

**UNIVERSITY OF MEDICINE AND PHARMACY OF  
CRAIOVA  
DOCTORAL SCHOOL**

**SUMMARY OF  
DOCTORAL THESIS**

**THYROID CANCER – CLINICAL, HISTOLOGIC  
AND IMMUNOHISTOCHEMICAL ASPECTS**

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## INTRODUCTION

Thyroid cancer (TC) is a rare oncologic entity, about 1% of all human malignant neoplasms, but representing the most common malignant endocrine neoplasia whose incidence has gradually increased over the past two decades, according to most National Cancer Registers. The data collected from Institutes of Epidemiology and Surveillance indicate a prevalence of thyroid cancer of 6,6/100.000 inhabitants with an average annual prevalence of 9,5/1000.000 for women and 3,5/100.000 for men with an annual growth >5% for both sexes, during the period 1975-2002 (Edwards BK, Brown ML, Wingo PA și colab, 2005; Giusti F, Falchetti A, Franceschelli F, și colab, 2010). In the US, thyroid carcinoma comprises about 1% of all cancers and represents 0,2% of cancer deaths (Enewold L, Zhu K, Ron E și colab, 2009; LiVolsi VA, 2011).

Potential etiological factors for thyroid cancer are exogenous factors (such as exposure to radiation, high intake of iodine, intake of nitrates, Western lifestyle or unknown environmental pollutants) and endogenous factors (such as high TSH level, the presence of Hashimoto's thyroiditis or obesity) (Pellegriti G, Frasca F și colab, 2013). However, it is still unclear which factor/ the most important factors would be contributed mainly to the recent increase in the incidence of thyroid cancer.

All these data in the medical literature underline the seriousness of thyroid cancer, the current stage of development of the human society. Therefore, in this doctoral thesis we proposed to conduct a clinical-statistical study on cases of thyroid cancer diagnosed and hospitalized in the Endocrinology Clinic of the Clinical Emergency Hospital of Craiova, during the period 2010-2015 and surgically treated in the same hospital. Also, we proposed to perform a histopathological and immunohistochemical examination of pieces of surgical excision in order to identify histological and immunohistochemical characteristics of these tumors.

Keywords: thyroid cancer, clinical study, histological study, histological staining, immunohistochemical markers, immunohistochemical reaction.

## **STATE OF KNOWLEDGE**

### **CHAPTER I – ANATOMY AND HISTOPHYSIOLOGY OF THYROID**

In this chapter are presented the embryology, anatomy, histology and physiology of the thyroid gland, being described the synthesis, secretion, transport and metabolism of thyroid hormones, mechanisms regulating thyroid gland and thyroid hormone effects.

### **CHAPTER II – BENIGN THYROID AFFECTIONS**

The second chapter of the thesis shows inflammatory diseases of the thyroid gland, thyroid hypertrophies and benign tumor lesions.

Inflammatory diseases are represented by:

-acute or chronic suppurative or non-suppurative infectious thyroiditis , rare disease including all forms of infection, other than viral and is caused by invasion of the thyroid by bacteria, mycobacteria, fungi or protozoa.

-subacute thyroiditis or De Quervain's thyroiditis, having as distinctive feature the granuloma, being defined by some authors as tuberculous thyroiditis.

-Autoimmune or lymphocytic Hashimoto's thyroiditis, having as main cause the non-iatrogenic primary hypothyroidism.

-Riedel's thyroiditis, extremely rare, histological examination highlighting the replacement of normal tissue with inflammatory cells, predominantly lymphocytes, plasma cells and eosinophils, and glandular tissue being arranged in a densely hyalinized connective tissue.

Thyroid hypertrophies having as representative the goiter – dystrophic hyperplasia with a diffuse or nodular growth, having as cause the iodine deficiency, parasitic, infectious, inflammatory, tumor diseases, gusogene food, drugs, growth factors, autoimmune diseases, stress, etc.

Benign tumor lesions of the thyroid, follicular adenomas, being defined as benign tumors, well-differentiated, surrounded by a capsule, of variable color and consistency, without invasion phenomena. Most patients are euthyroid and asymptomatic, yet about 1% of follicular adenomas can turn into “toxic adenomas”,

causing symptomatic hyperthyroidism.

### **CHAPTER III – THYROID CANCER – GENERAL DATA**

This chapter shows epidemiological data of thyroid cancer, highlights the increasing incidence worldwide, both by improving screening but also real increase in the number of thyroid cancers globally.

In the genesis of thyroid cancer, a lot of risk factors interfere: radiation, iodine intake, gland diseases already existing, and genetic factors.

Histologically, thyroid cancer was divided into:

- well-differentiated thyroid cancer: papillary, follicular, medullary
- poorly differentiated thyroid cancer
- undifferentiated thyroid cancer
- other types of cancer
- metastasis at thyroid level

Each type of cancer is described in this thesis in terms of clinical, macroscopic, microscopic, TMN staging, histological and immunoistochemical examination, and time evolution.

### **OWN CONTRIBUTIONS**

#### **CHAPTER IV – CLINICAL-STATISTICAL STUDY ON CASES OF THYROID CANCER CASES**

In this doctoral thesis we proposed to conduct a clinical-statistical study on cases of thyroid cancer diagnosed and hospitalized in the Endocrinology Clinic of the Clinical Emergency Hospital of Craiova, during the period 2010-2015 and surgically treated in the same hospital.

From 52 patients studied, the vast majority were women, male/female ratio being 7.66/1. Our data, correlated with recent studies published by some researchers, show an increased incidence of thyroid cancer particularly in females, without identifying a preponderant etiopathogenic agent.

Another feature of the group studied by us was a higher incidence of thyroid cancer in the countryside, especially since these patients have a lower

degree of intellectual training and less access to medical imaging examinations. One of the possible explanations could be reduced amount of iodine to food in rural areas.

Regarding the age of patients with thyroid cancer, we found that this type of cancer mostly occurs after the age of 40, but can occur in younger people under 20 years. Reducing the number of patients over 70 years with thyroid cancer it does not mean that the decade of age decreased risk factors, but rather that some patients with thyroid tumors die of other diseases.

Our study confirms that most thyroid cancers were papillary, followed by medullary ones. We believe that identifying follicular forms of thyroid cancer was complicated by follicular differentiation of papillary cancer. Fortunately, both forms have a reduced aggressiveness and they do not influence too much positive diagnosis and treatment of patient with thyroid cancer.

## **CHAPTER V – HISTOPATHOLOGICAL STUDY OF THYROID CANCER**

Histopathological study included a total of 38 fragments of surgical excision, coming from all of the many cases of thyroid cancers operated during 2010-2015 in Surgical Clinics of the Hospitals of Craiova.

From 38 fragments of thyroid tumors harvested after surgery, a number of 18 cases were diagnosed histopathologically as classic papillary carcinomas, 11 were diagnosed as papillary carcinomas – follicular form, 5 were interpreted as papillary carcinomas – tall cell form, 3 cases of papillary carcinoma – solid variant and 1 case – oncocytic variant.

Stromal changes in papillary thyroid cancer are extremely varied. Tumor stroma takes the most various aspects, from the appearance of loose connective tissue in the dense connective tissue with calcifications and even bone metaplasia. Most papillary carcinomas contain a central fibrovascular core, coated with one or sometimes multiple layers of columnar or cubic cells, with oval nuclei, crowded.

We must emphasize that we believe that most cases of papillary carcinomas are outbreaks that represent true proliferations of some independent cell clones.

## **CHAPTER VI – IMMUNOHISTOCHEMICAL STUDY OF THYROID CANCER**

The study was conducted on the same histopathological material included in paraffin, used also in the classic histopathological study, described in the previous chapter.

After preliminary preparation of biological material, all times of the immunohistochemical technique were fulfilled, namely, antigenic recovery, blocking endogenous peroxidase activity, blocking nonspecific sites, adding the primary antibody, proper signal detection and assessment of immunohistochemical reactions.

In order to characterize the types of thyroid cancers, we used the following immunohistochemical markers: Anti-Ki-67, Anti-p53, Anti-p63, Anti-E-cadherin, Anti-CD56, Anti-calretinin, Anti- BCL2, Anti-tyroglobulin.

In our study, we analyzed some aspects of tumor stroma, in papillary thyroid cancer, both in terms of histological and immunohistochemical and using Anti-vimentin, Anti-alpha-SMA, Anti-CD34 antibodies.

In our study, in order to assess the proliferative capacity of papillary thyroid cancer we have used anti-Ki-67 antibody; immunohistochemical evaluation has shown that the Ki-67 antigen expression was very low, less than 5% of the nuclei of tumor cells were positive for this antigen which explains the relatively low capacity for growth of this type of neoplasm.

Overexpression of p53 protein was found to (44,73%) while the remaining 21 cases (55,27%) p53 protein expression was negative.

In our study, p63 was intensely positive in only 5 cases (13,15%), moderately positive in 8 cases (21%) and weakly positive in 25 cases (65,85%).

I also noticed an intense reaction of E-cadherin in well-differentiated papillary carcinomas and very weak to his absence in poorly differentiated carcinomas, which shows that E-cadherin can be used as a marker of cell differentiation in thyroid cancers.

In all cases of papillary thyroid carcinoma in the study,

immunohistochemical expression of CD56 and calretinin was negative.

## **CHAPTER VII – CONCLUSIONS**

In this doctoral thesis, we conducted three studies of thyroid cancer: clinical-statistical study, histopathological study and immunohistochemical study.

Clinical-statistical study was conducted on a total of 52 people who were admitted and readmitted in the Endocrinology Clinic of the Clinical Emergency Hospital of Craiova during 2010-2015, with different forms of thyroid cancer, operated in Surgery Clinics of Craiova or at the Institute of Endocrinology in Bucharest.

From 52 patients with thyroid cancer, the majority were women (88,64%), rural area representing 67,31%, and the age of 40-45 years having a percentage of 86,54%. Histologically, papillary cancers predominated (94,23), the rest being represented by medullary carcinomas (5,77%).

Histopathological study of thyroid cancer was conducted on a total of 38 pieces of surgical excision collected from as many patients diagnosed clinically, biologically and imagistically with thyroid cancer. All cases were represented by papillary thyroid carcinomas, with classic, follicular, tall cell, solid and oncocyctic variant.

The defining elements of tumor cells were represented by the aspect of nuclei. Nuclei appeared oval, in “coffee beans”, hypochromic, nucleolated, often arranged in basal cell pole, crowded or overlapping. Sometimes the nuclei appearance was “frosted glass” or “Orphan Annie-eye”, or with grooves and nuclear inclusions.

In the immunohistochemical study of thyroid cancer we used 11 antibodies for determining the proliferative activity, genetic modifications, cytokeratin modifications as well as stromal vascularization.

p53 protein expression was positive in 44,73% cases, and p63 protein was intensely positive in only 13,15% cases.

E-cadherin was intensely positive at the level of well-differentiated papillary thyroid carcinoma, instead calretinin and CD56 adhesion molecule were negative

in all cases of papillary carcinoma studied.

BCL-2 protein had an intensely positive reaction in 21% cases, with a negative expression of 55,25%.

Tyroglobulin and CK19 were expressed very intensely in papillary thyroid cancer analyzed in our study.