

# Staining characteristics of BCL-2, BAX, p53, p21, Ki-67 and C-erbB2 in thyroid carcinomas

*Tiroid kansinomlarında BCL-2, BAX, p53, p21, Ki-67 ve CerbB2 nin boyanma karakteristikleri*

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### Abstract

**Purpose:** This study aims at investigating the relation between papillary, follicular, medullar and anaplastic types of thyroid carcinomas and immunohistochemical markers such as bcl-2, bax, p53, p21, Ki-67, and c-erbB2.

**Material and Methods:** A total of forty thyroid carcinoma cases, 10 of which were papillary, follicular anaplastic and medullar were applied bcl-2, bax, p53, p21, Ki-67, and c-erbB2 using immunohistochemistry.

**Results:** Of these, positive staining was observed in all thyroid carcinoma types except for medullar carcinoma for bax, Bcl-2 showed 60% positive staining in papillary type, 80% in follicular type, 90% in medullar type and 60% in anaplastic type. The relation was that as tumor differentiation decreased positive staining increased both for p21 and p53. None of the medullar carcinoma cases had positive staining with these markers. While Ki-67 index was found to be low in the papillary and follicular carcinomas it was found to be significantly high in the medullary and anaplastic carcinomas. The positivity, which was 60% in the papillary carcinomas, reached 80% in anaplastic and 90% in the medullary carcinomas, with cerbB2.

**Conclusion:** It was established that the relation between thyroid carcinomas and bax, bcl-2 and c-erbB-2 was not significant, while their relation to p21, p53 and Ki67 showed a significant difference between well-differentiated and poor-differentiated tumors.

Key Words: **Apoptosis; Thyroid carcinoma .**

### Özet

**Amaç:** Bu çalışmada tüm kanserler içinde %1.5 oranında görülen papiller, folliküler, medüller ve anaplastik tipteki tiroid kansinomlarının bcl-2, bax, p53, p21, Ki-67, ve c-erbB2 gibi immunhistokimyasal belirleyicilerle ilişkisini araştırmayı amaçladık.

**Gereç ve Yöntemler:** 10 papiller, 10 folliküler, 10 medüller ve 10 adet anaplastik kansinomdan oluşan 40 tiroid kansinomu olgusuna immunhistokimyasal metotla bcl-2, bax, p53, p21, Ki-67, ve c-erbB2 uygulandı.

**Bulgular:** Medüller kansinom hariç tüm tiroid kansinomlarında bax için pozitiflik saptandı. Bcl-2 papiller tipte %60, folliküler tipte %80, medüller tipte %90 ve anaplastik tipte %60 pozitif bulundu. p53 ve p21'in kendi aralarında ve tümör diferansiyasyonu ile aralarında bir ilişki bulundu. Tümör diferansiyasyonu azaldıkça p53 ve p21 pozitifliği artmaktaydı. Ancak medüller kansinomlar bu antikolar için negatifti. Proliferatif indeks olarak Ki-67 Papiller (1.71) ve folliküler kansinomlarda (2.56) düşük bulunurken medüller (12.00) ve anaplastik kansinomlarda (13.29) belirgin derecede yüksekti. CerbB2 için papiller kansinomlarda %60 pozitiflik saptanırken anaplastik kansinomlarda %80, medüller kansinomlarda %90 pozitiflik bulundu.

**Sonuç:** Sonuç olarak tiroid kansinom tipleri ile bax, bcl-2 ve C-erb-B2 arasında anlamlı bir farklılık saptanmazken, iyi diferansiyasyonlu tümörlerle az diferansiyasyonlu tümörler arasında p53, p21 ve ki-67 açısından belirgin farklılıklar saptandı.

Anahtar Kelimeler: **Apoptosis; Tiroid kansinom.**

## Introduction

Thyroid carcinomas making up 1.5% of all cancers have four major histological subtypes. These are papillary (75-85%), follicular (10-20%), medullar (5%) and anaplastic (less than 5%) carcinomas in order of frequency. They are generally more common among women (1).

Apoptosis, also defined as programmed cell death, is a cell death mechanism that plays a role in immune regulation and homeostasis in its normal course (2). It is characterized by morphological, biological and molecular genetic modifications (3,4,5,6,7,8,9,10). It is also highly important in cell pathogenesis (2). Bcl-2, the anti-apoptosis gene, is localized in the 18<sup>th</sup> chromosome (1,11,12). The effect of bax is the opposite of that of bcl-2 and it is an apoptosis agonist. Proportional levels of these two genes regulate the survival of the cell (1,13,14). P53 is considered as the gene causing apoptosis and it is seen that it exercises this effect by increasing bax cloning (1,15,16). P21 mediates p53's function of regulating growth. It reflects the functional state of p53 more definitely than p53 accumulation does (17).

Ki-67 which can also be defined as the proliferation marker is a nuclear protein. While it forms reaction in the cells that are in the late phases, such as G1, S, M and G2 of the cell cycle, it is not seen in the G0 phase. It marks the proliferative cells (18,19,20).

As for the C-erbB2, it is one of the growth receptors. In various tumors, mutation of the normal forms and the production which is pathologically extreme can be seen. Extreme formation is seen more frequently in the growth factor receptors than it is in the mutations. C-erbB2 (or c-neu), which is an example of the extreme formation increases in 15-30% of the breast cancer, pulmonary, over and salivary gland carcinomas. In the previous studies, it was reported that high levels, particularly in breast carcinomas is the signal of the poor prognosis (1). The present study aims at determining the relation between bcl-2, bax, p53, p21, Ki-67, and C-erbB2 and histological types of thyroid carcinomas in regulating apoptosis.

## Materials and Methods

Forty thyroid carcinoma cases, including 10 papillary (PTC), 10 anaplastic (AC), 10 follicular (FC) and 10 medullary carcinomas (MC) diagnosed in the Pathology Department of Faculty of Medicine were included in this study. Bcl-2, bax, p53, p21, Ki-67 and C-erbB2 were

immunohistochemically applied on the section taken from all cases, with the method of avidin-biotin peroxidase method. For these markers (except Ki-67) were counted 1000 cells at the 10 high power field on the light microscopy. Proliferative index was calculated as the Ki-67 positive cell numbers in 1000 cells in the randomly selected areas.

Negative staining was considered to be zero and the positivities were evaluated in four categories: Score 0 = negative staining, Score 1 = the cell that is positive by less than 25 %, Score 2 = the cell that is positive by 26-50 %, Score 3 = the cell that is positive by 51- 75 %, Score 4 = the cell that is positive by more than 75 %. Chi Square Test was used to determine the frequency of staining scores for bcl-2, bax, p53, p21 and C-erbB2. Statistical analysis, "student t" was used to determine the extent of significance in the difference between Ki-67 scores obtained from thyroid carcinoma cases.

## Results

While various degrees of positive staining in all papillary, follicular and anaplastic carcinoma cases were obtained with bax, there was staining in only two medullar carcinoma cases ( $p < 0.01$ ) (table 1, figure A1-2). As for bcl-2, various degrees of positive staining scores were attained in 60% of papillary carcinoma cases, 80% of follicular carcinoma cases, 90% of medullar carcinoma cases and 60% of anaplastic carcinoma cases (Table I, figure A3). The frequencies of bcl-2 staining scores in all thyroid carcinoma cases were similar (Table I).

P21 and p53 antibodies had similar staining characteristics in the same cases in general. Both p21 and p53 shows various score levels of positive staining in 30% of papillary carcinomas, 20% of follicular carcinomas and 40% of anaplastic carcinomas. However, they were negative in all medullar carcinomas (Table 1, figure A4-B5).

A statistically significant difference ( $p < 0.05$ ) was found between the staining characteristics of medullar carcinomas where negative results were obtained in all cases and those of other carcinoma types with regard to the relation between both p21 and p53, and thyroid carcinomas.

The proliferative index determined with Ki-67 antibody which was used for the purpose of evaluating the proliferative activity was found to be  $2.02 \pm 0.99$  for

papillary carcinomas,  $2.25 \pm 1.13$  for the follicular carcinomas,  $12.00 \pm 1.79$  for medullary carcinomas and  $13.56 \pm 3.60$  for the anaplastic carcinoma (Table I, Figure B6). In the medullary carcinoma cases, c-erbB2 was positive at the level of score 1 in 40% of the cases while it was positive at the level of score 2 in 50% of the cases (Figure B7) and it was totally negative in 10% of the cases.

C-erbB2 antibody was positive at the level of score 1 in the 60% of the papillary carcinoma cases and 80% of the follicular carcinoma cases.

As for the anaplastic carcinoma cases, a mild c-erbB2 positivity was seen in 70% of the cases (score 1) and positive cells were seen at the level of score 3 in 10% of the cases (Table I, Figure B8).

In terms of the relation between the c-erbB2 and thyroid carcinomas, there was statistically significant difference ( $p < 0.05$ ) between the staining features of medullary carcinoma and anaplastic carcinoma and staining features of the papillary and follicular carcinomas with a lower positivity.

### Discussion

Neoplasms which develop in thyroid pose is an important clinical problem. There are differences in terms of prognosis and response to treatment even in the same histological type (21).

The increase and loss of cells which determine the growth rate of tumor in neoplasms are important in terms of neoplasm aggression. Cell loss is caused by necrosis brought about by physical damage or by genetically controlled apoptosis mechanisms (21).

Genes that regulate apoptosis have been individually researched in various studies carried out with thyroid carcinoma types (2,14-15,21-28). Manetto et al. (14) who studied the relation between bax and thyroid carcinomas reported that bax was generally slightly, mildly or severely positive in papillary carcinomas, particularly evidently in tall cell subtype and less evidently in other subtypes. In the same study, it was also stated that bax gave strongly positive staining results in all anaplastic carcinoma cases. In a study about medullar carcinoma cases by Wang et al. (28) it has been shown that bax was generally negative and bax expression in medullar carcinomas was not significant.

In another study by Brocker et al. (22), it has been reported that bax pro-apoptotic protein gave varying degrees of positive results in follicular carcinomas.

An overall evaluation of the relationship between bax and histological types of thyroid carcinomas demonstrates that staining is observed in all papillary, follicular, and anaplastic carcinoma cases, while negative results are obtained in 80% of medullar carcinomas, which points out that medullar carcinomas are statistically significantly different from other histological types in terms of this marker ( $p < 0.001$ ). Some of the studies concerned with the relation between bcl-2 and thyroid carcinomas reported that bcl-2 showed strong positive values in most papillary carcinoma cases, whereas other studies on the same topic stated that it produced a positive reaction only in some cases (2, 14, 15, 21, 22, 27).

In most of the studies, it was found that about follicular carcinomas that bcl-2 was positive in a vast majority of cases (21,27), while Brocker et al. (22) established that it was positive in a small number of cases (2,25).

Studies that were performed to demonstrate the relationship between medullar carcinomas and bcl-2 found that bcl-2 was positive in all cases.

As for the studies concerning the relation between anaplastic carcinomas and bcl-2, it was reported in these studies that bcl-2 was generally negative in anaplastic carcinomas, but produced a positive reaction in a small number of cases.

In this study, it was found that bcl-2 was positive at various score degrees in 60% of papillary carcinomas, 80% of follicular carcinomas, 90% of medullar carcinomas and 50% of anaplastic carcinomas. As it is seen, bcl-2 and medullar carcinomas display the highest rate of staining, while anaplastic carcinomas have the lowest rate of staining, which is consistent with the source information.

The difference between medullar carcinomas, anaplastic and papillary carcinomas was statistically significant ( $p < 0.05$ ). However, there was no significant difference between follicular carcinomas and medullar carcinomas. The fact that bcl-2 had the lowest rate of positive staining in case of anaplastic carcinomas among histological types suggests that bcl-2 brought about the expected result as an anti-apoptosis gene. However, it seems difficult to

comment on the difference between follicular and papillary carcinomas in terms of positive staining. Although it may be considered that the expected result was achieved in case of follicular carcinomas, positive staining results obtained with bcl-2 in papillary carcinomas were in fact quite unexpected in the light of our current knowledge on this topic. This result may be attributed to our number of cases' being low.

In another study aimed at demonstrating the relation between p21 and thyroid carcinomas, Basolo et al. (29) reported that p21 was negative in almost half of papillary carcinoma cases, whereas it was positive at various degrees in the rest of the cases, though only slightly. Soda et al., (30) on the other hand, established p21 was positive in 12.5% of papillary carcinomas. In another study on the same topic, Mochizuki et al. (31) stated that p21 was expressed to a large extent in papillary and follicular carcinomas and that this expression was greater in follicular carcinomas. It was reported in the same study that p21 expression in medullar and anaplastic carcinomas was very little, if any. Lam et al. (32) stated in their study about anaplastic carcinomas that p21 was positive at a rate of 3%. Johnson et al. (33) who studied neoplastic and non-neoplastic tissues found that p21 was more positive in papillary and follicular carcinomas than in benign lesions, was less positive in anaplastic carcinomas when compared to other carcinomas and there was very little staining in medullar carcinomas.

The fact that mostly score 1 positive staining was achieved with p21 in 30% of papillary and 20% of follicular carcinomas in our study seems consistent with source information.

Interestingly, rate of positive staining in anaplastic carcinomas was 40% and staining in anaplastic carcinomas was intense. As for medullar carcinomas, p21 was negative for these cases and the difference between that and other histological types had statistical significance ( $p < 0.05$ ). An aspect of our study that should be noted is that positive staining characteristics of p21 and p53 were similar for all histological types.

In studies, using p53 antibody, it was reported that p53 was generally negative in well-differentiated tumors like papillary and follicular carcinomas and in medullar carcinomas, but it was strongly positive in anaplastic carcinomas (2, 21, 28).

In our study positive staining was achieved in 30% of papillary, 20% of follicular and 40% of anaplastic carcinomas with p53, whereas all medullar carcinoma cases were found negative.

There was a statistically significant difference between medullar carcinomas and other histological types with regard to positive staining with p53 ( $p < 0.05$ ).

Positive staining in papillary and follicular carcinomas which was found in a case who had tall cell variant and blood vessel invasion, which indicate poor prognosis, and in another case who had necrosis was generally score 1, whereas positive staining in anaplastic carcinoma cases was score 3. In our study, tumor cells located in the vicinity of necrosis regions in anaplastic carcinomas in particular had intense positive staining, while there was no positive staining in tumor cells there were not close to necrosis regions. In addition, staining was not observed in sarcomatous component regions, which are among components forming anaplastic carcinomas.

It is possible to attribute the high rate of negative results obtained in anaplastic carcinoma cases to the fact that the tumor in some of these cases was composed almost entirely from sarcomatous component. However, presence of intense staining in cells located close to necrosis regions and cells where anaplastic characteristic is evident shows that p53 is correlated with tumor anaplasia, as emphasized in previous studies.

Many studies have been conducted so far in order to determine whether there is relation between the c-erbB2, which is known as an indicative of poor prognosis, and the thyroid carcinomas. In a study that was conducted by Vitale et al. (34), it was reported that c-erbB2 display membranous and diffuse cytoplasmic staining in 52% of the papillary carcinoma cases and that it is cytoplasmic granular positive in all of the medullary carcinomas. In the same study c-erbB2 was found to be negative in the follicular and anaplastic carcinoma. In addition, in a study conducted by Haugen et al. (35), c-erbB2 was reported to be positive in 24.4% of the papillary carcinoma cases while it was found to be negative in the follicular and medullar carcinoma. In another study made by Yang et al. (36) on the same subject, it has been stated that there is no significant relation between c-erbB2 and medullary carcinoma.

In this study, it was detected that there was mild c-erbB2 positivity in 60 % of the papillary carcinoma cases and 80 % of the carcinoma cases while there was mild and medium degree cytoplasmic and pale staining in 90% of the medullary carcinoma cases. C-erbB2 was positive in 80% of the anaplastic carcinoma cases.

However, staining was intensive in one of the cases (score 3), while it was mild (score 1) in the other ones. A statistically significant difference ( $p < 0.05$ ) was found between the positive staining which was at a lower rate in the papillary carcinomas and the positive staining in the other histological types of thyroid carcinoma.

Different results have been achieved by the studies aiming at the determination of the relation between c-erbB2 and thyroid carcinomas shows that c-erbB2 does not have an indicating feature for the thyroid carcinomas. Similarly, in this study, c-erbB2 positivity was found in the form of pale staining in a couple of cells whose number does not exceed the number of the 1-2% of the tumor cell number.

Therefore, this study confirms the finding that c-erbB2 is not a marker for all the histological types of the thyroid carcinomas. In our study, 60% positivity was obtained in the papillary carcinoma with c-erbB2. This rate was 80% in the follicular and anaplastic carcinomas, 90% for the medullary carcinoma. The relation between the last carcinoma with c-erbB2 was found to be more significant when compared with the relation between the c-erbB2 and papillary carcinoma ( $p < 0.05$ ). However, the fact that negativity was detected in all of the histological types and different findings have been obtained by different studies so far makes it difficult to claim that there is sound relation between c-erbB2 and histological types of thyroid carcinoma. For this reason, it seems to be necessary to conduct new studies on larger case groups in order to reach a judgement on the issue.

Having conducted a study on ki-67, Yoshida et al. (37) reported that the Ki-67 positivity was lower in papillary and follicular carcinomas than it was in anaplastic carcinomas.

In this study, Ki-67 index was found to be  $0.4 \pm 0.3$  for papillary and follicular carcinomas and  $1.8 \pm 1.5$  for the anaplastic carcinomas. Ki-67 index, was low for the papillary carcinomas ( $2.02 \pm 0.99$ ) and follicular carcinomas ( $2.25 \pm 1.13$ ) which constitute the well-differentiated group of thyroid carcinomas in our study and it was considerably high for the medullary carcinomas ( $12.00 \pm 1.79$ ) and anaplastic carcinomas ( $13.56 \pm 3.60$ ). This finding indicates that cell proliferation increased at an significant extent ( $p < 0.001$ ) in both the anaplastic carcinoma and medullary carcinoma. As a result, in this study, a significant increase was detected in the Ki-67 while the tumor differentiation was decreasing. It was also found that the relation between Ki-67, which is a proliferation marker, and the histological type of thyroid carcinomas isare significant.

However no significant relationship was detected between c-erbB2, which is considered to be the indicator of poor prognosis, and thyroid carcinomas.

As a result, in this study, there were no significant relation between types of thyroid carcinomas and apoptotic markers such as bcl-2, p21 and p53 but only he relation was which as tumor differentiation decreased positive staining increased p53.

A significant increase was detected in the Ki-67 while the tumor differentiation was decreasing. It was also found that the relation between Ki-67, which is a proliferation marker, and the histological type of thyroid carcinomas is significant.

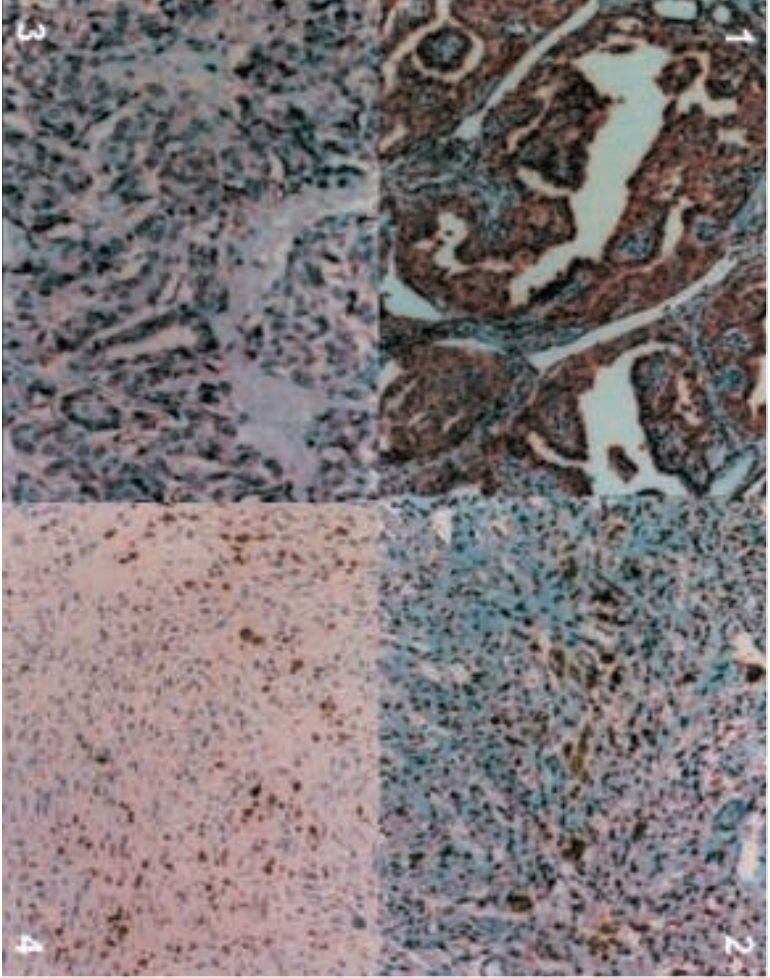
Different results have been achieved by the studies aiming at the determination of the relation between c-erbB2 and thyroid carcinomas shows that c-erbB2 does not have an indicating feature for the thyroid carcinomas that's why c-erbB2 is not a marker for all the histological types of the thyroid carcinomas

**Table I.** Results of immunohistochemistry staining

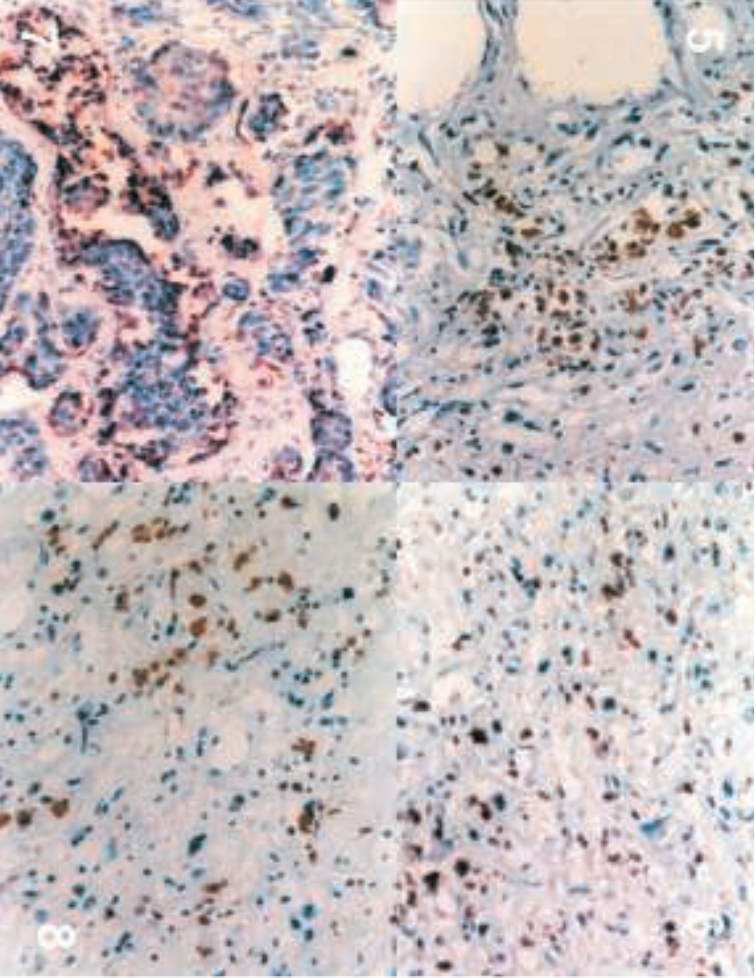
IHC	Score	PTC (n=10)	FC (n=10)	MC (n=10)	AC (n=10)
<b>Bcl-2</b>	Score 0	4	2	1	4
	Score 1	5	6	5	2
	Score 2	1	1	4	4
	Score 3	0	1	0	0
	Score 4	0	0	0	0
	X <sup>2</sup> p <	10,4 0,406	10,4 0,406	10,4 0,406	14,8 0,14
<b>Bax</b>	Score 0	0	0	8	0
	Score 1	0	4	2	0
	Score 2	0	5	0	0
	Score 3	5	1	0	2
	Score 4	5	0	0	8
	X <sup>2</sup> p <	23,6 0,009	10,4 0,406	19,2 0,038	19,2 0,038
<b>CerbB2</b>	Score 0	4	2	1	2
	Score 1	6	8	5	7
	Score 2	0	0	4	0
	Score 3	0	0	0	1
	Score 4	0	0	0	0
	X <sup>2</sup> p <	19,2 0,038	19,2 0,038	10,4 0,406	10,4 0,406
<b>P53</b>	Score 0	7	8	10	6
	Score 1	3	2	0	2
	Score 2	0	0	0	2
	Score 3	0	0	0	0
	Score 4	0	0	0	0
	X <sup>2</sup> p <	19,2 0,038	19,2 0,038	32,4 0,001	14,8 0,14
<b>P21</b>	Score 0	7	8	10	6
	Score 1	3	2	0	3
	Score 2	0	0	0	1
	Score 3	0	0	0	0
	Score 4	0	0	0	0
	X <sup>2</sup> p <	19,2 0,038	19,2 0,038	32,4 0,001	10,4 0,406
<b>Ki-67</b>	PI	2.02±0.99	2.25±1.13	12.00±1.79	13.56±3.60

**IHC** : Immunohistochemistry  
**PI** : Proliferating Index  
**n** : Case number

**PTC** : Papillary Thyroid Carcinoma  
**FC** : Follicular Carcinoma  
**AC** : Anaplastic Carcinoma  
**MC** : Medullary Carcinoma



**Figure A.** Papillary Carcinoma-Bax positivity (Score 4,X200), (1), Anaplastic Carcinoma-Bax positivity (Score 3, X2400),( 2), Follicular Carcinoma-Bcl-2 positivity (Score 1,X200),(3), Anaplastic Carcinoma-p21 positivity (Score 2, X200),(4)



**Figure B.** Anaplastic Carcinoma-p53 positivity (Score 2 X200) (5), Anaplastic carcinoma –Ki-67 positivity X 200 (6), Medullary carcinoma –c-erbB2 positivity X 200 (7), Anaplastic carcinoma –c-erbB2 positivity X 200 (8).

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