Tissue Microarray Construction and Immunohistochemical evaluation of Bcl-2 Gene Expression in Iraqi and Italian Breast Cancer Samples.

التقييم الكيميائي المناعي النسيجي لتعبير جين Bcl-2 في نماذج سرطان الثدي عراقية وايطالية باستخدام المصفوفة الدقيقة للنسيج

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Abstract

Breast cancer is a heterogeneous disease and remains one of the most leading causes of death among women world wild. Anti-apoptotic Bcl-2 family members inhibits cell death and promote proliferation of cancer cells. Overexpression of Bcl-2 has been shown to inhibit the initiation of apoptosis, in the presence of some stimuli including anticancer drugs in a number of systems. To study the expression of Bcl-2 tissue microarray (TMA) investigation was designed and constructed. Using 3 TMAs; the second for the 50 Iraqi breast cancer cases, one for the 30 Italian cases and the third for the 10 benign cases. Each sample represented in a triplicate in the recipient block, all these TMAs expressed to the same condition for construction and IHC staining. TMAs are a powerful, fast and economic technique. IHC was used to study the Bcl-2 expression. The results are represented as differences in the number of samples expressed the Bcl-2 and the intensity of the expression between the Iraqi and the Italian groups. Bcl-2 expression was found elevated in 26 (52%) of the 50 Iraqi breast cancer samples and 11 (36.66%) of the 30 Italian samples compared to the control samples. And in which only 1 (10%) out of 10 samples. The overexpression of Bcl-2 associated with poor prognosis for the patients. Level of Bcl-2 expression can be used as prognostic marker to monitor patient therapy. For the Iraqi cases the overexpression may be attributed to many factors like exposure to depleted uranium and bad food quality during the 90th when Iraq was under the siege of the United Nation (UN).

Key Words: Bcl-2 expression, Breast cancer, Iraqi and Italian samples, Tissue microarray, Immunohistochemistry.

الملخص

سرطان الثدي مرض متعدد الانواع ويبقى واحد من اكثر الاسباب المؤدية للوفاة في النساء حول العالم, اعضاء عائلة Bcl-2 المضادة للموت المبرمج تثبط موت الخلايا وتقود الى تضاعف الخلايا السرطانية. التعبير الفائض لجين Bcl-2 تم ملاحظته لمنع البداية في الموت المبرمج, في حال وجود بعض المحفزات بما فيها العقاقير المضادة للسرطان في عدة انظمة. لدراسة تعبير جين Bcl-2 تم تصميم وبناء المصفوفات الدقيقة للنسيج, تم بناء ثلاث مصفوفات الاولى تشمل 50 عينة سرطان ثدي انساء عراقيات والثانية تشمل 30 عينة سرطان ثدي لنساء ايطاليات والثالثة تحوي 10 عينات السيطرة (الحالات الحميدة). لقد تم تمثيل كل عينة بثلاث مكررات من العينة المستلمة للنماذج, لقد تم تعريض عينات المصفوفات لنفس الظروف من ناحية البناء والتصبيغ المناعي الكيميائي للنسيج, تقنية المصفوفة الدقيقة للنسيج كانت فعالة, وسريعة, واقتصادية. تم استخدام التصبيغ المناعي الكيميائي للنسيج لدراسة تعبير جين Bcl-2. النتائج اوجدت ان عائلة بروتين Bcl-2 لعبت دور تنظيمي مهم خلال القرارات الخلوية اما ان تموت او تتجاهل الموت, لقد وجد تعبير Bcl-2 مرتفعا في 26 (52%) من الخمسين عينة لسرطان الثدي العراقي, بينما للعينات الايطالية فكانت 11 (36,66%) من اصل 30 عينة ولوحظت عينة واحدة (10%) من اصل 10 عينات كانت موجبة لعينات السيطرة, التعبير الفائض ربما يعطي انطباع سيء عن المرضى او في بعض الاحيان يعطي معلم ابتدائي مرغوب به مع معلمات اخرى تستخدم في علاج المرضى. بالنسبة للعينات العراقية عالية التعبير ربما يعزى هذا الشيء الى عدة عوامل منها التعرض لليورانيوم المنضب خلال سنوات الحروب الطويلة على العراق او نوعية الغذاء السيئة خلال تسعينات القرن الماضي عندما كان العراق يقبع تحت الحصار المفروض من الامم المتحدة.

الكلمات الدالة: تعبير جين Bcl-2, سرطان الثدي, نماذج عراقية وايطالية, المصفوفة الدقيقة للنسيج, الكيمياء المناعية النسيجة.

Introduction

Tissue microarray (TMA) technology appeared in the late 1990`s and revolutionized the study of tissue morphology, protein, gene expression and chromosomal aberrations using different techniques, such as immunohistochemistry (IHC), in situ hybridization, study thousands of new genes and mRNA by molecular biology. The combination of TMAs with clinical samples is an economic, fast and cost-effective approach for

studying panels of biomarkers under identical experimental conditions and to investigate the prognostic or predictive patterns of patient outcomes. TMA consists of at least two different specimens per slide [1,2].

The Bcl-2 (B-cell leukemia/lymphoma 2) gene was first identified at the breakpoint of a chromosomal translocation t(14:18) in B-follicular lymphoma. It encodes a 26 kDa protein that protects cells against apoptosis. Bcl-2, is an oncogene is an oncogene that is frequently linked to an immunoglobulin locus by the chromosome translocation t(14:18) in follicular lymphoma [3]. Bcl-2 is a first example of an oncogene that inhibits cell death rather than promoting proliferation. For the first time it was shown that the pathway toward tumorigenesis depends not only on the ability to escape growth control but also depends on the ability to prevent apoptosis [4]. Overexpression of Bcl-2 has been shown to suppress the initiation of apoptosis in response to a number of stimuli, including anticancer drugs in a number of systems [5]. Bcl-2 family members, such as bax, also promote apoptosis and play a great role in cell death, Bcl-2 family members fall into two categories on the basis of their ability to either promote or suppress apoptosis, other findings have linked these proteins to caspases. It seems that Bcl-2 proteins influence cell survival by regulating the activation of key caspases [6]. One possible explanation is that the acquisition of bcl-2 expression creates a restrictive environment for the expansion of genetically unstable and potentially malignant p53-deficient cells, causing a delay in tumor progression and explaining the different prognostic value of bcl-2 and p53 [7]. In addition, bcl-2 is known to be up-regulated by estrogen and to be down-regulated by p53. However, reports are contradictory concerning whether bcl-2 is an independent predictive marker for responses to primary chemotherapy [8] [9]. Bcl-2 has been shown to inhibit chemotherapy-induced apoptosis, and chemotherapy resistance has been reversed in cancer cells treated with Bcl-2-targeting therapy [10]. Although Bcl-2 is an anti-apoptotic protein, high Bcl-2 expression has been observed in ER-positive breast cancers as well as in progesterone receptor (PR)-positive breast cancers, and has been associated with improved survival in breast cancer [11, 12]. Bcl-2 antanogene-1 (Bag-1) binds the antiapoptotic mediator Bcl-2, and enhances its activity. Bcl-2 and Bag-1 are associated with chemotherapy resistance in cancer cells. Drugs that target Bcl-2 are currently in clinical development. The assessment of the expression patterns of Bag-1 in a large cohort of breast tumors and evaluate the association with Bcl-2, estrogen receptor, progesterone receptor and Her2/neu, and other clinical/pathological variables studied and confirmed by [13].

This study conducted to evaluate the TMA assessment of breast biomarkers Bcl-2 expression in Iraqi and Italian samples using immunohistochemical analysis.

Materials and Methods

Tissues and histopathological processing

Fifty Iraqi breast cancer paraffin blocks were collected from patients attended to Jenin Private Laboratory, Baghdad, Iraq between 2007-2009. The clinical record for each patient was obtained, included the clinicopathological data, patient age, tumor type, grade and stage. Cases included invasive ductal carcinoma (45 cases) and invasive lobular carcinoma (5 cases). Hematoxylin and eosin (H and E) stained slides were prepared from the paraffin embedded blocks and reassessed by a specialist pathologist for tumor type, grade (WHO and Nottingham grading). Thirty paraffin blocks were taken from patients attended to the Department of Biomorphological and Functional Science, Policlinico, University of Naples Federico II, Naples, Italy, in 2009, clinical and pathological data were also recorded. Ten samples from Iraqi patients with benign breast lesions were used in this study as a control group [1].

Tissue Microarrays (TMAs)

Hematoxylin and Eosin (H and E)

The samples were sectioned into 5µm sections; the staining was prepared using H and E staining system (LEICA ST5020, LEICA, and Germany). The stained slides were examined under the double headed light microscope by pathologist and areas containing the most representative tumor tissues were marked on both the slides and corresponding paraffin of the donor blocks for tissue microarray construction.

Design and Construction of the Tissue Microarrays (TMAs)

Tissue Microarrays (TMAs) were constructed from formalin-fixed, paraffin embedded blocks. The corresponding H&E-stained slides were overlaid for TMA sampling. The optimal method used was to mark the area of interest on the H and E slides, and then the slides fitted to the donor block. Triplicates of 0.6-mm

diameter cylinders were punched from the representative tumor areas of the individual donor tissue block and re-embedded into a recipient paraffin block at a predefined position using the TMA machine (Minicore, Alyphelys, Plaisir, France), then 5µm sections from the TMA recipient block were made, and processed for H&E and IHC staining. It is recommended to perform all the sections at once thus eliminating the losses due to block facing each time. Three TMAs were constructed; the first included 10 samples from fibroadenomas and mastopathy; the second included 50 malignant samples and the third TMA constructed by using 30 Italian malignant breast samples taken from the Department of Biomorphological and Functional Sciences, Policlinico, University of Naples "Federico II", Naples, Italy. TMAs allowed the distribution and positioning of the cores to be easily examined by the histopathologist.

Immunohistochemistry

The protocol used according to the CEINGE Biotecnologie Avanzate, University of Naples Federico II. For the present study, Bcl-2 was detected using Clone 124 antibody (diluted 1:200, Dako, Glostrup, Denmark), after pretreatment of the slides with citrate buffer for antigen retrieval, IHC staining was performed using the Envision System with diaminobenzidine (Dako, Glostrup, Denmark). A negative control was obtained by replacing the primary antibody with a normal murine IgG. For Bcl-2, Intensity was graded as negative (0), weak (1+), moderate (2+) and strong/ intense (3+) according to the manufacturing kit (Dako, Denmark). Cut off values for Bcl-2 antibody done with the help of a pathologist. Data analysis was performed with SPSS v17.0 (SPSS Inc., UK) (using Chi-sequare and t test).

Results and Discussion

The study involved 80 cases of breast cancer, 50 Iraqi breast cancer patients and 30 Italian breast cancer patients and 10 benign cases between 2007 and 2009, all the patients were female, and their median age is 48 years (range 28-70 years). The clinicopathological data shown in Table (1). TMAs constructed successfully then used to evaluate the Bcl-2 expression in the three TMAs. The results of TMAs represented in figure (1), the TMA represented a whole section analysis for the breast cancer, the results agreed with the data published by [14], who used the tissue microarray to study IHC and in situ and molecular techniques, used different types of arrays, macroarray and microarray. TMA technique used to study the Cytokeratin-19 in breast cancer, and studied different aspects of TMAs the construction, the source of tissue specimen, dimension of the specimen and other factors may affect the design and construction of TMAs [15].

Table (1): Clinicopathological parameters of breast cancer samples.

Parameter	Iraqi Samples (50)		Italian Samples(30)	
Age (Years)	No.	%	No.	%
<40	5	10	2	6.66
40-49	20	40	10	33.33
50-59	17	34	11	36.66
≥60	8	16	7	23.33
Tumor Type	No.	%	No.	%
Invasive Ductal carcinoma	45	90	28	93.33
Invasive Lobular carcinoma	5	10	2	6.66
Total	50	100%	30	100%
Tumor Size (cm)				
<2	5	10	10	33.33
2-5	37	74	15	50
≥5	8	16	5	16.66
Histological Grade				
I	4	8	7	23.33
II	31	62	16	53.33
III	15	30	7	23.33
Axillary Lymph Node				
0	7	14	14	46.66
1-3	32	64	9	30
>3	11	22	7	23.33
	Control Samples			
Parameter	Control Samples (10)			
Age (Years)	No.		%	
<40	5		50	
40-49	3		30	
50-59	2		20	

TMA used for evaluating and assessing breast biomarkers using immunohistochemical analysis in a clinical histopathology laboratory. Who studied the performance parameters, inter observer variability, and concordance between TMA and whole section, and recommended to use TMAs because it is an economical replacement for whole section analysis for breast biomarkers for large number of samples [16].

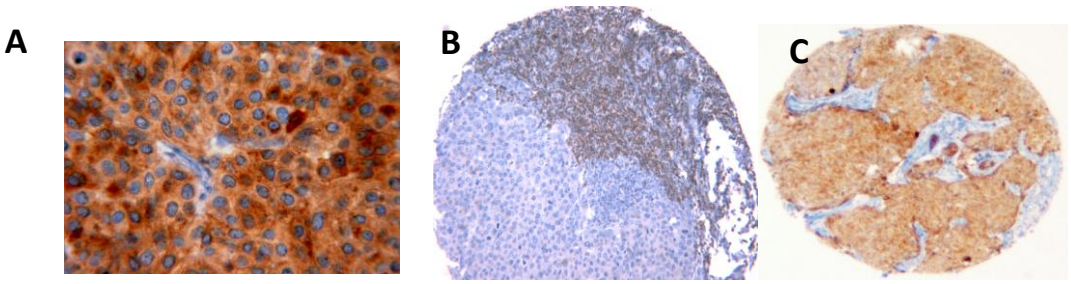

Fig.(1): Samples of Tissue Microarray TMA stained with immunohistochemistry for Bcl-2, breast cancer samples, A: strong positive 4X, B: Partially Positive Moderate 10X, C: Strong positive 40X.

Bcl-2 was expressed in Iraqi samples (52%) of 50 cases studied. No expression or negative was in 24 (48%) of the cases, while it was in11 Italian (36.66%) of 30 (10%) of 10 while 9 (90%) showed negative or no expression for Bcl-2. There were a significant differences (*P* value: 0.037) in the Bcl-2 expression between the studied groups, data shown in Table (2). The distribution of the intensity of Bcl-2 expression among the studied cases represented in Table (3), score 3 plus or strong found in 15(30%), score 2 plus or intermediate found in 8 (16%), score 1 plus in 3 (6%), while negative or no expression score 0 observed in 24 (48%) of the 50 studied Iraqi cases. In the 30 Italian studied cases strong or 3 plus found in 5 (16.66%), 2 plus in 3 (10%), 1 plus in 3 (10%), while score 0 or negative found in 19 (63.33%) of the 30 studied cases. In the control cases only 1 (10%) of 10 cases expressed Bcl-2 score 1 plus, the IHC study showed there was differences in the Bcl-2 expression in the sample numbers and the intensity of the expression between the Iraqi and the Italian cases, the data presented in Tables (2 and 3), the different intensity in staining illustrated in figure (2).

Table (2): Distribution of the immunohistochemical expression of the Bcl-2 in breast cancer samples.

Bcl-2	Bcl-2 N (%)			
Expression	Iraqi	Italian	Control	Total
Positive	26 (52%)	11 (36.66%)	1 (10%)	38
Negative	24 (48%)	19 (63.33%)	9 (90%)	52
Total	50 (100%)	30 (100%)	10 (100%)	90
P value		0.037		

Table (3): Distribution of the intensity of Bcl-2 expression in breast cancer samples.

Intensity	Iraqi N(%)	Italian N(%)	Control N(%)	Total
3	15 (30%)	5 (16.66%)	0	20
2	8 (16%)	3 (10%)	0	11
1	3 (6%)	3 (10%)	1 (10%)	7
0	24 (%)	19 (63.33%)	9 (90%)	52
Total	50	30	10	90

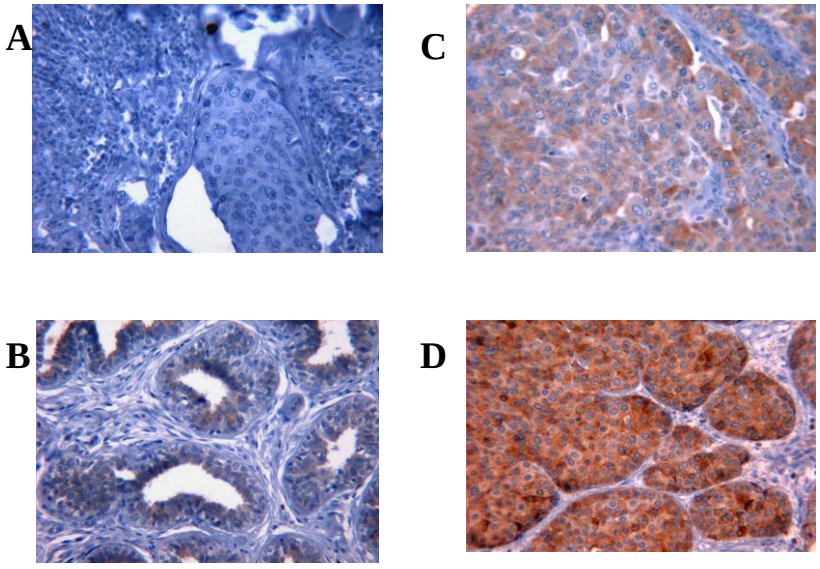

Fig. (2): Intensity of Bcl-2 Expression in Breast Cancer stained with IHC; A: Negative, B: IHC intensity=1, C: IHC intensity= 2, D: IHC intensity=3, 20X magnification.

Overexpression of Bcl-2 could be attributed to many factors and need for further studies, Bcl-2 expression may lead to good outcome in case if use for antisense therapy. There were some differences in the number of positive expression and the intensity of the expression between the two population but that is not a reason to attribute that for a specific effect maybe the low number of cases or the tissue processing different the quality of materials and the timing of the tissue embedding in the paraffin plays crucial role in the immunohistochemistry studies. The importance of use the Bcl-2 as a prognostic biomarker alone in breast cancer, the prognostic power of Bcl-2, strongly supporting the validity of the results obtained is emphasized in the study of [17]. The dysregulation of Bcl-2 gene expression is potentially involved in the pathogenesis of breast cancer. Using gene expression analysis would be a powerful tool in the diagnosis and prognostic evaluation of the breast cancer for Bcl-2 based therapy projects [18]. Bcl-2 expression has been shown to inversely parallel cell turnover or the modeling of tissues by apoptosis [19]. Bcl2 is an independent prognostic marker in two large series of breast cancer. Moreover, urgent need of testing whether Bcl2 and multiple other markers can provide prognostic information in addition to currently used standards and to establish if Bcl-2 has clinical utility [20].

The present results are in agreement with the data obtained by [6]. Who revealed that Bcl-2 is a cytoplasmic protein belonging to the bcl-2 family; it is overexpressed in 25–50% of breast cancers, so overexpression of Bcl-2 linked with the progression of breast cancer and worse outcome for the patients. Bcl-2 overexpression enhances both tumorigenicity and metastatic potential of MCF7 ADR cells by inducing metastasis-associated properties [21].

In spite of its role opposing tumor cell death, and association of Bcl2 expression in tumors with better intrinsic prognosis, Bcl-2 overexpression was associated with an increased risk of local recurrence in patients with early stage breast cancer. These results support the combination of radiation and pro-survival Bcl-2 family inhibitor as a potential novel therapeutic strategy in the local-regional management of breast cancer [22].

Overexpression in Iraqi breast cancer cases maybe attributed to many factors, weakness in the medical education so the patients not attending to the hospital till late stage leading then to progression of metastasis in the body. The Iraqi population exposed to ionic radiation and others stresses as a result of wars and different weapons used during the wars, depleted uranium is one of the reasons that leads to cancer so it may induced different types of cancer in Iraqi population like breast, blood and central nervous system cancers (23). A recommended study needed to link the exposure of the uranium and cancer induction in Iraqi population. Overexpression of Bcl-2 for some studies correlate with other biomarkers like ER is directly involved in controlling the prosurvival protein Bcl-2 by modulating its transcript and HDAC inhibitor-mediated induction of several apoptotic proteins and reversal of Bcl-2 up-regulation through ER ensues cell death, and concluded that their model implicates high

expression of ER and Bcl-2 plays a key drivers of anti-estrogen resistance, which can be modulated by epigenetic through HDAC inhibition [24].

The overexpression of the Bcl-2 in Iraqi cases would be a good tool for targeted therapy as the data confirmed by other studies like [11] they revealed that targeted therapy against Bcl-2 protein with the use of other enhancers might effects of chemotherapy in patients with breast cancer. Our results concerning the Bcl-2 expression which fall within the limits of overexpression of Bcl-2 in breast cancer and in agreements with the data published by [25] Bcl-2 overexpression occurs in 40% to 80% of human breast tumors, this overexpression could be attributed to ionizing radiation. Bcl-2 alone is not an independent prognostic marker in breast cancer patients, in part because most Bcl-2-positive breast cancers express estrogen and/or progesterone receptors. This positive association of Bcl-2 with hormone receptors in breast cancer may explain its apparent correlation with response to hormone therapy [26]. The proto-oncogene bcl-2 appears to serve a critical antiapoptotic function. Its broad expression in tumors coupled with its role in resistance to chemotherapy-induced apoptosis make bcl-2 a rational target for anticancer therapy [27].

There is some confusion of some studies about the Bcl-2 expression some thought it inhibits apoptosis and therefore delayed cancer cells death which related to worse outcome. In other hand some studies revealed the expression of bcl-2 in breast cancer found to be associated with favorable prognostic factors such as smaller tumor size, ER positivity, and low nuclear grade, in this case Bcl-2 predicts a good outcome and favorable prognostic factors in metastatic disease associated with early breast cancer patients who received heterogeneous adjuvant chemo- and hormonal therapies [28], this confusion agreed with the some of the data obtained in our study some of the data referred to worse outcome for the patients and other predicts a favorable prognostic factor associated with the patients therapy.

Conclusions

The current study concluded that, using TMAs was a useful, economic, reliable, feasible and fast technique for immunohistochemistry, Bcl-2 overexpression found in Iraqi samples more than the Italian cases, study the Bcl-2 expression of different populations Iraqi and Italian open the way for more studies and compare the data between the two populations. In Iraq the costs for IHC and other *in situ* technique very expensive we are firmly recommending to use TMA technique for our local studies to reduce the cost and allow more samples to be added in the studies, also to archive some of the predictive and prognostic markers in the Iraqi population for different types of cancer.

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References

1. Kononen, J., Bubendorf, L., Kallioniemi, A., Barlund, M., Schraml, P., Leighton, S., Torhorst, J., Mihatsch, M.J., Sauter, G., Kallioniemi, O.P. (1998). Tissue microarrays for high throughput molecular profiling of tumor specimens. Nat Med. 4, 844-847.
2. Zlobec, I., Koelzer, V.H., Dawson, H., Perren, A., Lugli, A. (2013). Next-generation tissue microarray (ngTMA) increases the quality of biomarker studies: an example using CD3, CD8, and CD45RO in the tumor microenvironment of six different solid tumor types. J Transl Med. 11, 104.
3. Tsuijimoto, Y., Gorham, J., Cossman, J., Jaffe, E. and Croce, C.M. (1985). The t(14:18) chromosome translocation involved in B-cell neoplasms results from mistake in VDJ joining. Science. 299 1390–1393.
4. Vaux, D.L., Cory, S. and Adams, J.M. (1988). "Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells." Nature. 335(6189): 440-442.
5. Teixeira, C., Reed, J.C. and Pratt, M.A. (1995). Estrogen promotes chemotherapeutic drug resistance by a mechanism involving Bcl-2 protooncogene expression in human breast cancer cells. Cancer Res. 55:3902-3907.
6. Van Slooten, H.J., Clahsen, P.C., van Dierendonck, J.H., Duval, C., Pallud, C., Mandard, A.M., Delobelle-Deroide, A., van de Velde, .C.J. and van de Vijver, M.J. (1996). Expression of Bcl-2 in node-negative breast cancer is associated with various prognostic factors, but does not predict response to one course of preoperative chemotherapy. Br J Cancer.74:78-85.
7. Reed, J.C. (1997). Double identity for proteins of the Bcl-2 family. Nature. 387:773-776.
8. Elledge, R.M., Green, S., Howes, L., Clark, G.M., Berardo, M., Allred, D.C., Pugh, R., Ciocca, D., Ravdin, P., O'Sullivan, J., Rivkin, S., Martino, S. and Osborne, C.K. (1997). Bcl-2, p53, and response to tamoxifen in

estrogen receptor positive metastatic breast cancer: a Southwest Oncology Group study. J Clin Oncol. 15:1916-1922.

9. Gurova, K.V., Kwek, S.S., Koman, I.E., Komarov, A.P., Kandel, E., Nikiforov, M.A. and Gudkov, A.V. (2002). Apoptosis inhibitor as a suppressor of tumor progression: expression of Bcl-2 eliminates selective advantages for p53-deficient cells in the tumor. Cancer Biol Ther. 1:39-44.
10. Bottini, A., Berruti, A., Bersiga, A., Brizzi, M.P., Brunelli, A., Gorzegno, G., DiMarco, B., Aguggini, S., Bolsi, G., Cirillo, F., Filippini, L., Betri, E., Bertoli, G., Alquati, P. and Dogliotti, L. (2000). P53 but not bcl-2 Immunostaining is predictive of poor clinical complete response to primary chemotherapy in breast cancer patients. Clin Cancer Res.
11. Emi, M., Kim, R., Tanabe, K., Uchida, Y. and Toge, T. (2005). Targeted therapy against Bcl-2-related proteins in breast cancer cells. Breast Cancer Res. 7:R940-R952.
12. Bilalovic, N., Vranic, S., Hasanagic, S., Basic, H., Tatarevic, A., Beslija, S. and Selak, I. (2004). The Bcl-2 protein: a prognostic indicator strongly related to ER and PR in breast cancer. Bosn J Basic Med Sci. 4:5-12.
13. Nadler, Y., Camp, R.L., Giltnane, J.M., Moeder, C. and Rimm, D.L. (2008). Expression patterns and prognostic value of Bag-1 and Bcl-2 in breast cancer. Breast Cancer Research. 10:R35.
14. Pinder, S.E., Brown, J.P., Gillett, C., Purdie, C.A., Speirs, V., Thompson, A.M., Shaaban, A.M. (2013). The manufacture and assessment of tissue microarrays: suggestions and criteria for analysis, with breast cancer as an example. J Clin Pathol. 66: 169-177.
15. Callau, C., Lejeune, M., Korzynska, A., García, M., Bueno, G., Bosch, B., Jaén, J., Orero, J., Salvadó, T. and López, C. (2014). Evaluation of Cytokeratin-19 in Breast Cancer Tissue Samples: A Comparison of Automatic and Manual Evaluations of Scanned Tissue Microarray Cylinders. Proceedings IWBBIO 2014. Granada. 7-9 April, 2014.
16. Avninder, S., Ylaya, K. and Hewitt, SM. (2008). Tissue microarray: a simple technology that has revolutionized research in pathology. J Postgrad Med. 54(2):158–162.
17. Grace, M.C., Mark, J. W., Paul, D.P. and Carlos, C. (2008). Meta-analysis confirms BCL2 is an independent prognostic marker in breast cancer, BMC Cancer. 8:153.
18. Martinez-Arribas, F., Nooez, M.J., Lucas, A.R., Sanchez, J., Tejerina, A. and Schneider J. (2003). Immunofluorometric study of Bcl-2 and Bax expression in clinical fresh tumor samples from breast cancer patients. Anticancer Res. 23: 565-568.
19. Ali, H.R., Dawson, S.J., Blows, F.M., Provenzano, E., Leung, S., Nielsen, T., Pharoah, P.D. and Caldas, C. (2012). A Ki67/BCL2 index based on immunohistochemistry is highly prognostic in ER-positive breast cancer. J Pathol. Jan; 226(1):97-107
20. Leila, R., Ashraf, F., Vahid, M., Mehrdad, A., Estiar, Mohammad, N., Somayyeh, H., Masoud S. and Ebrahim, S. (2013). Bcl-2 Gene Expression in Human Breast Cancers in Iran. Asian Pacific Journal of Cancer Prevention, Vol 14.
21. Smerage, J.B., Budd, G.T. and Doyle, G.V. (2013). Monitoring apoptosis and Bcl-2 on circulating tumor cells in patients with metastatic breast cancer. Molecular Oncology. 7, 680-692.
22. Moul, J.W. (2012). Angiogenesis, p53, Bcl-2 and Ki-67 in the progression of prostate cancer after radical prostatectomy. Eur Urol. 35, 399-407.
23. Al-Azzawi, S.N. (2006). Depleted uranium radioactive contamination in Iraq: An overview. Global Res., 1August 31. P4.
24. Del Bufalo, D., Biroccio, A., Leonetti, C. and Zupi G. (1997). Bcl-2 overexpression enhances the metastatic potential of a human breast cancer line. FASEB J. 1997 11(12):947-953.
25. Wu, h., Schiff, D.S., Lin, Y., Neboori, H., Goyal, S., Feng, Z. and Haffty, B.G. (2014). Ionizing Radiation Sensitizes Breast Cancer Cells to Bcl-2 Inhibitor, ABT-737, through Regulating Mcl-1. Radiation Research. 182(6):618-625.
26. Raha, P., Thomas, S., Thurn, KT., Park, J. and Munster, P. (2015). Combined histone deacetylase inhibition and tamoxifen induces apoptosis in tamoxifen-resistant breast cancer models, by reversing Bcl-2 overexpression. Breast Cancer Research. 17:26
27. Nahta, R. and Esteva FJ. (2003). Bcl-2 antisense oligonucleotides: a potential novel strategy for the treatment of breast cancer. Semin Oncol. 30(5 Suppl 16):143-149.
28. Gjertsen, B.T., Bredholt, T., Anensen, N. and Vintermyr, O.K. (2007). Bcl-2 antisense in the treatment of human malignancies: a delusion in targeted therapy. Curr Pharm Biotechnol. 8(6):373-381.