eISSN 2255-0569

ORIGINAL

Gene expression of *COX-2*, *MLH1* and *MSH2* in papillary thyroid carcinoma: a retrospective analysis

Expresión génica de COX-2, MLH1 y MSH2 en carcinoma papilar de tiroides: un análisis retrospectivo

Edgar García-Torres[1], Graciela Zambrano- Galván[2], Juan Carlos Cuevas- González[3], Víctor Hiram Barajas Pérez[1]

Research professors

1. Faculty of Dentistry. Juarez University of the State of Durango

2. Nutriepigenomics and Clinical Nutrition Laboratory, Faculty of Medicine and Nutrition. Juarez University of the State of Durango

3. Department of Stomatology, Institute of Biomedical Sciences, Autonomous University of Ciudad Juárez

Corresponding author
Dra. Graciela Zambrano- Galván
E-mail: gzambrano@ujed.mx

Received: 20 - VII - 2022
Accepted: 4 - VIII - 2022

doi: 10.3306/AJHS.2022.37.05.111

Abstract

Objetives: To determine the mRNA expression of the *COX-2, MLH1* and *MSH2* genes in Papillary Thyroid Carcinoma.
Methods: This is comparative cross-sectional clinical molecular study. The study was constituted by 26 biopsies with histopathological diagnosis of Papillary Thyroid Carcinoma, from the 450 General Hospital of Durango State. The relative gene expression of the *COX-2, MLH1* and *MSH2* was performed by qPCR.
Results: The mean age of patients was 46 ± 15.22. They were observed differences in the gene expression of *COX-2* ($p = 0.001$), *MLH1* ($p = 0.06$) and *MSH2* ($p = 0.09$) in the total Papillary Thyroid Carcinoma sample. The correlation between gene expression and patient age was $r^2 = 0.441$ ($p = 0.024$) for the *MSH2* gene. The correlation of mRNA expression between *COX-2* and *MLH1* was $r^2 = 0.69$ ($p = 0.001$); for *COX-2* and *MSH2* of $r^2 = 0.47$ ($p = 0.01$), while for *MSH1* and *MSH2* of $r^2 = 0.94$ ($p = 0.001$).
Conclusions: The results obtained in the present study showing the presence of gene expression of molecular targets such as the *COX-2, MLH1* and *MSH2* genes, contributing to the understanding of the molecular physiopathology of the Papillary Thyroid Carcinoma.

Key words: Papillary thyroid carcinoma, gene, expression.

Resumen

Objetivos: Determinar la expresión del mRNA de los genes *COX-2*, *MLH1* y *MSH2* en Carcinoma Papilar de Tiroides.
Métodos: Se trata de un estudio clínico- molecular transversal comparativo. El estudio estuvo constituido por 26 biopsias con diagnóstico histopatológico de Carcinoma Papilar de Tiroides, del Hospital General 450 del Estado de Durango. La expresión génica relativa de *COX-2*, *MLH1* y *MSH2* se realizó mediante qPCR.
Resultados: La edad media de los pacientes fue de 46 ± 15,22 años. Se observaron diferencias en la expresión génica de *COX-2* ($p = 0,001$), *MLH1* ($p = 0,06$) y *MSH2* ($p = 0,09$) en la muestra total de Carcinoma Papilar de Tiroides. La correlación entre la expresión génica y la edad del paciente fue de $r^2 = 0,441$ ($p = 0,02$) para el gen *MSH2*. La correlación de la expresión de ARNm entre *COX-2* y *MLH1* fue $r^2 = 0,69$ ($p = 0,001$); para *COX-2* y *MSH2* de $r^2 = 0,47$ ($p = 0,01$), mientras que para *MSH1* y *MSH2* de $r^2 = 0,94$ ($p = 0,001$).
Conclusiones: los resultados obtenidos en el presente estudio muestran la presencia de expresión génica de dianas moleculares como los genes *COX-2*, *MLH1* y *MSH2*, contribuyendo al conocimiento de la fisiopatología molecular del Carcinoma Papilar de Tiroides.

Palabras clave: Carcinoma papilar de tiroides, gen, expresión

Introducction

Thyroid cancer (TC) is the main malignant neoplasm of the endocrine system, which in the United States accounts for 6% of tumors in general[1], of which 80% corresponds to papillary thyroid carcinoma (PTC)[2], and of which it has manifested greater incidence in the last decades in different populations around the world[3-6]. It affects men and women, predominating in the female sex[7]. The PTC belongs to the well-differentiated TC originated from follicular cells of the thyroid gland[8], usually follows a slow and non-aggressive behavior that generally shows a low mortality; however[9],; there is knowledge of variants with different morphological and molecular manifestations that confer a high potential for aggressiveness[10]. There are risk factors such as age, sex, exposure to ionizing radiation and gene alterations so its clinical manifestation[11-13], approach and prognosis depends largely on the histopathological variants, which are identified using hematoxylin and eosin staining[10,14]. It should be mentioned that although histopathological assessment is the gold standard for diagnosis, the implementation of molecular tools has become important in recent years, since previous studies have shown the relationship between altered levels of gene expression with the manifestation and development of several types of cancer including PTC[15-17].

In this regard, it has been proposed that cyclooxygenases belonging to the family of enzymes that catalyze arachidonic acid resulting in prostaglandins have pain and inflammation regulatory functions, there are two isozymes *COX-1* and *COX-2*, of these the *COX-2* (cyclooxygenase 2) represents an inducible inflammatory initiating enzyme that is mediated under normal conditions, but under pathological stimuli such as inflammatory mediators and kinases shows an increased activity. Different studies have shown an overexpression of this protein in a large variety of tumors in humans, and its altered presence suggests an important action on progression, transformation and even tumor angiogenesis[18-22].

Similarly, the alterations that the repair mechanism of the DNA called Reparative System or Mismatch Repair[23] controlled by the *MLH1, MSH2, MSH6* and *PMS2* genes can undergo mainly[24]. When there is damage to the DNA, the reparative system acts by activating the cell cycle, stopping it or sending the cell to a programmed death. However, it has been observed that the inactivation or alteration of this system is related to the presence of hereditary or spontaneous cancer[25] because the main function of *MLH1* is to form a ternary complex with DNA in mismatch and the MutSα complex, increasing discrimination between heteroduplexes and homoduplexes, which also works in meiotic recombination; whereas *MSH2* recognizes the mismatches between base-base and the insertion-deletion loops[26]. It is for all of the above that in the present study the objective was to determine the mRNA expression of the *COX-2, MLH1* and *MSH2* genes in papillary thyroid carcinoma.

Methods

A comparative cross-sectional study was carried out, in which a total of 26 samples included in paraffin blocks (previous signature of informed consent and biopsy), corresponding to patients with a histopathological diagnosis of PTC in the period 2007-2014 were included. Those samples included in paraffin that did not present a complete clinical file were excluded. From the clinical files, sociodemographic data were collected such as age, sex, place of birth and residence, the definitive diagnosis of PTC, time of evolution, type of biopsy used, size of the lesion and its statification according to the tumor- nodule-metástasis system (TNM). This work was approved by the ethics committee of the General Hospital 450 of the Health Services of the State of Durango with a unique registration number assigned 405/014.

Procedures

Tissue Obtention

A histological cut was made with a thickness of 4 µm which was placed on a slide to proceed with the mRNA extraction.

mRNA extraction

To obtain the mRNA, the QuickExtract ™ FFPE RNA Extraction Kit mRNA extraction kit from EPICENTRE (Madison, WI, USA) was used according to the manufacturer's instructions.

Synthesis of cDNA.

The cDNA has obtained by the iScript ™ cDNA Synthesis Kit from BIO-RAD (Hercules, CA, USA), following the manufacturer's instructions.

Analysis of COX-2, MLH1 and MSH2 gene expression

The sense and antisense primers were designed in the PRIMER BLAST program of NCBI (Bethesda, MD, USA), and were synthesized by Integrated DNA Technologies (Coralville, IA, USA). The sequence and the size of the product for each gene are shown in **table I**. The PPIA gene was applied as a normalizing gene. The intercalating dye used was QuantiFast SYBR Green PCR Kit from BIO-RAD (Hercules, CA, USA) according to the manufacturer's instructions. The evaluation of the relative quantification of gene expression was carried out in an Eco Real-Time PCR System of Illlumina (San Diego, CA, USA). The polymerase activation it started at 95ºC for 15 min, followed for the initial denaturalization at 95ºC for 15s, and anneling-extension at 65ºC for 15s for 40 qPCR cycles.

Table I: The sequence and the amplicon size of the product of *COX-2, MLH1, MSH2* genes.

GENE	MOLECULAR LOCUS	EXÓN	OLIGONUCLEÓTID SECUENCE	AMPLICON SIZE
COX-2	1 q25.2-q25.3	10	F'TCT CAG ACG CTC AGG AAA TAG A-3' R'GTC GTT GAC CTC GTC TGT TAT G-3'	113 bp
MLH1	3p21.3	21	F'GTC CAC TGT AAC CTG CCT AAT C-3' R'CCA GCC CAA GAT GTC TCT TAA C-3'	99 bp
MSH2	2 p21	19	F'GAA GAT GGT GAG TGA GGA TAG G-3' R'GTG GAC TGA AAC TGT GCT AAT G-3'	94 bp

Statistical analysis.

Frequencies and central tendency measures were used for the analysis of the descriptive data. To determine the differences between means, the relationship and correlation between variables, the Student's t-test, Chi-square test, and Pearson's correlation were used, respectively. The statistically significant differences were estimated with a p <0.05 value. The statistical program IBM SPSS version 22 (Chicago, Inc.) was used. For the analysis of relative expression, the REST program of QIAGEN v.2009 was used.

Results

Twenty six samples of PTC were analyzed. The mean age in patients was 46.1 ± 15.52, being the youngest of 22 and the oldest of 82 years; 63% of the patients were born in the capital of the State of Durango, Mexico; of which 81% had a permanent residence in the mentioned location. The clinical manifestations of PTC in patients younger than 45 years of age are shown in **table II**. It is important to mention that the approach for biopsy was 46% thyroidectomy, 31% hemi-thyroidectomy, 19% excisional approach and only 4% incisional.

The reference *PPIA* gene allowed to observe differences in the expression of mRNA of *COX-2* (p = 0.001), *MLH1* (p = 0.06) and (p = 0.09) in CPT, presented in **figure 1**.

A statistically non significant correlation was observed (r^2 = 0.037, p = 0.85) between the expression of *COX-2* mRNA and the age of the patient at the time of

Table II: Clinical manifestation of the papillary thyroid cancer in patients younger than 45 years of age.

CPT Clinical Manifestatión	<45 years %(n=13)	>45 years %(n=13)	Total %(n=26)	P≤ 0.05*
Tumor Clinical Extention				
≤ 1cm	---	---	---	---
> 1- ≤ 4cm	30(4)	70(9)	50(13)	0.05
> 4cm	70(9)	30(4)	50(13)	
Diameter of CPT				
< 1cm	15(2)	15(2)	16(4)	0.67
1-2cm	47(6)	62(8)	54(14)	
> 2 - ≤4cm	38(5)	23(3)	30(8)	
> 4cm	---	---	---	
Metastasis to lymph nodes				
Present	23(3)	8(1)	16(4)	0.27
Ausent	77(10)	92(12)	84(22)	
TNM Estadificacion**				
TNM I	77(10)	8(1)	42(11)	0.001
TNM II	23(3)	62(8)	42(11)	
TNM III	---	30(4)	16(4)	
TNM IV	---	---	---	

*Fisher´s exact test for statistical analysis. **Tumor-nodule- metastasis.

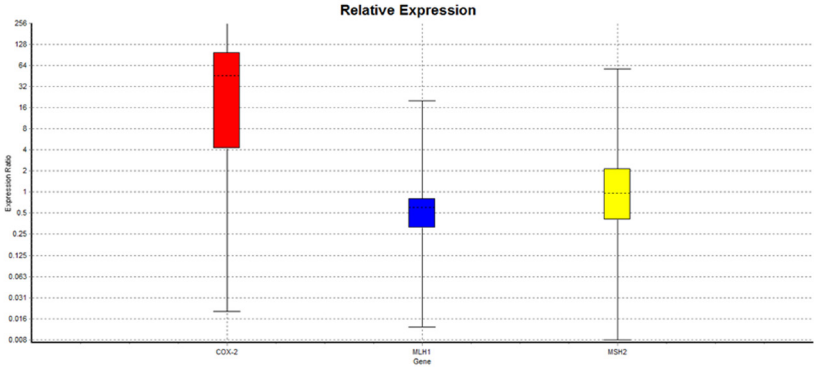
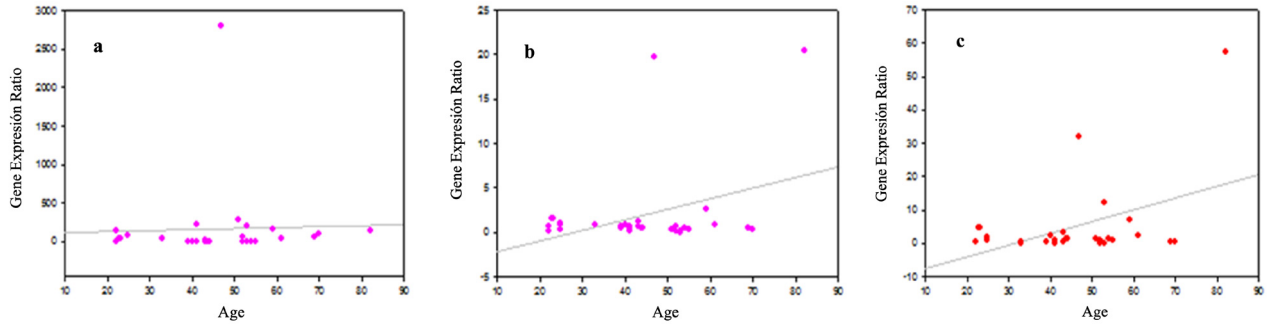


Figure 1: Results of the mRNA expression of *COX-2, MLH1, MSH2; PPIA** mRNA expression was used to reference gene.

*The results are calculated according to the average of the Ct values. Whiskers represent mean ± standard deviation for each group. Boxes represent mean ± SEM (standard error of mean). ǂT-Student test.

Figure 2: Correlational analysis between the age patient's and gene expression of (a) *COX-2* (r2= 0.037, p=0.85*), (b) *MLH1* (r2= 0.34, p=0.08) and (c) *MLH2* (r^2=0.441, p= 0.02).



diagnosis (**Figure 2a**), while the expression of *MLH1* mRNA and age of the patient at the time of diagnosis shows a statistically non significant correlation (r^2 = 0.34, p = 0.08) as seen in **figure 2b**. On the other hand, the expression of *MSH2* mRNA against the age of the patient at the time of diagnosis shows a mean and statistically significant correlation (r^2 = 0.44, p = 0.02), which is observed in **figure 2c**.

When the age was evaluated based on the clinical extension of the PTC, a statistically significant inverse correlation was found, since the patient's older age showed a smaller clinical lesion size (r^2 = -0.393, p = 0.04).); otherwise, when evaluating the age and diameter of PTC (r^2 = 0.08, p = 0.67).

Statistically significant difference was found between the clinical extension of the PTC and the age groups (p=0.05), **table II**. On the other hand, the diameter of the PTC was not statistically different between patients older and younger than 45 years (p= 0.67); **table II**. A correlation of r^2= 0.69 (p = 0.001) was obtained between the expression of mRNA of *COX-2* and *MLH1*; for *COX-2* and *MSH2* of r^2= 0.47 (p = 0.01), and for *MSH1* and *MSH2* of r^2= 0.94 (p = 0.001).

Discussion

The papillary thyroid carcinoma (PTC) is the most frequent malignancy of the thyroid gland, in general it tends to have a good biological behavior, however it is a disease that has a wide range of histological variants which confers changes in the prognosis of the lesion and it is important to focus your study with a molecular point of view. There are limitations for early diagnosis of PTC, which makes it difficult to understand how the lesion is differentiated in histological variants, resulting in late and radical treatments that compromise the patient's health.

The results found in this study show that the average age of the patients is consistent with the results shown in other studies where they are 45 years old[27], as the most frequent at the time of diagnosis compared to the one found in this study that was 46 years old. The age of the patient at the time of diagnosis has been established by the scientific literature as a risk factor for the development of thyroid carcinoma and in the same way as a prognostic factor, within the risk factor the presence of thyroid nodules is frequent in individuals that exceed the fourth decade of life and shows an increase in the incidence in patients within 40-50 years, it is worth mentioning that there have been cases in patients under the age of 16 years[28,29]. As a prognostic factor, age is used by the TNM system to stage the lesion solely and exclusively in thyroid carcinoma, and it is known that after 45 years, local aggressiveness and metastatic capacity increase at a distance[11,30,31]. Some studies conducted in the United States show that women are mostly affected by PTC at a female: male ratio that ranges from 2: 1 to 4: 1[28], while in Mexico the ratio is 4: 1 as reported by the National Institute of Cancerology[27]. In addition to being the most frequent PCT in women, they also have a better prognosis compared to men[17,32,33]. It should be mentioned that the literature reports that ethnicity plays an important role as a risk factor, in the United States the white race has greater affectation than the black race at a ratio of 11: 5 respectively[34]. In Hispanic, Hawaiian, Chinese and Japanese populations, women are more affected, and such differences can be attributed to genetic factors or to the type of diet without being completely clear[32,35]. In the present study, 63% of the patients affected with PTC were born in the capital of the State of Durango, Mexico; and that 81% had a permanent residence in the mentioned location. The importance of the *COX-2* gene in the development of malignant neoplasms is well established and its presence promotes cell invasion and growth. It can be present in many cancers such as colon, pancreas, stomach, cervix, esophagus, breast, lung and melanoma; some studies mention a high expression of *COX-2* in thyroid carcinomas compared with thyroid adenomas, similarly there was greater expression in undifferentiated thyroid carcinomas than in CPT in a study conducted in the Chinese population[36]. Regarding the results obtained in this study, they indicate that there is an overexpression of *COX-2* in 77%. Likewise, the presence of an increase in the *COX-2* gene has been reported in patients aged>

50 years suffering from papillary thyroid carcinoma[37]. DNA repair genes are important in the prevention of genetic instability, *MLH1* and *MSH2* have been detected with over expression in neoplasms with a tendency to malignancy more than in benign neoplasms[38]. The results of this study show that only about *MLH1* expression was found in only 15% while *MSH2* was present in 35% of the analyzed samples, In addition, this study allows us to identify the behavior of this disease in the population of Durango, Mexico, which will lead to future studies for a better understanding and management of the lesion in patients susceptible to this susceptible to this condition[39]. That is why the use of molecular tools that serve for the identification, diagnosis and monitoring of patients with susceptibility to the development of PTC will allow a timely and appropriate intervention in order to offer the best prognosis and provide quality care[40]. Despite the size of the small sample used in the present study, we were able to identify gene expression levels of importance for the development of the disease. The results obtained coincide with what is reported in the scientific literature, which is extremely important. The behavior of the disease in the population of Durango, Dgo. Mexico, which will allow future studies for a better understanding and management of the lesion in patients susceptible to this condition.

Conclusions

The results obtained in the present study show that gene expression of molecular targets regulating inflammation and DNA repair, such as *COX-2, MLH1* and *MSH2* genes, are involved in thyroid tumor development, which allows us to contribute to the understanding of the molecular pathophysiology of PTC.

Acknowledgements

To the Dr. Jorge Sanchez and Dr. Teodoro Gurrola medical pathology specialists of the General Hospital 450 of the Health Services of the State of Durango.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014; 64(1):9-29.

2. Schneider DF, Chen H. New developments in the diagnosis and treatment of thyroid cancer. CA Cancer J Clin. 2013; 63(3):374-94.

3. Pathak KA, Leslie WD, Klonisch TC, Nason RW. The changing face of thyroid cancer in a population-based cohort. Cancer Med. 2013,2(4):537-44.

4. Cramer JD, Fu P, Harth KC, Margevicius S, Wilhelm SM. Analysis of the rising incidence of thyroid cancer using the surveillance, epidemiology and end results national cancer data registry. Surgery. 2010; 148(6):1147-52.

5. Figge, J. J. Epidemiology of thyroid cancer. En: Thyroid Cancer .New York, NY: Springer; 2016.p.9-15. DOI 10.1007/978-1-4939-3314-3_2

6. Kitahara, C. M., Sosa, J. A. The changing incidence of thyroid cancer. Nature Reviews Endocrinology. 2016; 12(11): 646–653. doi:10.1038/nrendo.2016.110

7. LiVolsi VA. Papillary thyroid carcinoma: an update. Mod Pathol. 2011; Suppl 2: S1-9.

8. Sipos JA, Mazzaferri EL. Thyroid cancer epidemiology and prognostic variables. Clinical oncology, 2010; 22(6):395-404.

9. Neta G, Brenner AV, Sturgis EM, Pfeiffer RM, Hutchinson AA, Aschebrook-Kilfoy B, et al. Common genetic variants related to genomic integrity and risk of papillary thyroid cancer. Carcinogenesis. 2011; 32(8):1231-7.

10. Girardi FM, Barra MB, Zettler CG. Variants of papillary thyroid carcinoma: association with histopathological prognostic factors. Braz J Otorhinolaryngol. 2013; 79(6):738-44.

11. Zhao Y, Zhang Y, Liu XJ, Shi BY. Prognostic factors for differentiated thyroid carcinoma and review of the literature. Tumori. 2012; 98(2):233-7.

12. Sak, S. D. Variants of papillary thyroid carcinoma: multiple faces of a familiar tumor. Turk Patoloji Derg.2015; 31(Suppl 1): 34-47. doi: 10.5146/tjpath.2015.01313

13. Pellegriti, G., Frasca, F., Regalbuto, C., Squatrito, S., & Vigneri, R. Worldwide increasing incidence of thyroid cancer: update on epidemiology and risk factors. Journal of cancer epidemiology.2013. http://dx.doi.org/10.1155/2013/965212

14. Gonzalez Gonzalez R, Bologna Molina R, Carreon Burciaga RG, Gómez Palacio-Gastelum M, Molina Frechero N, Salazar Rodríguez S. Papillary thyroid carcinoma: differential diagnosis and prognostic values of its different variants: review of the literature. ISRN Oncol. 2011; 2011: 915925.

15. Rodríguez A, Jiménez T, Vidal-Ríos P. Marcadores moleculares para la valoración clínica del nódulo tiroideo. Endocrinología y Nutrición, 2003; 50(2):66-75.

16. Xing M. Molecular pathogenesis and mechanisms of thyroid cancer. Nat Rev Cancer. 2013;13(3):184-99.

17. Legakis I, Syrigos K. Recent advances in molecular diagnosis of thyroid cancer. J Thyroid Res. 2011;2011:384213.

18. Li GM. Mechanisms and functions of DNA mismatch repair. Cell Res. 2008;18(1):85-98.

19. Payá Romá A, Alenda González C, Jover Martínez R, Aranda López FI. Carcinoma colorrectal con alteración de la vía reparadora. Claves para su identificación y relevancia clínica. Rev Esp Patol. 2006;39:201-8.

20. Peltomäki P. Role of DNA mismatch repair defects in the pathogenesis of human cancer. J Clin Oncol. 2003;21(6):1174-9.

21. Jiricny J. The multifaceted mismatch-repair system. Nat Rev Mol Cell Biol. 2006; 7(5):335-46.

22. Clària J. Cyclooxygenase-2 biology. Curr Pharm Des. 2003; 9(27):2177-90.

23. Chandrasekharan NV, Simmons DL. The cyclooxygenases. Genome Biol. 2004; 5(9):241.

24. Roelofs HM, Te Morsche RH, van Heumen BW, Nagengast FM, Peters WH. Over-expression of COX-2 mRNA in colorectal cancer. BMC Gastroenterol. 2014; 14:1.

25. Tsujii M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell. 1995; 83(3):493-501.

26. Sawaoka H, Tsuji S, Tsujii M, Gunawan ES, Sasaki Y, Kawano S, et al. Cyclooxygenase inhibitors suppress angiogenesis and reduce tumor growth in vivo. Lab Invest. 1999;79(12):1469-77.

27. Hughes DT, Haymart MR, Miller BS, Gauger PG, Doherty GM. The most commonly occurring papillary thyroid cancer in the United States is now a microcarcinoma in a patient older than 45 years. Thyroid. 2011; 21(3):231-6.

28. Greco A, Borrello MG, Miranda C, Degl'Innocenti D, Pierotti MA. Molecular pathology of differentiated thyroid cancer. Q J Nucl Med Mol Imaging. 2009;53(5):440-53.

29. Valdez C. Aspectos histológicos del cáncer diferenciado de la tiroides. Cancerología, 2009;4:73-83.

30. Duntas L, Grab-Duntas BM. Risk and prognostic factors for differentiated thyroid cancer. Hell J Nucl Med.2006;9(3):156-62.

31. Granados García M, Estrada Lobato E, Apodaca Cruz Á. Cáncer Diferenciado de la Tiroides: Aspectos Generales. Cancerología, 2009;4:65-71.

32. Giusti F, Falchetti A, Franceschelli F, Marini F, Tanini A, Brandi ML.Thyroid cancer: current molecular perspectives. J Oncol. 2010;2010:351679.

33. Pacini F, DeGroot LJ. Thyroid Cancer. [Updated 2013 Mar 27]. In: De Groot LJ, Chrousos G, Dungan K, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK285559/

34. González H, Mosso L. Cáncer papilar de tiroides: visión actual. Bol Esc Med, 2006;31:87-91.

35. Chen H, Izevbaye I, Chen F, Weinstein B. Recent advances in follicular variant of papillary thyroid carcinoma. NAJ Med Sci. 2012;5(4):212- 216.

36. Ji B, Liu Y, Zhang P, Wang Y, Wang G. COX-2 expression and tumor angiogenesis in thyroid carcinoma patients among northeast Chinese population-result of a single-center study. Int J Med Sci. 2012;9(3):237-42.

37. Siironen P, Ristimäki A, Narko K, Nordling S, Louhimo J, Andersson S, et al. VEGF-C and COX-2 expression in papillary thyroid cancer. Endocr Relat Cancer. 2006;13(2):465-73.

38. Ruschenburg I, Vollheim B, Stachura J, Cordon-Cardo C, Korabiowska M. Analysis of DNA mismatch repair gene expression and mutations in thyroid tumours. Anticancer Res. 2006;26(3A):2107-12.

39. Santos JC, Bastos AU, Cerutti JM, Ribeiro ML. Correlation of MLH1 and MGMT expression and promoter methylation with genomic instability in patients with thyroid carcinoma. BMC Cancer. 2013;13:79.

40. Lopes JP, Fonseca E. BRAF gene mutation in the natural history of papillary thyroid carcinoma: diagnostic and prognostic implications. Acta Med Port. 2011; Suppl 4:855-68.