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PAPILLARY THYROID CANCER GENDER DISPARITY

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SOMMARIO/SUMMARY

SOMMARIO/RIASSUNTO

Il carcinoma papillare della tiroide (PTC) è il più frequente tipo di cancro differenziato della tiroide, e rappresenta circa l'80% dei casi. Esso ha un'incidenza di circa tre volte maggiore nelle donne rispetto agli uomini e rappresenta il settimo cancro per frequenza nella donna mentre nell'uomo esso è al quindicesimo posto. Tale aumento di incidenza esiste solo durante l'età fertile, difatti dopo gli 80 anni di età, non si evidenzia più una differenza per quanto riguarda l'incidenza di questo tumore tiroideo tra i due sessi.

Recentemente è stato ipotizzato un ruolo degli ormoni sessuali, in particolare degli estrogeni, nell'induzione della tumorigenesi per lo sviluppo del cancro tiroideo, ma i meccanismi che regolano questi eventi non sono ancora stati ben identificati [1-6].

I recettori degli steroidi sono caratterizzati da un'elevata affinità e specificità in rapporto ai loro leganti. Il recettore dell'estrogeno umano (ER) è una proteina dimerica con peso molecolare di 65 kDa, localizzata principalmente sulla membrana del nucleo cellulare e appartenente a una classe di proteine attivatrici, che stimolano cioè la trascrizione legandosi a elementi specifici del DNA, detti anche elementi di risposta ormonale.

Legandosi con l'estrogeno, il recettore ER è indotto a stimolare la trascrizione genica; per questo motivo è noto anche come fattore enhancer inducibile.

Studi storici hanno dimostrato che lo stato di ER è correlato direttamente con la prognosi (ad es. positivamente nel tumore della mammella invasivo ben differenziato) e alla risposta alla terapia anti-ormonale, ad es., tamoxifen.

È stato riscontrato che gli estrogeni sono principalmente concentrati negli organi bersaglio degli estrogeni di animali e nei tumori umani della mammella ed è ben documentato che gli effetti mitogenici dell'estrogeno sono mediati dall'ER. Indagini eseguite riguardo i meccanismi biologici per la crescita del tumore della mammella hanno dimostrato che la velocità della crescita dipende dalla presenza di estrogeni o progesterone o di entrambi in gran parte dei tumori della mammella. Pertanto, lo stato del recettore estrogeno nei carcinomi mammari è considerato uno strumento di previsione convalidato e un fattore predittivo per il trattamento della paziente con terapia anti-ormonale.

Lo scopo del nostro studio è stato quello di analizzare i pazienti sottoposti a chirurgia per PTC e confrontare le femmine (F) vs i maschi (M) per quanto riguarda le variabili cliniche ed istopatologiche per determinare se, come nel carcinoma mammario, vi sono delle differenze significative e vi sia effettivamente un ruolo degli estrogeni nella patogenesi del PTC.

In una piccola quota di pazienti è stata valutata la presenza di recettori per gli estrogeni sul pezzo operatorio per poter eventualmente identificare anche la possibilità di una terapia medica oncologica mirata per questo tipo di tumore. La ricerca di tali recettori è stata fatta per valutare quali recettori erano presenti e in che quantità e per determinare se la presenza o l'assenza di questi determinava un impatto sulla prognosi.

Abbiamo considerato 658 pazienti sottoposti a tiroidectomia per PTC dal 2007 al 2011 presso la Clinica Chirurgica II dell'Università degli Studi di Padova e l'Unità Operativa Complessa di Chirurgia Generale dell'Università degli Studi di Trieste. Abbiamo analizzato retrospettivamente i dati clinici ed istopatologici e abbiamo confrontato F vs M per quanto riguarda le seguenti variabili: età, estensione della chirurgia, tipo di dissezione linfonodale, stadi azione sec. TNM, mono/plurifocalità, l'associazione con tiroidite cronica, la presenza di mutazione BRAF ed il follow-up. Inoltre nelle donne sono state analizzate, l'età del menarca e della menopausa, il numero e l'età delle gravidanze, le eventuali interruzioni di gravidanza e la terapia estro-progestinica. Un valore di p inferiore a 0.05 è stato considerato statisticamente significativo.

Di 10 pazienti selezionati abbiamo analizzato il livello di estrogeni nel siero perioperatorio e l'espressione dei recettori ER-alpha ed ER-beta mediante studi immunoistochimici per la rilevazione semi-quantitativa del recettore dell'estrogeno umano in sezioni di tessuto fissate in formalina, incluse in paraffina di tumore della tiroide.

Questi 10 pazienti, tutte femmine, sono stati suddivisi in due gruppi da cinque in base all'età maggiore o minore di 40 anni e questi due gruppi sono stati confrontati tra di loro. Confrontando F vs M abbiamo riscontrato una differenza statisticamente significativa per quanto riguarda il sesso, difatti le donne sono state il 74% mentre il sesso maschile ha rappresentato il 26% dei casi ($p < 0,015$) e l'associazione con tiroidite cronica autoimmune più frequente nelle donne.

La necessità di eseguire una dissezione linfonodale laterocervicale è stata maggiore nel sesso maschile, mentre non è stata riscontrata differenza statisticamente significativa riguardo il tasso di recidiva tra i due sessi.

Per quel che concerne lo studio del profilo ormonale nelle donne abbiamo riscontrato che uno stadio relativamente più avanzato era correlato con un menarca precoce, età più avanzata della prima gravidanza e menopausa tardiva.

Le femmine sono state 489 (74%) vs 169 maschi (26%) ($p < 0,015$), l'età media è stata di 46 anni (range 11-86) nelle F vs 46 anni (range 11-83) nei M ($p < 0,44$). La tiroidectomia totale è stata effettuata in 474 F (97%) mentre è stata effettuata in 161 M (95%) ($p < 0,72$). La dissezione linfonodale è stata realizzata in 387 (79%) F vs 132 (78%) M ($p < 0,99$). La linfadenectomia del compartimento centrale è stata effettuata in 340 (88%) F vs 94 (71%) M ($p < 0,75$) mentre la dissezione laterocervicale è stata eseguita in 47 (12%) F vs 38 (29%) M ($p < 0,011$).

Per quanto concerne la stadiazione: 315 (64%) F vs 87 (51,5%) M ($p < 0,19$) presentavano uno stadio I di neoplasia, 15 (3%) F vs 4 (2,5%) M ($p < 0,51$) uno stadio II, 126 (26%) F vs 54 (32%) M ($p < 0,24$) uno stadio III mentre 33 (7%) F vs 24 (14%) M ($p < 0,22$) avevano uno stadio IV.

Il tumore era monofocale in 279 (57%) vs F 89 (52,7%) M ($p < 0,64$), mentre era plurifocale in 210 (43%) F vs 80 (47,3%) M ($p < 0,76$).

L'associazione con tiroidite cronica autoimmune era presente in 132 F (27%) e 25 M (15%) ($p < 0,001$). Per quanto riguarda la mutazione BRAF, la mutazione V600E è stata identificata in 143 (29%) F vs 52 (30,7%) M ($p < 0,84$).

Il follow-up è stato possibile in 395 casi. Il tempo medio di follow-up è stato di 37 mesi (range 1-343) nelle femmine rispetto a 45 mesi (range 4-156) nei maschi ($p < 0,24$). 251 F (88%) si sono sottoposte a I^{131} radioiodioterapia vs 91 M (83%) ($p < 0,74$) e la dose media è stata di 132.63 mCi (range 50-500) nelle F vs 139.42 mCi (range 50-350) nei M ($p < 0,27$).

I livelli medi di Tireoglobulina (Tg) erano 7 mU/L (range 0,1-485.3) nelle F vs 19.83 (range 0,1-593) nei M ($p < 0,11$). 274 F (96%) vs 102 M (93.5%) erano libere da malattia al follow-up ($p < 0,64$). Abbiamo riscontrato una differenza statisticamente significativa per quel che riguarda il numero di pazienti, la dissezione linfonodale laterocervicale e l'associazione con tiroidite cronica autoimmune.

Riguardo all'espressione dei recettori per gli estrogeni sul pezzo operatorio, sono stati studiati 10 pazienti, con un'età media di 48 anni (range: 31-77) Il follow-up medio è stato di 3 mesi (range 1-12). 10 preparati chirurgici tiroidei sono stati analizzati con tecnica immunoistochimica e sono stati testati gli anticorpi per gli estrogeni alpha e beta.

I recettori per gli estragoni erano presenti nella maggior parte del campione (80%) e non c'erano nel tessuto peritumorale.

In conclusione, l'identificazione dei meccanismi di carcinogenesi del PTC nella donna rispetto all'uomo rappresenta un passo veramente importante ed innovativo per lo studio del carcinoma tiroideo, in particolare il ruolo degli estrogeni nello sviluppo del PTC, una volta identificati i precisi meccanismi e steps della tumori genesi, potrebbe aprire la strada, come nel cancro mammario, per una potenziale nuova terapia medica antirecettoriale.

SUMMARY

Cancer gender disparity in incidence, clinical presentation, disease aggressiveness, and prognosis has been observed for a variety of cancers

The more aggressive types of thyroid cancer, anaplastic thyroid cancer and medullary thyroid cancer have similar incidence in men and women. Meanwhile, differentiated thyroid cancer of follicular cell origin, such as follicular thyroid cancer and papillary thyroid cancer are more common in women.

Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer, accounting for approximately 80% of cases. Its incidence has nearly doubled over the last 30 years and is thought to be due in part to earlier diagnosis of subclinical disease.

The rate of PTC among woman is nearly three times higher than men but in most studies males gender is associated with a lower disease free survival and higher mortality [1-6].

Our intention was to consider our experience about the gender disparity in patients underwent surgery for PTC comparing females (F) vs males (M) as regards a series of clinical, histopathologic, molecular, and hormonal variables to determine if, as in breast carcinoma , there is actually a role of estrogens in the pathogenesis of PTC.

Then, in a little series of patients we evaluated the presence of estrogen receptors on the surgical specimen to identify the eventual medical targeted therapy for this type of tumor.

We considered 658 patients underwent surgery for PTC from 2007 to 2011 at the Institute of 2nd Clinical Surgery , University of Padova, and General Surgery Department,

University of Trieste. We revised clinical and histopathologic documents and we compared F vs M as regards the following variables: age, extension of surgery, node dissection, TNM, mono/plurifocality, BRAF mutations, and outcome. A p value less than 0.05 was considered statistically significant.

In 10 selected patients, we analyzed the expression of ER- alpha receptors and ER –beta by immunohistochemical studies in the sections of formalin-fixed tissue , paraffin-embedded

tumor of the thyroid. We have divided patients in two categories: < 40 years old and > 40 and then we compared the two groups.

Comparing F vs M we observed: the F was 489 (74%) vs 169 M (26%) ($p < 0.015$), the mean age was 46 years (range 11-86) in F vs 46 years (range 11-83) in M ($p = < 0.44$), total thyroidectomy was realized in 474 F (97%) vs 161 M (95%) ($p < 0.72$), lobectomy was realized in 13 females (3%) and 8 males (5%) ($p < 0.83$), node dissection was realized in 387 (79%) F vs 132 (78%) M ($p < 0.99$), central node dissection in 340 (88%) F vs 94 (71%) M ($p < 0.75$), laterocervical node dissection in 47 (12%) F vs 38 (29%) M ($p < 0.011$), stage I was in 315 (64%) F vs 87 (51.5%) M ($p < 0.19$), stage II in 15 (3%) F vs 4 (2.5%) M ($p < 0.51$), stage III in 126 (26%) F vs 54 (32%) M ($p < 0.249$), stage IV in 33 (7%) F vs 24 (14%) M ($p < 0.223$), monofocality was in 279 (57%) F vs 89 (52.7%) M ($p < 0.64$), plurifocality was in 210 (43%) F vs 80 (47.3%) M ($p < 0.76$), tumor size was 14.13 mm (range 0.5-80), it was 13.97 mm (range 1-80) in F vs 14.62 (range 0.5-70) in M ($p < 0.42$), association with thyroiditis was observed in 157 cases, in 132 (27%) F vs 25 (15%) M ($p < 0.001$), BRAF – V600E mutation was identified in 143 (29%) F vs 52 (30.7%) M ($p < 0.849$).

We revised the follow up in 395 cases. The mean time of follow up was 37 months (range 1-343) in F vs 45 months (range 4-156) in M, 251 (88%) F underwent I131radioiodinetherapy vs 91 (83%) M ($p < 0.74$), the median dose was 132.63 mCi (range 50-500) in F vs 139.42 mCi (range 50-350) in M ($p < 0.27$), the median time of follow up was 37 months in F and 45 months in M ($p < 0.24$), the median value of Tiroglobulin (Tg) was 7 mU/L (range 0.1-485.3) in F vs 19.83 (range 0.1-593) in M ($p < 0.11$), F was free of disease in 274 (96%) vs 102 (93.5%) M ($p < 0.64$), 2 patients deceased, 1 was F (0.2%) vs 1 (1%) M ($p < 0.24$).

Regarding the expression of estrogen receptors in thyroid tissue, 10 patients, with a median age of 48 years (range: 31-77) were analyzed. Mean follow-up time was 3 months (range 1-12). 10 thyroid glands specimens have been examined using immunohistochemical assays with ERs antibodies. We did not find any significant difference in the incidences of positive staining for E2 between the two groups.

There was no expression of antibodies in non-neoplastic cells or in adjacent tissues.

In conclusion, the identification of mechanisms of carcinogenesis of PTC in women vs men is a really important and innovative step for the study of thyroid cancer.

The estrogen hormones may play an important role as a promoting factor in the development of PTC. The role of estrogen in the progress of PTC, once identified the

precise mechanisms and steps of tumorigenesis, could open up areas, as in breast cancer, of developing potential new medical antireceptorial therapies to prevent and treat thyroid malignancies. This indication needs further study.

INTRODUCTION

Thyroid cancer is the most common and prevalent of all endocrine-based malignancies accounting for more than 95% of all endocrine-related cancers [1]. Thyroid carcinomas can be classified as two groups based on their morphological variations: the differentiated cancers as papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), and medullary thyroid carcinoma (MTC) and on the other hand undifferentiated cancers as anaplastic thyroid carcinoma (ATC) and thyroid lymphoma and sarcoma [1-3].

PTC and FTC are both classified as differentiated thyroid carcinomas and represent 90–95% of thyroid cancers [1-3]. PTC, ATC and FTC arise from thyroid follicular cells, while MTC arises from C-cells (parafollicular cells). Treating advanced metastatic all thyroid cancer remains one of the greatest challenges in cancer research. I^{131} is an effective treatment for advanced thyroid cancer; however, its uptake is too low or absent in many cases in particular when the cancer is more aggressive and in addition the individual responses to radioiodine therapy is highly variable. Therefore, there is a pressing need for new treatment options for thyroid cancer and to be able to do so, increased knowledge about the biology and pathogenesis of thyroid cancer, as well as the understanding of various factors that contribute to thyroid cancer risk and development, is critical.

1.1.PAPILLARY THYROID CARCINOMA

Papillary thyroid carcinoma is the most common type of thyroid cancer,[1-2] representing 75 percent to 85 percent of all thyroid cancer cases [3]. It occurs more frequently in women and presents in the 20–55 year age group. It is also the predominant cancer type in pediatric age, and in patients with thyroid cancer who have had previous radiation to the head and neck [4].

Lymphatic spread is more common than hematogenous spread and also the multifocality is common.

Although papillary carcinoma has a propensity to invade lymphatics vessels, it is less likely to invade blood vessels. These types of tumors are most commonly encapsulated but sometimes have more aggressive pattern and may present thyroid capsule invasion. They have a high tendency to metastasize locally to cervical lymph nodes, which may produce cystic structures near the thyroid that are difficult to diagnose because of the paucity of malignant tissue. Furthermore, papillary tumors may metastasize to the lungs and produce a few nodules or the lung fields may exhibit a snowflake appearance throughout.

Moreover, the overall survival is very good but papillary carcinomas can have also an indolent growth, and 40 percent of cases spread out of the capsule.

Mutations associated with papillary thyroid cancer are mainly two forms of chromosomal translocation and one form of point mutation. These alterations lead to activation of a common carcinogenic pathway—the MAPK/ERK pathway [2].

Among different genetic factors involved in the pathogenesis of the PTC, rearrangements of RET protooncogene (RET/PTC), as well as rearrangements of NTRK1 protooncogene are best known. The resulting hybrid oncogenes are found in PTCs with variable frequency, depending on the examined population. The relationship between these chromosomal aberrations and clinical outcome of PTCs remains still controversial.

Chromosomal translocations involving the RET proto-oncogene (encoding a tyrosine kinase receptor that plays essential roles in the development of neuroendocrine cells) located on chromosome 10q11 occur in approximately a fifth of papillary thyroid cancers. The fusion oncoproteins generated are termed RET/PTC proteins (ret/papillary thyroid carcinoma), and constitutively activate RET and the downstream MAPK/ERK pathway [2].

The frequency of RET/PTC translocations is significantly higher in papillary cancers

arising in children and after radiation exposure. The gene NTRK1 (encoding the TrkA receptor), located on chromosome 1q, is similarly translocated in approximately 5 percent to 10 percent of papillary thyroid cancers [2].

Approximately a third to a half of papillary thyroid carcinomas harbor point mutations in the BRAF oncogene, also activating the MAPK/ERK pathway [2]. In those cases the BRAF mutations found were V600E mutation. After performing a multivariate analysis, it was found that the absence of tumor capsule was the only parameter associated ($P=0.0005$) with BRAF V600E mutation. According to recent studies, papillary cancers carrying the common V600E mutation tend to have a more aggressive long term course. BRAF mutations are frequent in papillary carcinoma and in undifferentiated cancers that have developed from papillary tumors.

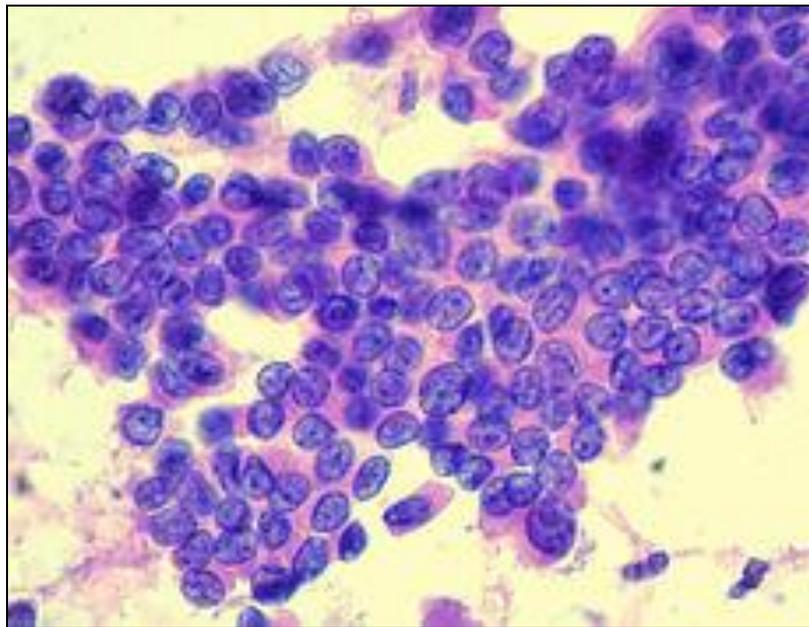


Figure 1. Papillary Thyroid Carcinoma

Papillary thyroid carcinoma is the most common thyroid cancer. About 80% of all thyroid cancers cases are papillary thyroid cancer.

PTC is more common in women than in men. It may occur in childhood, but is most often seen in adults between ages 30 and 50.

The cause of this cancer is unknown. A genetic defect may be involved.

- *Risk factors in Papillary Thyroid Carcinoma*

Radiation increases the risk of developing thyroid cancer. Exposure may occur from:

- High-dose external radiation treatments to the neck, especially during childhood, used to treat childhood cancer or some non-cancerous childhood conditions
- Radiation exposure from nuclear plant disasters

Radiation given through a vein (through an IV) during medical tests and treatments does not increase the risk of developing thyroid cancer.

- *Clinical presentation of Papillary Thyroid Carcinoma*

Regarding the clinical presentation, papillary carcinoma typically arises as an irregular, solid or cystic mass that comes from otherwise normal thyroid tissue. This cancer has a high cure rate with 10-year survival rates for all patients with papillary thyroid cancer estimated at 80% to 90%. Cervical metastasis are present in 50% of small papillary carcinomas and in more than 75% of the larger papillary thyroid carcinomas.

The presence of lymph node metastasis causes a higher recurrence rate but not a higher mortality rate.

Distant metastasis is uncommon, but lung and bone are the most common sites if the papillary carcinoma does spread. Tumors that invade or extend beyond the thyroid capsule have a much worse prognosis because of a high local recurrence rate.

Thyroid cancer usually begins as a small lump (nodule) in the thyroid gland, which is located at the center of the front of the neck.

While some small lumps may be cancer, most (90%) thyroid nodules are harmless and are not cancerous.

Most of the time, there are no other symptoms.

- *Characteristics of Papillary Thyroid Carcinoma*

- Peak onset ages are 30 to 50 years old
- Papillary thyroid cancer is more common in females than in males by a 3:1 ratio
- The prognosis directly related to tumor size (less than 1.5 cm [1/2 inch] is a good prognosis)
- This cancer accounts for 85% of thyroid cancers due to radiation exposure
- In more than 50% of cases, it spreads to lymph nodes of the neck
- Distant spread (to lungs or bones) is uncommon
- The overall cure rate is very high (near 100% for small lesions in young patients)

- *Diagnosis of Papillary thyroid carcinoma*

Thyroid function tests are usually normal in patients with thyroid cancer.

The diagnosis is or clinical by palpation of thyroidal mass or by individuation of cervical node metastasis.

The suspicion of cancer presence can be confirmed by cervical ultrasound.

If the cervical ultrasound shows that the lump is bigger than 1.0 centimeter, a special procedure called a fine needle aspiration biopsy (FNAB) will be performed. This test helps determine if the thyroid's nodule or lump is cancerous.

The management of patients with any thyroid nodules are illustrated in the table 1.

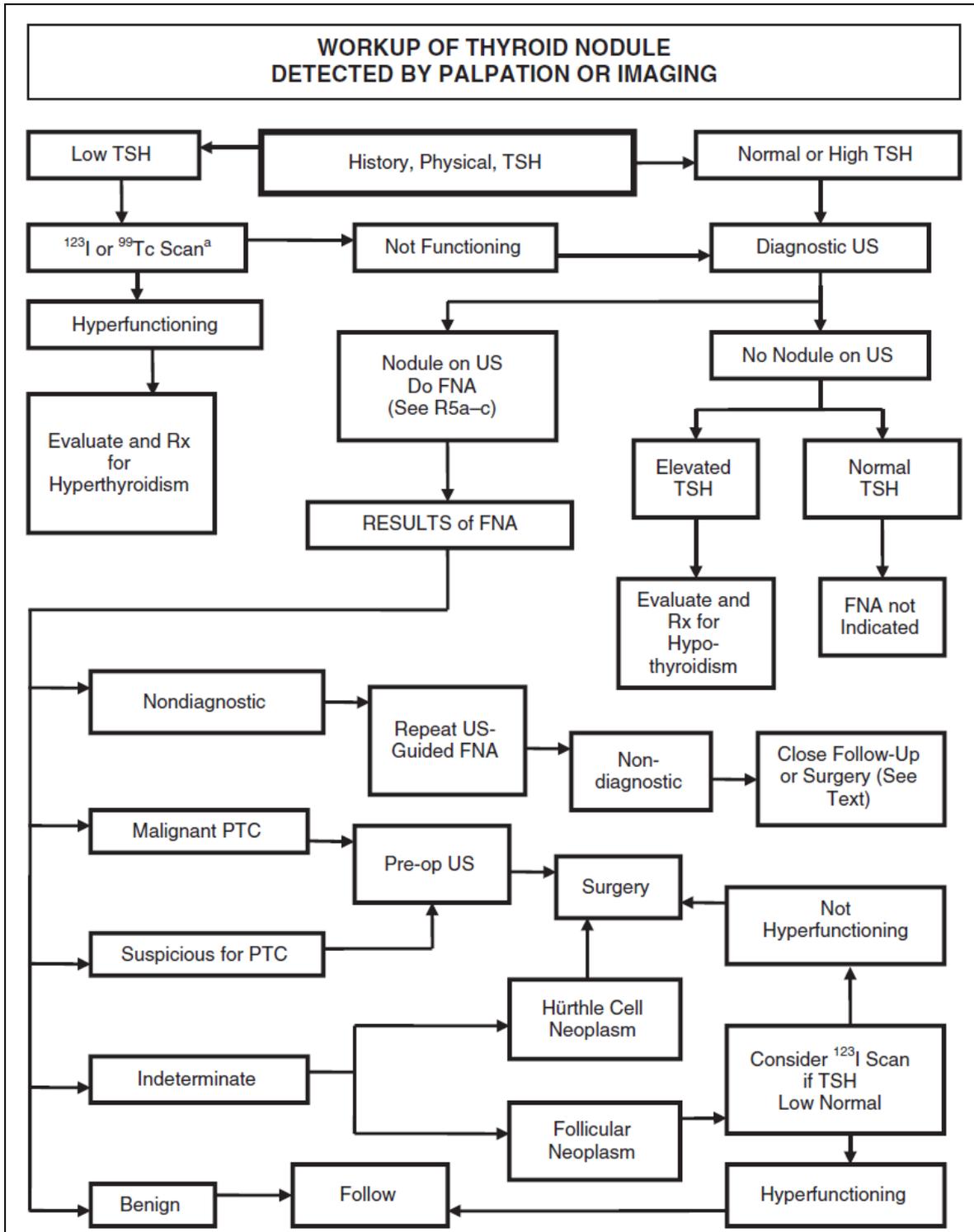


Table 1. Revised American Thyroid Association Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer. THYROID Volume 19, Number 11, 2009.

- *Management of Papillary Thyroid Carcinoma*

There are three types of thyroid cancer treatment:

- Surgery
- Radioactive iodine
- Medical treatment

Surgery is done to remove as much of the cancer as possible.

Considerable controversy exists when discussing the surgical management of well-differentiated thyroid carcinomas (papillary and even follicular). Some Authors suggest the hemithyroidectomy one lobe where are located the tumor. Several experts and the major Surgical Guidelines contend that if these tumors are small and not invading other tissues the intervention indicates is the total thyroidectomy.

In experienced hands, the incidence of recurrent nerve injury and permanent hypoparathyroidism are quite low (about 2%).

Total thyroidectomy and removal of any enlarged lymph nodes in the central or lateral neck areas are the best surgical approach to cure the PTC.

- *Major possible complications after surgery:*

- Hypoparathyroidism or transient or definitive: due to temporary devascularization of the parathyroid glands or due to accidental removal of the parathyroid gland which helps regulate blood calcium levels
- Laryngeal (recurrent) nerve palsy: due to damage to a nerve that controls the vocal cords

If surgery is not an option, external radiation therapy can be useful.

- Table 2. TNM Staging in Thyroid Carcinoma

Stage grouping			
Separate stage groupings are recommended for papillary or follicular (differentiated), medullary, and anaplastic (undifferentiated) carcinoma			
<i>Papillary and follicular thyroid cancer (age < 45y):</i>			
Stage	T	N	M
I	Any T	Any N	M0
II	Any T	Any N	M1
<i>Papillary and follicular; differentiated (age ≥ 45y):</i>			
Stage	T	N	M
I	T1	N0	M0
II	T2	N0	M0
III	T3	N0	M0
IVA	T1-3	N1a	M0
	T4a	N1b	M0
IVB	T4b	Any N	M0
IVC	Any T	Any N	M1
<i>Anaplastic carcinoma (all anaplastic carcinomas are considered stage IV):</i>			
Stage	T	N	M
IVA	T4a	Any N	M0
IVB	T4b	Any N	M0
IVC	Any T	Any N	M1
<i>Medullary carcinoma (all age groups):</i>			
Stage	T	N	M
I	T1	N0	M0
II	T2, T3	N0	M0
III	T1-T3	N1a	M0
IVA	T4a	N0	M0
	T4a	N1a	M0
	T1	N1b	M0
	T2	N1b	M0
	T3	N1b	M0
	T4a	N1b	M0
	T4a	N0, N1b	M0
	T1-T4a	N1b	M0
IVB	T4b	Any N	M0
IVC	Any T	Any N	M1

- Radioactive Iodine therapy in Papillary Thyroid Carcinoma

Thyroid cells have the cellular mechanism to absorb iodine. The iodine is used by thyroid cells to make thyroid hormone. No other cell in the body can absorb or concentrate iodine. Physicians can take advantage of this fact and give radioactive iodine to patients as a treatment option for papillary thyroid cancer.

After the surgery, most patients receive radioactive iodine, which is usually taken by mouth. This substance kills any remaining thyroid tissue. It also helps make medical images clearer, so doctors can see if there is any cancer left behind or if it comes back later.

There are several types of radioactive iodine, with one type being toxic to cells. Papillary thyroid cancer cells absorb iodine; therefore, they can be destroyed by giving the toxic isotope (I-131). Again, not everyone with papillary thyroid cancer needs this treatment, but those with larger tumors, tumors that have spread to lymph nodes or other areas, tumors that are aggressive microscopically, and older patients, may benefit from this treatment.

This is an extremely effective type of "chemotherapy" with little or no potential downsides (eg, no hair loss, nausea, or weight loss).

Uptake is enhanced by high thyroid-stimulating hormone (TSH) levels; thus, patients should be off thyroid replacement and on a low iodine diet for at least 1 to 2 weeks before being treated with radioiodine. It is usually given 6 weeks after surgery.

- Thyroid Hormone Replacement after thyroidectomy for Papillary Thyroid Cancer

Regardless of whether a patient has just one thyroid lobe and the isthmus removed, or the entire thyroid gland removed, most experts agree they should be placed on thyroid hormone replacement for the rest of their lives. This replaces the hormone in those who have no thyroid left, and to suppress further growth of the gland in those with some tissue left in the neck.

After surgery or radioactive iodine, patients will need to take medication called levothyroxine sodium for the rest of their life. This replaces the hormone the thyroid would normally make.

There is good evidence that papillary carcinoma responds to TSH secreted by the pituitary, therefore, exogenous thyroid hormone is given, which results in decreased TSH levels and a lower impetus for any remaining cancer cells to grow. Recurrence and mortality rates have been shown to be lower in patients receiving suppression.

- *Long-term Follow-up in PTC*

Most patients who had thyroid cancer need to have a blood test every 6 - 12 months to check thyroid levels. Other follow-up tests that may be done after treatment for thyroid cancer include:

- Ultrasound of the thyroid
- An imaging test called a radioactive iodine (I-131) uptake scan

In addition to the usual cancer follow up, patients should undergo serial checks of thyroglobulin levels. Thyroglobulin is not useful as a screening for initial diagnosis of thyroid cancer, but it is quite useful in follow up of a well-differentiated carcinoma (if a total thyroidectomy has been performed). A high serum thyroglobulin level that had previously been low following total thyroidectomy, especially if gradually increased with TSH stimulation, is virtually indicative of recurrence. A value of greater than 10 ng/ml is often associated with recurrence even if an iodine scan is negative.

- *Prognosis of Thyroid Papillary carcinoma*

The survival rate for papillary thyroid cancer is excellent. More than 95% of adults with this cancer survive at least 10 years. The prognosis is better for patients who are younger than 40 and for those with smaller tumors.

The following factors may decrease the survival rate:

- Age over 45
- Cancer that has spread to distant parts of the body
- Cancer that has spread to soft tissue
- Large tumor

1.2 GENDER DIFFERENCES IN THYROID CANCER

The gender difference in cancer susceptibility is one of the most consistent findings in cancer epidemiology.

Cancer gender disparity in incidence, clinical presentation, disease aggressiveness, and prognosis has been observed for a variety of cancers [1, 5-9].

Thyroid cancer is the most common malignancy of the endocrine system and the seventh most common malignancy in women, but it is not among the most common 15 cancer in men [10,11].

The gender disparity in thyroid cancer is also specific to the histologic subtype of thyroid cancer. The more aggressive types of thyroid cancer, anaplastic thyroid cancer and medullary thyroid cancer have similar incidence in men and women. Meanwhile, differentiated thyroid cancer of follicular cell origin, such as follicular thyroid cancer and papillary thyroid cancer are more common in women [10,11].

Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer, accounting for approximately 80% of cases and its incidence has nearly doubled over the last 30 years and is thought to be due in part to earlier diagnosis of subclinical disease [1,5].

The rate of PTC among woman is nearly three times higher than men but in most studies males gender is associated with a lower disease free survival and higher mortality [8,10,11].

As the incidence of thyroid cancer increases, in particular PTC, the gender differences observed are likely to be even more dramatic.

Hematologic malignancies are generally more common in males and this can be generalized to most other cancers. Similar gender differences in non-malignant diseases including autoimmunity, are attributed to hormonal or behavioral differences. Even in early childhood, however, where these differences would not apply, there are differences in cancer incidence between males and females. In childhood, few cancers are more common in females, but overall, males have higher susceptibility. In Hodgkin lymphoma, the gender ratio reverses toward adolescence. The pattern that autoimmune disorders are more common in females, but cancer and infections in males suggests that the known differences in immunity may be responsible for this dichotomy. Besides immune surveillance, genome surveillance mechanisms also differ in efficiency between males and females. Other obvious differences include hormonal ones and the number of X chromosomes. Various

risk factors aid in the progression and dissemination of the disease such as age, radiation, genetic lesions, iodine deficiency or excess iodine consumption, however the causes of most thyroid cancers is yet to be elucidated.

a. Radiation exposure:

Post-Chernobyl epidemiologic studies have showed that, although the incidence of papillary thyroid cancer increased in the exposed population, there was a decrease in the female to male ratio. Based on the current evidence, ionizing radiation does not appear to play a role in the gender disparity of papillary thyroid cancer, with both genders being at equal risk of developing thyroid cancer after exposure.

b. Dietary and nutritional factors:

Dietary and nutritional factors have been implicated as risk factors for differentiated thyroid cancer of follicular cell origin. Iodine deficiency is a well-established risk factor for developing follicular thyroid cancer, and iodine supplementation has been implemented in most regions with endemic goiter. By contrast, iodine excess has been associated with an increased risk of papillary thyroid cancer. Chronic iodine deficiency may have a protective effect in females [12], but there are no equivalent studies looking at male gender.

In the San Francisco Bay Area Thyroid Cancer study group, a protective effect of dietary iodine and phytoestrogens was found in women, but the study did not have any male subjects [13]. The role of fish (high in iodine) and cruciferous vegetables on thyroid cancers have been debated. However, there are no studies that show a gender difference in the effects of fish and cruciferous vegetables in thyroid cancer.

c. Common somatic genetic changes associated with thyroid cancer

The three most common types of somatic mutations observed in papillary thyroid cancer are rearranged in transformation/papillary thyroid carcinomas (RET/PTC) and neurotrophin receptor tyrosine kinase (NTRK)1 rearrangements, and an activation mutation in the mitogen signaling protein BRAF. These genetic alterations are present in

approximately two-thirds of papillary thyroid cancer and are specific to papillary thyroid cancer. The RET/PTC rearrangement is found in 20–40% of adult sporadic papillary thyroid cancer. The NTRK rearrangement is less common and it is found in up to 13% of papillary thyroid cancer [14,15].

The BRAF V600E point mutation accounts for over 97% of the BRAF mutations in papillary thyroid cancer. The BRAF mutation is present in approximately 40% of papillary thyroid cancer [14,15].

d. BRAF mutation

There have been several studies that have demonstrated that the presence of BRAF V600E mutation is associated with an aggressive tumor phenotype in papillary thyroid cancer, including a larger tumor size, extrathyroidal invasion, poorly differentiated histology, lymph node and distant metastases, and it is also associated with older age, higher recurrence rates and gender [16].

The BRAF (V600E) mutation, the most frequent genetic mutation in papillary thyroid carcinoma (PTC), is widely considered to have an adverse impact on PTC outcome however its real predictive value is not well stated.

Xu and colleagues observed that BRAF V600E mutation in men with papillary thyroid cancer was 64% compared with 27% in women [16]. In another study of 203 patients with papillary thyroid cancer, BRAF V600E mutation was present in 91% of men compared with 70% of women [17].

Besides, a meta-analysis of twelve studies with a total of 1168 patients (male:female ratio 259:909) did not show a significant difference in mutation rate by gender, with 50% of the males and 49% of the females with papillary thyroid cancer having the BRAF V600E mutation [18].

e. RET/PTC & NTRK rearrangement

Multiple studies have looked at the association of clinicopathological features of papillary thyroid cancer with the presence of this rearrangement. Although the association of RET/PTC rearrangements with radiation is well established, there is limited evidence supporting any association with tumor phenotype and gender [16,18].

Therefore, RET/PTC rearrangement in papillary thyroid cancer does not appear to be different by gender.

f. Reproductive factors

The fluctuation of sex hormones during a woman's menstrual cycle and pregnancy has been hypothesized as the reason for the gender disparity in papillary thyroid cancer. In particular, the peak incidence of papillary thyroid cancer has been observed in women aged 40–49 years, this being the age group at which most women approach or enter menopause [19].

There have been several studies looking at the association of papillary thyroid cancer with reproductive factors such as age of menarche, menopause, number of pregnancies and other parameters [19-24]. However, the evidence for hormonal, menstrual or reproductive roles in thyroid cancer in women is nowadays inconclusive. Late age of menarche, late age of first child birth, artificial menopause and miscarriage for the first pregnancy were significant in a study conducted by Negri E. et al. [19].

The age of menarche, age at menopause, number of pregnancies or oral contraceptive use were not associated with a higher risk of developing thyroid cancer. There was no association observed with irregular menstrual cycles, history of miscarriage or induced abortion, time since last birth, age and outcome of first pregnancy or breastfeeding.

g. Sex hormones

The important role of sex hormones in cancer is well documented for breast and prostate cancers. Sex hormone effects are mediated by hormone-specific nuclear receptors that regulate gene expression and tumor cell biology. The α - and β -estrogen receptors mediate the effect of estrogen and are expressed in papillary thyroid cancer [25].

It has been hypothesized that the polymorphism in estrogen receptors could be a risk factor for thyroid cancer [26]. Inoue et Al. demonstrated that although papillary thyroid cancer cells have low levels of estrogen receptor- α , with physiologic estrogen stimulation the receptor level is significantly upregulated and cell proliferation is promoted [27].

Furthermore, estrogen can dramatically increase the rate of cell proliferation in thyroid cancer cell lines compared with male sex hormones [25,28]. It also modifies the expression of estrogen receptor subtypes in thyroid cancer cell lines [25,28,29].

The effects of estrogen on thyroid cancer cell lines are dependent on the histological type of thyroid cancer, and estrogen dramatically increases estrogen receptor- α levels in papillary thyroid cancer, whereas in anaplastic thyroid cancer and follicular thyroid cancer the receptor levels are not significantly altered [28, 29]. The expression of estrogen receptor- β in papillary thyroid cancer cells was increased in anaplastic thyroid cancer cell lines. In addition, estrogen treatment promoted the growth of papillary thyroid cancer cells in culture but inhibited growth in anaplastic thyroid cancer. Rajoria S. et al also documented that estrogen is associated with increased adherence, invasion and migration in thyroid cancer cell lines [30].

Based on these in vitro studies, in papillary thyroid cancer, estrogen receptor status is affected by sex hormones. This may play a role in the gender disparity observed in papillary thyroid cancer, and can serve as a potential target for the treatment of advanced papillary thyroid cancer.

Similar molecular observations have been made during pregnancy. In particular, chorionic gonadotropin, which has been demonstrated to have close homology with thyroidstimulating hormone, has been shown to cause rapid growth of thyroid tumors (benign or malignant) during pregnancy [31,32].

h. family history of endocrine-related diseases

Family history of endocrine-related malignancies, such as breast cancer and goiter, are also known to predispose one to thyroid cancer.

According to American Thyroid Association (ATA), the incidences of thyroid proliferative diseases (TPD), which includes thyroid cancer, are four to five times more prevalent in women than in men [33-36].

The risk of developing thyroid disorders in women is one in eight which is comparable to that of sporadic breast cancer in women, and is significantly enhanced with family history of endocrine-related diseases, age, use of oral contraceptives, autoimmune diseases and pregnancy [37,38]. With respect to pregnancy, there is approximately 5% chance of developing a thyroid disorder in new mothers within 6 months of birth. An increased risk is also noted with the use of estrogen for gynecological problems, with a noted decrease in

the incidences of thyroid malignancies after menopause [39,40]. Reproductive challenges and infertility have all been associated with thyroid hormone abnormalities and the goitrogenic effects of iodine are more pronounced in women than in men. This marked gender bias in distribution of TPDs, with the incidence of thyroid disorders being much higher in women than in men, suggests that being a female might be the highest risk for developing TPDs. Indirect effects of estrogen on thyroid hormone synthesis and release have been demonstrated in the past [41,42], but the direct effects of estrogen on thyroid diseases are still not fully explored.

Several studies have been performed worldwide in an effort to investigate the role of estrogens in various cancers but the precise mechanism for estrogenopathies in TPDs remains poorly understood.

1.3 ESTROGEN AND ITS RECEPTORS

Estrogens play crucial roles in growth, differentiation, and function of not only sexual and reproductive organs, but also cardiovascular, musculoskeletal, immune and central nervous system in both men and women [43,44]. Estrogens consist of a group of three biochemically distinct hormones, estrone, 17 β -estradiol, and estriol, which are produced naturally by the female body and are metabolized into estrogen metabolites such as 2-hydroxyestrone (2-OHE1) and 16-alpha-hydroxyestrone (16-OHE1) [45,46]. These estrogen metabolites have stronger (16-OHE1) or weaker (2-OHE1) estrogenic ability and their relative concentration in females can increase the risk of developing breast, uterine and other cancers [46,47]. 17 β -estradiol, commonly denoted as E2, is the central and most potent player of the estrogens on the human body due to its highest affinity for estrogen receptors [48].

Estrogen signaling is mediated primarily through two isoforms of the estrogen receptor (ER), ER- α & ER- β , which are members of a large ligand dependent nuclear receptor superfamily of transcriptional factors [49,50]. Human ER- α protein consists of 595 amino acids with a molecular weight of 66 kDa while ER- β protein has 530 amino acids and a molecular weight of 55 kDa [51- 53]. The cellular signaling is initiated by binding of estrogen to these receptors by at least three different mechanisms, leading to a ‘multifaceted cascade of events’ [41, 54]. Genomic or classical estrogen signaling involves entry of estrogen in the target cell via passive diffusion, leading to a conformational change(s) of the estrogen receptor. This conformational change allows the dissociation of the receptor from its chaperone proteins, followed by nuclear translocation and homo- or hetero-dimerization of E2-ER. The complex then binds to a nucleotide sequence located in the promoters of target genes, known as estrogen response element (ERE). The activated DNA bound ER then recruits transcriptional co-activators resulting in an assembly of transcriptional complex and subsequent gene expression. This mechanism of E2 action has a slow transcriptional response from hours to days for maximal gene activation [55-57]. However, one-third of the genes regulated by estrogen in humans do not contain ERE-sequences [57,58]. In such cases, estrogen can regulate gene expression by modulating the functions of other transcriptional factors in the nucleus, by protein–protein interactions, leading to alteration of chromatin, histone unwinding and interactions with basal transcription machinery complex components and is known as ERE-

independent genomic actions of estrogen [59]. Estrogen also exerts a variety of cellular responses in bone, breast, vasculature and nervous system which are rapid and do not depend on E2–ER mediated gene transcription and protein synthesis [59-60]. These actions are referred are believed to be mediated through activation of ER located in or adjacent to the plasma membrane or through other plasma membrane-associated estrogen binding proteins [61,62]. These actions lead to regulation of various genes involved in a wide array of cellular processes such as proliferation, differentiation, cell motility and apoptosis [61].

1.4. EXPRESSION OF ESTROGEN RECEPTORS IN THYROID TISSUES

ER- α is predominantly expressed in breast, uterus, cervix, vagina, pituitary and several other organs including thyroid, while ER- β is predominantly expressed in ovary, prostate, testis, spleen, lung, hypothalamus, thymus. The presence of ER in thyroid was first reported in early 1980s using synthetic estrogens as a radioligand and has been since reported by several groups including ours in normal thyroid tissues as well as benign and malignant thyroid carcinomas [63-66]. ER- α mRNA has also been reported in both non-neoplastic and neoplastic thyroid tissues. The presence of both ER- α and ER- β in thyroid tissue, similar to that observed in breast carcinoma, would suggest that the relative ratio of ER- α and ER- β in thyroid carcinoma could have an important role in the pathophysiology of thyroid cancer [61,67,68]. The studies of Cho et al. demonstrated an increased ratio of ER- α /ER- β in human medullary thyroid cancer validating the contention [69].

Furthermore, Zeng et al. observed that the agonist and antagonist for ER- α and ER- β regulate the expression levels of the receptors. They demonstrated that ER- α agonist propylpyrazole-triol (PPT) enhances thyroid carcinoma proliferation and expression of antiapoptotic protein, Bcl-2, while ER- β agonist diarylpropionitrile (DPN) inhibits cell proliferation and enhanced the expression of Bax [70]. In addition, siRNA mediated knockdown of ER- α significantly attenuated Bcl-2 expression while ER- β silencing enhanced Bcl-2 expression [70], further supporting the notion that the imbalance between ER- α and ER- β expression may contribute to thyroid cancer pathogenesis.

Indeed some reports have shown a comparable ER- α expression in normal and tumor thyroid tissue [71,72], however all these reports rely on immunohistochemistry (IHC) to determine the presence or absence of ER- α expression, and it is therefore interesting to note such a difference in the ER- α expression from different laboratories. A recent study [73] investigated the possibility of measuring ER- α in PTC samples by different molecular biology techniques. The best approach to individuate ER- α expression was found to be Laser-capture microdissection (LCMD), followed by real-time PCR or Western blot in the study.

Therefore, the different and opposing results regarding the expression of ER- α in normal thyroid tissue could depend, in the case of a whole tissue analysis, on the ratio between tumor and normal cells in the sample analyzed. In this study, ER- α expression could be

detected in human biopsy specimens obtained from PTC and at much lower level from goiter [73].

1.5. EFFECTS OF ESTROGEN ON THYROID HORMONE ACTIVITY

The normal physiological functions of the thyroid gland involve the synthesis and secretion of two principal metabolic hormones triiodothyronine (T3) and thyroxine (T4) and their metabolism and action on the peripheral tissue. The mechanism of action for thyroid hormones' activity involves hormone binding to the nuclear thyroid hormone receptor (TR), resulting in formation of a complex which then binds to thyroid hormone response elements in promoter regions of the target genes. The activated receptor can then either stimulate or inhibit transcription of the target gene depending on the nature of regulatory element, bringing in effect its various functions. Both ERs and TRs are members of the nuclear receptor superfamily and they share an identical half site at the consensus sequence of nucleotide bases which constitute ER response element and thyroid hormone response element. This allows the TR to bind ERE and vice versa, and such interactions between these nuclear receptors can possibly lead to an infinite number of gene regulatory events, leading to a greater array of transcriptional response to environmental stimuli. There are discrepancies in the literature in regards to effects of estrogen on activation of pituitary-thyroid axis essential for the normal physiological functions of thyroid gland.

A study by Banu et al. implicates that the stimulatory effects of estrogen on human NPA-87-1 papillary thyroid carcinoma are independent of thyroid-stimulating hormone (TSH) activity. In this study, they observed that estrogen enhances the proliferation of thyroid cells potentially by upregulating the ERs and these proproliferative effects of estrogen were independent of TSH administration [74]. Furlanetto et al. demonstrated similar proliferative effects of estrogen on rat FRTL-5 thyroid follicular cells which were independent of TSH administration. Moreover, they observed that estrogen attenuates TSH-induced sodium/iodide symporter (NIS) expression in the thyroid cells while cotreatment with estrogen and fulvestrant restored the TSH induced NIS expression [75]. The same group later showed that estrogen treatment led to a decreased iodide uptake from the same FRTL-5 thyroid cells [76]. Estrogen may be directly acting on the NIS system modulating its ability to carry iodide or may be leading to production of growth factors which leads to decreased iodide uptake, but this needs further investigation. Lima et al. demonstrated that estradiol administration in prepubertal and adult rats leads to a significant increase in the thyroid weight but they observed no significant changes in

serum T3, T4 and TSH levels, suggesting a more direct action of estrogen in the thyroid gland [77].

1.6. ESTROGEN ON AND TUMOR PROGRESSION

In a study of Zeng Q et al. [29] it has been investigated how 17beta-oestradiol (E2) influenced, promoting the proliferation and growth of thyroid cancer cells. Imbalance between ERalpha and ERbeta may contribute to thyroid carcinogenesis.

Tumor metastases accounts for around 90% of all the cancer related deaths [78]. The process of metastasis involves sequential steps and the tumoral tissue invades the circulation through the process called intravasation. After intravasation, individual tumor cells migrate to the distant sites, make transient adhesion to the vessel wall and start invading the new organ, a process called extravasation. The last step is colonization of the tumor cells at the new site and initiating the formation of new blood vessels—angiogenesis—allowing them to establish and expand the newly formed metastatic foci [78,79].

For reasons still unknown, certain cancer types have a high probability to metastasize while others never metastasize.

Secondary metastatic lesions are a major complication in patients with thyroid cancer. The secondary disseminated metastasis causes many patients to relapse despite the improved surgical and therapeutic processes, causing further complications. Several factors aid in a number of molecular mechanisms involved in the malignant transformation and development of metastatic disease, including estrogen. Kousidoua et al. have observed a positive correlation between ER- α expression and the effects of estrogen on matrix metalloproteinase (MMP) gene expression in ER+ breast cancer cells [80]. Nilsson et al. have also shown that estrogen regulates the metastatic propensity in breast cancer by regulating the activity of MMP-2 and MMP-9 [81], but there are not much evidences linking estrogen with thyroid cancer metastasis.

1.7. EFFECTS OF ESTROGEN ON ON THYROID CANCER METASTASIS

Well-differentiated thyroid cancer, including papillary and follicular thyroid cancer, can metastasize to the nearby lymph nodes in over 50% of the cases [82,83], with secondary disseminated metastasis being the biggest complication in the majority of thyroid cancer patients. Rajora S et al has recently shown that estrogen enhances thyroid cancer cell adhesion, migration and invasion, potentially due to downregulation of tumor suppressive proteins such as catenins and cadherins [66,67]. A defective cadherin/catenin complex has been correlated with increased cancer proliferation and progression in vitro and in vivo, while restitution of the cadherin/catenin complex has shown to suppress metastasis in several cancers including estrogen responsive cancers. Rajora S have recently shown that cotreatment with an antiestrogen, fulvestrant, restores catenin protein levels, further validating the effects of estrogen on cadherin/catenin proteins in thyroid cancer cells. Besides cell–cell adhesion proteins, proteolytic enzymes such as MMPs also play a critical role in tumor cell invasion. It was recently demonstrated that estrogen treatment leads to enhanced activity and secretion of MMP-2 and MMP-9 in human thyroid cancer cells, which was attenuated by ER antagonists [67].

Roudabush et al. have demonstrated that activation of ERK1/2 requires the involvement of estrogen regulated MMPs in monkey kidney fibroblast COS-7 cells [84]. Later, Song et al. demonstrated in human MCF-7 breast cancer cells that estrogen leads to a sequential activation of MMPs and MAPK [85]. A study by Ocharoenrat et al. showed that growth factors were able to stimulate MMP 9 activation in head and neck squamous cell carcinoma and elevated levels of MMPs have been demonstrated in thyroid carcinoma [86]. In a later study, Yeh et al. further demonstrated that MMPs act as critical effectors of invasion in papillary and follicular thyroid cancer cell lines [87]. A recent study showed that silencing of ER- α using siRNA attenuates estrogen-mediated migration and invasion of human thyroid cancer cells [67].

1.8. ANTIESTROGENS AS THERAPEUTIC AGENTS FOR THYROID DISEASES

The observations that thyroid cells are estrogen responsive and estrogen can directly and indirectly modulate thyroid cancer proliferation, metastasis and angiogenesis opens up the possibility of using antiestrogens. Several antiestrogenic synthetic compounds such as tamoxifen and ICI 182,780 (fulvestrant) exists but unfortunately, these compounds have undesirable side effects such as ICI 182,780 results in a complete blockade of the activation pathways of ER and tamoxifen resulting in detrimental uterotrophic effects and can act as an agonist for breast cancer cell growth [88,89]. These damaging effects warrant the research for safer alternatives such as natural compounds present in diet, for thyroid disease treatment and prevention.

Many studies has shown that exposure of estrogen responsive cancers, such as breast cancer, to I3C and its in vivo dimeric product DIM trigger anti-proliferative and/or apoptotic signals [67, 90-94].

Despite its beneficial roles, especially against hormone responsive cancers such as thyroid and breast, the exact anti-cancer mechanism of DIM has not been fully elucidated. Studies indicate that DIM affects estrogen mediated effects on cancer cells by activating several individual and interdependent transcriptional, cell signaling, enzymatic and metabolic cascades that result in the abrogation of estrogen-induced cell proliferation. A recent study by Rajora S group has revealed that DIM acts in a similar fashion to the antiestrogenic compound fulvesterant by inhibiting estrogen-induced proliferation and clone formation of thyroid cancer cells [67]. DIM was also observed to interfere with essential events involved in cancer cell metastasis as evidenced by a decrease in in vitro cell adhesion, migration, and invasion [67,95]. Of these three crucial steps involved in initiating metastasis, invasion is the most essential one. An agent that could efficiently inhibit the ability of cancer cells to form secondary metastatic foci would be an ideal candidate to suppress cancer progression. DIM has a unique antiinvasive property, with as high as 78% inhibition of invasion observed in papillary thyroid cancer cells. The observed antiestrogenic property of DIM is in accordance with its ability to modulate E2-ER interactions and E2-mediated signal transduction pathways such as the pAKT and ERK pathways, as described earlier by us and others, which provide evidence that DIM shows a strong potential for treatment and prevention of thyroid diseases including cancer.

AIM OF THE STUDY

Our intention is to consider our experience about the gender disparity in patients underwent surgery for PTC comparing females (F) vs males (M) as regards a series of clinical, histopathologic, molecular, and hormonal variables to determine if, as in breast carcinoma , there is actually a role of estrogens in the pathogenesis of PTC.

Then, we have evaluated the presence of estrogen receptors on the little series of surgical specimen to identify the eventual medical targeted therapy for this type of tumor.

MATERIALS AND METHODS

a. Patients selection

We considered 658 patients underwent surgery for histologically proven Papillary Thyroid Carcinoma (PTC) from 2007 to 2011 at 2nd Clinical Surgery, University of Padova, and General Surgery Department, University of Trieste.

The patients were given a detailed explanation of aim of our study and all patients gave their informed consent to participate.

b. Variables evaluated

We revised clinical and histopathologic documents and we compared F vs M as regards the following variables: age, extension of surgery, node dissection, TNM, mono/plurifocality, association with lymphocitary chronic thyroiditis, BRAF mutations, and outcome.

c. Reproductive factors analysed in women

In the women, the hormonal variables, as: age of menarche, age and type of menopause, number and age of pregnancy, interruption of pregnancy, and the assumption of oral estrogen-progestin therapy, were investigated to determine the effective role of reproductive factors and hormonal effects in the pathogenesis of this tumor.

All female patients investigated for the variables cited below, were divided in two groups: < 40 years (childbearing) old and age > 40 years old. For each stage the various variables were tested to determine if there were any correlation with a specific reproductive factor.

d. Evaluation of expression of ER- alpha and ER-beta receptors in thyroid tissue

In a little series of patients, we prospectively analyzed the perioperative serum level of estrogens and the expression of ER-alpha receptors and ER – beta by immunohistochemical studies in the sections of formalin-fixed tissue , paraffin-embedded tumor of the thyroid. Then, the patients selected, all women, were divided in two groups: age < 40 years (childbearing) old and age > 40 years old. These two groups were compared regarding stage and outcome.

e. Follow-up evaluation

At follow up the patients underwent physician exam, thyroid function testing, serum Thyroglobulin (Tg) levels, and circulating anti-Tg antibodies, measured 4 weeks after operation before levothyroxine suppression therapy.

The completeness of the operations was determined also by neck US exam and by iodine-131 (¹³¹I) whole body scan (WBS) 3 to 6 months after surgery.

After total thyroidectomy, the treatment with ¹³¹I to ablate postsurgical thyroid remnant were performed, in hypothyroidism conditions (TSH > 30μUI/mL, obtained by withdrawing thyroid replacement hormone), on the basis of stage and prognostic/risk factors according to the American Thyroid Association Guidelines [9].

WBS was repeated in these patients 4 days after ¹³¹I administration to visualize any thyroid remnant or metastases, followed by thyroid hormone supplementation.

The mean period of time elapsed between the thyroidectomy and ¹³¹I administration for postsurgical remnant ablation was 3–4 months. Patients who did not receive radioiodine treatment underwent long-term follow-up at 6- to 12-month intervals, at which time ultrasonographic examinations were performed, and serum Tg levels were measured while the patients were on levothyroxine. Patients whose WBS revealed no thyroid remnants or metastases were also placed on this follow-up protocol. The criteria for successful thyroid ablation were defined as the disappearance of any visible area of uptake in the thyroid bed ($\leq 1\%$), and undetectable serum Tg levels off levothyroxine (TSH >30μUI/mL).

f. Statistical analysis

The statistical analysis was performed by using the following tests: the Mann-Whitney test for age, and the χ^2 test for gender, surgical procedure/extension of surgery, node dissection, TNM, mono/plurifocality, association with thyroiditis, BRAF mutations, and outcome.

The software used was the “Statistica released 7”.

A p value less than 0.05 was considered statistically significant.

RESULTS

Comparing F vs. M there was a statistically significant difference with regard to sex, in fact, women were 74% while the male sex accounted for 26% of cases ($p < 0.015$), the association with chronic autoimmune thyroiditis (more frequent in women), and the lateral cervical lymph node dissection (necessary in most men).

Comparing females with men regarding the rate of recurrence, we did not find a significant difference.

In our series BRAF^{V600E} mutation was identified in 29% of women and in 30.7% of men confirming the absence of significant difference among two sexes.

In our series, regarding the reproductive factors in the women, relatively more advanced stage was correlated with early menarche, older age of first pregnancy, late menopause; moreover, a higher incidence of tumor was registered in childbearing age stressing the role of estrogen to promoting the PTC.

Moreover, our data confirm that, comparing males vs females, there was a significant difference concerning the number of patients affected by PTC with a preponderance of women. Also the number of central node dissection performed was dissimilar among the two groups of patients, in fact it was performed in only 12% of females and in 29% of males confirming the major aggressiveness of this tumor in the men. The association with thyroiditis was most important in the women confirming the better outcome in female gender, whereas the others characteristics as age, TNM stage, mono and plurifocality, BRAF mutations, and outcome were not statistically diverse between two sexes.

Of the 658 patients considered in this study, the F was 489 (74%) vs 169 M (26%) (**$p < 0.015$**), the mean age was 46 years (range 11-86), in particular 46 years (range 11-86) in F vs 46 years (range 11-83) in M ($p = < 0.44$), total thyroidectomy was realized in 635 cases, 474 F (97%) vs 161 M (95%) ($p < 0.72$), loboistmectomy was realized in 21 cases, 13 F (3%) and 8 M (5%) ($p < 0.83$), node dissection was realized in 519 cases, 387 (79%) F vs 132 (78%) M ($p < 0.99$), central node dissection in 434 cases, 340 (88%) F vs 94 (71%) M ($p < 0.75$), laterocervical node dissection in 85 cases, 47 (12%) F vs 38 (29%) M (**$p < 0.011$**), stage I was defined in 402 cases, 315 (64%) F vs 87 (51.5%) M ($p < 0.19$), stage

II in 19 cases, 15 (3%) F vs 4 (2.5%) M ($p<0.51$), stage III in 180 cases, 126 (26%) F vs 54 (32%)M ($p<0.249$), stage IV in 57 cases, 33 (7%) F vs 24 (14%) M ($p<0.223$), monofocality was in 368 cases, 279 (57%) F vs 89 (52.7%) M ($p<0.64$), plurifocality was in 290 cases, 210 (43%) F vs 80 (47.3%) M ($p<0.76$), tumor size was 14.13 mm (range 0.5-80), it was 13.97 mm (range 1-80) in F vs 14.62 (range 0.5-70) in M ($p<0.42$), association with thyroiditis was observed in 157 cases, in 132 (27%) F vs 25 (15%) M (**$p<0.001$**), BRAF – V600E mutation was identified in 195 cases, 143 (29%) F vs 52 (30.7%) M ($p<0.849$).

We revised the follow up in 395 cases (60%). The mean time of follow up was 38 months (range 1-137), 37 months (range 1-343) in F vs 45 (range 4-156) in M ($p<0.24$), 342 patients underwent ^{131}I radioiodinotherapy, 251 (88%) F vs 91 (83%) M ($p<0.74$), the median dose was 134.33 mCi (50-500), 132.63 mCi (range 50-500) in F vs 139.42 mCi (range 50-350) in M ($p<0.27$), the median postoperative value of serum Tg level was 10.5 mU/L (range 0.1-593), 7 mU/L (range 0.1-485.3) in F vs 19.83 (range 0.1-593) in M ($p<0.11$), 376 patients was free of disease, 274 (96%) F vs 102 (93.5%) M ($p<0.64$), 2 patients deceased , 1 (0.2%) F vs 1 (1%) M ($p<0.24$).

These findings are summarized on table 3.

- Reproductive factors analyzed in women:

Regarding hormonal profile in the women, relatively more advanced stage was correlated with early menarche, older age of first pregnancy, late menopause; moreover, a higher incidence of tumor was registered in childbearing age.

These findings are summarized on table 4.

Table 3:

Variables	total	Femaless	Maless	P value
Number of patients	658	489 (74%)	169 (26%)	p<0.015
Age	46 (11-86)	46 (11-86)	46 (11-83)	P<0.44
Total thyroidectomy	635	474 (97%)	161 (95%)	p<0.72
Loboistmectomy	21	13 (3%)	8 (5%)	p<0.83
Node dissection	519	387 (79%)	132 (78%)	p<0.99
Central compartment	434	340 (88%)	94 (71%)	p<0.75
Lateral compartment	85	47 (12%)	38 (29%)	p<0.011
TNM				
I	402	315 (64%)	87 (51,5%)	p<0.19
II	19	15 (3%)	4 (2,5%)	p<0.51
III	180	126 (26%)	54 (32)	p<0.249
IV	57	33 (7%)	24 (14)	p<0.223
monofocality	368	279 (57%)	89 (52,7)	p<0.64
plurifocality	290	210 (43%)	80 (47,3)	p<0.76
Tumor size in mm	14,13 (0,5-80)	13,97 (1-80)	14,62 (0,5-70)	p<0.42
Association with LCT (thyroiditis)	157	132 (27%)	25 (15%)	p<0.001
BRAF mutation				
V600E	195	143 (29%)	52 (30,7)	p<0.849
WT	184	142 ((29%)	42 (25%)	p<0.433
Follow up	395(60%)	286 (58%)	109 (64%)	p<0.18
Time of follow up	38 (1-343)	37 months (1-343)	45 months (4-156)	p<0.24
I ¹³¹ radiotherapy	342	251 (88%)	91 (83%)	p<0.74
Dose	134,33 mCi (50-500)	132,63 mCi (50-500)	139,42 mCi (50-350)	p<0.27
N° of radioiodine treatment	1,13 (1-3)	1,09 (1-3)	1,23 (1-3)	p<0.14
TG levels	10,5 (0,1-	7 (0,1- 485,3)	19,83 (0,1-	p<0.11

	593)		593)	
Free of disease	376	274 (96%)	102 (93,5%)	p<0.64
Not free of disease	17	11 (3,8%)	6 (5,5%)	p<0.76
Deceased	2	1 (0,2%)	1 (1%)	p<0.24

Table 4:

More aggressive stage was correlated with: (histotype and tumor diameter)	
Parameters	<i>P value</i>
Group A age <40 years	
Group B age > 40 years	
early menarche (stage III)	<i>P<0,026</i>
older age at first pregnancy (stage III)	<i>P<0,047</i>
late menopause (stage III)	<i>P<0.038</i>
> incidence of thyroid tumor in reproductive age (in group having < 40 years old)!!!	

Any correlation was found with the number of pregnancy, eventual interruption of pregnancy or with assumption of oral estrogen-progestin therapies.

- *Evaluation of expression of ER- alpha and ER-beta receptors in thyroid tissue:*

In a little series of patients, we prospectively analyzed the perioperative serum level of estrogens and the expression of ER- alpha receptors and ER –beta by immunohistochemical studies in the sections of formalin-fixed tissue , paraffin-embedded tumor of the thyroid.

We have immunohistochemically examined estrogen receptors (ER) on papillary thyroid carcinoma in 10 females. Of the specimens obtained from the 10 patients, 80% (8/10) showed positive staining for ER.

Totally 10 patients, with a median age of 48 years (range: 31-77) were analyzed. Mean follow-up time was 3 months (range 1-12). Regarding the age, we have divided patients in two categories: young < 40 years old and > 40.

We did not find any significant difference in the incidences of positive staining for E2 between the two groups. 10 thyroid glands specimens have been examined using immunohistochemical assays with ERs alpha and beta antibodies. There was no expression of antibodies in non-neoplastic cells or in adjacent tissues.

Tumor size ranged from 1,5 mm to 30 mm (median 13,5 mm). Micro PTC (diameter < 1cm) were found in 3 (30%) cases. BRAF mutation was present in 5 cases.

Six patients underwent to central lymph node dissection (CLND). Of those, three (50%) had metastatic lymph nodes. Final histology showed the neoplasms to be in group A (<40 years) 3 stage I and 2 stage III whereas in group B (>40 years old) there were 2 neoplasms stage I and three stage III [p < 0,36].

The group A resulted composed by a more aggressive histological variants than group B (see table I) and the tumor size was greater [p<0,0746].

At the follow-up any neck recurrence occurred. Mean serum postablative Tg levels after levothyroxine withdrawal resulted a little higher in group A (1,6 ng/mL vs 1,1 ng/mL) [p=N.S]. The mean radioactive iodine ablation doses was 142,63 mCi in group A vs 139,42 mCi in group B [p= N.S].

DISCUSSION

Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer, accounting for approximately 80% of cases. Its incidence has nearly doubled over the last 30 years and is thought to be due in part to earlier diagnosis of subclinical disease [1- 6].

Diagnostic sensitivity and opportunities for detection have improved over the past decades with the introduction of thyroid ultrasound in the early 1980s and fine-needle aspiration cytology in the late 1980s. Probably, these technologies could have potentially impacted the secular trends more in one gender than the other, but the gender disparities in PTC incidence is very high.

Thyroid cancer incidence rates have increased worldwide for decades, although more for papillary carcinomas than other types and more for females than males.

Cancer gender disparity in incidence, clinical presentation, disease aggressiveness, and prognosis has been observed for a variety of cancers [1-8].

There are few known thyroid cancer risk factors except female gender, and the reasons for the increasing incidence and gender differences are unknown.

The pathogenesis of thyroid cancer is complex. Iodine deficiency, genetic factors, sex, older age, irradiation in childhood, thyroid growth stimulating antibodies and epithelial growth factor possibly affect its development. Recent reports, also epidemiological, have shown that differentiated thyroid cancer (papillary, follicular, medullary) is dependent on sex hormones, especially estrogens. This has prompted research into the presence of estrogens and progesterone receptors (ER and PR), as well as androgen receptors (AR) in normal and neoplastic thyrocytes and estradiol content in thyroid tissue. The results of these investigations imply that thyroid cancers are estrogen-dependent. There is, however, no agreement in reports about correlation between tumor malignancy and ER, PR and AR expression.

Several epidemiological studies have suggested a possible correlation between incidences of thyroid malignancies and hormones but the precise contribution of estrogen in thyroid proliferative disease initiation, and progression is not well understood [1-11]

The rate of papillary thyroid cancer among woman is nearly three times higher than men,

with a widening gender gap over time [1,8].

Kilfoy Briseis A. et Al [8] reported that the age-specific rates were higher among women than men across all age groups analyzed, and the female-to-male rate ratio declined quite consistently from more than five at ages 20–24 to 3.4 at ages 35–44 and approached one at ages 80+. These results confirmed that gender is an age-specific effect modifier for papillary thyroid cancer incidence.

The less aggressive histologic subtypes of thyroid cancer are more common in women, whereas the more aggressive histologic subtypes have similar gender distribution. In females, the age-specific incidence rate rises sharply at the beginning of the reproductive years, with increasing age peaking at 40–49 years, while in men the peak is at 60–69 years [8]. The incidence rates equalize by 85 years of age [8,10-11]. Women have an earlier age of onset but men tend to have more aggressive disease at diagnosis.

The gender disparity in incidence, aggressiveness and prognosis is well established for thyroid cancer but the origin of the disparity is poorly understood.

Several causes as, radiation exposure, nutritional, genetic, molecular, reproductive, hormonal and environmental factors it has been hypothesized that may account for this difference [1-11].

As described by Rahbari R et al. [11] in the review of thyroid cancer gender disparity, regarding the ionized radiation exposure, it does not appear to play a role in the gender disparity of PTC, with both genders being at equal risk after exposure as demonstrated by Moysich Kb et al.[96].

As concerns the nutritional factors, in the San Francisco Bay Area Thyroid Cancer Study Group a protective effect of dietary iodine and phytoestrogens was found in women (608), but the study did not have any males subjects [13].

Regarding the genetic factors, although some studies suggest BRAF - V600E mutation is more common in men [16] and this may be results in an more aggressive prognosis, a meta analysis of 12 studies with a total of 1168 patients didn't show a significant difference in mutation rate by gender [18]. Besides, Sadetzki S et al. [97] described a higher rate of *RET/PTC* rearrangements in papillary thyroid cancer with male gender. About NTRK rearrangement, Brzezianska E. et al. analyzed the presence of an NTRK rearrangement and gender and no significant difference were found [98].

In our series BRAF - V600E mutation was identified in 29% of women and in 30.7% of men confirming the absence of significant difference among two sexes.

In our series, although BRAF - V600E mutation was demonstrated to be an adverse

prognostic factor for the aggressiveness of the disease, but it not resulted independent from other clinical-pathological features in low-risk intrathyroid PTC patients, however it could be useful in the workup of low-risk PTC patients, as supplementary prognostic factor to assess the preoperative risk stratification with the aim to avoid unnecessary central neck node dissection and complementary I¹³¹-therapy .

Although thyroid cancer occurs much more frequently in females, the role of sex hormones in thyroid carcinogenesis is unknown.

According to American Thyroid Association (ATA) [26], the incidences of thyroid proliferative diseases (TPD), which includes thyroid cancer, are four to five times more prevalent in women than in men. The risk of developing thyroid disorders in women is one in eight which is comparable to that of sporadic breast cancer in women, and is significantly enhanced with family history of endocrine-related diseases, age, use of oral contraceptives, autoimmune diseases and pregnancy. With respect to pregnancy, there is approximately 5% chance of developing a thyroid disorder in new mothers within 6 months of birth. An increased risk is also noted with the use of estrogen for gynecological problems, with a noted decrease in the incidences of thyroid malignancies after menopause [11,30].

Several studies have been performed worldwide in an effort to investigate the role of estrogens in various cancers but the precise mechanism for estrogenopathies in TPDs remains poorly understood.

There have been several studies looking at reproductive and hormonal factors and the risk of thyroid cancer with inconclusive results. The interpretation of these population-based studies is hampered by the fact that the rate of thyroid cancer varies dramatically among populations. The suggestion that the rates of thyroid cancer may differ due to reproductive and menstrual factors in these populations would support the hypothesis that these factors influence rates of thyroid cancer. However, based on clinical and epidemiology studies, there is no conclusive association between menstrual, reproductive or hormonal history with thyroid cancer risk.

Hormonal differences are hypothesized to contribute to the approximately ≥ 2 -fold higher thyroid cancer incidence rates among women compared with men worldwide [99]. As regards the hormonal factors, recently, several studies described the role of estrogens in the pathogenesis of thyroid cancer in women and it was confirmed that sex hormone effects are mediated by hormone specific nuclear receptors that regulate gene expression and tumor cell biology [27,28, 68 100 - 102].

Estrogen is known to promote the proliferation of thyroid papillary carcinoma cells.

However, the molecular mechanism responsible is not well understood [68,70].

The pattern of the subcellular localization of ER-alpha and ER-beta differs between papillary and anaplastic cancer [68]. Upon E2 treatment, the level of ER-alpha increases in the nuclei of papillary cancer cells but ER-beta remains unchanged. The level of mitochondrial ER-beta surpassed that of ER-alpha in anaplastic cancer cells. The different locations of ER-alpha and ER-beta in PTC cells and in anaplastic carcinoma cells agreed with the finding that E2 promoted the proliferation of PTC but inhibited or did not affect that of ATC cells. Consequently, there are differences in epidemiology and responses to anti-tumour therapy between papillary and anaplastic cancer in terms of the subcellular localization of ER isoforms.

Estrogen signaling is mediated primarily through two isoforms of the estrogen receptor (ER), ER- α and ER- β , which are members of a large ligand dependent nuclear receptor superfamily of transcriptional factors.

The alpha and beta estrogen receptors (ER- α and ER- β) mediate the effect of estrogen and are expressed in PTC and it has been hypothesized that the polymorphism in estrogen receptors could be a risk factor for thyroid cancer [27,28, 68 100 - 102].

ER- α is predominantly expressed in breast, uterus, cervix, vagina, pituitary and several other organs including thyroid, while ER- β is predominantly expressed in ovary, prostate, testis, spleen, lung, hypothalamus, thymus.

ER- α mRNA has also been reported in both non-neoplastic and neoplastic thyroid tissues. An imbalance between the expression of ER subtypes α and β has also been proposed to have significance with higher expression of ER- α promoting cell proliferation and growth while higher expression of ER- β promoting cell apoptosis in thyroid cancer cells. The presence of both ER- α and ER- β in thyroid tissue, similar to that observed in breast carcinoma, would suggest that the relative ratio of ER- α and ER- β in thyroid carcinoma could have an important role in the pathophysiology of thyroid cancer [61,65,68].

Kumar A et al. [68] demonstrated that the estradiol-induced proliferation of papillary and follicular thyroid cancer cells is mediated by ER- α and ER- β .

Magri et al., using immunohistochemistry, evaluated ER α , ER β , and AR expressions in thyroid tumors and in its correspondent extra-tumor parenchyma and concluded that ER α positivity, ER β negativity, and Androgen Receptors expressions are associated with a more aggressive phenotype of small T1-differentiated thyroid carcinoma [103].

Hormonal differences are hypothesized to contribute to the approximately ≥ 2 -fold higher

thyroid cancer incidence rates among women compared with men worldwide. Although thyroid cancer cells express estrogen receptors and estrogen has a proliferative effect on PTC cells in vitro, epidemiologic studies have not found clear associations between thyroid cancer and female hormonal factors. Schonfeld SJ et al. [99] hypothesized that polymorphic variation in hormone pathway genes is associated with the risk of developing papillary thyroid cancer but the pathway genes analyzed do not appeared to be strongly related to PTC risk.

Concerning the reproductive factors, there have been few studies looking at reproductive and hormonal factors and the risk of thyroid cancer with inconclusive results [11,104]. Premenopausal women resulted at highest risk for papillary and follicular thyroid carcinoma, implicating a role for estrogens in thyroid cancer [68]. In our series, regarding the reproductive factors in the women, relatively more advanced stage was correlated with early menarche, older age of first pregnancy, late menopause; moreover, a higher incidence of tumor was registered in childbearing age stressing the role of estrogen to promoting the PTC.

Moreover, our data confirm that, comparing males vs females, there was a significant difference concerning the number of patients affected by PTC with a preponderance of women. Also the number of central node dissection performed was dissimilar among the two groups of patients, in fact it was performed in only 12% of females and in 29% of males confirming the major aggressiveness of this tumor in the men. The association with thyroiditis was most important in the women, whereas the others characteristics as age, TNM stage, mono and plurifocality, BRAF mutations, and outcome were not statistically diverse between two sexes.

Recently, several studies aimed to evaluate the expression of estrogen receptors, α and β , in normal and malignant thyroid tissues and their effects on different molecular pathways involved in growth and function of the thyroid gland [101]. These studies opened up the possibility of developing alternative therapeutic treatments and preventive approaches which employ the use of antiestrogen to treat thyroid malignancies.

Regarding the evaluation of the expression of estrogen receptors, α and β , in normal and malignant thyroid tissues, we did not find any significant difference in the incidences of positive staining for E2 between the two groups of patients analyzed.

In all specimen we find receptors ER-alpha and ER-beta. The presence of ER-alpha was major in patients who presented relative more aggressive stage but the results were non

statistically significant. It's throw that we should consider that the time for evaluate the rate of recurrence was very low.

Our preliminary results are in agreement with most available data. It seems, however, that the material so far examined by the investigators is too small. Furthermore, because of using various methods the results cannot be compared. Further studies are necessary to reveal if there is any true influence of sex hormones on the development of thyroid lesions and if the detection of sex hormone receptors may help in choosing adequate therapy.

It was verified by Rajoria S. et al. that the estrogen induced metastatic modulators MMP-3 and MMP-9 are targets of 3,3'-diindolylmethane (DIM) in thyroid cancer. Furthermore, DIM, natural dietary compound found in cruciferous vegetables, displays anti-estrogenic like activity by inhibiting estradiol enhanced thyroid cancer proliferation [95].

Moreover Rajora S. [30] in another review studied the estrogen activity as a preventive and therapeutic target in thyroid cancer. In this review there are the description of principal studies on estrogen receptors in thyroid tissue (table 5).

Principal studies on estrogen receptors in thyroid tissue.			
Study	Thyroid cells	Estrogen receptor type	Technique
Furlanetto et al., 1999 [58]	FRTL-5	ER- α	RT-PCR, Western Blot analysis
Manole et al., 2001 [59]	HTC-TSHr Goiter nodules	ER- α ER- β	RT-PCR, Western Blot analysis
Banu et al., 2001 [64]	NPA87 WRO	ER- α ER- β	Western Blot analysis
Vivacqua et al., 2006 [54]	WRO FRO ARO	ER- α GPR30	RT-PCR, Immuno-cytochemistry, Western Blot analysis
Zeng et al., 2007 [48]	FRO ARO	ER- α ER- β	Western Blot analysis
Rajoria et al., 2010 [42]	BCPAP Nthy-ori 3-1	ER- α ER- β	Western Blot analysis
Kumar et al., 2010 [46]	NPA87 WRO	ER- α ER- β	Immuno-fluorescence, Western Blot analysis
Di Vito et al., 2011 [52]	PTC tissue BCPAP	ER- α	RT-PCR, Western Blot analysis

ER: estrogen receptor; RT-PCR: reverse transcriptase-polymerase chain reaction technique; FRTL-5: Fischer rat thyroid cell line; HTC-TSHr: human thyroid carcinoma cell line lacking endogenous TSH receptor; NPA87 and BCPAP: human papillary thyroid carcinoma cell lines; WRO and FRO: human follicular thyroid carcinoma cell lines; ARO: human anaplastic thyroid carcinoma cell line; Nthy-3-1: human normal transformed thyroid cell line.

Table 5: Principal studies on estrogen receptors in thyroid tissue. [S. Rajora et Al. *Biomedicine & Pharmacotherapy* 66 (2012) 151–158].

Family history of endocrine-related malignancies, such as breast cancer and goiter, are also known to predispose one to thyroid cancer.

Somjen D. et Al., described the growth inhibition of human thyroid carcinoma and goiter cells in vitro by a novel isoflavone-derived anti-estrogenic compound developed in laboratory, the N-t-boc-hexylenediamine derivative of 7-(O)-carboxymethyl daidzein (cD-tboc) [105].

In this study we considered the clinical, histopathologic, molecular, and hormonal variables in our series of patients but our intention in the future is to evaluate the molecular and genetic characteristics and the presence of estrogen receptors on surgical specimens to identify also the possible medical targeted therapy for this type of tumor.

A strong link between estrogen and thyroid cancer is still missing but the studies and findings reviewed here (summarized in table 6), open a potential new avenue and clinical utility for ER as a target for prevention and treatment of TPDs as well as a prototypical antiestrogen that can be used for therapeutic and preventive purposes of TPDs by not only suppressing the proliferation of thyroid cancer cells but also by inhibiting metastasis and angiogenesis associated events. This provides the foundation for multiple lines of investigation to elucidate estrogen's biological functions in thyroid diseases, specifically in the evolution of an aggressive phenotype. The use of functional estrogen-ER antagonist that affects the multifaceted biological activity of estrogen in thyroid disease singly or in combination provide a reasonable therapeutic and secondary preventive option that is novel, effective and devoid of unwanted side effects.

Molecular and cellular effects of estrogen on thyroid proliferative diseases.		
Study	Genotypic effects	Phenotypic effects
Furlanetto et al., 1999 [58]	↓ NIS expression	↑ Proliferation
Furlanetto et al., 2001 [65]	↓ Iodide uptake	↑ Proliferation
Manole et al., 2001 [59]	↑ ER- α , ER- β and cyclin D1	↑ Proliferation
Vivacqua et al., 2006 [54]	↑ MAPK	↑ Proliferation
Chan et al., 2006 [105]	Low urinary 2-OHE/16-OHE1 ratio	
Rajoria et al., 2010 [42]	↓ Cell-cell adhesion proteins	↑ Metastasis associated events
Rajoria et al., 2011 [43]	↑ Matrix metalloproteinases	↑ Metastasis associated events
Kamat et al., 2011 [82]	↑ VEGF	↑ Angiogenesis

NIS: sodium/iodide symporter; ↑: increase; ↓: decrease; ER: estrogen receptor; MAPK: mitogen-activated protein kinase; 2-OHE/16-OHE1: 2-hydroxyestrone and 16- α -hydroxyestrone ratio; VEGF: vascular endothelial growth factor.

Table 6: Molecular and cellular effects of estrogen on thyroid proliferative diseases. [S. Rajora et Al. *Biomedicine & Pharmacotherapy* 66 (2012) 151–158].

CONCLUSIONS

The incidence of thyroid cancer is increasing worldwide. Women of a reproductive age are at a threefold higher risk of developing thyroid cancer.

Premenopausal women are at highest risk for papillary and follicular thyroid carcinoma, implicating a role for estrogens in thyroid cancer.

The gender disparity in thyroid cancer incidence is age dependent. Although thyroid cancer is less common in men, they have a worse survival and more aggressive disease at presentation.

The identification of mechanisms of carcinogenesis of PTC in women vs men is a really important and innovative step for the study of thyroid cancer.

The rates of thyroid cancer differ across populations, yet the gender differences are uniformly observed among different regions and continents. Dietary and environmental factors do not appear to have a significant role in thyroid cancer gender disparity. Common somatic mutation in BRAF, RET/PTC and NTRK also do not account for the gender disparity in thyroid cancer.

Clinical and epidemiologic studies show no consistent evidence in reproductive factors contributing to thyroid cancer gender disparity.

Recent estrogen receptor-status studies in thyroid cancer cells demonstrate a difference in the receptor subtypes expressed based on the histology of thyroid cancer. Recent studies suggest estrogen and estrogen hormone receptor status in thyroid cancer cells may have a role in thyroid cancer progression. Moreover, the response to estrogen is dependent on the specific estrogen receptor expressed in thyroid cancer cells.

In our opinion, oestrogen may play an important role as a promoting factor in the development of PTC. The role of estrogen in the progress of PTC, once identified the precise mechanisms and steps of tumorigenesis, could open up areas, as in breast cancer, of developing potential new medical antireceptorial therapies to prevent and treat thyroid malignancies. This indication needs further study.

Our understanding of the environmental and genetic susceptibility for thyroid cancer will improve and can serve to help identify factors influencing thyroid cancer gender disparity.

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