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# Immunohistochemical evaluation of Bcl-2 protein expression in thyroid follicular pathology: a retrospective observational study

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## Abstract

The use of chemotherapy in treating malignant thyroid neoplasms with aggressive biological behavior, such as doxorubicin, has been associated with an overall poor outcome. Therefore, it has been suggested that thyroid malignant cells are resistant to apoptosis induced by chemotherapy agents. The objective of this study was to compare the immunohistochemical expression of Bcl-2 in thyroid tissues obtained from follicular hyperplasia, follicular adenoma, and follicular carcinoma of thyroid glands. This retrospective observational study was conducted at the Department of Pathology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, covering the period from January 2013 to December 2023. The study focused on cases of thyroid follicular hyperplasia (20 cases), thyroid follicular adenoma (20 cases), and thyroid follicular carcinoma (12 cases) found in thyroidectomy specimens. Immunohistochemical analysis was performed on 4µm thick sections obtained from a paraffin block of tumor tissue to detect Bcl-2. Comparison of mean bcl-2 immunohistochemical score among study groups revealed that the median score was 2 in both follicular hyperplasia and follicular adenoma groups, whereas the median score was 3 in the group of follicular carcinomas, thus it was significantly higher in this later group in comparison with adenoma and hyperplasia ($p$ <0.001). There was no significant association between Bcl-2 immunohistochemical expression and invasiveness. Bcl-2 immunohistochemical expression can be detected in follicular thyroid neoplasms with the highest intensity in carcinoma cases, suggesting a role for Bcl-2 in marinating the growth of these neoplasms, but it does not correlate with the degree of tumor invasiveness.

**Keywords:** Immunohistochemical. Bcl-2. Thyroid, follicular.

## Introduction

Malignant thyroid neoplasms are the most common forms of endocrine malignant neoplasms. Anaplastic, poorly differentiated, and medullary histological types are associated with a relatively high rate of mortality **[1,2]**. The use of chemotherapy in treating malignant thyroid neoplasms with aggressive biological behavior, such as doxorubicin, has been associated with an overall poor outcome **[3,4]**. Therefore, it has been suggested that thyroid malignant cells are resistant to apoptosis induced by chemotherapy agents **[5-7]**. Inside the cell, the apoptotic signal is under the control of several types of molecules, and Bcl-2 is one of the major families of proteins that are involved in the control of this cellular biologic function **[9, 10]**. The family of Bcl-2 proteins

includes at least 20 members [10]. The principal members are represented by Bcl-2, Bcl-w, BclxL, and Mcl-1, which have the capacity to prevent a wide range of apoptotic triggers and stimuli, even those initiated by chemotherapy agents, thus promoting the process of carcinogenesis [11]. The Bcl-2 protein is encoded by the Bcl-2 proto-oncogene [12]. The gene is located on chromosome 18 (band q21.3). This protein blocks programmed cell death, leading to prolongation of cell survival [12]. The protein Bcl-2 has been found in a variety of tissues under physiological and pathological conditions [13].

Pilotti et al. were pioneers in evaluating the immunohistochemical expression of Bcl-2 in thyroid tissues obtained from follicular neoplasms, papillary neoplasms, and poorly differentiated neoplasms, and they detected the expression of this protein in various cell proportions in these various tissues [14]. Later on, Ahn et al. examined various forms of thyroid neoplasms, including follicular, papillary, medullary, and undifferentiated malignant neoplasms, and they described various patterns of Bcl-2 immunostaining in these types of thyroid tissues [15]. Manetto et al. have also evaluated the immunohistochemical expression of Bcl-2 in a series of thyroid neoplasms, and they detected Bcl-2 expression in poorly differentiated thyroid tumors and some cases of papillary carcinoma [16].

In this study, we examined the immunohistochemical expression of Bcl-2 in thyroid tissues obtained from follicular hyperplasia, follicular adenoma, and follicular carcinoma of thyroid glands, aiming to compare such expression among these varieties of thyroid pathologies.

## Methods

### Study Design and Settings

This retrospective observational study was conducted at the Department of Pathology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, covering the period from January 2013 to December 2023. The study focused on cases of thyroid follicular hyperplasia (20 cases), thyroid follicular adenoma (20 cases), and thyroid follicular carcinoma (12 cases) found in thyroidectomy specimens. Patients who had undergone hormone treatment, chemotherapy, or radiation therapy were not included in the study. Clinical information such as patients' age, sex, and histopathological features were obtained from pathology reports retrieved from the central laboratory of Al-Imamain Al-Kathimain Medical City, Baghdad, Iraq, with permission from the relevant authorities.

## Ethical Approval

The study received approval from the ethical approval committee of the Department of Pathology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, and written consent was not required as none of the patients were directly involved in the study.

## Analysis

Immunohistochemical analysis was performed on 4μm-thick sections obtained from a paraffin block of tumor tissue to detect Bcl-2. The IHC procedure involved antigen retrieval in citrate buffer using a microwave oven, blocking endogenous peroxidase with 3% hydrogen peroxide, incubating with primary mouse anticlonal antibody against Bcl-2 (Anti-bcl-2 oncoprot), linking with rabbit anti-mouse secondary antibody (Biogenex), enzyme labeling with streptavidin-horseradish peroxidase, developing chromogen with deaminobenzidine (DAB), and counterstaining with hematoxylin [17]. Positive staining for Bcl-2 protein was determined when specific cytoplasmic staining was observed. The percentage of tumor cells showing positive staining (positive tumor cells over total tumor cells) was calculated for each case. Staining intensity was semi-quantitatively assessed as negative (0), weak positive (1+), moderate positive (2+), and strong positive (3+) [18].

### Statistical Analysis

The obtained data, including age, sex, histopathological type, and Bcl-2 immunohistochemical expression, were introduced into a spread sheet of the statistical software (SPSS Version 16, SPSS Inc., Chicago, USA). The age variable was presented as the mean, standard deviation, and range. The sex variable was presented as a number and a percentage. BCL-2 scoring was treated as a qualitative ordinal variable, and it was expressed as the median, interquartile range, and range. A one-way ANOVA test was used to compare means of age among study groups. A chi-square test was used to compare proportions of sex among study groups. The Kruskall-Wallis test was used to compare Bcl-2 scores among study groups. The level of significance was considered at $p \leq 0.05$.

## Results

Table 1 includes demographic data. The mean age of patients with follicular hyperplasia was significantly ($p < 0.001$) lower than that of patients with follicular adenoma and follicular carcinoma, and there was no significant difference ($p > 0.05$) in mean age between the follicular adenoma group and the follicular

carcinoma group. The proportion of females was higher than the proportion of males in all histological types of thyroid pathology. The follicular hyperplasia group included 12 (60.0%) females and 8 (40.0%) males. The follicular adenoma group also included 12 (60.0%) females and 8 (40.0%) males, and there was no significant difference with respect to sex proportions between the follicular hyperplasia group and the follicular adenoma group ($p > 0.05$), but the follicular carcinoma group included only females, thus showing significant variation when compared to other groups ($p = 0.031$).

A comparison of the mean bcl-2 immunohistochemical score among study groups is shown in Table 2 and Figures 1-7. The median score was 2 in both follicular hyperplasia and follicular adenoma groups, whereas the median score was 3 in the group of follicular carcinomas, thus it was significantly higher in this later group in comparison with adenoma and hyperplasia ($p < 0.001$). There was no significant association between Bcl-2 immunohistochemical expression and invasiveness (Table 3).

Table 1. Demographic characteristics of enrolled patients.

| Characteristic | Follicular hyperplasia n = 20 | Follicular adenoma n = 20 | Follicular carcinoma n = 12 | p |
|---|---|---|---|---|
| Age (years) | | | | |
| Mean ±SD | 43.40±15.06b | 60.40±8.89a | 60.17±16.91a | <0.001O |
| Range | 20 -59 | 45 -70 | 33 -80 | *** |
| Sex | | | | |
| Male | 8 (40.0 %) | 8 (40.0 %) | 0 (0.0 %) | 0.031 C |
| Female | 12 (60.0 %) | 12 (60.0 %) | 12 (100.0 %) | * |

SD: standard deviation; *n*: number of cases; O: one way ANOVA; C: chi-square test; *: significant at $p \leq 0.05$; ***: significant at $p \leq 0.001$. Source: Own authorship.

Table 2. Comparison of mean bcl-2 immunohistochemical score among study groups.

| Characteristic | Follicular hyperplasia n = 20 | Follicular adenoma n = 20 | Follicular carcinoma n = 12 | p |
|---|---|---|---|---|
| Score | | | | |
| Median (IQR) | 2(1) | 2 (1) | 3 (0) | <0.001 K |
| Range | 1 -2 | 2 -3 | 2 -3 | *** |

IQR: inter-quartile range; *n*: number of cases; K: Kruskal Wallis test; ***: significant at $p \leq 0.05$. Source: Own authorship.

Table 3. Association between Bcl-2 immunohistochemical expression and invasiveness.

| Score | Total n = 12 | Invasiveness | | p |
|---|---|---|---|---|
| | | Minimal n = 7 | Moderate n = 5 | |
| Score 2 | 2 (16.7 %) | 2 (28.6 %) | 0 (0.0 %) | 0.470 F |
| Score 3 | 10 (83.3 %) | 5 (71.4 %) | 5 (100.0 %) | NS |

F: Fischer exact test; NS: not significant. Source: Own authorship.

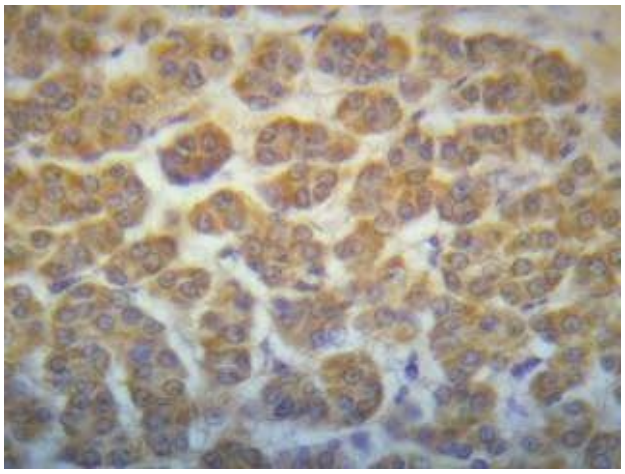

Figure 1. Positive immunohistochemical expression of Bcl-2 (cytoplasmic brown color; black arrow) in thyroid follicular adenoma of score2 (26% to 50% of cells stained) (IHC:20x). Source: Own authorship.

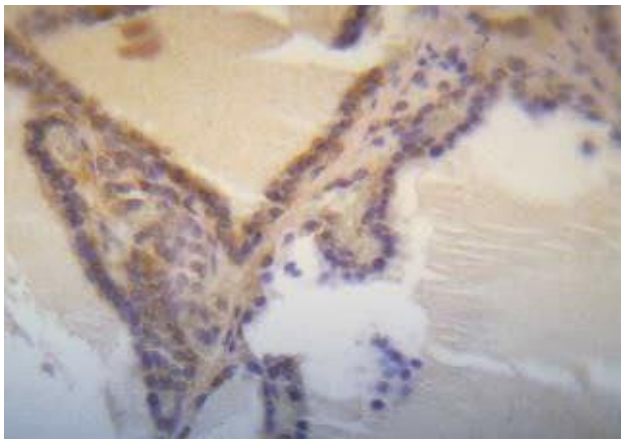

Figure 2. Positive immunohistochemical expression of Bcl-2 (cytoplasmic brown color; black arrow) in thyroid follicularadenoma of score3 (more than 75% of cells of cells stained) (IHC, 10X). Source: Own authorship.

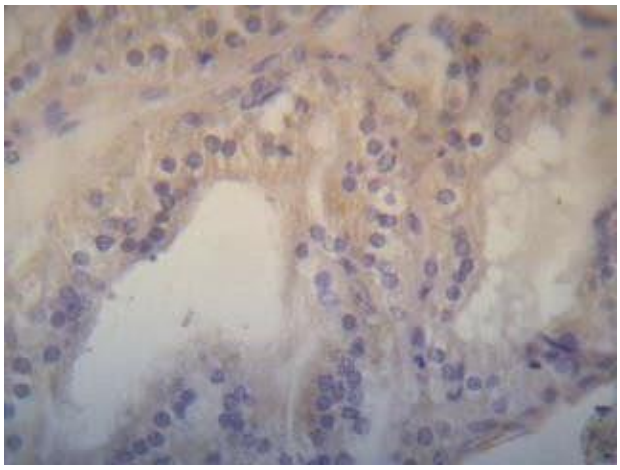

Figure 3. Positive immunohistochemical expression of Bcl-2 (cytoplasmic brown color; black arrow) in thyroid follicular hyperplasia of score 3(51% to 75% of cells stained) (IHC, 10X). Source: Own authorship.

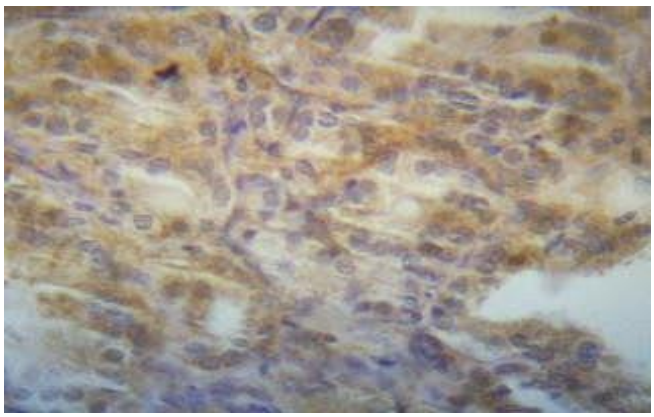

Figure 4. Positive immunohistochemical expression of Bcl-2 (cytoplasmic brown color; black arrow) in thyroid follicular hyperplasia of score 3(51% to 75% of cells stained) (IHC, 20X). Source: Own authorship.

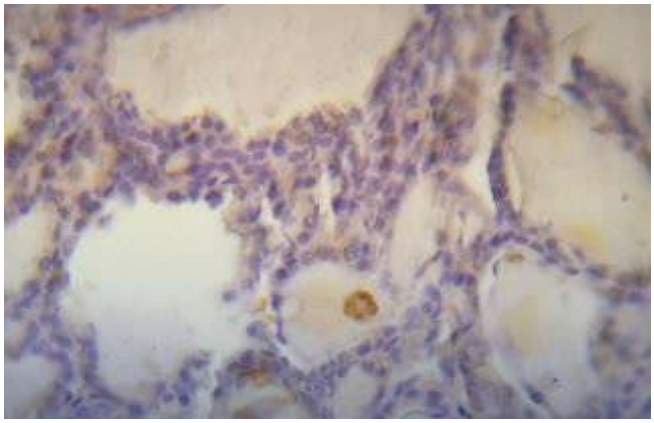

Figure 5. Positive immunohistochemical expression of Bcl-2 (cytoplasmic brown color; black arrow) in thyroid follicular hyperplasia of score 2(26% to 50% of cells stained) (IHC, 10X). Source: Own authorship.

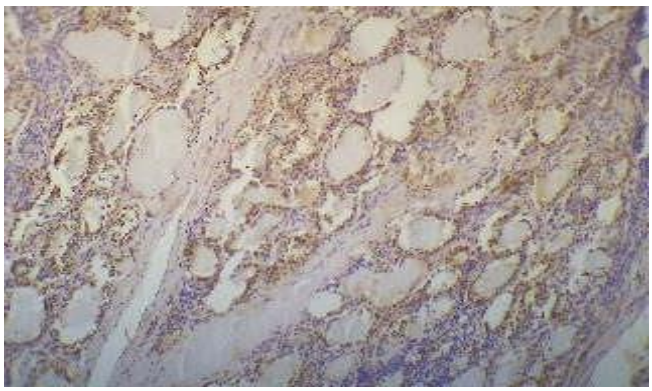

Figure 6. Positive immunohistochemical expression of Bcl-2 (cytoplasmic brown color; black arrow) in thyroid follicular carcinoma of score2 (26% to 50% of cells stained) (IHC, 10X). Source: Own authorship.

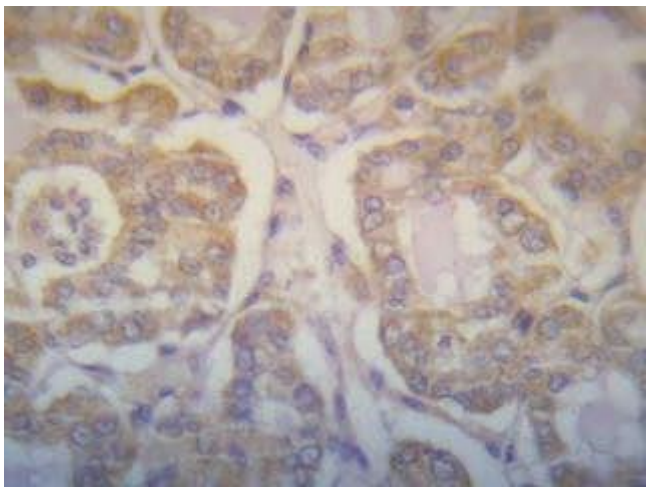

Figure 7. Positive immunohistochemical expression of Bcl-2 (cytoplasmic brown color; black arrow) in thyroid follicular carcinomaof score 3(51% to 75% of cells stained) (IHC, 20X). Source: Own authorship.

## Discussion

Thyroid carcinomas, as a rule, exhibit resistance to traditional DNA-damaging chemotherapeutic drugs, indicating an abundance of apoptosis inhibitors compared to apoptosis inducers **[19]**.It has been previously noted that the overexpression of Bcl-2 in thyroid carcinoma cells offers protection against a number of emerging anticancer medications, such as the histone deacetylase inhibitor suberoylanilidehydroxamic acid, the proteasome inhibitor bortezomib, and the heat shock protein 90 inhibitors 17-AAG and 17-DMAG **[20-22]**.

In the current study, we demonstrated strong immunohistochemical expression of Bcl-2 in follicular carcinoma in addition to moderate staining intensity in adenoma and hyperplasia of the thyroid gland. Previously, it has been shown that follicular adenoma expresses Bcl-2 protein to a moderate extent **[15]**. Gupta et al. **[23]** have reported that 88.9% of follicular carcinoma thyroid cases were positive for Bcl-2 and that the expression level was significantly higher in this particular type of tumor when compared to a papillary variant.

The significant involvement of Bcl-2 and other antiapoptotic family members in various cancers has sparked great interest in this area, leading to numerous current initiatives aimed at pharmacologically targeting these molecules **[19]**. In this study, we observed no significant difference in the intensity of Bcl-2 expression with respect to invasiveness so indirectly we can suggest that Bcl-2 expression plays a little role as a prognostic marker in follicular thyroid carcinoma. In line with this suggestion, Branet et al. **[24]** reported expression of

this marker in normal thyroid tissue, and in thyroiditis. Therefore, much research work should be directed toward the therapeutic targeting of Bcl-2 molecules in thyroid follicular neoplasms. However, it has been shown that loss of Bcl-2 expression in recurrent thyroid **[25]**. In addition, we observed higher level of expression in carcinoma when compare to hyperplasia and adenoma and this finding may be beneficial when staining fine needle aspiration samples of thyroid tissue with Bcl-2 immunohistochemistry, since more intense pattern will be correlated with possible malignancy.

## Conclusion

Bcl-2 immunohistochemical expression can be detected in follicular thyroid neoplasms with the highest intensity in carcinoma cases, suggesting a role for Bcl-2 in marinating the growth of these neoplasms, but it does not correlate with the degree of tumor invasiveness.

## CRediT

**Author contributions:** Conceptualization, methodology, investigation, data collection, formal analysis, data interpretation, supervision, writing—original draft: All authors.


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## Ethical Approval

The study received approval from the ethical approval committee of the Department of Pathology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, and written consent was not required as none of the patients were directly involved in the study.

## Informed Consent

Not applicable.


## Funding

Not applicable.


## Data Sharing Statement

The datasets generated and analyzed during the current study and available from the corresponding author upon reasonable request.

## Conflict of Interest

The authors declare that there are no competing interests or potential conflict related to the conduct or publication of this study.

## Similarity Check

It was applied by Ithenticate®.

## Application of Artificial Intelligence (AI)

Not applicable.

## Peer Review Process

It was performed.



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