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Correlation of KI-67/MIB-1 in the Papillary Macro and Micro-carcinomas of the Thyroid Gland with the Prognosis of the Patients

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ABSTRACT

The Papillary Thyroid Carcinoma (PTC) has the ability to show an aggressive clinical course and regional lymph node metastasis. This study was conducted to assess the Ki-67 labeling index (Ki-67.LI) as a prognostic factor to identify a high risk group of the macro- and micro PTC to elucidate this issue. All 98 patients with the thyroid mass that underwent thyroidectomy enrolled in this study, then people were divided into two equal groups of case (PTC) and control (Benign thyroid lesions). We evaluated the ki-67.LI in their primary lesions and revealed its relationship with the various features of prognostic variables. The patients, who were followed-up during 24 months and recurrence and distant metastasis was proved. The ki-67.LI had statistically significant correlation with the tumor size ($p = 0.003$), the vascular invasion and regional lymphatic involvement ($p < 0.001$). An extra thyroid tissue invasion and extensive tumor necrosis were detected, with the high LI ($p < 0.001$). The tumor multifocality was correlated with the $LI > 2.8\%$ ($p = 0.004$). Among the remaining with the $LI > 2.8\%$, one patient with the Papillary Thyroid Micro Carcinoma (PTMC) and three patients with the Papillary Thyroid Carcinoma (PTC) were with the recurrence after the surgery. The ki-67.LI revealed a close correlation with the vascular-lymphatic invasion, the nodal metastasis, extra-thyroid extension and an extension of the necrosis and the infiltrative tumor borders. An evaluation of the ki-67 could be helpful to detect the high risk patient especially for the patient with papillary thyroid micro carcinoma.

Key words: Thyroid papillary micro-carcinoma, ki-67 LI, prognosis

INTRODUCTION

Thyroid neoplasm is the account for nearly 95% of all endocrine tumors of the Papillary Thyroid Carcinoma (PTC) is the most common thyroid malignancy that tends to be a hidden biological standpoint (Theoharis *et al.*, 2012). Though, these carcinomas usually has a good prognosis, the tumor has the ability of lymphatic invasion; therefore, it can form multifocal lesions and regional lymph node metastasis (Hay *et al.*, 1992; Theoharis *et al.*, 2012). Currently no reliable indicators

exist to conclusively determine whether aggressiveness or recurrence of the disease. In contrast, the risk factors in the affected groups which are based on the clinical and pathological criteria, give the important information about prognosis (Siironen *et al.*, 2005; Theoharis *et al.*, 2012). The factors affecting on the prognosis of the patients that have been studied include age, tumor size, lymph node invasion, capsule invasion, vascular invasion and distant metastases (Siironen *et al.*, 2005; Theoharis *et al.*, 2012). In some studies the micro carcinoma is defined as tumor size less than 1 cm in greatest diameter (Theoharis *et al.*, 2012).

Several cell markers included the markers in the nucleus and the cytoplasm of tumor cells has been identified that are associated with the nature of biology and behavior of the tumor (Mitselou *et al.*, 2004; Urruticoechea *et al.*, 2005). The Ki-67 nuclear cell marker was introduced by Gerdes *et al.* (1991) as a nuclear non-histone protein and antibody processed against this antigen was named the MIB-1 (Urruticoechea *et al.*, 2005). Assessing the MIB-1 in evaluating proliferative activity and predicting the aggressiveness of the tumor types is very useful (Nakayama *et al.*, 2007; Pesutic-Pisac *et al.*, 2008; Pujani *et al.*, 2010; Ranjbari and Rahim, 2013). In spite of the fact the index of the Ki-67 cell marker (ki-67 labeling index = LI) for the diagnosis of thyroid neoplasms has been studied but the relationship between the papillary thyroid carcinoma and the ki-67 LI and the biological behavior is not well understood (Lim *et al.*, 2007; Pujani *et al.*, 2010). Some studies conducted on patients with the PTMC with the high ki-67 LI levels, obviously demonstrated that they had the lower disease-free survival and higher rates of the disease recurrence, compared with the PTMC and PTC (macro carcinoma) with Low Ki-67 LI (Hay *et al.*, 1992; Siironen *et al.*, 2005).

The study was aimed to determine the incidence of nuclear cell marker ki-67 tumor specimens of patients with the papillary thyroid carcinoma according to the clinical and pathological prognostic factors and to investigate the possible relationship between these markers and the prognostic factors of the disease.

MATERIALS AND METHODS

Study design and population: In this case-control study, a total of 98 patients referred to the Otolaryngology Department of Imam Khomeini Hospital, in the Ahvaz, situated in the western south of Iran, due to a thyroid mass from 2009 to 2012, were enrolled.

Inclusion criteria: All patients with the thyroid neoplasm in which papillary carcinoma was detected by a histopathological assessment.

Exclusion criteria: Other types (Medullary, Follicular, Hurtle cell ...) of the thyroid carcinoma.

Method: After recording the clinical data, regular physical examination and imaging and laboratory studies, thyroidectomy surgery (total or lobectomy) was performed and then, based on inclusion and exclusion criteria of the study, these patients were divided into two groups, including patients with the papillary thyroid carcinoma (case group) and patients with the benign thyroid lesions (goiter) (control group).

Immunohistochemical staining on 3 mm slides covered by the MIB-1 monoclonal antibody to detect the ki-67 using a standard method were reviewed by two pathologists. For MIB-1 monoclonal Ab staining, the metastatic lymph node tissue were chosen as positive control (Fig. 1a) and to evaluate the ki-67 levels, number 2.8% were embedded as the cut-off point to measure the increased level of the marker and the nuclear proliferation index (ki-67 labeling index = ki-67 LI) identified compared with the mentioned number (Fig. 1b).

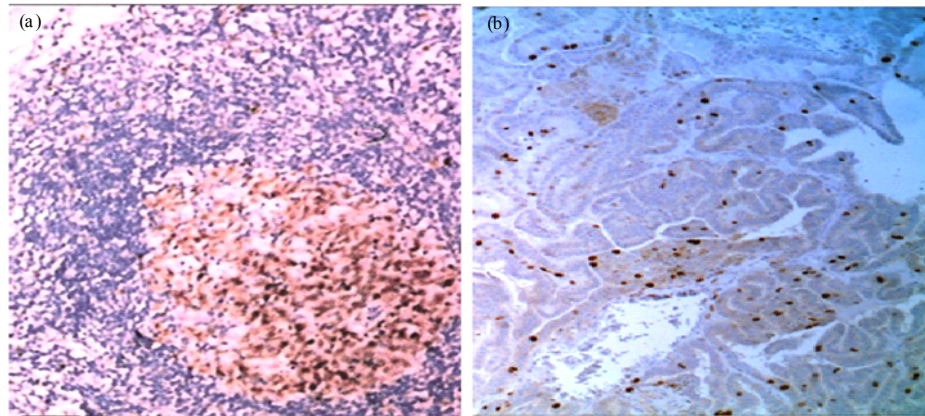


Fig. 1(a-b): (a) MIB-1 monoclonal Ab staining in lymph node as positive control tissue and (b) Actively cycling tumor cells within a papillary thyroid carcinoma are identified by their nuclear labeling with the MIB-1 monoclonal antibody in this immunoperoxidase preparation

The patients were periodically visited during a 24 month follow-up and the evidence of recurrence and distant metastasis, were assessed using the clinical examinations and para-clinical tools (ultrasonography of the thyroid bed and the TG level and whole body scan).

RESULTS

Among the 49 patients with the papillary carcinoma studied, 36 cases were female and 13 cases were male. The women were in the age range of 17-72 years and men were from 23 to 68 years of age. The largest age group referred with a thyroid mass was 23-44 years of age. In the study of tumor tissue's size in the thyroid cases of the control group, 20 cases of tumor's size were >1 cm that followed the papillary thyroid macro carcinoma and 19 cases of tumor's size were <1 cm which followed papillary thyroid micro carcinoma. The Ki-67LI (Labeling index) in 22 patients was <2.8% and in 27 patients with the PTC was >2.8% and the ki-67 level had statistically significant correlation with the tumor size ($p = 0.003$) (Table 1). The vascular invasion and regional lymphatic involvement were seen in correlation with high ki-67 LI >2.8% ($p < 0.001$) (Table 1, 2). An extra thyroid tissue invasion was diagnosed in 24 patients, that among them 22 cases had the LI >2.8% ($p < 0.001$). The tumor necrosis was diagnosed in 19 cases. A close correlation existed between the extent of the necrosis and the LI ($p < 0.001$) (Table 1, 2). Nineteen cases had multifocal tumor correlated with the LI >2.8% ($p = 0.004$). The PTC was encapsulated in 17 patients but it was not capsulated in 32 cases. No statistical correlation existed between having capsule and high LI ($p = 0.454$) (Table 1, 2).

In a systematic pursuit and survey of patients in a 2 year period of time after surgery, some signs of loco regional recurrence were revealed in four cases. In a detailed examination among these cases, recurrence after surgery was detected in a patient with the PTMC and in three patients with the PTC having LI >2.8%. In two female cases with the macro carcinoma distant metastasis was revealed that ki-67 index drastically rose compared to the other patients and the control group.

Table 1: Relationship of tumor variables and ki-67 LI

Variables	N	KI-67 mean	KI-67 mean rank**	p-value
Tumor size				
>1 cm	33	3.78	29.08	0.003*
<1 cm	16	0.92	16.59	
Vascular invasion				
Vascular invasion	25	4.84	33.98	<0.001*
No vascular invasion	24	0.76	15.65	
Lymphatic invasion				
Positive pathologic lymph node	24	4.98	33.77	<0.001*
Negative pathologic lymph node	25	0.79	16.58	
Multifocal				
Multifocal	19	4.73	31.97	0.004*
Unifocal	30	1.65	20.58	
Tumor necrosis				
Tumor necrosis	19	4.65	33.55	<0.001*
No necrosis	30	1.70	19.58	
Extrathyroid invasion				
Extrathyroid invasion	19	5.53	35.84	<0.001*
No invasion	30	1.14	18.13	
Capsule				
Encapsulated tumor	17	2.42	23.00	0.454
Non encapsulated	32	3.07	26.06	

*Significant difference, **On the basis of Mann-Whitney test

Table 2: Statistical results in different PTC tumor variables

Variables	KI-67 labeling index values (%)		
	LI<2.8 (%)	LI>2.8 (%)	Total
Tumor size			
<1 cm	13	10	23
>1 cm	3	23	26
Lymphatic invasion			
LN+	4	18	22
LN-	20	7	27
Vascular invasion			
Positive	3	19	22
Negative	22	5	27
Tumor focality			
Multifocal tumor	5	14	19
Unifocal tumor	17	13	30
Tumor necrosis			
Tumor necrosis	2	17	29
No necrosis	20	10	30
Extra thyroid tissues			
Invasion	2	17	19
No invasion	20	10	30

DISCUSSION

Papillary thyroid micro carcinoma has almost high relationship to the sex and age. The chance of the lymph node metastasis and the possibility of making multiple focuses on involvement in thyroid are high. The reviews of the morphologic findings are not able to predict the tumor policy and aggressive behavior. The more advanced methods of the growth of tumors are not only useful to foresee the biological behavior of the tumor cells, rather they help to choose a better way to cure and pursuit patients (Ito *et al.*, 2010; Siironen *et al.*, 2005). The significance of this subject will be more appear when it is about those patients who are selected from the dangerous group of intensity and process of malignancy and needs to a particular pursuit and attention (Ito *et al.*, 2010). In this

study, we measured the growth and propagation of tumor cells in the papillary carcinoma with the help of the ki-67 LI in both sorts of micro carcinoma and classic papillary carcinoma. In our former study, the scale of ki-67 in the papillary macro carcinoma was higher than the PTMC (Pujani *et al.*, 2010; Ranjbari and Rahim, 2013). However, in some cases, the PTMC with the aggressive behavior and invasive feature revealed high LI and loco regional recurrence. Thus, an evaluation of the ki-67 could be helpful to identify the high risk patient especially for the patient with the PTMC. Furthermore, the LI revealed a close correlation with the vascular-lymphatic invasion, nodal metastasis, extra thyroid extension and extent of necrosis and tumors with infiltrative borders compared to the other kinds that similar studies too revealed that (Kjellman *et al.*, 2003; Siironen *et al.*, 2005). Moreover, in those patients who revealed signs of tumor recurrence and distant metastasis in the clinical follow-up and survey (even PTMC), the ki-67 LI showed a rapid rise and the level of this scale was related to the Disease-Free Survival (DFS). In the other studies this relationship was evident (Ito *et al.*, 2010; Kjellman *et al.*, 2003).

CONCLUSION

Paying attention to this point that how the oncogenes can effect on measures of cell's growth and death gives us useful information about the biologic behavior of tumors and the results of this survey showed that the scale of the ki-67 is a valuable marker and has the ability to give us beneficial information/data about the growth of papillary thyroid carcinoma and clinical policy, especially the micro carcinoma.

REFERENCES

- Gerdes, J., L. Li, C. Schlueter, M. Duchrow and C. Wohlenberg *et al.*, 1991. Immunobiochemical and molecular biologic characterization of the cell proliferation-associated nuclear antigen that is defined by monoclonal antibody Ki-67. *Am. J. Pathol.*, 138: 867-873.
- Hay, I.D., C.S. Grant, J.A. van Heerden, J.R. Goellner, J.R. Ebersold and E.J. Bergstralh, 1992. Papillary thyroid microcarcinoma: A study of 535 cases observed in a 50-year period. *Surgery*, 112: 1139-1146.
- Ito, Y., A. Miyauchi, K. Kakudo, M. Hirokawa, K. Kobayashi and A. Miya, 2010. Prognostic significance of ki-67 labeling index in papillary thyroid carcinoma. *World J. Surgery*, 34: 3015-3021.
- Kjellman, P., G. Wallin, A. Hoog, G. Auer, C. Larsson and J. Zedenius, 2003. MIB-1 index in thyroid tumors: A predictor of the clinical course in papillary thyroid carcinoma. *Thyroid*, 13: 371-380.
- Lim, D.J., K.H. Baek, Y.S. Lee, W.C. Park and M.K. Kim *et al.*, 2007. Clinical, histopathological and molecular characteristics of papillary thyroid microcarcinoma. *Thyroid*, 17: 883-888.
- Mitselou, A., D. Peschos, P. Dallas, K. Charalabopoulos, N.J. Agnantis and T. Vougiouklakis, 2004. Immunohistochemical analysis of expression of BCL-2 protein in papillary carcinomas and papillary microcarcinomas of the thyroid gland. *Exp. Oncol.*, 26: 282-286.
- Nakayama, H., A. Yoshida, Y. Nakamura, H. Hayashi and Y. Miyagi *et al.*, 2007. Clinical significance of *BRAF* (V600E) mutation and Ki-67 labeling index in papillary thyroid carcinomas. *Anticancer Res.*, 27: 3645-3649.
- Pesutic-Pisac, V., A. Punda, I. Gluncic, V. Bedekovic, A. Pranic-Kragic and N. Kunac, 2008. Cyclin D1 and p27 expression as prognostic factor in papillary carcinoma of the thyroid: Association with clinicopathological parameters. *Croatian Med. J.*, 49: 643-649.

- Pujani, M., B. Arora, M. Pujani, S.K. Singh and N. Tejawani, 2010. Role of Ki-67 as a proliferative marker in lesions of thyroid. *Indian J. Cancer*, 47: 304-307.
- Ranjbari, N. and F. Rahim, 2013. The Ki-67/MIB-1 index level and recurrence of papillary thyroid carcinoma. *Med. Hypotheses*, 80: 311-314.
- Siironen, P., J. Louhimo, S. Nordling, A. Ristimäki, H. Maenpää, R. Haapiainen and C. Haglund, 2005. Prognostic factors in papillary thyroid cancer: An evaluation of 601 consecutive patients. *Tumour Biol.*, 26: 57-64.
- Theoharis, C., S. Roman and J.A. Sosa, 2012. The molecular diagnosis and management of thyroid neoplasms. *Curr. Opin. Oncol.*, 24: 35-41.
- Urruticoechea, A., I.E. Smith and M. Dowsett, 2005. Proliferation marker Ki-67 in early breast cancer. *J. Clin. Oncol.*, 23: 7212-7220.