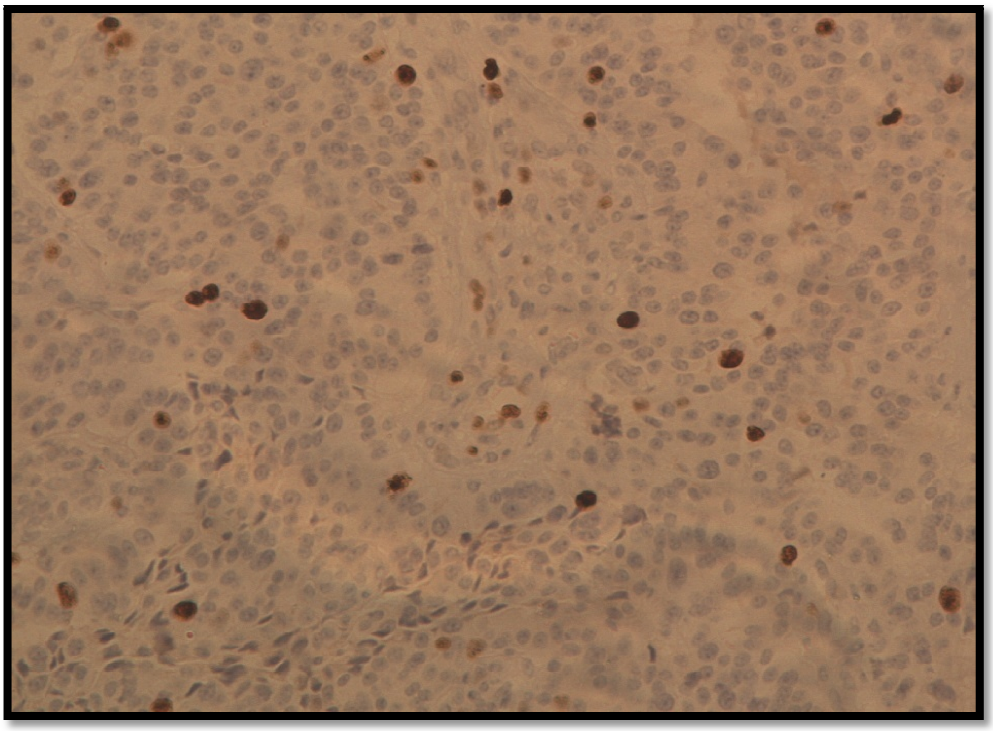


Figure.4.1. Low proliferation index (Ki-67) in papillary thyroid carcinoma (IHCX20) (Department of Pathology, Faculty of Medicine, Benghazi University).

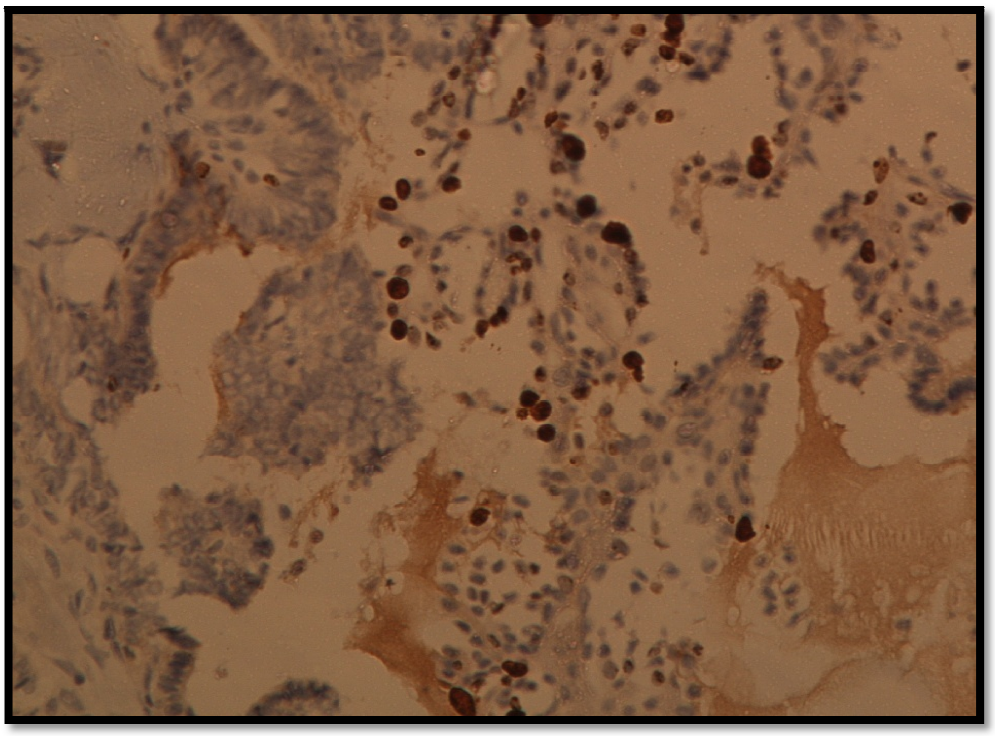


Figure.4.2. High proliferation index (Ki-67)in papillary thyroid carcinoma (IHCX40) (Department of Pathology, Faculty of Medicine, Benghazi University).

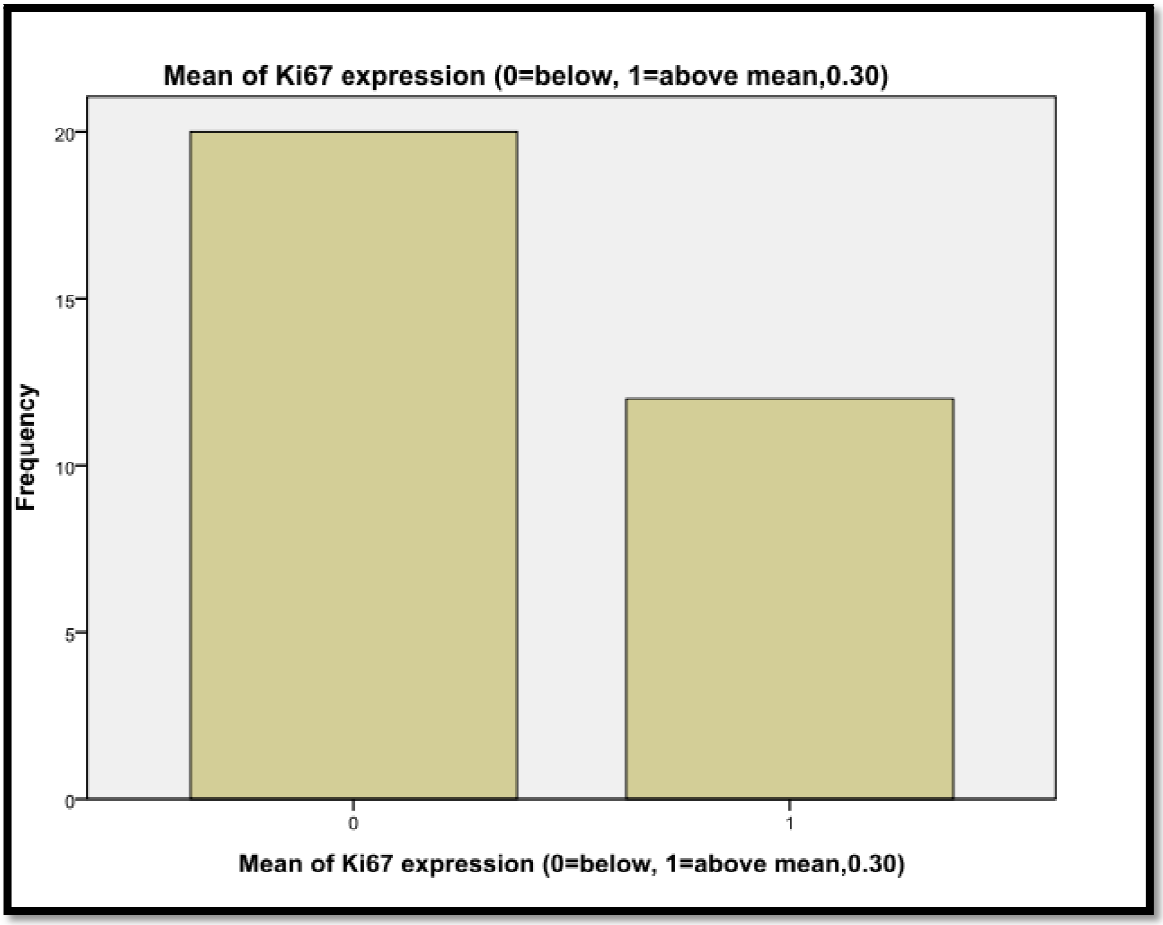


Figure.4.3 Frequency of Ki-67 expression in PTC.

Table.4.1. Correlation between Ki67 expression and clinicopathological features of PTC at mean as cut-off point.

Feature		Number%	Ki-67expression		P value
			Below mean(0.3)	Above mean(0.3)	
Age	≤36	16(51.6%)	10(50%)	6(54.5%)	0.809
	≥36	15(48.4%)	10(50%)	5(45.5%)	
gender	female	30(93.8%)	19(95%)	11(91.7%)	0.706
	male	2(6.3%)	1(5%)	1(8.3%)	
Histopathological type	classic	21(67.7%)	12(63.2%)	9(75%)	0.492
	follicular	10(32.3%)	7(36.8%)	3(25%)	
LN involvement	No	10(58.8%)	6(60%)	4(57.1%)	0.9
	Yes	7(41.2%)	4(40%)	3(42.9%)	
Tumor size	<2.5cm	16(51.6%)	9(47.4%)	7(58.3%)	0.55
	>2.5cm	15(48.4%)	10(52.6%)	5(41.7%)	
Extrathyroid extension	No	27(84.4%)	16(8o%)	11(91.7%)	0.37
	Yes	5(15.6%)	4(20%)	1(8.3%)	
Multifocality	No	20(62.5%)	11(55%)	9(75%)	0.25
	Yes	12(37.5%)	9(45%)	3(25%)	
Tumor localization	Left lobe	13(44.8%)	10(58.5%)	3(25%)	**0.028**
	Rightlobe	9(31%)	2(11.8%)	7(58.3%)	
	Both lobe	7(24.1%)	5(29.4%)	2(16.7%)	
Lymphatic invasion	No	29(90.6%)	18(90%)	11(91.7%)	0.87
	yes	3(9.4%)	2(10%)	1(8.3%)	
Pre-existence Hashimiotothyroiditis	No	13(40.6%)	14(70%)	5(41.7%)	0.1
	yes	7(41.2%)	6(30%)	7(58.3%)	

Table.4.2. Correlation between Ki-67 expression and clinicopathological features of PTC at cut-off point(2-tier score 0,1 vs. 2,3).

features		Number%	Ki67 expression 2-tier score (0,1 vs. 2,3)		Pvalue
			0,1vs	2,3	
age	≤36	16(51.6%)	15(50%)	1(100%)	0.32
	≥36	15(48.4%)	15(50%)	0(0,0%)	
gander	female	30(93.8%)	28(93.3%)	2(100%)	0.7
	male	2(6.3%)	2(6.75)	0(0,0%)	
Histopathological type	classic	21(67.7%)	19(65.5%)	2(100%)	0.31
	follicular	10(32.3%)	10(34.5%)	0(0,0%)	
LN involvement	No	10(58.8%)	9(60%)	1(50%)	0.78
	Yes	7(41.2%)	6(40%)	1(50%)	
Tumor size	<2.5cm	16(51.6%)	15(51.7%)	1(50%)	0.60
	>2.5cm	15(48.4%)	14(48.3%)	1(50%)	
Extrathyroid extension	No	27(84.4%)	2(100%)	25(83.3%)	0.53
	Yes	5(15.6%)	0(0,0%)	5(16.7%)	
Multifocality	No	20(62.5%)	18(60%)	2(100%)	0.8
	Yes	12(37.5%)	12(40%)	0(0,0%)	
Tumor localization	Left lobe	9(31%)	8(29.6%)	1(50%)	0.4
	Rightlobe	13(44.8%)	13(48.1%)	0(0,0%)	
	Both lobe	7(24.1%)	6(22.2%)	1(50%)	
Lymphatic invasion	No	29(90.6%)	27(90%)	2(100%)	0.63
	yes	3(9.4%)	3(10%)	0(0,0%)	
Pre-existence hashimiotothyroiditis	No	19(59.4%)	17(56.7%)	2(100%)	0.22
	yes	13(40.6%)	13(43.3%)	0(0,0%)	

expression patterns of p53 expression

The intracellular localization of p53 in tumor cells was predominantly nuclear in tumor area. The expression patterns of p53 in PTC lesions are illustrated in the following figures respectively (Figure 4.4, 4.5, 4.6). The mean value of staining p53 indice (NI) was (0.13). 75% of tumors express P53 were below the mean (24/32), and 25% above the mean (8/32) the frequency of P53 in PTC are illustrated in figure 4.7.

Correlation of p53 expression with clinicopathological characteristics

The distribution of p53 expression in tumor samples in relation to clinicopathological characteristics is presented in (Table 4.3, 4.4).

Using different cut-off points (mean, and 2-teir score (0 Vs 1,2,3). An interesting finding in our immunohistochemical study, p53 expression show a significant correlation with tumor localization ($p < 0,06$), tumor arising in both lobes show low expression of p53 (7/7), also tumor arising in left lobe or in right lobe express low P53(6/9) (8/13) respectively.

Moreover, P53 expression showed also border line significant correlation with lymphovascular invasion ($p < 0.08$) in that the tumors express P53 associated with lymphatic invasion (2/3).

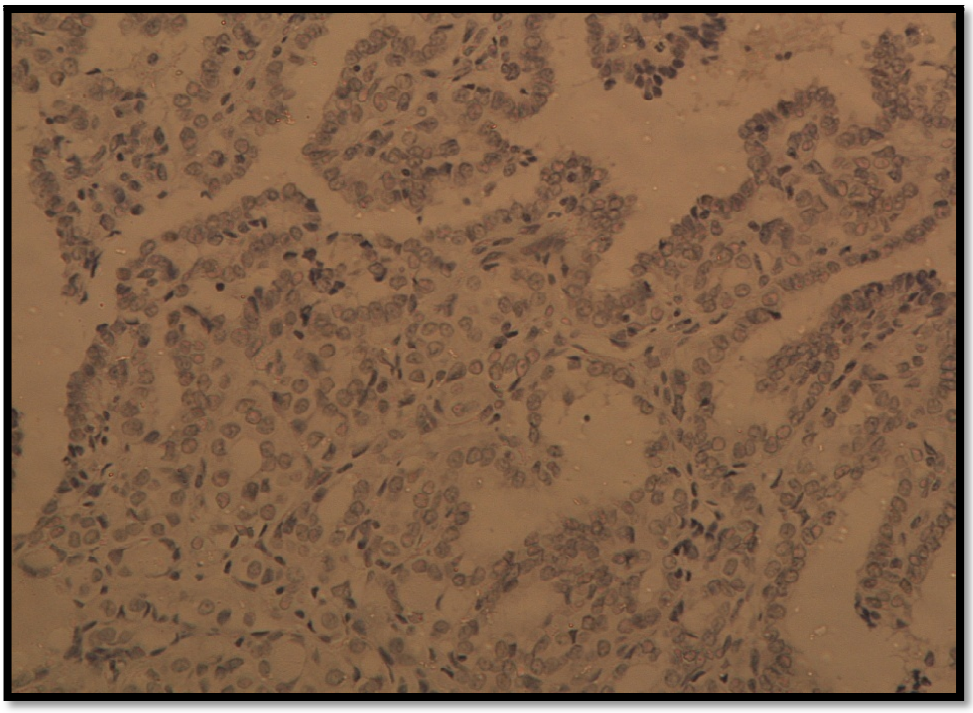


Figure.4.4. Negative nuclear expression of P53 in papillary thyroid carcinoma (IHCX20) (Department of Pathology, Faculty of Medicine, Benghazi University).

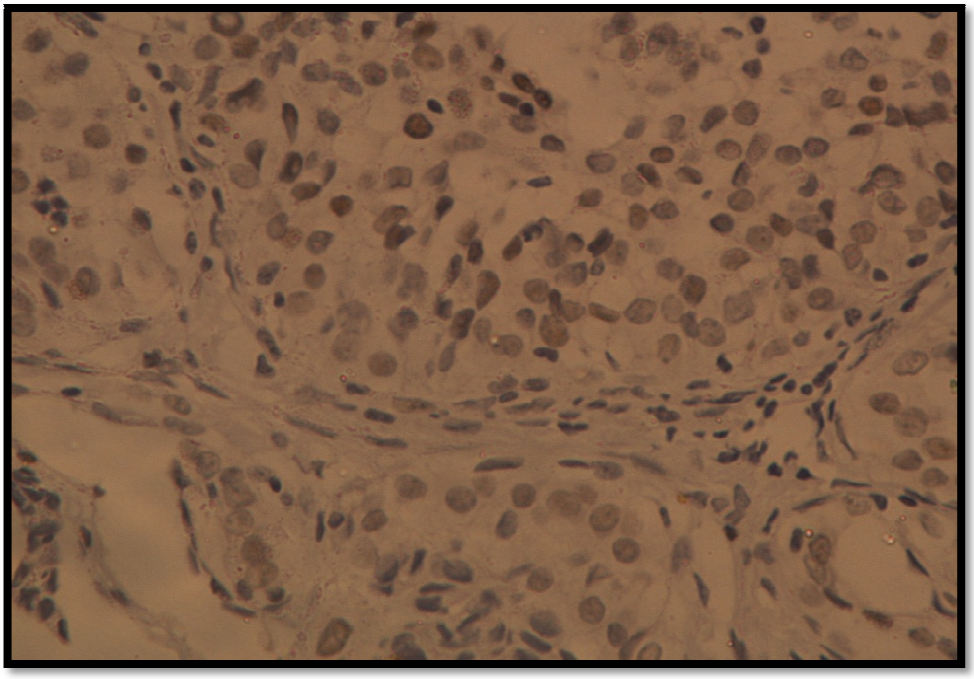


Figure.4.5.weak nuclear expression of P53 in papillary thyroid carcinoma (IHCX20) (Department of Pathology, Faculty of Medicine, Benghazi University).

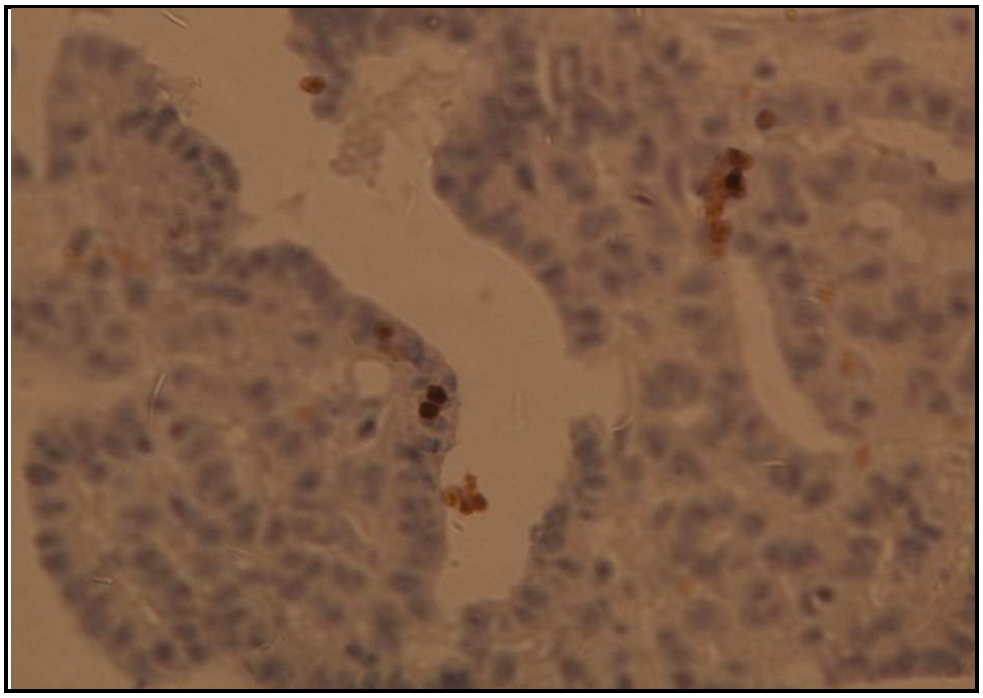


Figure.4.6. strong nuclear expression of P53 in papillary thyroid carcinoma (IHCX20) (Department of Pathology, Faculty of Medicine, Benghazi University).

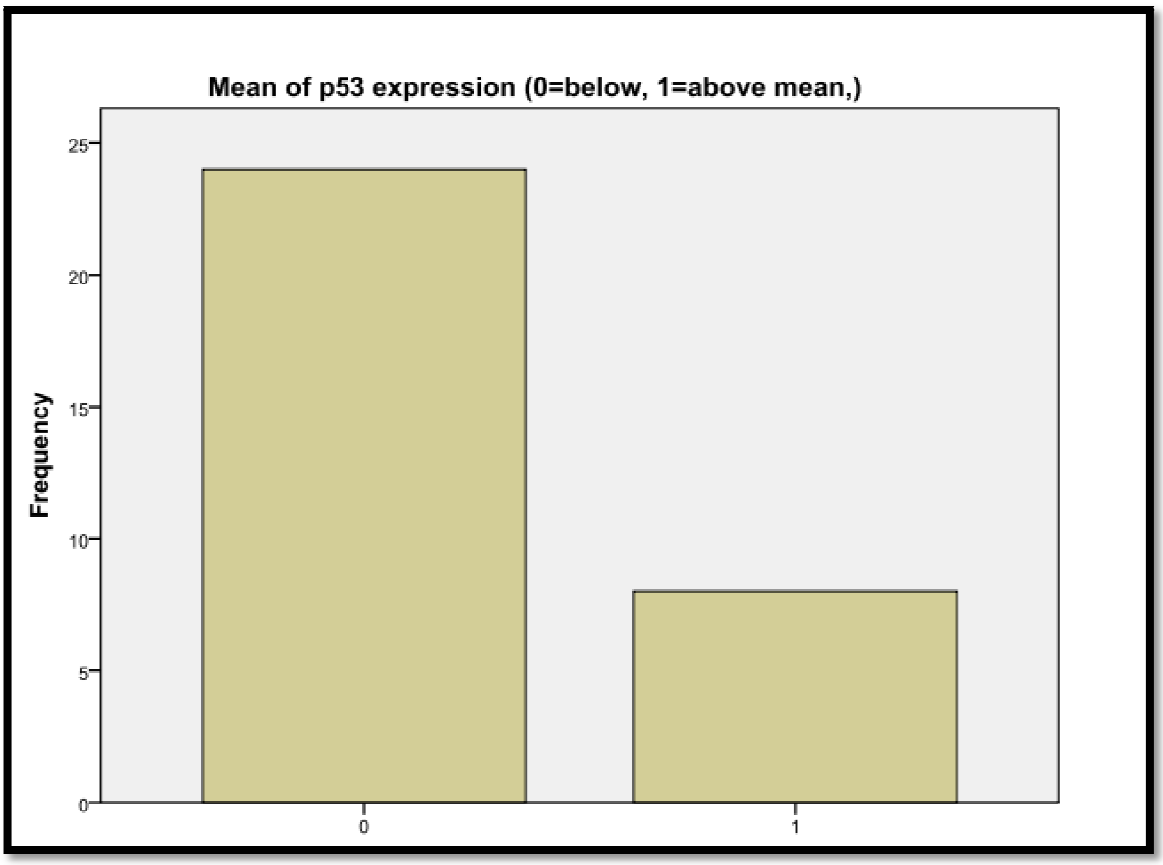


Figure.4.7. the frequency of P53 in PTC

Table.4.3 Correlation between P53 expression and clinicopathological features of PTC at mean as cut-off point.

features		Number%	expression of p53 (0=below, 1=above mean,)		P value
			0	1	
Age	≤36	16(51.6%)	12(52.2%)	4(50%)	0.916
	≥36	15(48.4%)	11(47.8%)	4(50%)	
gender	female	30(93.8%)	22(91.7%)	8(100%)	0.399
	male	2(6.3%)	2(8.3%)	0(.0%)	
Histopathological type	classic	21(67.7%)	16(66.7%)	5(71.4%)	0.813
	follicular	10(32.3%)	8(33.3%)	2(28.6%)	
LN involvement	No	10(58.8%)	7(70%)	3(42.9%)	0.362
	Yes	7(41.2%)	3(30%)	4(57.1%)	
Tumor size	<2.5cm	16(51.6%)	12(50%)	4(57.1%)	0.739
	>2.5cm	15(48.4%)	12(50%)	3(42.9%)	
Extrathyroid extension	No	27(84.4%)	20(83.3%)	7(87.5%)	0.779
	Yes	5(15.6%)	4(16.7%)	1(12.5%)	
Multifocality	No	20(62.5%)	14(58.3%)	6(75%)	0.399
	Yes	12(37.5%)	10(41.7%)	2(25%)	
Tumor localization	Left lobe	9(31%)	7(30.4%)	2(33.3%)	0.267
	Right lobe	13(44.8%)	9(39%)	4(66.7%)	
	Both lobe	7(24.1%)	7(30.4%)	0(.0%)	
Lymphatic invasion	No	29(90.6%)	23(95.8%)	6(75%)	**0.08**
	yes	3(9.4%)	1(4.2%)	2(25%)	
Pre-existence hashimiotothyroiditis	No	19(59.4%)	13(54.2%)	6(75%)	0.299
	yes	13(40.6%)	11(45.8%)	2(25%)	

Table.4.4 Correlation between P53 expression and clinicopathological features of PTC at cut-off point (2-tier score 0 vs. 1,2,3)

features		Number%	p53 expression 2-tier score (0 vs. 1,2,3)		P value
			0	1	
age	≤36	16(51.6%)	10(47.6%)	6(60%)	0.519
	≥36	15(48.4%)	11(52.4%)	4(40%)	
gender	female	30(93.8%)	20(90.9%)	10(100%)	0.325
	male	2(6.3%)	2(9.1%)	0(.0%)	
Histopathological type	classic	21(67.7%)	15(68.2%)	6(66.7%)	0.935
	follicular	10(32.3%)	7(31.8%)	3(33.3%)	
LN involvement	No	10(58.8%)	6(66.7%)	4(50%)	0.486
	Yes	7(41.2%)	3(33.3%)	4(50%)	
Tumor size	<2.5cm	16(51.6%)	10(45.5%)	6(66.7%)	0.283
	>2.5cm	15(48.4%)	12(54.5%)	3(33.3%)	
Extrathyroid extension	No	27(84.4%)	18(81.8%)	9(90%)	0.555
	Yes	5(15.6%)	4(18.2%)	1(10%)	
Multifocality	No	20(62.5%)	12(54.5%)	8(80%)	0.168
	Yes	12(37.5%)	10(45.5%)	2(20%)	
Tumor localization	Left lobe	9(31%)	6(28.6%)	3(37.5%)	**0.068**
	Right lobe	13(44.8%)	8(38.1%)	5(62.5%)	
	Both lobe	7(24.1%)	7(33.3%)	0(.0%)	
Lymphatic invasion	No	29(90.6%)	21(95.5%)	8(80%)	0.164
	yes	3(9.4%)	1(4.5%)	2(20%)	
Pre-existence hashimiotothyroiditis	No	19(59.4%)	11(50%)	8(80%)	0.109
	yes	13(40.6%)	11(50%)	2(20%)	

Chapter V

Discussion

5-Discussion

In 2008, the worldwide estimated age-standardised incidence rates for thyroid cancer incidence were 4.7 and 1.5 per 100,000 women and men, respectively. Thyroid cancer's overall contribution to the worldwide cancer burden is relatively small, but incidence rates have increased over the last three decades throughout the world. This trend has been hypothesized to reflect a combination of technological advances enabling increased detection, but also changes in environmental factors, including population exposure to ionizing radiation from fallout, diagnostic tests and treatment for benign and malignant conditions (Schonfeld et al, 2011). The death rate from thyroid cancer has been fairly stable for many years, and remains very low compared with most other cancers (American Cancer Society, 2013).

Thyroid cancer is within the top twenty most common cancers for UK females (number 18) (cancer research UK, 2011). The highest rates for thyroid cancer in the world occur in Northern America, where the female age-standardized rate is 15.1 per 100,000 females, compared with 1.2 per 100,000 females in Middle Africa. Incidence is low in all parts of Africa (Ferlay et al, 2010). In Saudi Arabia (SA) thyroid cancer is the second most common malignancy after breast cancer in female patients (Saudi Cancer Registry; 2008). Outside the Gulf region, Syria is the only country in which thyroid cancer ranks among the top five cancer sites in females. While in Yemen, the bulk of thyroid cancer is of the papillary type, in other countries like Algeria and Sudan, follicular cancer is the predominant form. In both the latter countries, the number of patients presenting with advanced stages of the disease is high (Ghzi omar et al., 2010).

In Western Libya, according to Sebrata Cancer Registry during 2006, thyroid cancers constitute 1.2% of all cancers reported from collective data.

The prognosis of PTC is excellent, with an overall 20- to 25-year cancer-specific mortality rate of 5% (Hay et al., 2002) Some patients, however, have a worse prognosis than others. In contrast to staging systems for other cancers, most of those for PTC take into account the patient’s age, which has been found to influence prognosis. In the TNM system, patients younger than 45 years are classified as having stage I or II

disease, with only the presence of distant metastases distinguishing the two. It is imperative to note that patients in this age group who have lymph node metastases are still classified as having stage I disease. Patients who are age 45 years or older are categorized into having stage I, II, II, or IV disease depending on tumor size or invasion as well as lymph node or distant metastases. In addition to the prognostic factors examined in these staging systems, other factors such as histologic subtype of PTC, as well as genetic factors, have been associated with a worse prognosis. The tall cell, columnar cell, diffuse sclerosing, and insular histologic variants of PTC, as well as PTCs that contain a mutation in the gene for the BRAF, are considered more aggressive (Sywak et al., 2004; Xing et al., 2005).

As shown in the current study, mean age of patients with PTC were 36 years, and 51.6% of our patients were 36 years or younger, this observation is a same as comparable to rates described by other investigators in Libya (El Mistiri et al, 2010) and Arab countries such as Egypt, Jordan (Zidan et al., 1997), Kuwait (Memon et al., 2002), Yemen (Abdulmughni et al., 2004). However in UK were rates peak in 35 to 39 year olds. Almost half (48%) of all cases occur in people aged less than 50 years (Cancer Research UK, 2011).

In our study, we observed that increased PTC in women compared with men (93.7%) (6.3%) respectively. In all geographic areas thyroid cancer is less common among males, with a female-to-male ratio ranging from 2:1 to 5:1 in most populations (Parkin et al., 2002). Gender bias in the incidence of thyroid cancer is well known, however, the underlying mechanism is largely unknown, Stanley JA et al. (2012) studied variations in the molecular characteristics of thyroid cancers between men and women. Testosterone levels in serum and thyroid cancer tissues were elevated in women while it decreased in men compared to respective control groups. Androgen receptor (AR) mRNA expression increased in a majority of men while it decreased in a majority of women except those with FTC. Their study suggested the varying pattern of testosterone level and AR status in thyroid tissues of men and women may predispose to the gender specific incidence of thyroid tumors and androgen mediated thyroid tumor growth. other explanation for disparities between males and females with sporadic thyroid cancers are biological sex differences where there is increased levels of female

hormones during reproductive years, due to pregnancy which increases TSH levels, potentially leading to thyroid dysplasia and then to cancer (Goodman et al., 1992; Negri et al., 1999; Memon et al., 2002; Zivaljevic et al., 2003). Experimental evidence shows that TSH stimulation of the thyroid gland can lead to proliferative changes of follicular cells including hypertrophy and hyperplasia, as well as ultimately neoplasia in rodents (Smith et al., 1987) .

In the current study, we found that 65.6% of PTC were classic papillary , which is comparable to previous Libyan finding (Sabratha cancer registry, 2006; El Mistiri et al., 2007).

Ki-67 is a useful tool for evaluating cell proliferative activity in various tumors. Although the utility of Ki-67 labeling index (LI) to diagnose thyroid neoplasm has been investigated, little is known regarding the relationship between Ki-67 LI and the biological behavior of papillary thyroid carcinoma. In this study, we examined Ki-67 in 32 patients with papillary thyroid carcinoma to elucidate this issue.It is know that the Ki-67 LI in PTC_s is lower than that in breast, lung, stomach and colon adenocarcinomas (Katoch et al., 1995). In this study we found low expression of Ki-67 protein, mean was 0.3 and 62.5% of patients were below mean (20/32). This observation is in aligment with study by Ito and Miyauchi (2012), who observed that In PTC, cell proliferating activity is generally low. The same observation was demonstrated by Ozolins et al. (2010), who reported the practical use of Ki-67 in PTC is difficult due to low values.

Ki-67 is aprotein expressed in the cell nuclei in all cells except those in the G0 phase(Cattoretti et al., 1992). In our result Ki-67 immunostaining gave brown granular nuclear reactivity, finely dispersed over the nucleus.

An interesting finding in our immunohistochemical studies is that the tumor arising from left lobe show proliferation index more than the right lobe ($p < 0.028$). No statistically significant correlation was found between Ki67 expression in tumors and other clinicopathological parameters (such as age, gender, histopathological type, tumor size, extrathyroid extension, multifocality, pre-existence hashimiotothyroiditis).

In this study, we examined the expression and localization of P53 protein in a subset of PTC, the results showed that a nuclear staining pattern. These results are in

general agreement with several studies because P53 protein is a DNA – binding protein localized to the nucleus (venot et al., 1998).

In current study the expression of p53 protein was low, mean value was 0.13, 75%of tumors were below mean(24/32), and This finding is consistent with finding of Ito et al. (1992), Donghi et al.(1993), Fagin et al.(1993), Zedenius et al.(1996), Park et al.(1998), Horie et al.(2001), Karlidag et al.(2007), they observed p53 immunoreactivity was present, faint (grade 1) staining in a small number of cells in PTCs, normal thyroid tissue had no immunoreactivity for p53. Although Dobashi et al. (1993), Chen et al. (1999), Naomi morital et al. (2008) reported the over expression of p53 protein in PTC.

Results from the present study indicate that the decreased P53 expression was significantly associated with tumor localization, tumor arising in both lobes show low expression of p53, also tumor arising in left lobe or in right lobe express low P53($p < 0,06$). However tumor express P53 associated with lymphatic invasion and show border line significant ($p < 0.08$).

No statistically significant correlation was found between P53 expression in tumors and other clinicopathological parameters (such as age, gender, histopathological type, tumor size, extrathyroid extension, multifocality, pre-existence hashimiotothyroiditis). Asimilar finding has been reported by Demet Corapcioglu et al. (2006). Also Szybińsk etal. (2001) reported no influence of expression on clinical course of the well differentiated thyroid carcinoma has been found. Worse prognoses have correlated with poor differentiated thyroid cancer cases and with strong expression of p53 protein.

Chapter VI

Summary and conclusions

6.Summary and conclusion

- Thyroid cancers are quite rare, accounting for only 1.5% of all cancers in adults and 3% of all cancers in children. The prognosis for papillary thyroid carcinoma is favorable.
- In Western Libya, thyroid cancers constitute 1.2% of all cancers reported from collective data.
- Among 32 patients involved in the current study 6.3 % were males and 93.7% females. The mean age of patients with PTC were 36 years.
- The tumor arising from left lobe show proliferation index (Ki-67) more than the right lobe ($p < 0.028$), also decreased P53 expression was significantly associated with tumor localization, tumor arising in both lobes show low expression of p53, also tumor arising in left lobe or in right lobe express low P53($p < 0,06$). However tumor express P53 associated with lymphatic invasion and show border line significant ($p < 0.08$).

Recommendations

- Establishment of electronic archives to facilitate collection of data for further studies in the future.
- Provision of the National cancer registry of Libya, to give more precise information.
- Provision of the National Guidline in histopathology reporting.
- The members of Oncology, Pathology, Surgon and Radiology must work as one team for more productive result.
- Iodine deficiency has been postulated to be one of the key factors in the rise of thyroid cancer cases. Salt iodization programs, thus, have been suggested as effective counteractive strategies.
- screening programme should be done for familial medullary thyroid cancers.
- Molecular testing is cost saving and should be done to cytologically indeterminate FNA because it predominantly lead to reduction in two-stage thyroidectomy.
- Further studies with larger numbers of patients to prove the significant effect of Ki-67 and P53 as markers of progression and prognosis among Libyan patients.

Chapter VII

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7.References

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جامعة بنغازى

كلية الطب

قسم علم الأمراض

دراسة عوامل التنبؤ (البروتينات القامعة للوارم ومؤشر تكاثر الخلايا) فى ورم الغدة الدرقية الحليمى وبيان تأثيرها على حياة المرضى

قدمت هذه الدراسة لقسم علم الأمراض استكمالا لنيل الإجازة العليا

اعداد

زينب مفتاح عبد الله بشير

بكالوريوس طب وجراحة

تحت أشراف

أ.د.عمران المهدى الفيتورى

أستاذ علم الأمراض-جامعة بنغازى/كلية الطب

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الملخص العربي

سرطان الغدة الدرقية الحليمى

الغدة الدرقية تقع تحت الغضروف الدرقى (تفاحة آدم) فى الجزء الأمامى من الرقبة .فى معظم الناس، لايمكن رؤية الغدة الدرقية أو الإحساس بها. وشكلها مثل الفراشة، وهى مكونة من فصين، فص أيمن وفص أيسر، يوصلهما ببعض برزخ ضيق.تحتوى الغدة الدرقية، بصفة رئيسية، نوعين من الخلايا,الخلايا الجرابية الدرقية، وخلايا سى (وتسمى أيضاً الخلايا نظيرة الجرابية),

حوالى 8 من كل 10 من سرطانات الغدة الدرقية هى ورم حليمى (يسمى أيضاً سرطان حليمى، ورم غدى سرطانى حليمى). الأورام الحليمية، عادة تنمو ببطء شديد، وهى تصيب فص واحد من الغدة الدرقية، ولكن أحياناً تصيب الفصين. رغم أنها تنمو ببطء شديد، ولكنها غالباً تنتشر إلى الغدد اللمفاوية فى العنق .ولكن فى معظم الأحيان يمكن علاجها ونادراً ما تشكل خطورة على المريض.
يمكن تمييز سبعة أنواع من الورم الحليمى تحت المجهر .من هذه النوع الجريبى (النوع الحليمى- الجريبى المختلط) وهو الأكثر حدوثاً, النوع العادى من الورم الحليمى والنوع الجريبى، لها نفس المظهر الإنذارى للبقاء، وتعامل بنفس الطريقة .الأنواع الأخر من الورم الحليمى ليست شائعة، وتميل إلى النمو والانتشار بسرعة أكبر.

ينتشر اكثر عن طريق الغدد الليمفاوية وعكس كل الاورام السرطانية الاخرى انتشاره الى الغدد الليمفاوية لا يعنى ضعف الفرصه فى الشفاء النهائى ولكن يجب استئصال الغدد الليمفاوية المتضخمة جراحيا سواء ظهرت مع المرض او فى صورة ثانويات متأخرة بعد علاجه (ارتجاع) ولا يمكن الاعتماد على اليود المشع فقط.وينتشر ايضا الى الرئة شائعة جدا خصوصا فى اورام الاطفال ويمكن شفاؤها باليود المشع لو كانت من النوع المتعدد الصغير الذى لا يظهر فى الاشعة العادية ولكن يظهر فى الاشعة المقطعية وفى هذه الحالة يحتاج يود مشع - إما ثانويات الرئة الوحيدة الكبيرة فيمكن استئصالها جراحيا , والأنواع المتعددة الكبيرة لا يمكن شفاؤها نهائيا ولكن يمكن السيطرة عليها بالعلاج باليود المشع المتكرر. العظام اقل شيوعا من الرئة , والمخ بنسبة اقل ويحمل نتائج سيئة وينصح بعلاجه باليود المشع واستعمال الهرمون المحفز للغدة المخلق صناعيا (المكلف جدا) حيث ان توقف هرمون الثيروكسين وخمول الغدة يزيد نشاط هذا النوع.

الصورة الاكلينيكية

- ورم صغير او عقيدة وحيدة - داخل الغدة
- غير مؤلم وأحيانا تظهر مع غدد ليمفاوية فى الرقبة
- ويكون متعدد البؤر داخل الغدة لذلك لا يمكن استئصال الورم فقط
- بالمقارنة بالأورام الخبيثة الاخرى يعتبر من افضلهم من حيث فرصة الشفاء التام مع العلاج السليم

العلامات التي تشير إلى صعوبة علاج هذا النوع

- السن اكبر من 40 سنة
- الورم اكبر من واحد ونصف سم
- انتشاره بالأنسجة المحيطة بالغدة ويتطلب استئصال الغدد الليمفاوية والأنسجة المحيطة المصابة واستكشاف الرقبة بالكامل فى ناحية الورم
- الانتشار البعيد - الرئة - العظام
- الرجال لهم نتائج اسوأ من السيدات لان الورم ينتشر اسرع فى الرجال

جدير بالذكر ، نجد أن معدل الاصابة في المملكة العربية السعودية و دول الخليج مشابهة لمعدل الاصابة في كل من : أميركا ، أوربا ، و استراليا.

يختلف سرطان الغدة الدرقية، عن كثير من سرطانات البالغين، فى أنه غالباً ما يتم تشخيصه فى أشخاص أصغر سناً .حوالى 2 من كل 3 حالات يتم تشخيصها فى أشخاص أعمارهم بين 20 إلى 55 سنة. ارتفعت فرص تشخيص سرطان الغدة الدرقية، وقد تضاعفت فى الفترة الأخيرة إلى مرتين عما كانت عليه عام 1990م .قد يرجع سبب هذه الزيادة، ربما إلى استعمال الموجات فوق الصوتية فى الفحص، والتى تظهر العقد الدرقية الصغيرة التى لم يكن ممكناً رؤيتها.

عوامل المخاطرة لسرطان الغدة الدرقية

لأسباب غير معروفة، فإن سرطان الغدة الدرقية (مثل كل أمراض الغدة الدرقية الأخرى الغير سرطانية) تصيب النساء بمعدل 3 مرات أكثر من الرجال.سرطان الغدة الدرقية يمكن أن يحدث فى أى عمر، ولكن أغلب حالات الإصابة بين النساء هى بين 45 و 49 عاماً. أما بالنسبة للرجال فهى بين 65 و 69 عاماً. إن الغذاء الفقير لليود، يزيد المخاطرة للإصابة بسرطان الورم الجريبى. التعرض للإشعاع من عوامل المخاطرة المثبتة، للإصابة بسرطان الغدة الدرقية. مصادر هذا الإشعاع تتضمن بعض العلاجات الطبية، وتسربات حوادث محطات الطاقة، والأسلحة النووية.
إذا تمت معالجة الرأس أو الرقبة بالإشعاع فى الطفولة،فهذا عامل مخاطرة للإصابة بسرطان الغدة الدرقية. تعتمد المخاطرة على كمية الإشعاع المستعملة وعمر الطفل. وعموماً فالخطورة تزيد كلما زادت كمية الإشعاع، وأيضاً تزيد كلما قل عمر الطفل عند العلاج. وعلاج بعض السرطانات، مثل الورم اللمفاوى، ورم ولمز، الأورام الجذعية العصبية، بالإشعاع فى فترة الطفولة، يرفع مخاطرة الإصابة بسرطان الغدة الدرقية. سرطانات الغدة الدرقية المرتبطة بعلاج إشعاعى سابق، ليست أكثر خطورة من سرطانات الغدة الدرقية الأخرى.

علامات وأعراض سرطان الغدة الدرقية

الانتباه الشديد للعلامات والأعراض هو أفضل طريقة لتشخيص سرطان الغدة الدرقية مبكراً. يمكن لسرطان الغدة الدرقية أن يسبب أى من هذه العلامات والأعراض:

عقدة، مرتفع (يشبه الانتفاخ)، أو ورم فى العنق، أحياناً ينمو سريعاً, ألم فى مقدمة العنق، أحياناً يمتد إلى الأذنين, خشونة أو بحة فى الصوت، وتكون مستمرة, صعوبة فى البلع, صعوبة فى التنفس (الشعور بأن الشخص يتنفس عبر قشة), سعال مستمر، بدون الإصابة بالبرد.

علاج سرطان الغدة الدرقية

- عادة ما يشخص عن طريق أخذ خزعة بالإبرة من الغدة أو استئصال جزء من الغدة الدرقية
- بعد أخذ العينة وفحصها تشكل نسبة تشخيص السرطان 1-5 % فقط
- تحدد العينة نوع السرطان وبالتالي طريقة علاجه
- عادة ما يتكون العلاج من استئصال كامل الغدة الدرقية مع أو بدون تجريف العقد اللمفاوية (بعد فحصها أثناء العملية)
- في حالات السرطان الحليمي أو المسامي، يتم تعريض المريض للعلاج باليود المشع بعد العملية بـ 4-6 أسابيع، وذلك عن طريق تناول كبسولات بالفم يتم حسابها لكل مريض حسب حالته) والذي يقوم بالقضاء على أي خلايا درقية بالجسم. يمنع المريض من الاختلاط بالناس لمدة يومين بعد تناول اليود المشع وذلك لحين التخلص منه عن طريق الفضلات.
- يتم بعد ذلك إعطاء المريض جرعة يومية مرتفعة من هرمون الغدة الدرقية بحيث تثبط إفراز الهرمون المحفز للغدة الدرقية TSH
- يجب عدم التوقف عن تناول الهرمون إلا بتوجيه من الطبيب (عادة قبل إجراء التصوير باليود المشع بأربعة أسابيع)، وهذا الهرمون هو دواء بسيط وليس له أضرار على الجسم ولا يمكن العيش بدونه (لأن الغدة قد استؤصلت)
- يتم عمل فحص للدم كل 6-12 شهر لقياس مستوى الهرمون وتعديل الجرعة.
- السرطان الجذعي لا يتأثر باليود المشع لذلك تشكل الجراحة العلاج الوحيد ويجب إجراؤها بدقة متناهية وتتكون من استئصال الغدة الدرقية وتجريف الرقبة من العقد اللمفاوية على الجانبين ومن منطقة النحر.
- السرطان مشوه النسج يعتبر من الأورام المتقدمة جدا والذي عادة لا ينفع معه أي علاج لأنه يؤدي بحياة المريض خلال 6 أشهر إلى سنة، ولذلك يقتصر علاجه على علاج الأعراض

العلاج الكيميائي

يتميز سرطان الغدة الدرقية بأنه السرطان الوحيد الذي يمكن القضاء عليه دون الحاجة للعلاج الكيميائي, ويعود السبب لتوفر علاج يسمى اليود المشع وهو عبارة عن مادة اليود ولكنها معالجة طبيا بحيث تكون سريعة الانشطار (مشعة) وبالتالي تتسبب في موت الخلايا التي تستقطبها وبما أن خلايا الغدة الدرقية هي الوحيدة في الجسم التي لديها خاصية استقطاب اليود فهي الوحيدة التي ستتأثر باليود المشع، دون الحاجة للعلاج الكيميائي لأنه يؤثر على جميع خلايا الجسم دون تفريق. يجب أن نتذكر أن اليود المشع هو علاج أمن جدا ولذلك إذا كان المريض في حاجته فيجب تناوله بدون تردد.

ملخص البحث

هدف هذه الدراسة البحثية هى تسليط الضوء على عوامل التنبؤ فى سرطان الغدة الدرقية الحليمى بواسطة الكشف عن البروتينات القامعة للورم(P53) ومؤشر تكاثر الخلايا(Ki67) وتقييم دورها فى حالات مرضى سرطان الغدة الدرقية الحليمى فى ليبيا وتقييم علاقة هذه البروتينات بعوامل التنبؤ التقليدية وأثرها على حياة المريض.

المرضى وطرق الدراسة

تم اخذ 32 مريض من مرضى سرطان الغدة الدرقية الحليمى فى ليبيا من واقع سجلات قسم علم الامراض بجامعة بنغازى وقد تم صبغ هذه العينات بالبروتين القامعة للورم (P53) ومؤشر تكاثر الخلايا(Ki67) واختبار هذه العينات احصائيا.

النتائج

وجدت علاقة بين مؤشر تكاثر الخلايا و سرطان الغدة الدرقية الموجود فى الفص الايمن. وكذلك وجدت علاقة بين البروتين القامعة للورم (P53) ومكان الورم ,وأيضا مع الورم الذى ينتشر الى الجهاز الليمفاوى. وعلى الرغم ان عدد حالات دراستنا كان صغير ,إلا انه يمكن استخدام البروتين القامعة للورم و مؤشر تكاثر الخلايا لتنبؤ بمستقبل المريض.