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Hypoxia-inducible factor 1α (HIF 1α) and monocarboxylate transporter 1 (MCT 1) in papillary thyroid carcinoma; A study in Sulaimani City/Iraq

Hadeel Adnan Yasseen*
Ari Mohammed Abdullah**

Abstract

Background and objectives: Papillary thyroid carcinoma is one of the most common cancers of human, monocarboxylate transporter 1 and hypoxia inducible factor 1 alpha expressions in many tumors have recently being targets for treatment, HIF-1α has been correlated with increased angiogenesis, aggressive tumor growth, and poor patient prognosis. Marked increases in the levels of monocarboxylate transporters are a hallmark of several human malignancies. The objectives of this study are to evaluate expression of MCT1 and HIF1α in papillary thyroid carcinoma and compare them to the expression in non-malignant thyroid tissue, also correlate their expression with poor outcome parameters as lymph node status, tumor size, capsular and vascular invasions. **Methods:** The study samples composed of 68 cases; 52 males, 16 females, age ranging from 15-73 years, that had been diagnosed as papillary thyroid carcinoma and compared with 78 cases of non-malignant thyroid tissue regarding the expression of monocarboxylate transporter 1 and hypoxia inducible factor 1 alpha. **Results:** Monocarboxylate transporter 1 was negative in 19.1% and positive in 80.9% of the tumors while hypoxia inducible factor 1 alpha was negative in 27.9% of the cases and was positive in 72.1%. Both markers show strong expression in tall cell variant and both are negative in non-malignant thyroid tissues with statistically significant different between both. **Conclusions:** monocarboxylate transporter 1 and hypoxia inducible factor 1 alpha expression are more intense with the aggressive variant of papillary thyroid carcinoma. They can be used as target therapy especially monocarboxylate transporter 1 as it is totally negative in non-malignant thyroid tissue.

Key words: Hypoxia inducible factor 1 alpha, Monocarboxylate transporter 1, Papillary thyroid carcinoma.

Introduction

Thyroid cancer, the most common and prevalent endocrine malignancy, represents approximately 0.5–1% of all human malignancies, papillary thyroid carcinoma (PTC) being the commonest[1,2]. With an annual increased incidence of about 5.5 and 6.6 in men and women respectively, and for the last three decades it has been the fastest growing cancer in the United States[3,4]. Hypoxia may occur when aberrant blood vessels are shut down by becoming compressed or obstructed by growth, a feature commonly observed during the rapid growth of tumors[5]. The most important mediator identified to date of the cell's response to reduced oxygen availability is the hypoxia inducible factor 1 (HIF-1) transcription factor[6,7]. The hypoxia inducible factor 1 activity in tumors depends on the availability of the HIF-1α subunit, the levels of which increase under hypoxic conditions and through activation of oncogenes and/or inactivation of tumor suppressor genes. Increased tumor HIF-1α has been correlated with increased angiogenesis, aggressive tumor growth, and poor patient prognosis, leading to the current interest in HIF-1α as a cancer drug target[6]. Cancer cells are programmed to rely on aerobic glycolysis to support their proliferation and anabolic growth, an observation known as the Warburg effect[8,9]. As a consequence of this switch, glucose is preferentially catabolized to lactate[10]. Lactate homeostasis in both normal cells and cancer cells requires its transport by four members of the solute carrier proton-linked monocarboxylic acid transporter: MCT1-4[11]. Marked increases in the levels of MCT1 and/or MCT4 are a hallmark of several human malignancies[12-15]. In muscle cells, increases in extracellular lactate that occur following high-intensity exercise induce MCT1 expression, apparently via the generation of reactive oxygen species ROS[16], and this response may be

* FIBMS (Path), Assist. Prof. Department of Pathology and Forensic Pathology/ College of Medicine/ University of Sulaimani
**MBChB, MSc path, Sulaimani Teaching Hospital/Ministry of Health
Email: ariabdullah1978@gmail.com

relevant to MCT1 control in highly glycolytic, oxidative tumor cells and in adjacent stromal cells that are bathed in lactate[10]. Aims of the study was to evaluate the expression of MCT1 and HIF1α in papillary thyroid carcinoma, correlate the expression of MCT1 and HIF1α with the histological parameters reflecting aggressiveness of the tumor like capsular invasion, vascular invasion and more aggressive variant and comparing the expression of MCT1 and HIF1 α to their expression in non-neoplastic thyroid tissue.

Materials and methods

In this retrospective study, paraffin-embedded tissue blocks along with the histopathological reports of 68 patients with papillary thyroid carcinoma and 78 histologically benign thyroid tissues including nodular goiter and thyroiditis were obtained from the Department of Surgical Pathology- Shorsh Hospital, Sulaimani Teaching Hospital and private histopathology laboratories for the period from January-April 2017. The research work was carried out in the department of Pathology and Forensic Pathology in the College of Medicine in Sulaimani University. Four µm thick sections were processed and stained by Hematoxylin and Eosin for re-evaluation. Other 4µm thick tissue sections were cut and mounted on positively charged slides. Primary monoclonal (Rabbit anti human HIF-1alpha (28b) mouse monoclonal IgG1 (kappa light chain) and anti MCT1 (H-1) mouse monoclonal IgG1 (kappa light chain) antibody (Santa Cruz Company Biotechnology, INC) antibodies were used for immunohistochemical study. The percentage of posi¬tive cells was scored as follows: 0, <5%; 1, 5-30%; 2, 31-70%; and 3, >71%. Staining intensity was scored as follows: 0, no staining; 1, weakly positive, 2, moderate stain¬ing; and 3, strongly positive. The immunostain¬ing score of each specimen was calculated by adding the staining percentage score and the staining intensity score. Then the quantity scores were calculated, and a final score of 0-2 indicated nega¬tive while a score of 3-6 indicated positive 17. Both antibodies staining were determined in cytoplasm of neoplastic cells. The expression of MCT1 and HIF1α were correlated with the all collected clinicopathological parameters including the gender of the patient, tumor variants, tumor size, tumor capsular invasion, vascular invasion and extra-thyroid extension of the tumor

Results

Out of 68 cases, fifty two (76.5%) papillary thyroid carcinoma (PTC) were females and 16 (23.5%) were males. Thirty six cases were conventional PTC, 26 cases were tall cell variant, 1 case was follicular and 5 cases were oncocytic variant, with no significant correlation between the type of PTC and the gender of the patient. The age ranged from 15-73 years with a mean of mean±SD of 40.6±14.63 The MCT1 staining was totally negative in 19.1% of the tumors and 80.9% were positive (22.1% were moderately positive and 58.8% were strongly positive. The expression of the MCT1 was comparable between female and male with non-significant p-value. The tall cell and the oncocytic variants show stronger positivity in comparison to the conventional one.

Figure (1) shows different MCT1staining intensities. According to the size of the tumor, 50 cases were ≤ 20 mm and 18 cases were > 20 mm with no significance correlation with the positivity of the tumor for MCT1. All other parameters show no significant correlation with MCT1 expression, Table (1).

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Table (1): Correlation of MCT1 expression with clinicopathological parameters.

Clinicopathological parameters	MCT1 stain						Total	p-value
	Negative stain		Weakly positive		Strongly positive			
	No.	%	No.	%	No.	%		
Gender								
Female	10	19.2	10	19.2	32	61.5	52	0.58
Male	3	18.8	5	31.3	8	50.0	16	
Total	13	19.1	15	22.1	40	58.8	68	
Type of the tumour								
Conventional type	8	22.2	11	30.6	17	47.2	36	0.100
Tall cell variant	2	7.7	4	15.4	20	76.9	26	0.048
Follicular variant	0	0.0	0	0.0	1	100.0	1	0.070
Oncocytic variant	3	60.0	0	0.0	2	40.0	5	0.044
Total	13	19.1	15	22.1	40	58.8	68	
Size of the tumour								
0-20mm	11	22.0	9	18.0	30	60.0	50	0.32
> 20 mm	2	11.1	6	33.3	10	55.6	18	
Total	13	19.1	15	22.1	40	58.8	68	
Capsular invasion								
Not present	10	18.9	11	20.8	32	60.4	53	0.86
Present	3	20.0	4	26.7	8	53.3	15	
Total	13	19.1	15	22.1	40	58.8	68	
Vascular invasion								
Not present	8	16.7	9	18.8	31	64.6	48	0.33
Present	5	25.0	6	30.0	9	45.0	20	
Total	13	19.1	15	22.1	40	58.8	68	
Extra-thyroid extension								
Not present	13	19.7	15	22.7	38	57.6	66	0.49
Present	0	0.0	0	0.0	2	100.0	2	
Total	13	19.1	15	22.1	40	58.8	68	

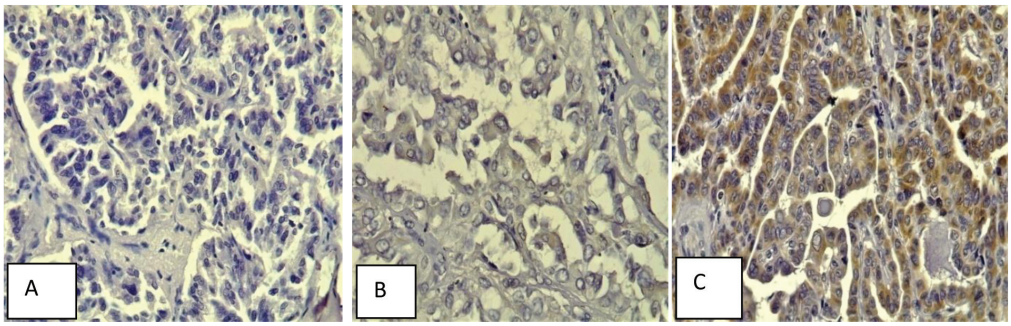

Figure (1): MTC1 staining intensity in PTC, A: negative (40X), B: moderate (40X), C: strong (40X).

Regarding the HIF1α; 27.9% of the cases were totally negative and 72.1% were positive for the stain (25% moderately positive and 47.1% strongly positive). Among all variant, tall cell papillary thyroid carcinoma showed more positivity for the stain with significant p-value <0.011. Figure (2) shows the different staining intensities of HIF1α. The other parameters were all non-significant when correlated with positivity of HIF1α and were comparable to the MCT1 stain, Table (2).

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Table (2): Correlation of HIF1alpha expression with clinicopathological parameters.

Gender	HIF1α stain						Total	P-value
	Negative stain		Weakly positive		Strongly positive			
	No.	%	No.	%	No.	%		
Gender								
Female	12	23.1	13	25.0	27	51.9	52	0.22
Male	7	43.8	4	25.0	5	31.3	16	
Total	19	27.9	17	25.0	32	47.1	68	
Type of the tumour								
Conventional type	13	36.1	11	30.6	12	33.3	36	0.054
Tall cell variant	3	11.5	5	19.2	18	69.2	26	0.011
Follicular variant	1	100.0	0	0.0	0	0.0	1	0.270
Oncocytic variant	2	40.0	1	20.0	2	40.0	5	0.822
Total	19	27.9	17	25.0	32	47.1	68	
Size of the tumour								
0-20mm	14	28.0	13	26.0	23	46.0	50	0.94
≥21	5	27.8	4	22.2	9	50.0	18	
Total	19	27.9	17	25.0	32	47.1	68	
Vascular invasion								
Not present	11	22.9	12	25.5	25	52.1	48	0.31
Present	8	40.0	5	25.0	7	35.0	20	
Total	19	27.9	17	25.0	32	47.1	68	
Extra-thyroid extension								
Not present	19	28.8	17	25.8	30	45.5	66	0.31
Present	0	0.0	0	0.0	2	100.0	2	
Total	19	27.9	17	25.0	32	47.1	68	

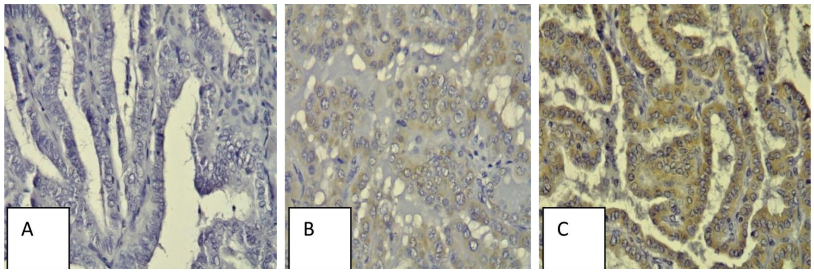

Figure (2): HIF1α staining intensity in PTC A: negative 40X, B: moderate 40X, C: strong 40X

When compared to the control cases which consist of the non-malignant thyroid tissue both MCT1 and HIF1α showed a highly significantly positivity in PTC meanwhile both stains show a total negativity in all of the follicular cells in the control cases (n=78, 100%), Tables (3) and (4).

The exceptional finding for the stromal Hürthle cells that showed some focal positivity for HIF1α, whereas the colloid material in the follicles show some reactivity for the MCT1 stain.

Table (3): Correlation between studied and control cases in regard to MCT1.

Categories	MCT1 stain				Total	P-value
	Negative		Positive			
	No.	%	No.	%		
Cases	13	14.3	55	100.0	68	≤0.01
Controls	78	85.7	0	0.0	78	
Total	**91**	**100**	**45**	**100.0**	**146**	

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Table (4): Correlation between studied and control cases in regard to HIF1α.

Categories	HIF1α stain				Total	P-value
	Negative		Positive			
	No.	%	No.	%		
Cases	19	19.6	49	100.0	58	≤0.01
Controls	78	80.4	0	0.0	78	
Total	97	100	49	100.0	146	

Discussion

Although papillary thyroid carcinoma is among commonest endocrine carcinoma, the mechanisms involved in the exact way of development and progression of this cancer is poorly understood. Here we want to highlight the potential role of MCT1 and HIF1α in papillary thyroid carcinoma and correlate these it with the parameters like tumor variants, tumor size, vascular invasion and extra-thyroid extension of the tumor.

We demonstrated that in papillary thyroid carcinoma there is high expression of MCT1 and HIF1α compared to the non-cancerous thyroid tissue, the high expression of these two markers can be used as a target for the treatment and they may have diagnostic values.

Monocarboxylate transporter 1 and hypoxia inducible factor 1 alpha has been reported to be expressed in many types of carcinoma such as lung, colorectal, breast, stomach, prostatic and ovarian cancers and has been suggested to be related to poor outcome features such as vascular invasion, lymph node metastasis and higher stages of the tumors[5,6.8-10].

In a study by Rossi et al, MCT1 expression in papillary thyroid carcinoma found in 76% to be positive and 24% were negative for MCT1[18]. This is comparable to the results of MCT expression in our study in which 80.9% of the cases were positive (between moderately and strongly positive) and 19.1% were negative.

In another study by Johnson et al, 13 out of 15 cases of anaplastic thyroid carcinoma showed high expression for MCT1 and 10 out of 12 (83.3%) of the cases of papillary thyroid carcinoma had expression for MCT1[19], which is also comparable to the results of our study that showed 80.9% overall positivity for MCT1.

In a study by Lee et al, expression of HIF1α was seen in 47 out of 225 (20.9%) and 178 cases (79.1%) were negative[20], which is almost opposite to the results of our study in which 72.1% were positive for HIF1α (between moderately-strongly positive) and 27.9% were totally negative, which may be due to the fact they used only papillary thyroid microcarcinomas in their study and they didn't incubate the antibody overnight as in our study.

In another study by Burrows et al[21], they found that HIF1α is not expressed in any cases of non-malignant thyroid tissue while detected only in cases of thyroid carcinoma and this is also comparable to the results of our study, in which none of the non-neoplastic thyroid tissue expressed HIF1α while most of the papillary thyroid carcinoma showed positivity for the stain.

Regarding both stains MCT1 and HIF1α the largest number of the cases that gave strong positivity for both of them is noticed in tall cell variant of papillary thyroid carcinoma which is generally thought to be more aggressive with significant p-value < 0.05.

Conclusions

Expression of MCT1 is seen in most cases of papillary thyroid carcinoma and especially strong in the aggressive tall cell variant. Expression of HIF1α is seen in most cases of papillary thyroid carcinomas and also correlated to the poor outcome parameter as more expressed in the tall cell variant (more aggressive variant). Both MCT1 and HIF1α are not expressed in non-neoplastic thyroid tissue and both markers can have diagnostic advantages as they are totally negative in non-neoplastic thyroid tissue.

References

1.Nguyen QT, Lee EJ, Huang MG, Park YI, Khullar A, Plodkowski RA. Diagnosis and treatment of patients with thyroid cancer. Am Health Drug Benefits. 2015;8(1):30–40.

2. Rosai and Ackerman's surgical pathology, Eleven Edition, John R. Goldblum, Laura W. Lamps, Jesse K. Mckenney and Jeffery L. Myers. Thyroid gland, tumors. 2017:297.

3. American Cancer Society. Available from: http://www.cancer.org/Research/CancerFactsFigures/CancerFactsFigures/cancer-facts-figures-2012.

4. Jemal A, Bray F, Center M, Ferlay J, Ward E, Forman D. Global Cancer Statistic. CA Cancer J Clin. 2011;61(2):69-90.

5. Powis G, Kirkpatrick L. Hypoxia inducible factor-1A as a cancer drug target. Mol Cancer Ther. 2004; 3:647-54.

https://doi.org/10.56056/amj.2019.100

6. Harris AL. Hypoxia—a key regulatory factor in tumour growth. Nat Rev. 2002; 2:38–47.

7. Semenza GL. HIF-1 and tumor progression: pathophysiology and therapeutics. Trends Mol Med.2002;8: S62–7.

8. Ward PS, Thompson CB. Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate. Cancer Cell. 2012;21(3):297–308.

9. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science. 2009;324(5930):1029-33.

10. Joanne R. Doherty, John L. Cleveland. Targeting lactate metabolism for cancer therapeutics. J Clin Invest. 2013;123(9):3685-92.

11. Halestrap AP. The monocarboxylate transporter family- Structure and functional characterization. IUBMB Life. 2012;64(1):1.

12. Miranda-Gonçalves V, Honavar M, Pinheiro C, et al. Monocarboxylate transporters (MCTs) in gliomas: expression and exploitation as therapeutic targets. Neuro Oncol. 2013;15(2):172–88.

13. Fang J, Quinones QJ, Holman TL, et al. The H+-linked monocarboxylate transporter (MCT1/SLC16A1): a potential therapeutic target for high-risk neuroblastoma. Mol Pharmacol. 2006;70(6):2108–15.

14. Pinheiro C, Longatto-Filho A, Ferreira L, et al. Increasing expression of monocarboxylate transporters 1 and 4 along progression to invasive cervical carcinoma. Int J Gynecol Pathol. 2008;27(4):568–74.

15. De Oliveira AT, Pinheiro C, Longatto-Filho A et al. Co-expression of monocarboxylate transporter 1 (MCT1) and its chaperone (CD147) is associated with low survival in patients with gastrointestinal stromal tumors (GISTs). J Bioenerg Biomembr. 2012;44(1):171-8.

16. Hashimoto T, Hussien R, Oommen S, Gohil K, Brooks GA. Lactate sensitive transcription factor network in L6 cells: activation of MCT1 and mitochondrial biogenesis. FASEB J. 2007;21(10):2602–12.

17. Nalwoga H, Ahmed L, Arnes J, Wabinga H, Akslen L. Strong Expression of Hypoxia-Inducible Factor-1α (HIF-1α) Is Associated with Axl Expression and Features of Aggressive Tumors in African Breast Cancer. PLoS One. 2016; 11(1): e0146823.

18. Rossi E, Bizzarro T, Granja S, et al. The expression of monocarboxylate transporters in thyroid carcinoma can be associated with the morphological features of BRAF V600E mutation. Endocrine. 2017; 56:379–87.

19. Johnson J, Lai S, Cotzia P et al. Mitochondrial Metabolism as a Treatment Target in Anaplastic Thyroid Cancer. Semin Oncol. 2015; 42(6): 915-22.

20. Lee J, Kim K, Sul H, et al. HIF-1α Expression in BRAFV600E-Positive Papillary Thyroid Microcarcinoma. J Korean Thyroid Assoc. 2011;4(2), 102-8.

21. Burrows N, Resch J, Cowen R, et al. Expression of hypoxia-inducible factor1a in thyroid carcinomas. Endocr Relat Cancer. 2010; 1761-72.

https://doi.org/10.56056/amj.2019.100