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Original Article

Investigating the role of minichromosome maintenance protein 2 in differentiating follicular adenoma from follicular carcinoma of thyroid

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Abstract. Thyroid nodules are often discovered incidentally in the clinical practice. Even though definite preoperative diagnosis of follicular thyroid lesions prevents unnecessary thyroid surgeries, fine needle aspiration (FNA) cannot definitely differentiate benign or malignant follicular lesions of the thyroid. Several proliferative markers including minichromosome maintenance proteins (MCM) have been evaluated for diagnosis of follicular lesion. In this study, the immunohistochemical expression of MCM2 in follicular adenoma and carcinoma of thyroid was investigated to determine whether it can distinguish between them and if there is a difference between follicular carcinomas diagnosed with capsular/vascular invasion. The correlation between intensity of MCM2 expression in follicular adenoma and carcinoma was evaluated. In this retrospective study, thirty cases of follicular carcinoma and thirty cases of follicular adenoma were obtained from pathology department in Yazd, Iran from 2005 to 2015. We subdivided the follicular carcinomas into three groups on the basis of vascular and capsular invasion. The data were analyzed using SPSS v. 16 and p-value<0.05 was considered meaningful. In this study, the average intensity of MCM2 expression was 73.16% in adenoma and 70.31% in carcinoma which was equally distributed in the invasion area of carcinoma and subcapsular area of adenoma. There is no meaningful relationship between marker expression of MCM2 and gender nor age. MCM2 expression in the thyroid follicular carcinoma is not significantly more than the MCM2 expression in follicular adenoma. MCM2 cannot be used for cytology diagnosis before surgical operations nor for confirming or ruling out histopathologic suspicious cases.

Keywords: Thyroid carcinomas, follicular adenoma, minichromosome maintenance proteins.

Introduction

Thyroid nodules are common and often discovered in the clinical practice or incidentally during various imaging procedures. As they have a malignant potential, they are clinically valued [1]. Cytological diagnosis by fine needle aspiration (FNA), determines the treatment and postoperative management of the thyroid nodules, whereas only 10% of the obtained lesions are definitely malignant [2]. A FNA biopsy specimen is only 20 % consistent with a follicular neoplasm [3, 4, 5]. Diagnosis between benign or malignant follicular lesion of the thyroid by FNA is difficult [6]; therefore, patients with this diagnosis are chosen for a thyroid lobectomy. Then, if the histologic result is accordant with malignancy the patient undergoes surgery again for a total thyroidectomy [7]. As mentioned the surgical approach to these lesion is necessary and causes anxiety and distress to the patients and social healthcare system as well [8]. Definite preoperative diagnosis of follicular thyroid lesions prevents unnecessary thyroid surgeries [9]. Sometimes, due to incomplete capsular penetration or equivocal vascular invasion, the term “follicular lesion” is used and the malignancy potential is unknown [10, 11]. Neoplasms are defined as dysregulation in the control of cell proliferation [12]. So, the evaluation of the cell proliferation activity by immunohistochemistry analysis is an important tool to provide information about the tumors [13]. Several proliferative markers including ki67 have been evaluated for diagnosis of follicular lesion [14], another one is the minichromosome maintenance proteins (MCM), are important regulators of the cell cycle and specific for the cell proliferation [15]. The MCM protein family consist of six major isoforms (MCM 2-7) that are important for chromosome replication after the activation of early origins of DNA replication [16]. In the normal cells, MCM proteins

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are expressed in the nucleus when the cell is proliferating but are rapidly degraded afterward. In the malignant and premalignant lesions, the MCM proteins are not degraded so they accumulate in the nucleus leading to the possibility that they could be used as biomarkers in malignancies [17].

In this study, the immunohistochemical expression of MCM2 in follicular carcinoma and follicular adenoma of the thyroid was investigated to determine whether it can distinguish between these two tumors and if there is a difference between follicular carcinomas diagnosed with capsular invasion, vascular invasion or both. Considering age and sex, the correlation of labeling MCM2 was also evaluated in the follicular adenoma and carcinoma.

Materials and Methods

Study design

In this retrospective study, thirty cases of follicular carcinoma and thirty cases of follicular adenoma were obtained from archives of the four hospital's pathology department in Yazd, Iran from 2005 to 2015. The diagnosis of follicular adenoma was made based on the presence of encapsulated mass with homogenous follicular proliferation, lack of papillary thyroid carcinoma (PTC) nuclear features and absence of vascular and/or capsular invasion. Follicular carcinoma was diagnosed based on the presence of follicular proliferation with a thick capsule and a full capsular penetration and/or vascular invasion, and atypical hyperchromatic nuclei that lacked features of PTC nuclei. Oxyphilic variant tumors, encapsulated papillary carcinoma were excluded. The surgically resected thyroid specimens were fixed in 10% buffered formalin, embedded in paraffin and 4 micron-thick sections stained with hematoxylin and eosin (H&E) for routine histological examination. All the H&E-stained sections were reviewed and adequate section in each case was selected. The follicular carcinomas were subdivided into three groups on this basis; Group 1, only capsular invasion was seen, but had no definite vascular invasion; Group 2 had only vascular invasion, but no definite capsular invasion; and Group 3, had both capsular invasion and vascular invasion. The vascular invasion, if present, was found usually in, or immediately around the capsule. The 30 FCs were then categorized as fourteen Group 1, ten Group 2 and six Group 3 tumors.

Immunohistochemistry

Tissues were deparaffinized in xylene and rehydrated, After antigen retrieving and washing with phosphate buffer, the tissue 4 micron-thick sections were incubated with a ready-to-use rabbit anti-human MCM2 monoclonal antibody (Cat No.AN585-5M, BioGenex, USA) for 60 minutes. The slides were then washed again in PBS and incubated with secondary antibody (mouse or rabbit monoclonal antibody, DakoCytomation, Carpinteria, Denmark) for 25 minutes. The staining was completed using a streptavidin biotin complex detection method, then were washed in PBS. Finally, the diaminobenzydin was used as a chromogenic agent substrate and was incubated for ten minutes in a dark place for antibody staining. The samples were then stained with hematoxylin for one minute, dehydrated and covered with a cover glass. Positive control was tonsil. The stained slides were examined by two pathologists (MA, ST) blindly and independently without knowing the original histologic diagnosis. The nuclei stained homogeneously for MCM2 were accepted as positive. The percentage of positive cells for MCM2 was calculated by counting 3000–4000 tumor cell nuclei in the most positive areas, at least 10 high-power fields (*400 magnification) in each case.

Statistical analysis

The obtained data was put into SPSS version 16 and undergone statistical analysis based on t-test for the relationship between age and marker expression, also for calculating the difference between the MCM2 expression in adenomas and carcinomas. P-value<0.05 was considered meaningful. Marker expression in three groups of carcinomas based on the kind of invasion was investigated by ANOVA statistical test. P-value<0.05 was considered meaningful. We used kappa coefficient for checking the agreement between two pathologists and Pearson correlation for the relationship between age and marker expression. Correlation was considered significant at level 0.01.

Ethical considerations

Authors have no conflicts of interest. Study protocol was in accordance with the latest Declaration of Helsinki for medical research involving human subjects. This article does not contain any studies with animals performed by any of the authors.

Results

In this study, we studied 60 thyroid follicular lesions, 30 cases of follicular adenoma and 30 cases of follicular carcinoma. These samples were immunostained using MCM2 proliferative marker on paraffin blocks, one case of carcinoma was lost during immunostaining. The results were reported by two pathologists, showing a meaningful agreement between the two pathologists` opinions. Correlation investigation of each groups of adenoma and carcinoma was done. We concluded that the results of the two pathologists in each group were acceptable. With regard to agreement of the two pathologists, the average of the two reported results for each sample was calculated separately and this average was used as the amount of MCM2 expression.

No meaningful difference between adenoma and carcinoma in MCM2 expression

In this study, the average intensity of MCM2 expression was 73.16% in adenoma (Fig. 1, A) and 70.31% in carcinoma which was equally distributed in the invasion area of carcinoma and subcapsular area of adenoma. Nuclear staining was not significantly different between the two mentioned groups. By using the statistical method of t-test and with regard to the p-value=0.620, there is no meaningful difference between adenoma and carcinoma in terms of immunohistochemistry marker expression.

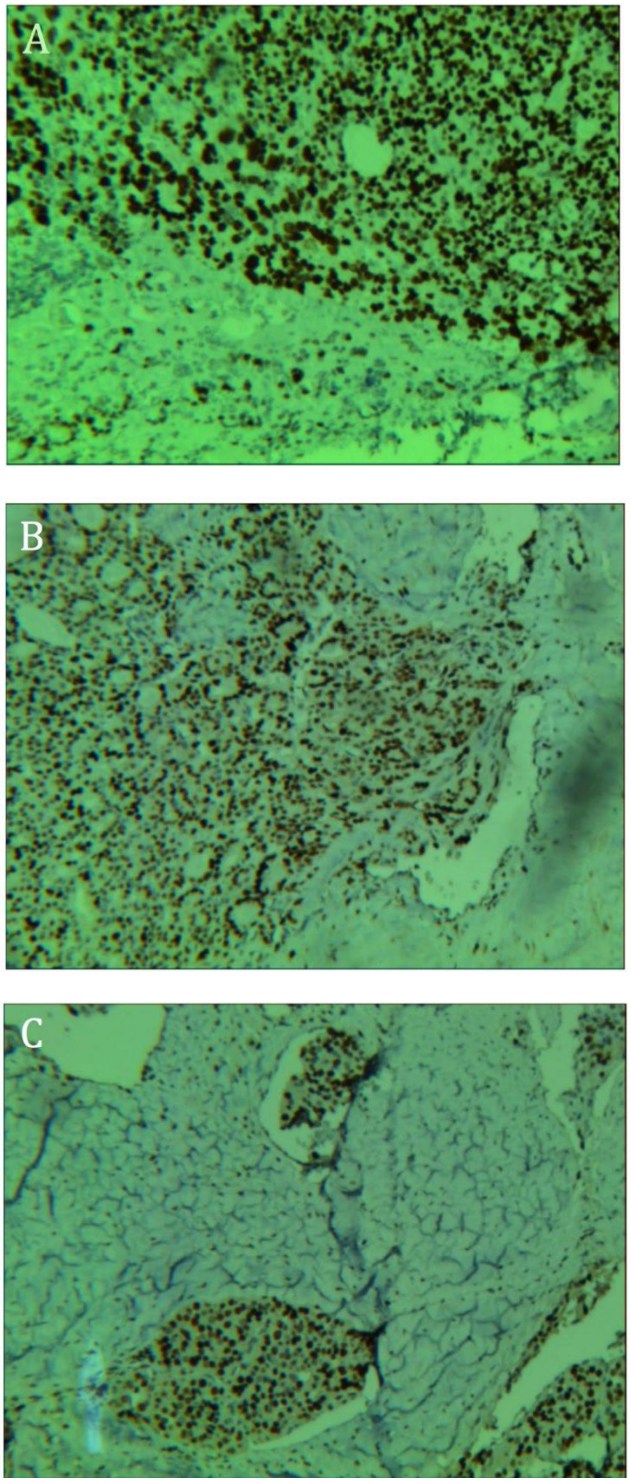

Figure 1 A, B, C, immunohistochemical stainings for MCM2.

No significant difference between the three carcinoma groups in MCM2 expression

Carcinomas were divided into three groups based on the capsular invasions (14 cases), vascular invasions (6 cases), capsular and vascular invasions (10 cases). Intensity of MCM2 expression was 68.10% for capsular invasion (Fig. 1, B), 79.66% for vascular invasion (Fig. 1C) and 70.3% for capsular and vascular invasion.

By using statistical test of ANOVA, intensity of MCM2 expression between three groups of carcinoma was compared and with regard to the p-value=0.511, we concluded that there is no significant difference between the three groups in terms of intensity of marker expression. Immunoreactivity of MCM2 in formalin-fixed follicular thyroid neoplasm (100 magnification). A follicular adenoma (Fig. 1A) with many epithelial cells immunostaining for MCM2 in whole of tumor. A follicular carcinoma with immunoreactivity for MCM2 in cells of tumor in capsular (Fig. 1B) and vascular invasion (Fig. 1C) which is partially similar to subcapsular area of adenoma

No significant differences in MCM2 expression by gender and age between the two groups of the adenoma and carcinomas

In this research, out of 30 studied adenomas, 24 cases were for women and 6 cases were for men. 24 Out of 30 carcinomas were for women and 6 cases were for men. By using the statistical study, p-value=0.359 for adenomas and p-value=0.808 for carcinomas were obtained. We concluded that there is no meaningful relationship between intensity of marker expression of MCM2 and gender. Also, the average age for the 30 studied adenomas was 41.06 and for carcinomas was 43.2. Coefficient correlation between age and intensity of marker expression MCM2 in each groups of adenoma and carcinoma was investigated and calculated. We concluded that there is no meaningful relationship between age and intensity of MCM2 expression (p-value=0.685, t-test).

Discussion

Thyroid nodules are pretty common clinical findings. Almost 40% of the population between ages 30 to 60 have thyroid nodules [18, 19]. FNA is not a reliable way to distinguish benign follicular tumors from malignant follicular tumors. These patients usually need surgical resection to recognize capsular and vascular invasions which differentiates benign and malignant lesions [20]. Even sometimes complete differentiation of the lesions in histology is not possible because of incomplete capsular or vascular invasion, the artifacts of the process and provision of the sections [21]. Because of these reasons, researchers have focused on molecular and immunohistochemical findings, which may be helpful in differentiating these lesions.

Immunohistochemistry is a cheaper and easier method and does not need special equipment and it is also accurate [22, 23]. One group of markers which is used for this purpose, is the markers corresponding to cellular proliferation. Cellular proliferation is necessary for growth of every kind of malignancy. Analyzing cellular proliferation by using antibodies specifically for members of the MCM protein family, the more common MCM2 is known as a new method for the assessment of cellular cycle which may have diagnostic and prognostic value for assessment of neoplasms. Other proliferative markers such as ki67 and PCNA (Proliferative Cell Nuclear Antigen), probably are not as helpful as MCM2 for this purpose, because ki67 exists in the early G1 phase and its

performance is unknown. The PCNA is less specific for determination cell proliferation, it also exists in DNA repair processes [24].

This research is one of the few studies which investigates the intensity of immunohistochemistry marker (MCM2) expression in two follicular neoplasm of thyroid (follicular adenomas and follicular carcinomas) and compares them. In our research, on average, MCM2 expression was 76.16% for follicular adenoma and 70.3% for follicular carcinoma. While in the study of Mehrota et al., this rate was 10.8% for follicular adenoma and 17.34% for follicular carcinoma [25]. Also, this rate was 10.7±45 for adenoma and 26.7±11 for carcinoma in Dr. Cho Mar's study [26]. In this study we investigated 30 cases of follicular carcinomas and 30 cases of follicular adenomas. In a similar study 22 carcinomas and 22 adenomas were compared. Regarding very few number of follicular carcinomas, we obtained this number of cases in four pathology centers during 10 years, which was significant .As same as the study of Mehrato et al. [25], in this study there was no significant difference between adenoma and carcinoma in MCM2 distribution which was unlike the study by Chomar et al. [26].

In our study, there was no difference between the intensity of MCM2 expression in follicular carcinoma and adenoma, which demonstrates that follicular adenomas are active in proliferation. This study shows that the proliferation activity of adenoma is partially high and equal with malignant lesions.

Follicular adenoma and follicular carcinoma are differentiated mainly based on the capsular or vascular invasion, probably other factors affect carcinoma invasion. This study shows that differentiating between follicular adenoma and carcinoma by using MCM2 expression is not helpful.

Other factors may affect positivity and expression of the marker, as it is seen that positivity of most of the nuclei in thyroid epithelial cells around areas with chronic inflammation in response to the cytokines generated by inflammatory cells, these factors was not considered in this study.

In this study, we concluded that the intensity of positivity of the nucleus for MCM2 proliferative marker was pretty high in all the investigated tumors. The accuracy of the results is undeniable, Since immunohistochemistry staining was in the pathology center of Shahid Sadoughi hospital, a center with 8000 IHC in year, this method was based on the brochure of the marker itself, its dilution of antibody was determined by the manufacturer and it was a ready-to-use antibody. The method of calculating numbers of positive cells for MCM2 was similar to the previous studies. The results were based on two pathologist opinion that make them less biased.

In this study, the follicular carcinoma cases were divided in 3 groups: 1- follicular carcinomas with the capsular invasion only (14 cases) 2- follicular carcinomas with vascular invasion only (6 cases) 3- follicular adenoma with capsular and vascular invasion (10 cases) and the positivity and expression of MCM2 was determined based on each of these groups. The conclusion was that there was no meaningful difference between these three groups in nuclear marker expression which was the same as the study conducted by Cho Mar et al. [26], stated that there is no meaningful difference regarding MCM2 among the three groups of MIFC. Also, we didn't see any meaningful difference in MCM2 expression in the mentioned groups.

Follicular carcinoma is 3 times more prevalent among women with the average age of 60 [27]. In this study, out of 30 cases, 24 were for women and 6 for men (approximately 4 to 1), while the average age for follicular carcinoma was 43.2 which was lower than the expected.

There was not a meaningful relationship between MCM2 expression and age of the patients which is similar to the conclusion of Mr. Ellen Cobra et al. [28]. They investigated MCM2 in lymphomas of large B cells. Also similar to their study, there was not a meaningful relationship between intensity of MCM2 expression and gender.

One of the limitations of this study was that other proliferative markers and tumor invasion markers were not concluded and compared. In the future studies we recommend that other interfering factors in interpretation the results of immunohistochemistry such as inflammation and proliferative factors inflammation be considered.

Conclusion

MCM2 is an effective marker in cellular cycles and was positive in all cases of the follicular adenomas and carcinomas. We showed that MCM2 expression in the thyroid follicular carcinoma is not significantly more than the MCM2 expression in follicular adenoma. With regard to overlapping and relatively equal marker expressions in the follicular lesions, this marker should not be used to confirm or rule out cytological diagnosis prior to surgical intervention or to identify or rule out suspicious histo-pathological cases.

Conflict of Interest

The authors declare no conflicts of interest.

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