

## Expression of Bc-l2 in precancerous endometrial lesion and endometrial carcinoma

Walaa Abd El-Mohsen Hassan<sup>1</sup>, Mohamed Arafa Adly<sup>2</sup>, Ahmed R.H. Ahmed<sup>3</sup>  
Departments of Zoology<sup>1,2</sup> and Pathology<sup>3</sup>, Faculty of Science<sup>1,2</sup> and Medicine<sup>3</sup>,  
Sohag University

### Abstract

The oncogene Bcl-2 is known as a unique gene that plays an important role in the carcinogenesis pathway by blocking apoptosis. Aims: To evaluate the expression of Bcl-2 protein in normal, hyperplastic and neoplastic endometrial tissues. Materials and methods: Eighty three cases including 7 cases of normal endometrial changes, 37 cases of endometrial hyperplasia and 39 cases of endometrial adenocarcinoma were enrolled for this study. The expression of Bcl-2, ER and PR was evaluated by immunohistochemistry using streptavidin-biotin technique. Results: Non-malignant endometrial lesions tend to express significantly higher level of Bc-l2 compared to malignant endometrial lesion ( $p=0.014$ ). The expression of glandular Bc-l2 was significantly higher in hyperplasia compared to carcinoma ( $p=0.006$ ). There was no significant association between Bcl-2 expression and tumors' histological type or stage or grade. There was a correlation between Bcl-2 expression and ER or PR expression in endometrial carcinoma. Conclusions: Bcl-2 expression might represent a key in understanding the progression of endometrial neoplastic lesions.

**Keywords:** Endometrial carcinoma, Endometrial hyperplasia, Bcl-2, ER, PR.

### Introduction

Endometrial carcinoma is one of the common malignant tumors of female genital tract. The incidence of endometrial carcinoma continued to increase annually and it has preceded cervical cancer in some countries as the most common malignant tumors of female genital tract [1]. Endometrial carcinoma has been classified into two different types. Type I includes endometrioid carcinomas often developing on top of complex and atypical hyperplasia, having lower malignancy, rare production of metastases, and are associated with estrogen stimulation. Type II is refers to non endometrioid carcinomas, largely papillary serous carcinomas, arising on top of endometrial atrophy. Type II includes estrogen-independent carcinomas, which are more aggressive and more frequently metastasizing [2]. Apoptosis is a genetically controlled cell suicide pathway which plays an essential role in deleting

excess, unwanted or damaged cells during development and tissue homeostasis. Dysfunction of apoptosis contributes to a wide variety of pathological conditions in humans, including carcinogenesis [3, 4]. The Bcl-2 family of proteins comprises pro-apoptotic and anti-apoptotic proteins that play a pivotal role in the regulation of apoptosis, especially via the intrinsic pathway as they reside upstream of irreversible cellular damage and act mainly at the mitochondrial level [5]. Bcl-2 is a proto-oncogene encoding a protein located on the mitochondrial and the nuclear membranes as well as the endoplasmic reticulum. The product of the Bcl-2 (B-cell lymphoma/leukemia 2) gene was first identified from the t (14:18) translocation occurring in most cases of follicular lymphoma. It prevents apoptosis induced by a number of agents and is believed to be under

hormonal control, as demonstrated by Wang et al[6, 7].

### **Material and methods:**

#### ***Patient's selection***

Formalin-fixed paraffin-embedded endometrial tissue blocks of 83 patients were retrieved retrospectively from the archive of Pathology Department, Sohag University Hospitals. These samples aged between 19 and 80 years. Different types of hyperplastic and malignant endometrial lesion were presented in this study in addition to four cases of proliferative and three cases of secretory endometrial changes.

#### ***Immunohistochemistry***

The used primary antibodies and secondary chromogen detection system were purchased from Thermo Scientific. Mouse monoclonal anti-Bcl-2 (Clone 100/D5, Cat#MS-123-P0), rabbit monoclonal anti-ER (Clone SP1, Cat#RM-9101-S0) and mouse monoclonal anti-PR (Clone hPRa 2+hPR 3, Cat#MS-298-P0) antibodies were used to evaluate expression of Bcl-2, ER and PR respectively by immunohistochemistry. Sections of 4µm thick were de-paraffinized in xylene then rehydrated in graded alcohol and incubated in 0.5% hydrogen peroxide to block endogenous peroxidase activity. The antigens were retrieved by boiling in 10 mM citrate buffer, pH 6.0, using a microwave followed by cooling down to room temperature. Sections were then incubated with anti-Bcl-2, anti-ER or anti-PR antibodies for overnight at 4°C. Next day, the sections were incubated with biotinylated secondary antibody for 10 minutes followed by

incubation with streptavidin for 10 minutes. The sections were then exposed to 3, 3'-diaminobenzidine tetra hydrochloride solution (DAB) to yield insoluble brown deposits. The sections were counterstained with hematoxylin then dehydrated and mounted as usual. Sections from tonsil were used as positive controls for Bcl-2 expression.

#### ***Scoring of immunoreactions and statistical analyses***

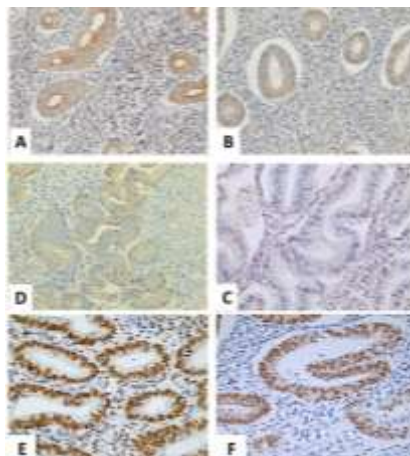
The expression level of Bcl-2 was measured by the histoscore system that combines intensity of immunoreactions with percentage of positive cells. Cells present in four 400X high power fields were counted and scored in each case. The intensities of immunoreactions were stated as negative, weakly positive, moderately positive, or strongly positive. These four categories are weighed as 0, 1, 3, and 10, respectively. The final score ranged between 0 when all scored cells were negative to 1000 when all scored cells were strongly positive[8]. The commercially available statistical software (IBM-SPSS version 16.0 for Windows; IBM Inc) was used for data analysis. The correlation of Bcl-2 expression with expression of either ER or PR was evaluated by Pearson's correlation Test. Mann-Whitney Test was used to compare the mean rank of Bcl-2 expression in simple and complex hyperplasia, in atypical and non-atypical hyperplasia and in malignant and non-malignant lesions. *Ap* value of less than 0.05 was considered as statistically significant.

### **Result**

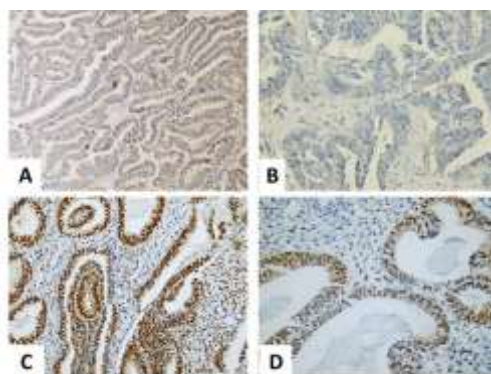
This study included 37 cases of endometrial hyperplasia, 14 cases were non-atypical simple hyperplasia (NSH), 7 were atypical simple hyperplasia (ASH), two cases were non-atypical complex hyperplasia (NCH) and 14 cases were atypical complex hyperplasia (ACH). On the other hand the 39 cases of endometrial carcinoma

included 34 endometrioid adenocarcinomas versus five serous endometrial adenocarcinomas.

Bcl-2 protein expression appeared as a brownish cytoplasmic staining. Bcl-2 expression was evaluated in glands of the non-neoplastic and neoplastic endometrial tissue. Bcl-2 was expressed in all cases of hyperplasia (Figure 1 A, B, C, D) and in 30 cases of endometrial carcinoma (Figure 2 A, B). Non-malignant endometrial lesions tend to express significantly higher level of Bcl-2 compared to malignant endometrial lesion ( $p = 0.014$ ). Expression of both PR and ER in hyperplastic (Figure 1 E, F) as well as neoplastic endometrial changes (Figure 2 C, D) was directly proportional with Bcl-2 expression.



**Figure 1:** Expression of Bcl-2 in non-atypical simple hyperplasia (A), atypical simple hyperplasia (B), non-atypical complex hyperplasia (C) atypical complex hyperplasia (D), expression of ER in endometrial hyperplasia (E) and expression of PR in endometrial hyperplasia (F). Original magnification is 200X in A, B, C and 400X in others.

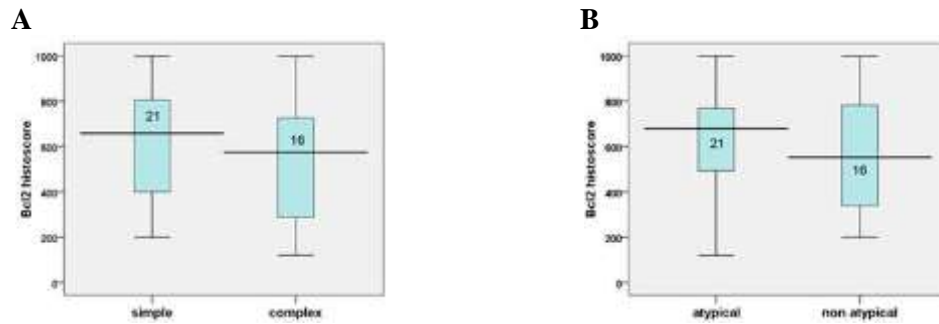


**Figure 2:** Expression of Bcl-2 in endometrioid adenocarcinoma (A), serous endometrial carcinoma (B), expression of ER in endometrial carcinoma (C) and expression of PR in endometrial carcinoma (D). Original magnification is 400X in D, B and 200X in the others.

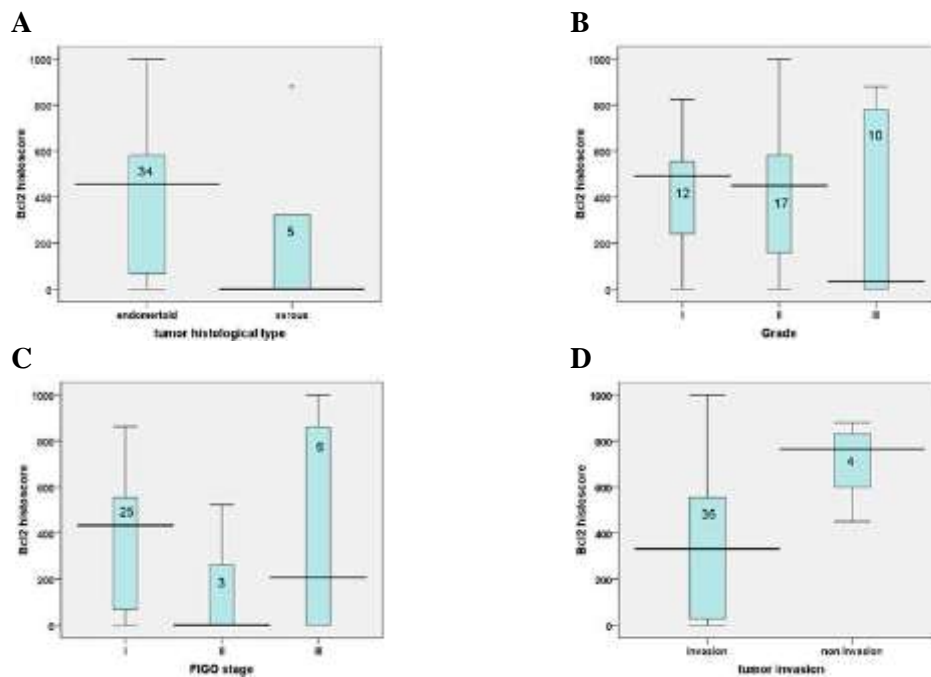
Although demonstration in all cases of endometrial hyperplasia; the histoscore of Bcl-2 showed no significant changes among atypical and non-atypical hyperplasia nor among simple and complex hyperplasia (Figure 3A, B).

In malignant neoplastic conditions, the glandular expression of Bcl-2 was significantly higher in endometrial hyperplasia compared to endometrial carcinoma in the same gland ( $p = 0.006$ ). Bcl-2 histoscore was higher in endometrioid compared to serous adenocarcinomas but the difference was not statistically significant (Figure 4 A). Bcl-2 histoscore showed a decline as tumor grade increases particularly in grade III tumors but this relationship did not reach the significant level (Figure 4 B). Tumors with different FIGO stages showed no significant

difference of Bcl-2 expression (Figure 4 C); but is has been noticed that Bcl-2 histoscore showed a significant decrease with invasion of tumor into muscle ( $p = 0.04$ , Figure 4 D).



**Figure 3:** Expression of Bcl-2 in simple and complex endometrial hyperplasia (A) and in atypical and non-atypical endometrial hyperplasia (B). The horizontal bars represent median values; the boxes represent 50<sup>th</sup> percentiles; whiskers represent range of data and the numbers refer to total number of cases in each group.



**Figure 4:** Expression of Bcl-2 in different histological types(A), different grades (B), different FIGO stages (C) and different invasive potential (D) of endometrial carcinomas. The horizontal bars represent median values, the boxes represent 50<sup>th</sup> percentiles; and whiskers represent range of data; the circles refer to outlier values and the numbers refer to total number of cases in each group.

## Discussion

Bcl-2 family proteins are known to play a crucial role in regulation of cyclic endometrial changes, as Bcl-2/BAX ratio keeps the proliferation balance of the endometrial cells. However, the definite role of this family in initiation, differentiation and invasiveness of endometrial carcinoma has not been well understood[9].The present study tried to address this gap.It showed that

the Bcl-2 expression in non-neoplastic endometrial lesions was detected in all cases. There was a relative decrease of Bcl-2 expression in complex compared to simple hyperplasia. Non atypical hyperplasia expressed slightly higher level of Bcl-2 compared to atypical hyperplasia. The differences of Bcl-2 expression among simple and complex and among non-atypical and atypical endometrial lesion did not reach the

significant level which is consistent with previous reports[10]. In contrast; Laban et al. reported a significant lower expression level of Bcl-2 in simple compared to complex hyperplasia. The limited number of cases with simple and complex endometrial hyperplasia in the current study could explain the non-significant association among these two lesions[11].

Among malignant endometrial lesion Bcl-2 was detected in 86% of cases and the expression was mainly glandular while hyperplastic endometrial lesion showed mainly mixed stromal and glandular expression (p value = 0.001) this could help to differentiate between endometrial hyperplasia from early carcinomatous changes. Bcl-2 histoscore showed decline as tumor grade increase with no significant difference statistically. This was in agreement with Mourizikou et al. [9] who showed that Bcl-2 expression was more frequent in low-grade carcinoma where as it was much reduced in the high grade tumors. There was no statistical significant correlation between Bcl-2 expression and FIGO stage or Bcl-2 expression in various histological type which was concordant to the results of Erkanli et al. [12].

Kounelis et al. and Appel et al. reported that there was no significant difference between Bcl-2 expression in various histopathological type, tumor grade and staging [12-14]. Invasion and metastasis are general hallmarks of tumor progression. In this study low expression of Bcl-2 was strongly associated with invasion of tumor into myometrium (p<0.05). in concomitance Sakuragi et al. have demonstrated that expression of Bcl-2 was inversely correlated with depth of myometrial invasion[15]. In this study, there was a reduction of Bcl-2

expression in carcinoma compared to hyperplasia which was concordant to result of Amalinei [16]. The expression of ER and PR was strongly associated with Bcl-2 expression in endometrial hyperplasia and these relationships were preserved in endometrial carcinoma. This finding implies that the expression of Bcl-2 could be regulated by hormonal factor[17]. Sivridis et al reported a strong association between Bcl-2 expression and PR expression in endometrial carcinoma and Yamauchi et al.[18] suggested that Bcl-2 expression in endometrial carcinomas is regulated in a hormone dependent manner. In conclusion Bcl-2 expression is significantly different in malignant and non-malignant endometrium. This might represent a key in understanding the progression of endometrial neoplastic.

## References

1. Jemal, A., R. Siegel, and E. Ward, Cancer statistics. *Annals of Diagnostic Pathology*, 2007. **11**: p. 297-311.
2. Monika, P., et al., Estrogen Receptor  $\beta\Delta 6$  (ER $\beta\Delta 6$ ) Isoform in Human Endometrial Hyperplasia and Adenocarcinoma. *CANCER INVESTIGATION*, 2004. **22**: p. 211-218.
3. Sara, M.E.O., et al., Current strategies to target the anti-apoptotic Bcl-2 protein in cancer cells. *Curr Med Chem*, 2004. **11**(8): p. 1031-1039.
4. Osford, S., et al., Current strategies to target the anti-apoptotic Bcl-2 protein in cancer cells. *Curr. Med. Chem.*, 2004. **11**(8): p. 1031-1039.
5. Gross, A., J.M. McDonnell, and S.J. Korsmeyer, Bcl-2 family members and the mitochondria in apoptosis. *Genes Dev*, 1999. **13**: p. 1899-1911.
6. Wang, T. and J. Phang, Effects of estrogen on apoptotic pathways in human breast cancer cell line MCF-7. *Cancer Res.*, 1995. **55**: p. 2487-2489.
7. Morsi, H.M., et al., The Patterns of Expression of an Apoptosis-Related CK18 Neopitope, the Bcl-2 Proto-

- Oncogene, and the Ki67 Proliferation Marker in Normal, Hyperplastic, and Malignant Endometrium. *Journal of Gynecological Pathology*, 2000. **19**: p. 118-126.
8. Ahmed, R.H., et al., TFF3 Is a Normal Breast Epithelial Protein and Is Associated with Differentiated Phenotype in Early Breast Cancer but Predisposes to Invasion and Metastasis in Advanced Disease. *The American Journal of Pathology*, 2012. **180**(3): p. 904-916.
  9. Mourizikou, A., et al., The use of an immunocytochemical double-labellingstaining can display the distribution of Bcl-2/Ki-67 cells in endometrial adenocarcinoma as well as in normal endometrium. *Clin. Lab.*, 2012. **58**: p. 133-144.
  10. Mora, L., et al., Differential diagnosis of endometrial hyperplasia and carcinoma by computerized imagecytometry of cell proliferation, apoptosis and Bcl-2 expression. *Ann. Clin. Lab. Sci.*, 1999. **29**: p. 308–315.
  11. Laban, M., et al., Bcl-2 may play a role in the progression of endometrial hyperplasia and early carcinogenesis, but not linked to further tumorigenesis. *Journal of Microscopy and Ultrastructure*, 2015. **3**: p. 19-24.
  12. Erkanli, S., et al., Bcl-2 and P53 expression in endometrial carcinoma. *J. Exp. Clin. Cancer Res.*, 2004. **23**: p. 97–103.
  13. Kounelis, S., N. Kapranos, and E.e.a. Kouri, Immunohistochemical profile of endometrial adenocarcinoma: a study of 61 cases and review of the literature. *Mod. Pathol.*, 2000. **13**: p. 379-388.
  14. Appel, M., M. Edelweis, and J.e.a. Fleck, P53 and Bcl-2 as prognostic markers in endometrial carcinoma. *Pathol. Oncol. Res.*, 2008. **14**: p. 23-30.
  15. Saegusa, M., et al., Bcl-2 expression is correlated with a low apoptotic index and associated with progesterone receptor immunoreactivity in endometrial carcinomas *The Journal of pathology*, 1996. **180**(3): p. 275-282.
  16. Amalinei, C., et al., Immunohistochemical analysis of steroid receptors, proliferation markers, apoptosis related molecules, and gelatinases in non-neoplastic and neoplastic endometrium *Annals of Anatomy* 2011. **193** p. 43–55.
  17. Sivridis, E., A. Giatromanolaki, and M.e.a. Koukourakis, Endometrial carcinoma: association of steroid hormone receptor expression with low angiogenesis and Bcl-2 expression. *Virchows Arch.*, 2001. **438**: p. 470-477.
  18. Yamauchi, N., et al., Immunohistochemical analysis of endometrial adenocarcinoma for Bcl-2 and p53 in relation to expression of sex steroid receptor and proliferative activity *Int J. Gynecol Pathol.*, 1996. **15**: p. 202–208.