**DETAILED DESCRIPTION OF RESEARCH PROPOSAL**

**Scientific Area:** Laboratory Histology - Histopathology

**Keywords:** Hashimoto's Thyroiditis, Thyroid Carcinoma, Clinicopathological Characteristics, Molecular Markers, Risk Factor, Prognosis

**University / Department / Sector / Laboratory:** University of Western Attica, Department of Biomedical Sciences, Department of Medical Laboratories, Histology - Histopathology Laboratory

**Language:** Greek

|  |
| --- |
| **PROPOSED TITLE OF DOCTORAL THESIS:** Correlation of Hashimoto's Thyroiditis (HT) with Papillary Thyroid Carcinoma (PTC): Clinicopathologic and Molecular Indicators in the Study to Promote HT as a Possible Predisposing and Prognostic Indicator of Disease |
| **Detailed description of research proposal** |
| **Thyroid Gland**The thyroid gland is one of the largest in the human body. Located in the anterior cervix consists of two lobes that are connected to each other by a thin zone of thyroid tissue. Each pod is 1-1.5 cm thick, 1.5-2 cm wide and 2.5-4 cm long. The main function of the thyroid is the uptake of iodine through the diet and its utilization for the production of thyroid hormones thyroxine T4 and T3. Thyroglobulin (TG) and thyroid peroxidase (TPO) proteins are involved in the synthesis of these hormones, while thyroid hormone (TSH) controls all stages of synthesis. The biological activity of these hormones is due to their free molecules (FT3, FT4) which bind to almost all cells of all tissues of the body, their blood levels being particularly important for the normal functioning of all systems (1-3 , 4, 5, 6).**Hashimoto's Thyroiditis** Hashimoto's thyroiditis (autoimmune or chronic lymphocytic thyroiditis, HT) is one of the most common autoimmune diseases, the most common inflammatory thyroid disease and the most common cause of hypothyroidism in the general population. It affects people of all ages but has an increased impact on women (7 times more than men) and is more common in middle age (7, 8, 9).It is characterized by progressive replacement of healthy thyroid gland parenchyma by lymphocytes and fibrous tissue. Anti-thyroid antibodies (anti-Tg) and anti-thyroid peroxidase (anti-TPO) antibodies are detected in the serum of patients. The disease usually progresses slowly and in the early stages is asymptomatic. HT has a gradual loss of thyroid function, resulting in patients developing hypothyroidism, often a bronchial hernia (9, 10).The factors that contribute to the appearance of HT are genetic and environmental. In the thyroglobulin gene, many point mutations have been identified that are also associated with HT, and racial differences have been identified in the detection of these mutations. Also, mutations and polymorphisms of HLA class I and HLA class II genes have been associated with the development of autoimmune thyroid disease. Environmental contributing factors are high levels of iodine and selenium deficiency, smoking, stress, infections, medications, alcohol consumption and low levels of vitamin D (11, 12, 13, 14).Hashimoto's clinically becomes apparent when hypothyroidism develops. Patients with severe hypothyroidism show symptoms of almost all systems including chronic fatigue, lack of mood, depression, muscle cramps, muscle weakness, hallucinations, arthralgia and bradycardia, cold skin, oily skin. Deficiency of thyroid hormones reduces the synthesis of hemoglobin and causes malabsorption of folic acid, iron and vitamin B12 (9, 10, 15).The diagnosis is mainly laboratory and includes detection of anti-thyroid antibodies (anti-Tg and anti-TPO) in the patient's serum, measurement of thyroid hormones TSH and FT4, as well as ultrasound imaging which shows the characteristic heterogeneous presentation of thyroid parenchyma. Treatment of thyroiditis is symptomatic and consists of administration of thyroxine in subjects with elevated TSH levels and long-term substitution doses (16).**Papillary thyroid carcinoma (PTC)**The most important types of thyroid cancer are Papillary Carcinoma (PTC), Follicular Carcinoma (FTC), Myeloid Carcinoma (MTC), Low Thyroid Carcinoma (PDTC) and Atypical Cancer (ATC).Papillary carcinoma (PTC) is the most common type of cancer, accounting for 75% to 85% of all cases of thyroid cancer. It comes from follicular cells, is characterized by highly differentiated cells with a papillary architecture and features that include the oval, elongated nucleus as well as grooves. It occurs more frequently in women with a 3: 1 ratio than men and more specifically in the 30-50 age group. The incidence of carcinoma is associated with iodine uptake in each region, with predisposing hereditary factors and with prior radiation exposure. It is also found in patients with Hashimoto's thyroiditis (17).Often patients are asymptomatic and present with the first manifestation of cervical disease, either in the form of thyroid nodules or in the form of cervical lymph node infiltration. The disease is diagnosed by taking into account the patient's history, clinical and laboratory findings, thyroid ultrasound and FNAC biopsy.Clinicopathologic staging of the tumor is performed according to TNM criteria in accordance with the American Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) based on the largest tumor diameter (T) and the presence or absence of extravascular invasion or not of the lymph nodes (N) and any distant metastases (M) it has given (18, 19).The prognosis of the disease is related to the staging of the tumor, gender and age. In general, papillary carcinoma has a very good prognosis, even complete treatment; in particular 90% of patients have ten years survival. Of these, about 15% have recurrence after the first decade and the mortality rate reaches 5% (17).The treatment of papillary carcinoma is usually total thyroidectomy especially in cases of multifocal cancer. In some cases, total lobectomy or hemithyroidectomy is recommended. A typical treatment method is the administration of radioactive iodine and thyroxine to high-risk patients (20).**Correlation of Hashimoto's Thyroiditis with Papillary Carcinoma of the Thyroid**The relationship between HT and PTC was first studied in 1955 since then, many studies have been conducted on whether their coexistence is accidental or whether the role of HT in the development of PTC is predisposing, protective or aggravating.Many theories have been formulated about the mechanism of the link between the two diseases. HT is a chronic inflammatory condition associated with the development of cancer given the damage caused to cellular DNA through the production of reactive oxygen species (ROS) (21).HT leads to hypothyroidism and therefore to elevated TSH levels. Elevated TSH levels have been shown to induce the fastest proliferating rate of thyroid cells, indicating HT as a risk factor for PTC development (22, 23, 24).Complementary to the above work hypotheses and the conclusions that lead to the predisposing role of HT in PTC development and in contrast to them the coexistence of HT seems to be related to better outcome of the disease, in particular smaller and better targeted tumors. , milder lymph node invasions, lower metastasis rates, increased life expectancy and greater disease free time. This protective effect of HT may be due to the body's immune response to both diseases. Tg and TPO are the major antigenic targets for the cellular immune response to HT leading to and ultimately destroying the thyroid gland. The same seems to be the case in PTCs where Tg and TPO are also antigenic targets, so it is likely that anti-thyroid antibodies attack cancer cells just as in healthy ones (25, 24, 22).The study of molecular and genetic mechanisms has revealed other interesting and unclear points concerning the coexistence of the two diseases. More specifically the mutation of the BRAF V600E oncogene is known to be associated with an adverse and more aggressive manifestation of PTC. However, it is significantly less frequently found in patients with PTC co-existing with HT, a fact that may be due to its protective effect (26, 25).A particularly important chromosomal translocation of the RET oncogene, encoding a tyrosine kinase receptor, is the RET / PTC rearrangement that is found in the majority of HT-free tissues without detectable PTC, which could link cancer development to macrophage 26 , 21, 23).The above makes the MAP kinase signaling pathway mutations particularly interesting and indefinable in relation to the association of HT and PTC. The same is true for the PI3K / AKT signaling pathway involved in the balance between pro- and anti-apoptotic cell signals and therefore carcinogenesis and appears highly activated with increased expression in HT and PTC tissues. Research shows that patients with Hashimoto's disease are three times more likely to be diagnosed with differentiated thyroid cancer than those who do not. The above finding is also supported by immunohistochemical findings demonstrating increased expression of phosphorylated Akt, Akt1 and Akt2 kinases in tissue regions with HT and PTC compared to regions surrounded by healthy tissue (24, 26).Equally important is the expression of p63, an homologous protein of the p53 suppressor protein after alternative splicing, which is found in the vast majority of samples with HT and PTC exclusively, as it is not detected in specimens with other malignancies (21, 26 ). **Purpose of the research project**This proposal will examine the mechanisms through which Hashimoto's thyroiditis and papillary thyroid carcinoma may be linked. It will also attempt to identify the role of HT as a risk factor for thyroid cancer development and evaluate it as an indicator of the course of progression and progression of cancer, as it has not yet been established whether the two diseases, whose prevalence is significant especially in the female gender, they are actually correlated or just coincidental.**Research project methodology*** **Selection of N = 100 patients all of whom underwent thyroid surgery**
* **Health History collection for each patient**
* **Performing ultrasound of the thyroid gland**
* **Type of thyroid disease and confirmation of malignancy were determined by histopathological analysis of tissues**
* **The histological criteria used for the diagnosis of HT include: diffuse lymphoblastic cell infiltration, enlarged large nuclei epithelial cells, and eosinophilic cytoplasm**
* **The following variables were studied:**
* **Age**
* **Gender**
* **Cytological examination** **with fine needle aspiration cytology (FNAC)**
* **The size and the differentiation of the tumor**
* **The multifocality of cancer**
* **The extrathyroidal extension**
* **The extent of surgical removal**
* **Metastasis to local lymph nodes**
* **Distant metastases, Staging of the disease is by TMN**
* **Conduct thyroid function hormone tests, in particular:**
* **Thyroxine T4**
* **Triodothyronine T3**
* **Free T4**
* **Free T3**
* **Thyroglobulin TG**
* **Thyroid Peroxidase Antibodies (Anti-TPO)**
* **Thyroglobulin Antibodies (Anti-Tg)**
* **Thyroid hormone TSH**
* **Use of immunohistochemical techniques in a portion of tissue preparation to identify proteins as gene expression products associated with papillary carcinoma and Hashimoto. Specific study of HMBE-1, CK-19, TTF-1, Thyroglogoulin, BRAF, p53, PTEN**
* **Isolation of genetic material from selected thyroid tissues**
* **Amplification of the desired gene sequence using polymerase chain reaction (PCR) and study / analysis of the BRAF V600E gene mutation**
* **Statistical analysis of the results using the IBM SPSS statistical program**

**The contribution to theoretical and / or applied scientific knowledge**The present study will examine in depth the mechanisms that appear to link PTC development in HT patients and elucidate the role of HT in the development and prognosis of cancer. Several carcinogenic mechanisms will be studied separately and in combination, such as the chronic inflammatory state that causes damage to cellular genetic material, increased levels of hormones that induce strong cellular proliferation, MAP kinase signaling pathways, important oncogenes but also tumor suppressor genes and the localization of the expression of their oncogenic protein-related protein molecules in patients who will be grouped based on numerous variables such as gender, tumor size and stage of disease.The remarkable research interest in the subject and the study of the literature indicate the relationship between the two diseases, but the way in which they interact and the effects of this interaction have not been sufficiently documented in order to use this valuable information in more effective management and assessment of the relevant incidents. |
| The study is expected to be completed in 36 months. |
| **Bibliography** |
| 1. Linder HH. The thyroid gland. In: Clinical Anatomy. Appleton &Lange 19892. Dumont J, Opitz r, Christophe D, Vassart G, Roger PP, Maenhaut C. Ontogeny, Anatomy, Metabolism and Physiology of the Thyroid. 2015 Jul 15. In: De Groot LJ, Beck-Peccoz P, Chrousos G, Dungan K, Grossman A, Hershman JM, Koch C, McLachlan R, New M, Rebar R, Singer F, Vinik A, Weickert MO, editors. Endotext. South Dartmouth (MA): MDText.com, Inc.; 2000-. PMID: 25905409.3. Francis S. Greenspan. The thyroid gland. In: Basic Endocrinology. Francis S. Greenspan & David G. Gardner. 7th edition. Lange p 215-291 20044. Miot F, Dupuy C, Dumont J, Rousset B. Chapter 2 Thyroid Hormone Synthesis and Secretion. 2015 Sep 2. In: De Groot LJ, Beck-Peccoz P, Chrousos G, Dungan K, Grossman A, Hershman JM, Koch C, McLachlan R, New M, Rebar R, Singer F, Vinik A, Weickert MO, editors. Endotext. South Dartmouth (MA): MDText.com, Inc.; 2000-. PMID: 259054055. Dunn JT, Dunn AD. Thyroglobulin: chemistry, biosynthesis, and proteolysis. In: Braverman LE, Utiger R, editors. The Thyroid. Philadelphia: Lippicott Williams & Wilkins, 2000: 91-1046. Brent GA. Mechanisms of thyroid hormone action. J Clin Invest. 2012 Sep;122(9):3035-43. doi: 10.1172/JCI60047. Review. PMID: 229456367. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab. 2002 Feb;87(2):489-99. PMID:118362748. McLeod DS, Cooper DS. The incidence and prevalence of thyroid autoimmunity. Endocrine. 2012 Oct;42(2):252-65. Review. PMID: 22644837.9. Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: clinical and diagnostic criteria. Autoimmun Rev. 2014 Apr-May;13(4-5):391-7. doi: 10.1016/j.autrev.2014.01.007. Review. PMID: 2443436010. Radetti G. Clinical aspects of Hashimoto's thyroiditis. Endocr Dev. 2014; 26:158-70. doi: 10.1159/000363162. Review. PMID: 2523145111. Dong YH, Fu DG. Autoimmune thyroid disease: mechanism, genetics and current knowledge. Eur Rev Med Pharmacol Sci. 2014;18(23):3611-8. Review. PMID: 25535130.12.Wiersinga WM. Thyroid autoimmunity. Endocr Dev. 2014;(26):139-57. doi: 10.1159/000363161. Review. PMID: 25231450.13. Effraimidis G, Wiersinga WM. Mechanisms in endocrinology: autoimmune thyroid disease: old and new players. Eur J Endocrinol. 2014 Jun;170(6):R241-52. doi: 10.1530/EJE-14-0047. Review. PMID: 24609834.14. Wiersinga WM. Clinical Relevance of Environmental Factors in the Pathogenesis of Autoimmune Thyroid Disease. Endocrinol Metab (Seoul).106 2016 Jun;31(2):213-22. doi: 10.3803/EnM.2016.31.2.213. Review. PMID: 27184015.15. Davis JD, Tremont G. Neuropsychiatric aspects of hypothyroidism and treatment reversibility. Minerva Endocrinol. 2007 Mar;32(1):49-65. Review. PMID: 17353866.16. Lee JH, Anzai Y. Imaging of thyroid and parathyroid glands. Semin Roentgenol. 2013 Jan;48(1):87-104. doi: 10.1053/j.ro.2012.09.003. Review. PMID: 23158053.17.Ανθούλη – Αναγνωστοπούλου Φ., Ιστοπαθολογία με Στοιχεία Ογκολογίας, 2009:Εκδόσεις Πασχαλίδης18. Sobin, L.H., M.K. Gospodarowicz, and C. Wittekind, TNM Classifiaction og Malignant Tumours. 7th ed. 2009, New York: John Wiley & Sons.19. Edge, S.B., et al., AJCC Cancer Staging Manual. 7th ed. 2010, New York:Springer20. Kυρούδη Α, Φλάρης Ν, Αβραμίδης Α. Καρκίνος του θυρεοειδή. Θηλώδες καρκίνωμα του θυρεοειδή. Σε: Αβραμίδης Α. Ενδοκρινολογία. University Studio Press 2000. Τόμος Α’ θυρεοειδής, 28: 253-262. 21. Barbora Uhliarova a\* , Andrej Hajtman b. Hashimoto's thyroiditis-an independent risk factor for papillary carcinoma. Braz J Otorhinolaryngol. 2018;84(6):729-73522. J. LIANG, W. ZENG, F. FANG, T. YU, Y. ZHAO, X. FAN, N. GUO, X. GAO. Clinical analysis of Hashimoto thyroiditis coexistent with papillary thyroid cancer in 1932 patients. ACTA OTORHINOLARYNGOLOGICA ITALICA 2017;37:393-400: doi: 10.14639/0392-100X-1709.23. Maria Trovato, Giuseppe Giuffrida, Antonino Seminara, Simone Fogliani, Vittorio Cavallari, Rosaria Maddalena Ruggeri, Alfrdo Campenni. Coexistance of diffuse large B-cell Lymphoma and papillary thyroid carcinoma in a patient affected by Hashimoto's thyroiditis. Arch Endocrinol Metab..2017;61/6 24. Ctherine Kapnopoulou, Nikolaos Thalassinos, Despina Dessou, Maria Venetikou, Fragiski Anthouli-Anagnostopoulou. Correlation of Hashimoto's Thyroiditis with Thyroid Cancer: Is it a random Coexistance or a particular Risk factor? Review clinical pharmacology and pharmacokinetics, international edition 29: 5-12 (2015) 25. Shinje Moon, Hye Soo Chung, Jae Myuang Yu, Hyuang joon Yoo, Jung Hwan Park, Dong Sun Kim, Young Joo Park. Assotiations between Hashimoto Thyroiditis and Clinical Outcomes of Papillary Thyroid Cancer: A Meta-Analysis of Observational Studies. Endocrinol Metab 2018;33:473-48426. Csaba Molnar, Sarolta Molnar, Judit Bedekovics, AttilaMokanszki, Ferenc Gyory, Endre Nagy, Gabor Mehes. Thyroid Carcinoma Coexisting with Hashimoto's Thyreoiditis: Clinicopathological and Molecular Characteristics Clue up Pathogenesis. Pathology & Oncology Research 2019 |