Iranian Journal of Pathology (2009)4 (1), 32 - 37

Original Article

BCL2 Family Related Genes Expression and Chemotherapy Response in Diffuse Large B-Cell Lymphoma

Kazem Anvari[1], Mahmoud Reza Kalantari[2], Abdolazim Sedighi Pashaki[1], Mehdi Seilanian Toosi[1], Mohammad Reza Ghavam Nasiri[1], Hamid Reza Raziee[1]

1. Dept. of Radiotherapy Oncology and Cancer Research Center, Mashhad Univiversity of Medical Sciences, Mashhad, Iran.
2. Dept. of Pathology, Mashhad University of Medical Sciences, Mashhad, Iran.

ABSTRACT

Background and Objective: **Approximately half of patients with diffuse large B-cell lymphoma are cured with current chemotherapy regimens. The purpose of this study was to evaluate Bax and Bcl2 expression and their relationship with the response to chemotherapy.**

Materials and Methods: **This study was a prospective analysis on 44 patients with diffuse large B-cell lymphoma. Their specimens were stained with immunohistochemistery method for Bax and Bcl2. The relationship between Bax/Bcl2 expression and the response to chemotherapy as well as some other prognostic factors were assessed.**

Results: **Out of 44 patients, 29 were Bax+ and 15 Bax-, 31 Bcl2+ and 13 Bcl2-. We found a statistically significant relationship between IPI score and the response to chemotherapy (*P* = 0.002). The response rates were relatively better (but not significant) in cases with Bax + compared to Bax- and in patients with Bcl2- compared to Bcl2 + tumors. The combination of immunohistochemistery results for Bcl2 and Bax could predict relatively higher response rates in a way that those with Bax+ Bcl2- had a higher response compared to Bax- Bcl2+ (57%% *VS*.22%, *P*=0.15).**

Conclusion: **Although we found a relatively higher responses in our cases with Bax+vs. Bax- and in those with Bcl2- vs. Bcl2 +, the differences were not statistically significant. We suggest further studies to confirm whether the Bcl2 and Bax expressions have any effect on the response to chemotherapy and whether they could be considered as predictor factors for chemotherapy response.**

Keywords: Diffuse large B-Cell lymphoma, Bax, Bcl2, Chemotherapy

Received: 12 July 2008
Accepted: 6 November 2008
Address communications to: Dr Kazem Anvari, Department of Radiotherapy Oncology and Cancer Research Center, Mashhad Univiversity of Medical Sciences, Mashhad, Iran.
Email: anvarik@mums.ac.ir

Introduction

Diffuse large B cell lymphoma (DLBCL) is a neoplasm of large, transformed B cells with a diffuse growth pattern. It is the most common type of non Hodgkin's lymphoma (NHL) (31% of all cases) (1). Almost 50% of DLBCL cases are cured with CHOP standard regimen (CTX, Adriamycin, VCR, prednisolone). However, the remaining 50% are chemoresistant and this regimen is not able to cure them. The latter group usually recurs after CHOP-induced remission with a short disease free interval. Most of these patients finally succumb to their disease. Alternatives treatment strategies such as combination chemotherapy and mononuclear antibodies or more intensive chemotherapy with bone marrow transplantation may be effective to them (2).

Therefore, it would be highly valuable if we could be able to detect upfront which patients need more intensive treatments. According to that, researchers have been attempting to find prognostic factors to help predict who will experience good or unsatisfactory response.

The most important prognostic factor in DLBCL is IPI (International Prognostic Index) score, but it is not able to distinguish curable chemosensitive from incurable chemoresistant patients (3). Apoptosis is one of the mechanisms which affect chemotherapy response, and has been thoroughly investigated. Evidence has accumulated in recent years showing that many and perhaps all chemotherapeutic agents affect tumor cell killing by inducing apoptosis (4). Intrinsically chemoresistant tumor are unable to activate the apoptotic machinery and may therefore are fundamentally resistant to induction of cell death by chemotherapeutic agents. The Bcl2 protein, due to its antiapoptotic affects, is considered to be an important multidrug resistance agent. Bcl2 is a member of a related and interacting family of proteins, some of which (e.g, Bcl-xl) are antiapoptotic as well, whereas others (e.g., Bax, Bcl-xs, Bik and BID) have proapoptotic functions(5) . The molecules of these proteins are known to dimerize with themselves or with each other, and it is proven that if this balance favors the production of free Bcl2, apoptosis is inhibited, whereas when Bax predominates, apoptosis is initiated. Thus, the ratio of anti/pro apoptotic proteins determines whether a cell will response or ignore an apoptotic stimulant (6).

Several studies have assessed the association between Bcl2 expression and disease free survival (7-9). In aggressive NHL, these studies have led to the belief that Bcl2 family proteins have an important role in chemosensitivity or chemoresistance of DLBCL.

The purpose of our study was to evaluate Bcl2 family genes expression and its relationship with the response to chemotherapy.

Materials and Methods

Patients Selection

This prospective study was conducted from February 2006 to December 2008. All patients were included with diffuse large B-cell lymphoma referred to radiation Oncology Department of Omid and Qaem hospitals in Mashhad, Iran.

The DLBCL pathology was confirmed by Omid Hospital Lymphoma Group and the pathologist of this study by sample review and immunophenotyping.

The treatment plan consisted of six courses of CHOP chemotherapy or four courses plus involved-field radiotherapy. Chemotherapy courses were 3 weeks apart.

Forty nine DLBCL cases were selected for this study, all with adequate pathological specimens available. We excluded 3 patients that did not have sufficient biopsy specimens, and 2 other patients whose first lymphoma diagnosis were rejected by the second pathological review. Forty four remaining specimens were assessed by IHC. One patient who did not begin treatment was excluded from further analysis. Detailed history taking and physical examination with particular emphasis on response to chemotherapy were performed before each chemotherapy course and the results were recorded. The protocol were reviewed and approved by Mashhad University of Medical Sciences Ethic Committee before patient enrollment. After treatment completion patients were visited each 2-3 months.

Immunohistochemistry

Formalin-fixed, paraffin embedded biopsy specimens were collected. Sections with 4 micron thickness were prepared. Microscopic slides with H&E staining were reviewed revised if necessary by our pathologist. After confirming the diagnosis, IHC staining for Bcl2 (Fig.1) and Bax (Fig. 2) by monoclonal antibodies were performed using avidin-biotin technique (Dako, Carpenteria, California, USA). Hyperplastic human tonsillar tissue was used as positive control.

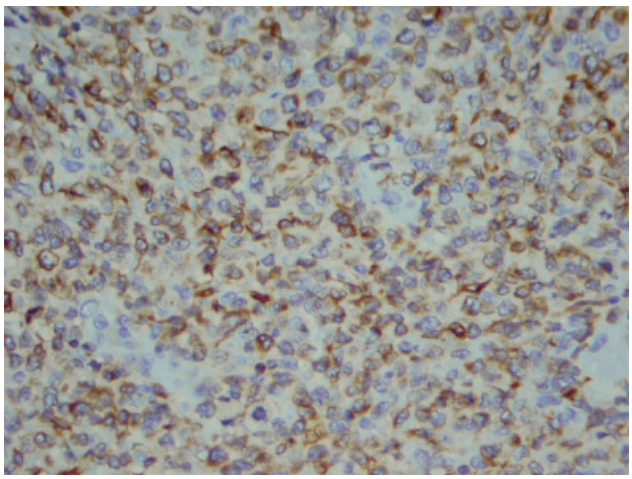

Fig. 1. Immunostaining for Bax, nearly all of neoplastic cells show strong cytoplasmic reactivity. (×400)

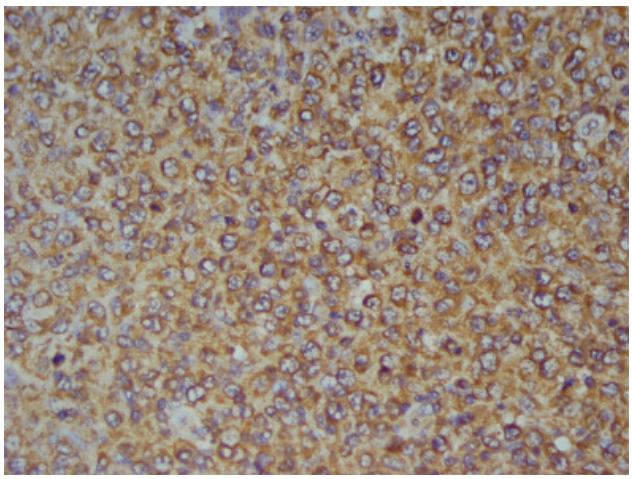

Fig. 2. Immunostaining for Bcl2, most of neoplastic cells show moderate to strong cytoplasmic reactivity. (×400)

Immunohistochemical scoring

Stained specimens were evaluated for stain intensity and also for percentage of immunoreactive cells. Stain intensity was classified as weak, moderate and strong. Percentages of immunoreactive cells were categorized as less than or equal to 10% and more than 10%. Only staining that was moderate or strong and also more than 10% were considered positive.

Statistical Analysis

Statistical Analysis was done by SPSS statistical software program (version 11.5). We used the Kaplan-Meier formula for survival calculation and Log-rank test for the comparison of survival curves between different groups. Chi-square test was performed for the comparison between groups.

Results

Forty four patients (23 males, 21 females) with median age of 59 (range 14-95 years) were studied. Median of follow up was 8 months (range 2-31). One patient, who was lost to follow up, was excluded. Thirty patients were in early (I, II) and 13 in advanced stages (III, IV) at presentation. Twenty eight patients had nodal and 16 had extranodal disease .Thirty nine (88. 6%) cases had measurable disease after diagnosis and therefore were suitable for chemotherapy response assessment.

Regarding chemotherapy response, patients were divided into 3 categories including response to less than 3 chemotherapy courses, 3 or more than chemotherapy courses, and chemotherapy resistant.

Fifteen patients responded to less than 3 chemotherapy courses, 13 in more than 3 courses and 11 patients were chemoresistant. Out of 43 patients, 26 (60%) had no evidence of recurrence during follow up.

IPI score was between 0-2 in %72 of patients, and there was a statistically significant relationship between the score and response to chemotherapy (P=0.02). There was also a statistically significant relationship between stage at presentation and response to chemotherapy (P=0.04).

Bax and Bcl2 expression

After immunohistochemistry the reactive neoplastic cells in stained slides, revealed cytoplasmic reactivity for BCL2 (figure1) and also Bax (Fig. 2*)*.

Out of 44 patients, 29 were Bax positive (Bax+) and 15 Bax negative (Bax-), 31 Bcl2 positive (Bcl2+) and 13 Bcl2 negative (Bcl2-) .

Association between Bax and Bcl2 and response to chemotherapy:

We assessed the relationship between Bax and Bcl2 expression with response to chemotherapy but we didn't find a statistical significant relationship between them (P values were respectively 0.48 and 0.32) (Table1).

Table 1: Probable prognostic factors

Prognostic factors		Rapid response*		Slow/resistance**		Total	*P* value
		Number	Percent	Number	Percent		
IPI	**0,1,2**	15	100	0	0	15	0.002
	3,4	13	54	11	46	24	
Bax	**Positive**	11	42	15	58	26	0.48
	Negative	4	30	9	69	13	
Bcl2	**Positive**	9	33	18	67	27	0.32
	Negative	6	50	6	50	12	

IPI= International Prognostic Index
*Rapid Response= complete Response to less than 3 chemotherapy courses
** Slow=complete Response to 3 or more than chemotherapy courses
Resistance= chemotherapy resistant.

We did not find any association between Bax and Bcl2 expression with stage at presentation. No association was found between concomitant expression of Bcl2 +, Bax-, with response to chemotherapy (*P*= 0.38).

We also assessed the relation between Bax+Bcl2- group with Bax -Bcl2+ group. 4 out of 7 (57%) in former group had rapid response to chemotherapy and 3(43%) had slow response or were resistant .In Bax-, bcl2+ group only 2 out of 9(22%) had rapid response to chemotherapy and 7(78%) had slow response or were resistant to chemotherapy, but these differences did not reach statistical significance.

There was not an association between more than 80% bcl2 positivity with response to chemotherapy.

Discussion

In this project, we studied the expression of two proteins of Bcl2 family (Bax,Bcl2) in DLBCL Bcl2 is an antiapoptotic protein which has a role in resistance to chemotherapeutic agents. Some studies have indicated that high expression of Bcl2 may be associated with short survival(10,11) and high expression of Bcl2 is a prognostic factor that is independent of IPI score (10,12,13) and other indicators of poor clinical outcome in patients with several types of cancer, including aggressive NHL, acute myelogenons leukemia (14) and prostatic adenocarcinoma (15). In one study by Mounier and *et al*. an analysis of the GELA trial demonstrated that the benefit of rituximab appeared limited to patients with lymphoma that overexpressed bcl2 on IHC (16). Another study by Hermine *et al.* has shown that Bcl2 is an independent prognostic factor on disease free survival (17).

In *in-vitro* studies have been conducted so far have proven that Bax is a proapoptitic protein (18, 19) and seem to induce accelerated cell death. In contrast, other studies such as the Gascoyne's *et al*. have reported that low percentage (≤10) of Bax staining in tumoral cells in Bcl2+ patients, had an association with improved disease free survival (DFS) , overall survival (OS) and relapse free survival (RFS)(20).

We assessed the association of Bax and Bcl2 with response to chemotherapy. We had limitation in overall survival and disease free survival estimation due to short follow up duration.

In this study, in spite of a statistical significant relation between IPI score and response rate, we did not find a statistical significant relation between Bax and Bcl2 expression with response to chemotherapy.

It seems possible that the number of enrolled patients were not enough to reach statistically significant results. However, we found some results that can come into consideration. The ratio of Bax+ patients in chemosensitive group is higher than chemoresistant group (74.4% vs 25.6% respectively, P=0.29). This finding indicates Bax+ patients have better response to chemotherapy. As per Bcl2, there was not statistically significant relation between Bax+ with chemotherapy response.

In our study staining intensity or overexpression of Bcl2 (more than 80%) obviously was associated with slow response or resistance to chemotherapy, but did not reach statistical significance, (81% *vs* 18.2% *P*=0.1)

We believe this is a reflection of small number of patients that were enrolled in the study.

In our analysis, Bax+ and Bcl2- patients had better

response to chemotherapy compared to Bax- Bcl2 + group (57% vs 23.2% respectively) but again this difference did not reach statistical significant value.

Conclusion

In summary, we found a statistically significant relation between IPI score and response to chemotherapy. However, despite some relationship between Bax, Bcl2 expression with chemotherapy response, these differences were not statistically significant. These results may be because of small numbers of patients. Overall it seems that Bax positivity associated with better response, and Bcl2 is related to resistance to chemotherapy that may be considered for more aggressive treatment. However, these results should be confirmed in the future with larger treated patient's studies.

Acknowledgments

This project was supported by a grant from Vice Chancellery for Research, Mashhad University of Medical Sciences, Iran. We would also like to thank Dr. Taghi Shakeri for statistical analysis and personnel of Departments of Pathology (Omid and Qaem Hospitals) for their cooperation.

References

1. Armitage JO, Weisenburger DD. New approach to classifying non-Hodgkin's lymphomas: clinical features of the major histologic subtypes. Non-Hodgkin's Lymphoma Classification Project. J Clin Oncol 1998; 16(8):2780.

2. Fisher RI. Autologous stem-cell transplantation as a component of initial treatment for poor risk patients with aggressive non-Hodgkin's lymphoma: resolved issues versus remaining opportunities. J Clin Oncol 2002; 20(22):4411.

3. Haioun C, Lepage E, Gisselbrecht C, Salles G, Coiffier B, Brice P, *et al*. Survival benefit of high-dose therapy in poor-risk aggressive non-Hodgkin's lymphoma: final analysis of the prospective LNH87-2 protocol - a Groupe d'Etude des Lymphomes de l'Adulte study. J Clin Oncol 2000; 18(16) :3025.

4. Jonthan W, Sriedberg, Ter M. Mauch. Lisa M. Rimsreda. Non-Hodgkin's Lymphoma. In: Vincent T. DeVita, Theodore S. Lawrence, Stewen A. Rosenberg; Cancer principle & practice of oncology. 8th ed. Philadelphia: Lippincott, 2008; pp: 2099-2143.

5. Reed JC. Bcl-2 family proteins. Oncogene 1998; 17:3225.

6. Burlacu A.Regulation of apoptosis by Bcl2 family proteins. J Cell Mol Med 2003; 7(3):249-57.

7. Tang SC, Visser L,Hepperle B, Hanson J, poppema S. Clinical significance of bcl2- MBR gene rearrangement and protein expression in DLBCL: an analysis of 83 cases. J Clin Oncol 1994; 12(1):149-54.

8. Piris MA, Pezzella F, Martinez-Montero JC, Orradre JL, Villuendas R, Sanchez-Beato M. P53 and bcl2 expression in high grade B cell lymphomas: correlation with survival time. Br J Cancer. 1994; 69(2): 337-41.

9. Kramer MH, Hermans J, Parker J, Krol AD, Kluin-Nelemans JC, Haak HL, *et al*. Clinical significance of bcl2 and P53 protein expression in diffuse large B – cell lymphoma: a population based study. Blood 1998; 92(4): 257-63.

10. Lossos IS, Czerwinski DK, Alizadeh AA, Wechser MA, Tibshirani R, Botstein D, *et al*. Prediction of survival in Diffuse Large B Cell lymphoma based on the Expression of six Genes. N Engl J Med 2004; 350(18): 1828 –37.

11. Iqbal J, Neppalli VT, Wright G, Dave BJ, Horsman DE, Rosenwald A, *et al*. Bcl2 Expression is a prognostic Marker for the Activated B Cell Like Type of Diffuse large B Cell lymphoma. J Clin Oncol. 2006; 24(6): 961 –8.

12. Veelken H, Vik Dannheim S, Schulte Moenting J, Martens UM, Finke J, Schmitt-Graeff A. Immunophenotype as prognostic factor for diffuse large B Cell lymphoma in patients undergoing clinical risk adapted therapy. Ann Oncol 2007; 18(5): 931-9.

13. Barrans SL, Carter I, Owen RG, Davies FE, Patmore RD, Haynes AP, *et al*. Germinal center phenotype and bcl2 expression combing with the international prognostic index improves patient risk stratification in diffuse large B Cell lymphoma. Blood 2002; 99(4): 1136-43.

14. Campos L, Rouault JP, Sabido O, Oriol P, Roubi N, Vasselon C, *et al*. High expression of bcl2 protein in acute myeloid leukemia cells in associated with poor response to chemotherapy. Blood 1993; 81(11):3091-6.

15. Bonbendo F, Saunter G, *et al*. Prognostic significance of Bcl2 in clinically localized prostate cancer. Am J Pathol. 1996; 148(5):1557-65.

16. Mounier N, Briere J, Gisselbrecht C, Emile JF, Lederlin P, Sebban C, *et al*. Rituximab plus Chop (R – Chop) overcomes bcl2 associated resistance to chemotherapy in elderly patients with diffuse large B Cell lymphoma (DLBCL). Blood 2003;101(11): 4279 –84.

17. Hermine O, Haioun C, Lepage E, d'Agay MF, Briere J, Lavignac C, *et al*. Prognostic significance of bcl2 expression in aggressive non-Hodgkin's lymphoma. Groupe d' Etade des lymphomas de l'Adulte GELA.Blood 1996; 87(1); 265-72.

18. Strobel T, Swanson L, Korsmeyer S, Cannistra SA. Bax enhances paclitaxel induced apoptosis through a p53 independent path way. Proc Nat Acad Sci USA 1996; 93 (24): 14094 –9.

19. Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl2 hetero dimerizes in vivo with a conserved homo log, Bax that accelerates programmed cell death. Cell 1993; 74(4):609-19.

20. Gascoyne RD, Krajewska M, Krajewski S, Connors JM, Reed JC. Prognostic significance of Bax protein Expression in Diffuse aggressive non Hodgkin's lymphoma. Blood 1997; 90(8): 3173 – 80.