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The Expression Profile of Bcl-2 Protein in Prostate Cancer Patients

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Abstract

Background: The Bcl-2 oncoprotein inhibits programmed cell death or apoptosis. Over production of Bcl-2 protein occurs in a wide variety of human cancers including prostate cancer and presumably contributes to tumor expansion by prolonging cell survival through inhibition of apoptosis.

Objectives: This study aims to evaluate the Bcl-2 expression in benign prostatic hyperplasia and prostate cancer tissues. In addition, it focuses on finding the association of Bcl-2 protein expression in prostate malignant tissues with the clinical data of the patients such as age and tumor stage.

Materials and Methods: Tissue samples from 49 patients with prostate diseases included 40 adenocarcinoma and 9 benign prostate hyperplasia cases. The patients underwent curative surgical prostatectomy or prostate true cut biopsy at National Cancer Institute (NCI)-Misurata at the period from 2016 to 2018. Immunoreactivity for Bcl-2 expression was examined by immunohistochemistry method. The clinical and histopathological information included age and Gleason score was collected from the patients' files.

Results: Among the studied 40 cases of adenocarcinoma 22/40 (55%) showed positive immunostaining of Bcl-2 protein. The statistical analysis of the results revealed that there was no significant correlation between Bcl-2 expression level in benign and in cancer prostate tissues (p-value =0.485). In addition, no significant association between Bcl-2 expression and the age or the tumor stage of patients.

Conclusion: Our data revealed that the predictive and prognostic role of Bcl-2 protein in prostate cancer was not noticed in this study.

Keywords: Prostate cancer; Bcl-2 protein; Apoptosis; Immunohistochemistry

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Introduction

Bcl-2 family proteins are crucial regulators of pathways involved in cell death, acting to either inhibit or promote programmed cell death or apoptosis. It is induced by a wide variety of stimuli and leads to the elimination of cells without releasing harmful substances into the surrounding area [1]. Bcl-2 family proteins can be divided (according to their apoptotic function) into two groups: Antiapoptotic proteins protect cells against apoptosis, and proapoptotic proteins that actively kill cells [2]. Imbalance between antiapoptotic and proapoptotic molecules may affect tumor development [3]. Bcl-2 is an inner mitochondrial membrane protein that suppresses apoptosis, providing a survival advantage on cells expressing this oncoprotein [4]. Cellular proliferation and programmed cell death (apoptosis) are involved with tumour growth in general, and with prostate cancer growth in particular. Over expression of Bcl-2 protein has been detected frequently in prostate cancer tissues [5]. In prostate cancer it has been suggested that elevated levels of Bcl-2 protein may play a role in progression of prostate cancers to a metastatic state characterized by poor responses to chemotherapy [6].

Prostate cancer is a heterogeneous disease in which prostate tumors can be indolent or very aggressive so the prognosis following diagnosis is greatly variable [7]. It has been revealed that protein expression of the proto-oncogene Bcl-2 is useful prognostic indicator of the progression of prostate cancer [8]. A study by Tolonen et al. [9] revealed that normal epithelium of cancerous prostates contain multiple foci with high expression of Bcl-2. Pollack et al. [10] using immunohistochemical

analyses have showed that abnormalities in the expression levels of Bcl-2 was associated with increased therapy failure after patients were treated for prostate carcinoma with radiotherapy. Colombel et al. [11] also had a similar result whereas their results showed over expression of Bcl-2 in advanced prostate tumors, also confirmed Bcl-2 involvement in the regulation of apoptosis and tumour response to anti-androgen therapy. A study carried by Asmarinah et al. [12] demonstrated that transcript expression of the anti-apoptotic Bcl-2 protein increased significantly in prostate cancer tissues compared to normal ones and established that the increased Bcl-2 mRNA expression was associated with progression of prostate cancer. In other trials, high Bcl-2 expression was significantly associated with high Gleason score of tumors [7,13]. Those results suggest a crucial role for Bcl-2 protein and altered apoptosis in prostate carcinogenesis and its therapy.

This study aims to evaluate Bcl-2 expression as an effective investigative marker in prostate cancer tissues using immunohistochemistry method. The expression of Bcl-2 protein in prostate cancer patients is correlated with the age and the tumor stage of cases.

Materials and Methods

Tissue samples

This study included 49 tissue samples from patients with prostate diseases including 40 patients with adenocarcinoma and 9 patients with benign prostate hyperplasia. The patients underwent curative surgical prostatectomy or prostate true cut biopsy at National Cancer Institute (NCI)-Misurata at the period from 2016 to 2018. Tissues were available as Formalin Fixed Paraffin Embedded (FFPE) sections and were obtained from the archive of Histopathology Department at NCI-Misurata. The study was approved by the ethical committee of the NCI-Misurata. The relevant consents were signed by patients.

Clinical records

The clinical and histopathological information of the obtained tissues were collected from the medical records of the patients which included age, Gleason score, and histologic type of cancer.

Tissue microarray (TMA)

Tissue blocks were stored at room temperature in the archive of Histopathology Department at NCI-Misurata before being used for TMA construction. All reserved samples were diagnosed by proficient pathologists in NCI-Misurata according to the World Health Organization classification. Histological slides for each sample included in the TMA were reviewed. TMAs were constructed using a manual tissue microarray 'Quick Ray'.

Sectioning

After construction, all TMA blocks were cut into 2 µm thick sections using a histone rotary microtome (Thermo Fisher Scientific, USA). The tissue sections were floated in a warm water bath, collected and mounted on slides, then exposed to hot plate and let to dry. From each block, two sections were collected; one of these sections was mounted on an ordinary slide and stained with hematoxylin and eosin for histopathological observation and examination. The other section was mounted on coated glass slides for immunohistochemistry staining to detect the expression of Bcl-2 protein.

Hematoxylin and eosin staining

The sections on an ordinary slide were first put in incubator for 20 min, and then dipped in two changes of xylene. Sections were immersed in decreasing grades of ethyl alcohol (100%, 95%, and 60%), washed under indirect running tap water, and stained with hematoxylin and with eosin. Tissue sections were then dehydrated in increasing grades of ethyl alcohol (60%, 95% and 100%), and finally mounted with 1 to 2 drops of Distrene Plasticizer Xylene (DPX), then quickly covered with a cover slip and left to dry.

Immunohistochemistry staining for Bcl-2

Immunohistochemistry for Bcl-2 carried out using the Novacastra™ polymer detection system method (Leica Biosystem Newcastle Ltd, United Kingdom) according to the manufactory instructions.

Evaluation of immunostaining was done through the histological observation with light microscope as positive and negative cases, in which prostate tissue samples immunostaining will give cytoplasmic brown color as a sign of positive reactivate. To calculate the staining index, the intensity of staining and the fraction of positively stained cells were taken into account using the following formula: ($I=0 \times f0 + 1 \times f1 + 2 \times f2 + 3 \times f3$). Where "I" is the staining index and f0-f3 are the fractions of the cells showing a defined level of staining intensity (from 0 to 3). Theoretically, the index could vary between 0 and 3.

Statistical analysis

Differences in categorical variables, which is age and Gleason score between patients and Bcl-2 expression were evaluated for significance with chi-squared test. The data was performed using SPSS version 20.0 statistical software. P value <0.05 was considered to indicate a statistically significant difference.

Results

A total of 49 tissues from patients with prostate gland diseases, consisting of 9/40 (18.4%) benign prostate hyperplasia, and 40/49 (81.6%) prostate adenocarcinoma were selected for systemic analysis.

The prostate adenocarcinoma cases were aged between 60 and 90 years (average 76.3 years). Moreover, the most recurrence rate was found in 70 years and over; 33/40 (82.5%) which including 18/33 (54.5%) under age of 80 years, and 15/33 (45.4%) under 91 years of age, while patients with ages under 70 years were 7/40 (17.5%). Concerning the Gleason score of the tissues, the study showed 15/40 (37.5%) were in score 4 (2+2), 11/40 (27.5%) in score 6 (3+3), 2/40 (5%) in score 7 (3+4), 7/40 (17.5%) in score 8 (4+4), 1/40 (2.5%) was in score 9 (4+5) and 4/40 (10%) were in score 10 (5+5). The grading of tissues in the study group showed 26/40 (65%) in well differentiation, 2/40 (5%) in moderate differentiation and 12/40 (30%) in poor differentiation.

All the 49 prostate tissues were immunostained for Bcl-2 detection. Among the 9 benign prostatic hyperplasia samples: 4/9 (45%) were negative stained for Bcl-2 expression, and 5/9 (55%) were positive. The Bcl-2 positive were: 3 weakly stained (+), and 2 were moderately stained (++). On the other hand, of the 40 cases of adenocarcinoma, overall 22/40 samples (55%) were positive for Bcl-2 protein; including 19 tissues showed weakly positive (+), and 3 were moderately positive stained (++). The remaining 18/40 (50%) were entirely negative for Bcl-2 staining (Table 1). Figure 1 showed examples of different staining intensities and detection of Bcl-2 protein in prostate tissues by IHC method.

Bcl-2 expression was detected in (5/9, 55%) of the studied benign

Table 1: Bcl-2 stain intensity of samples.

	Bcl-2 stain index			Total
	Negative	Weak	Moderate	
Adenocarcinoma	18	19	3	40
Benign prostate hyperplasia	4	3	2	9
Total	22	22	5	49

Table 2: Distribution of clinical factors according to Bcl-2 expression.

	Bcl-2 stain index			P value
	Negative	Weak	Moderate	
Number of patients	18	19	3	
Age (years)				**0.327**
60-69	3	4	0	
70-79	6	11	1	
>80	9	4	2	
Gleason score (differentiation)				**0.704**
Well	10	13	3	
Moderate	1	1	0	
Poor	7	5	0	

prostatic hyperplasia samples, and it was found in (22/40, 55%) of the prostate adenocarcinoma cases. Statistical analysis of results showed that there is no significant correlation between Bcl-2 expression level in benign and in cancer prostate tissues, (p-value =0.485). This indicates that the predictive role of Bcl-2 protein in prostate cancer was not prominent in our study.

At the 40 prostate adenocarcinoma cases, the statistical analysis showed no significant correlation between Bcl-2 expression and the studied clinical factors of patients (Table 2). The age of patients was found to be not statistically significant for Bcl-2 status, 18/22 (81.8%) of the patients with positive Bcl-2 immunostaining were aged 70 years and over of age. This indicates that patients with positive Bcl-2 expression are more likely to present with the advanced age (Figure 2). However, this data did not translate to statistical significance (p value =0.327). In addition tumor differentiation (Gleason score) was not statistically significant factor for Bcl-2 expression, (p value =0.704) (Figure 3).

Discussion

One of the most main health problems in men is prostate cancer [14]. Prostate cancer has a slow growth rate but it may develop into metastatic disease. The surviving rate for patients diagnosed with metastatic prostate cancer remains poor [15]. While pathological stage and tumour volume are perhaps the most established prognostic factors, they cannot be used preoperatively [16]. There is a demand to develop immunohistochemistry test for prognostic markers correlating with biological aggressiveness of prostate tumors [17]. Regulators of the apoptotic pathway, including Bcl-2 have been evaluated as prognostic markers. Functional studies and immunohistochemical data presented evidence that Bcl-2 plays a critical role in prostate cancer progression. This evidence is based on the results showed that Bcl-2 is expressed in normal prostatic epithelium and in prostatic tumors [18]. The expression is more common in tumors that exhibit malignant features, such as the invasive growth type, thus, it has been suggested as a biomarker for prostate cancer [11].

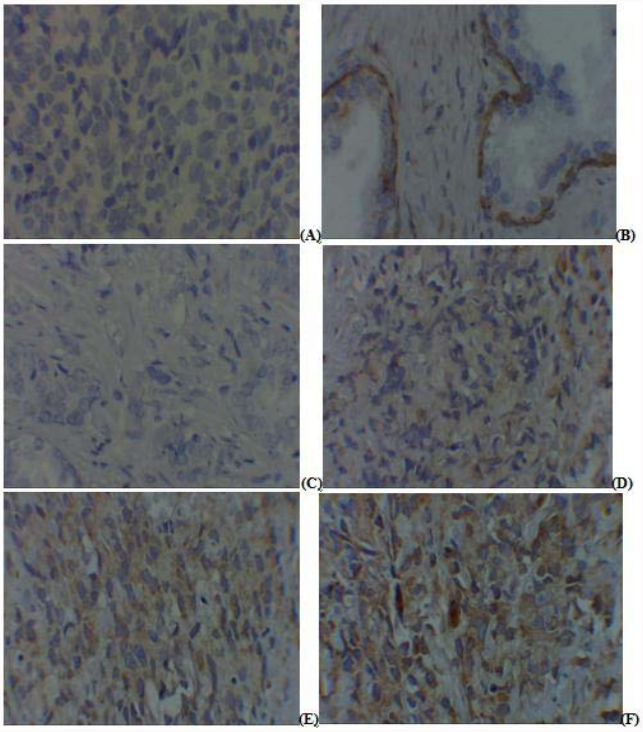

Figure 1: Immunohistochemical analysis in prostate tissue samples. (A): BPH, Negative of Bcl-2 staining, (B): BPH, positive of Bcl-2 staining. (C): adenocarcinoma, Bcl-2 negative. (D): adenocarcinoma, Bcl-2 positivity +1 (weak). (E): adenocarcinoma, Bcl-2 positivity +2, (moderate). (F): adenocarcinoma, Bcl-2 positivity + (strong).

Here we have examined prostate tumors from Libyan patients. The expression of Bcl-2 protein has been detected in 55% of the analyzed prostate cancer patients. In our study, we found no significant association between Bcl-2 expressions in Benign Prostatic Hyperplasia (BPH) and cancer prostate tissues. This result was in agreement with previous studies conducted by Asmarinah et al. [12], Ma et al. [19]. In contrary Colombel et al. [11] and Iacopino et al. [20] reported that Bcl-2 was over expressed in prostate cancer tissues compared to benign prostate hyperplasia tissue with statistical significant correlation. There are relatively many factors that could be linked to the variations in the detected levels of Bcl-2 expression. Among these, different populations of the studies may be the most important factor that may explain the different results of Bcl-2 expression at prostate adenocarcinoma patients [21]. Moreover, immunostaining variations may be refers to differences in the antibodies used and the qualified preservation of antigen epitopes and other technical procedures. Our data showed no significant association between Bcl-2 expression and the age of patients. However, our results showed a trend for a higher Bcl-2 expression in ages >70 years. This trend was in agreement with the idea that the risk of prostate cancer goes up with advanced age [21].

The role of Bcl-2 in tumor proliferation and its association with Gleason score have been investigated by earlier studies. These studies reported that higher frequency of Bcl-2 immunostaining in prostate tumor samples was statistically associated with more advanced Gleason scores [8,19,22]. This was in contrast with data reported by other studies done by Bubendorf et al. [23], Missaoui et al. [24] and Lin et al. [25] who found no significant correlation between the expression of Bcl-2 anti-apoptotic protein and Gleason

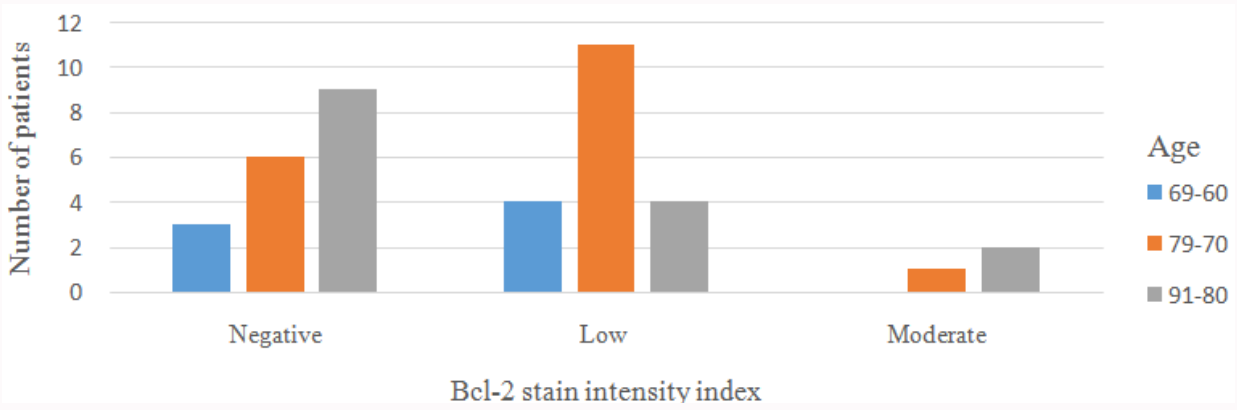

Figure 2: The frequency of age groups among the studied prostate adenocarcinoma patients with Bcl-2 stain intensity index.

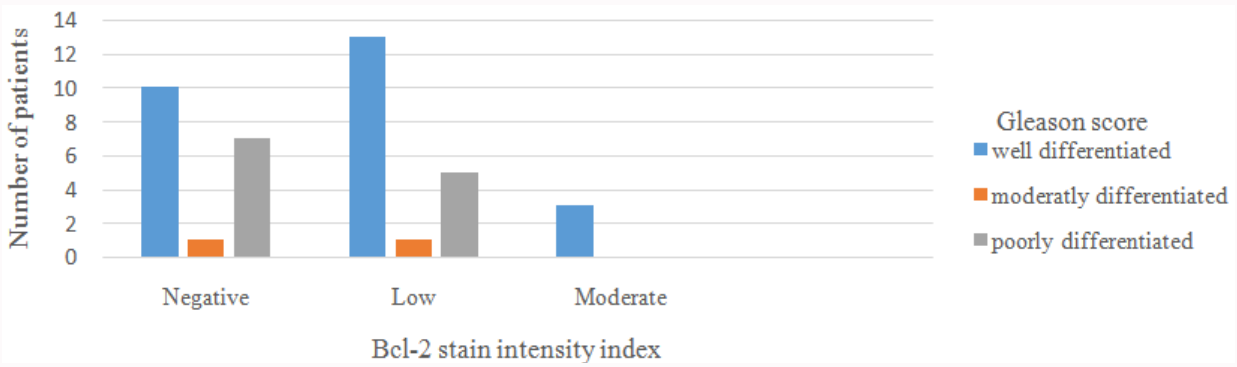

Figure 3: The frequency of Gleason grading among prostate adenocarcinoma patients with Bcl-2 stain intensity index.

score. Similarly, we could not find an association between Bcl-2 expression and Gleason score of our studied patient group. Iacopino et al. [20] attributed this difference to homogenous structure of prostate tissues and lack of homogeneity of the tissues obtained by different procedures. Additionally, the race and life-style factors may contribute to such variations.

In conclusion, our data showed high Bcl-2 expression rate in the studied prostate cancer patients. The rate of the expression was at the range of other studies on non-Libyan patients. The lack of association between Bcl-2 expression in benign and malignant tumor tissues indicates that predictive role of Bcl-2 was not strong. This may be explained by the small size of the prostate cancer patients. Furthermore, we could not find a significant association between Bcl-2 expression and the clinical parameters of prostate cancer patients such as: age and the tumor grade.

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