Medical Journal of Babylon
Vol. 12- No. 3: 721-729, 2015
http://www.medicaljb.com
ISSN 2312-6760©2015 University of Babylon

Original Research Article

Prognostic Significance of Immunohistochemical Overexpression of P53 And Bcl-2 Proteins in Patients with Non-Hodgkin'sLymphoma

Farah Amer Albejow[1*] Hadeel Abdul-Elah Kerbal[2] Najlaa Bader Alawadi[2]
Ahmed Raji Kharulla[2]
[1]Merjan Teaching Hospital , Babylon Health Directorate , Hilla, IRAQ
[2] College of Medicine, Babylon University, Hilla , IRAQ

[*]E-mail: dr_mkm2000@yahoo.com

Accepted 20 May , 2015

Abstract

Non-Hodgkin's lymphomas are the hematologic malignancy with the most prevalence worldwide, they are generally classified as B-cell or T-cell lymphoma .Theincidence rate of NHL for the total population is 5.16 % according to Iraqi cancer registry (ICR) in 2010.

This study was designed to asses'bcl-2 and p53 overexpression in Iraqi patients with NHL and correlation with different prognostic indices (age, sex, stage, grade, and International prognostic index).

Samples included in this study represented as 50 formalin-fixed paraffin embedded lymph node blocks of patients with NHL,during the period from September 2013 till November 2014,and these blocks collected from the histopathology laboratories of Al-Hilla Teaching Hospital as well as many private histopathology laboratories in Hilla city, These blocks stained Immunohistochemically for bcl-2 and p53 protein expression by using L-SAB+ staining methods.

Results revealed Significant correlation between bcl-2, p53overexpression and different grades of NHL (p-value < 0.05), while there are no significant relationship between bcl-2, p53 and age, sex, stage, and IPI score (p-value > 0.05).

In conclusion there are up regulation of P53 protein associated with down regulation of bcl2 protein in patients with high, intermediate to low grades of NHL while no significant association with IPI score& factors.

Key words: Non-Hodgkin lymphoma, P53, Bcl-2.

أهمية التكهن في زيادة التعابير المناعية الكيميائية المفرطة لبروتينات (p53 , bcl -2) في مرضى ورم الغدد اللمفاوية اللاهودجكين

الخلاصة

تعد اورام الغدد اللمفاوية اللاهودجكين من الاورام الدموية الخبيثة الواسعة الانتشار في العالم . يصنف الورم بصورة عامة الى اورام الخلايا نوع B او T.

ان معدل السكان الإجمالي لحدوث ورم الغدد اللمفاوية اللاهودجكين هو ٥.١٦% طبقا الى التسجيل العراقي لأمراض السرطان لسنة ٢٠١٠.

صممت دراستنا لتقييم زيادة التعابير المفرطة للبروتين p53 و bcl– 2 للمرضى المصابين بورم الغدد اللمفاوية اللاهودجكين في مرضى العراق وعلاقتهما مع مختلف عوامل التطور (العمر،الجنس ،المرحلة،الدرجة، دليل التكهن الدولي).

العينات المصممة في هذه الدراسة تمثل ب(٥٠) عينة من الخزعات النسيجية من الغدد اللمفاوية المحفوظة بالفورمالين والمطمورة بشمع البارافين للمرضى المصابين بورم الغدد اللمفاوية اللاهودجكين كذلك (٥٠) عينة على شكل قوالب شمعية للمرضى المصابين بالتهاب الغدد اللمفية، خلال الفترة من ايلول سنة ٢٠١٣ الى كانون الأول سنة ٢٠١٤ هذه العينات تمت جميعها من مختبر الأنسجة في مستشفى الحلة التعليمي والعديد من مختبرات

الأنسجة الخاصة في مدينة الحلة وهذه القوالب تم صبغها ومعاملتها مع الطريقة المناعية النسيجية لتحديد التعبير المناعي للبروتينp53 و bcl-2وذلك باستخدام طريقة صبغة SAB-L+.

نتائجنا تبين وجود علاقة ذات دلالة بين الزيادة المفرطة لبروتين (p53,bcl-2) ومختلف درجات ورم الغدد اللمفاوية اللاهودجكين (P-value < 0.05) بينما لا توجد علاقة ذات دلالة بين (p53,bcl-2) والعمر، الجنس، المرحلة، درجة دليل التكهن الدولي (P-value > 0.05) .

نستنتج انه يوجد زيادة في البروتين p53 مع نقصان في بروتين bcl-2 خلال الدرجات (العالية، الوسطى، القليلة) في مرضى ورم الغدد اللمفاوية اللاهودجكين بينما لا توجد علاقة مع عوامل ودرجات دليل التكهن الدولي.

Introduction

Non-Hodgkin's lymphomas are the hematologic malignancy with the most prevalence worldwide, they are generally classified as B-cell or T-cell lymphoma depending on which type of lymphocyte becomes malignant, B-cell type is more common than T-cell type, NHL can be classified as either slow growing (low grade or indolent lymphoma) or rapid growing (high grade or aggressive lymphoma)[1]. The incidence of NHL has increased significantly in the last few decades, but causes for these increments were unclear [2].NHL is slightly more common in males than females [3]. In our country according to Iraqi cancer registry (ICR) in 2010 the incidence rate of NHL for the total population 5.16 %[4].

P53 is a tumor suppressor gene; which is situated on short arm of chromosome 17 at position p1.4.1[5].P53 gene encoded a protein that regulated cell cycle checkpoint and stimulation of programmed cell death (apoptosis) in response to the DNA damage, cell stress or the abnormal expression of some oncogenesis [6].Mutation inP53 is the most frequently detected genetic alteration in most types of human cancers including lymphomas[7].

In NHL, P53 mutation was associated with poor prognosis [8]. P53 overexpression has been found to be a fairly common feature in high grade lymphomas in the majority of tumor cells. The results vary from series to series, from 25% to 33% of cases[9].

Bcl-2 is the acronym (bcl-2) lymphoma/leukemia-2 gene; this gene was first discovered because of its involvement in chromosomal translocations t (14; 18) found in the majority of follicular B-cellnon-Hodgkin's lymphomas[10]. BCL-2 is an anti-apoptotic molecule generally expressed in pre-B cells, resting B cells including normal mantle and marginal zone lymphocytes, also certain types of proliferating B cells, so that bcl-2 can be noticed in non-neoplastic lymphoid tissue exactly hyperplastic lymph node and it is down-regulated in normal germinal center B cells. Almost all of the small B-cell neoplasms are expressed bcl-2[11]. However, bcl-2 is a type of proto-oncogenes that block cell death without enhancing cell proliferation, by controlling the mitochondrial membrane permeability [10].

The initial evaluation of NHL helps to found the correct diagnosis and extent of disease. Prognostic models have been developed for predicting outcome in patients on the basis of the patient's clinical characteristics before treatment. These models have been designed for the most common lymphomas and include the International Prognostic Index (IPI)[12],which divide NHL in to 4 risk groups Box(1) depending on the international prognostic factors which include age, stages, number of extranodal site, LDH, and performance status.

Box (1): International Prognostic Index [12]

Risk group	Risk factor	5-Year OS (%)
Low	0–1	73%
Low intermediate	2	51%
High intermediate	3	43%
High	4–5	26%

The current study investigated the role of apoptosis related proteins, such as bcl-2, and p53 immuno-histochemical expression, with respect to international prognostic index and clinical characteristics in patients with NHL.

Patients and Methods

From September 2013 to November 2014, 50 patients with histologically confirmed diagnoses of NHL were collected from histopathology laboratory of Al-Hilla Teaching Hospital. Their corresponding Paraffin stained Immunohistochemically for bcl-2, and p53 protein expression. The clinical parameters and outcome of patients included in this study were retrospectively reviewed. The prognostic data were analyzed according to known clinical parameters, including grades, International Prognostic Index (IPI) correlated with p53 & bcl2 protein over expression.

Immunohistology

Two tissue sections of (5µm) thickness were cut from each paraffin blocks; and adhered to charged slides. These slides were then subjected to a deparaffinization and rehydration process and washed with distilled water. Epitope retrieval was done by incubating the tissue sections into target retrieval solution (code no.S2367), PH 9 which was already placed in water bath rise its temperature for 93^{o}C. Incubation in retrieval solution last for 30min. After that the antigen was expressed, the slides were left at room temperature for 20 min to cool gradually. Next, the slides were washed with phosphate buffered saline(PBS), treated with hydrogen peroxide (code K0679)for5 minutes, and then washed with PBS three more times. Then the tissue samples were reacted with primary antibodies for bcl-2 (DAKO, 1:50), and p53 (DAKO, 1:50) for 30 min. Then, the slides washed with PBS three times and reacted with Biotinylated link for 20min. another washing in PBS and streptavidin-peroxides were applied for 20min followed by washing in PBS for 3 times, their colors developed with Chromogen solution (codeK0679) (for each 1ml of buffer add 1 drop of DAB Chromogen) for 10 min. Finally, after being cleaned with distilled water, Haematoxylin was used as a counter stain for 2 min, and then the slides were sealed.

ImmunohistologicalScoring

The scoring in relation to Sophia K.*et al*. [13] at X40 for P53 protein expression was divided into four positive scoresdepending cut of value ≥5%of cancer cells positive for staining.While the positive peripheral cytoplasmic staining of cancer cells for bcl-2 protein expression at X40 were divided into three positive score[14] depended cut of value ≥25%of cancer cells positive for staining.

Statistical Analyses

The data were analyzed with the SPSS version 18 software package (SPSS Inc, Chicago, IL, USA). Definite variables were summarized as frequencies and percentages also analyzed by Chi-square or Fisher's exact tests where appropriate. Continuous variables were summarized as mean. The data were measured significant if the P-

value ≤ 0.05(where P is the probability value).

Results

This study included 50 patients with NHL, the patient's age were ranged from 3 -80 years, with mean age was (44.18), and divided into four groups but the highest incidence occur at age group of (40- 60)year which account for 19 cases(38%), and more common in male than female, alsothe highest incidence was found in cases with intermediate grade (grade II) and stage II, as showed in table (1).

Table 1: Characteristic of 50 patients with Non-Hodgkin Lymphoma.

	No. of patients	%
Age(years):		
Mean	44.18	
20≥	9	18%
20-40	11	22%
40-60	19	38%
60-80	11	22%
Sex:		
Males	34	68%
Females	16	32%
Grade:		
Low	4	8%
Intermediate	28	56%
High	18	36%
Stage:		
I	8	16%
II	32	64%
III	10	20%
IV	0	0%

While Immuno-histochemical findings of the patients are listed in table (2) and table (3) which found nuclear staining for positive p53protein expression and peripheral cytoplasmic staining for positive bcl-2 protein expression respectively. Both p53 and bcl-2 have significant correlation with grades of NHL (P-value 0.014, 0.016) respectively, while other parameters included (age, sex, stage, and international prognostic index (IPI)) have no significant correlations with p53 and bcl-2(P-value > 0.05).

Table 2: Characteristic of non-Hodgkin lymphoma patients according to p53 protein expression

	P53 expression		
	Positive NO. (%)	**Negative NO. (%)**	**P-Value**
Age			
≤60	36 (94.7%)	2 (5.3%)	1.000
>60	11(91.7%)	1 (8.3%)	
Sex			
Males	31 (91.2%)	3 (8.8%)	0.542
Females	16 (100.0%)	0 (0.0%)	
Grade			
Low	2 (50.0%)	2 (50.0%)	0.014
Intermediate	27 (96.4%)	1 (3.6%)	
High	18 (100.0%)	0 (0.0%)	
Stage			
I, II	38 (95.0%)	2(5.0%)	0.496
III,IV	9 (90.0%)	1(10.0%)	
IPI			
Low	27 (96.4%)	1 (3.6%)	0.691
Low intermediate	14 (87.5%)	2 (12.5%)	
High intrmediate	5 (100.0%)	0 (0.0%)	
High	1 (100.0%)	0 (0.0%)	

Table 3: Characteristic of non-Hodgkin lymphoma patients according to bcl-2 protein expression

	Bcl-2 expression		
Positive NO. (%)	**Negative NO. (%)**	**P-Value**	
Age			
≤60	25 (65.8%)	13 (34.2%)	0.304
>60	10 (83.3%)	2 (16.7%)	
Sex			
Males	24 (70.6%)	10 (29.4%)	1.000
Females	11 (68.8%)	5 (31.3%)	
Grade			
Low	4 (100.0%)	0 (0.0%)	0.016
Intermediate	23 (82.1%)	5(17.9%)	
High	8 (44.4%)	10 (55.6%)	
Stage			
I,II	29 (72.5%)	11 (27.5%)	0.462
III,IV	6 (60.0%)	4 (40.0%)	
IPI			
Low	18 (64.3%)	10 (35.7%)	0.762
Low intermediate	12 (75.0%)	4 (25.0%)	
High intrmediate	4 (80.0%)	1 (20.0%)	
High	1 (100.0%)	0 (0.0%)	

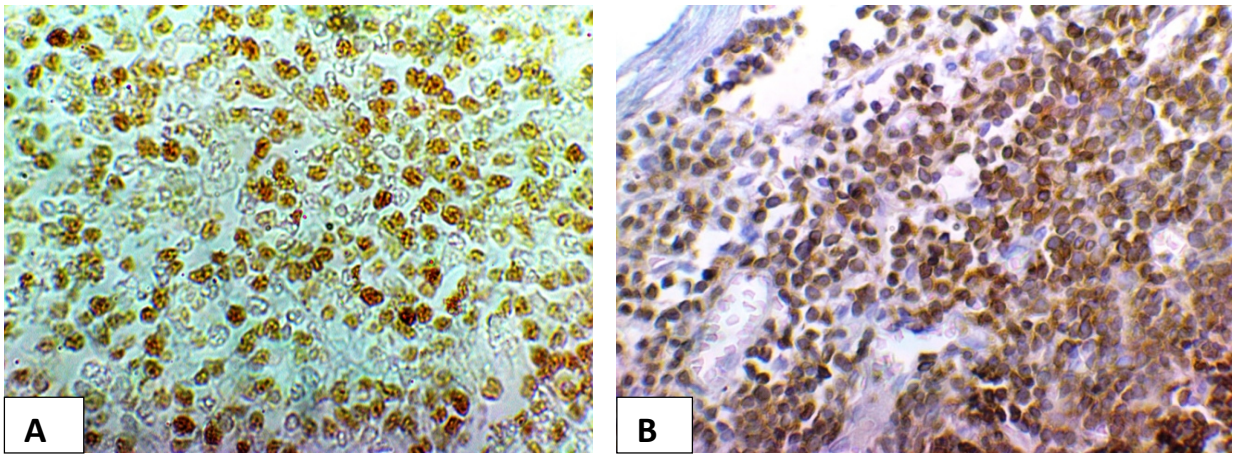

Figure (A): Histological section of diffuse large lymphoma appear strong positive P53 (score 4) (x40)**.**
Figure (B): Histological section of small lymphocytic lymphoma appear strongpositive Bcl-2 (score 3) (x40).

Discussion

In present study, the mean of patient's age was (44.18). According to table(1) NHL more frequently diagnosed in older age groups with highest incidence in age groups of (40- 60) year corresponding to 19 cases (38%). Our results reliable with other studies carried in the same region (Iraq and Jordan) by Al- Obiadi[15] and Al-Masri*et al*[16] respectively, while disagree with study carried in western region by Jong *et al.*[17]and Kait*et al.*[18]with highest mean age were (68, 63) respectively. These differences in age groups could be contributed to the differences in environmental and geographical risk factors affecting each study groups making NHL affecting relatively middle age group in Iraq and neighboring countries.

In this studythe incidence of NHL in males (68%) was greater than in females as in table (1). In our country the incidence of NHL in males is (6.33%) and females are (4.15%) [9]. Similar results obtained by two studies done in Pakistan by La*et al.*[19] and Naz*et al.*[20]who found M:F ratio (2:1 and 2.6:1, respectively).Alsothe present study demonstrated that highest incidence was found in cases with intermediate grade (56%) and high grade (36%),while the lowest incidence was found in cases with low grade (8%) as in table (1).Our results reliable with study carried in United Arab Emirate (UAE) revealed that the percentage of high grade, intermediate grade and low grade were (34%, 59% and 7% respectively)[21], also similar with those reported in Iraq who found that the highest incidence rate in the intermediate grade[22] .While our results are incompatible with those reported in western area like in Argentina (39.7% and 38.25% for high and low grades, respectively)[23]. Also these differences could be contributed to different geographical and risk factors in these areas. In addition to that our results revealed the highest incidence occur in stage II (64%) and lowest incidence occur in stage IV as in table (1).These results are agree with those reported by Lorenzo Leoncini*etal.*[24]and Sang KyunSohn*etal.*[25], who found that the high incidence of NHL occur in stage II in different percentage according to their samples size.

P53 is a protein that regulated cell cycle checkpoint and stimulation of programmed cell death (apoptosis) in response to the DNA damage, cell stress or abnormal expression of some

oncogenesis[6]. In current study was reported positive (100%) in high grade, (96.4%)in an intermediate grade, while in low grade p53 overexpression is 50%, there are significant difference betweenthegrades of non-Hodgkin's lymphoma and p53 overexpression. P-value< 0.05. Tables (2).These results showed an agreement with M. R. Hussein *et al.*[26]and Myung - JuAhn[27], revealed that gradual up-regulation of p53 staining values with the transition from low to intermediate- to -high- grade NHL with significant correlation between them (P-value < 0.05). This up-regulation may be due to the presence of p53 gene mutations that stabilize the mutant p53 protein, and increase its half-life leading to its accumulation in the cells[28]. In present study, there are no significant correlation between p53 and age, sex, stage and IPI (P-value > 0.05). The above study results were consistent with Kramer[29]and kucukzeybek *et al*.[30],who stated that there was no significant correlation between p53 expression and one or more subgroups of the IPI. Also our results agree with MyungJuAhn[27] which found no significant correlation between p53 expression and any clinicopathological factors and IPI score,these results may be due to small sample size.

Bcl-2 is a type of proto-oncogenes that block cell death without enhancing cell proliferation, by controlling the mitochondrial membrane permeability[10]. In present study was reported positive (100%) in low grade and (20%) in high grade, there are significant difference between the grades of NHL and bcl-2 (P-value< 0.05) as in table (3).These results showed an agreement with Hussein *et al.*[26] and Kiberu*et al.*[31], revealed that gradual down-regulation of bcl-2 staining values with the transition from low to intermediate- to -high- grade NHL with significant correlation between them (P-value < 0.05). These down-regulations may be due to: epigenetic mechanisms like bcl-2 promoter hyper-methylation; another prosurvival molecule takes over its role; and up-regulation of bcl-2 antagonists[31]. While there are no significant association were found between bcl-2 IHC expression and age, sex, stage and IPI (P-value > 0.05). In my opinion this could be contributed to theheterogeneityand smallsamples size. This study similar to other studies like Kramer M.H.H.[29] and kucukzeybek BB.*et al*.[30].

Conclusion

From the present study we can conclude that gradual down-regulation of bcl-2 and up-regulation of p53 protein expression with the conversion from low to intermediate to high- grade NHL. Also there are no significant correlation of bcl-2 and p53 with IPI this could be attributed to small study group involved in this study. However;the high percentage of bcl-2 and p53 detection among NHL groups suggests that bcl-2 and p53 proteins play essential role in pathogenesis of NHL.

Reference

1. National Cancer Institute. Adult non-Hodgkin lymphoma treatment: Cellular classification of adult non-Hodgkin lymphoma, 2010: pages Accessed.
2. Shankland KR., Armitage JO., Hancock BW. Non-Hodgkin lymphoma. Lancet; 2012, 380 (9844): 848-57.
3. American Data were provided by ISD Scotland on request. (2012).
4. Iraqi cancer registry. 2010:37-219.
5. Gasco M., Shamis S., Crook T. The P53 Pathway in breast cancer, Cancer Research 2002; 4: 70-76.
6. Richard A. Thomas J, Barbara A, Jains K: Immunology; 6th edition (2004); 22:499-522.
7. Sathiya M., Muthuchelian K. Significance of Immunologic Markers in

the Diagnosis of Lymphoma. Academic Journal of Cancer Research 2009; 2 (1): 40-50.
8. Jack AS., Burnett AK. Procedures for the primary diagnosis and follow-up of patients with lymphoma. In: Mauch PM., Armitage JO., Coiffier B, Dalla-Favera R., Harris NL., eds. Non-Hodgkin's Lymphomas. Philadelphia, PA: Lippincott Williams & Wilkins; 2004:81-95.
9. Ross DD., Joneckis CC., Ordonez JV., *et al*. Estimation of cell survival by flow cytometric quantification of fluorescein diacetate/propidium iodide viable cell number. Cancer Res. 1989; 49:3776-3782.
10. Reed JC. Bcl-2 and the regulation of programmed cell death. The journal of cell Biology 2004; 124: 1-6.
11. Wang T., Lasota J., Hanau CA., Miettinen M. Bcl-2 oncoprotein is wide-spread in lymphoid tissue and lymphomas but its differential expression in benign versus malignant follicles and monocytoid B-cell proliferations is of diagnostic value. APMIS. 1995; 103:655–662.
12. The International Non-Hodgkin lymphoma prognostic factors Project (1993). A predictive Model for aggressive non-Hodgkin s lymphoma: Eng. J med 329:987-994.
13. Sophia K. Apple, J. Randolph Hecht, W. David. IHC evaluation of K-ras, P53, and HER-2/neu expression in hyperplastic, dysplastic, and carcinomatous lesions of the pancreas: evidence for multistep carcinogenesis. Human Pathology. 1999, Vol. 30, No.2:123-130.
14. Shirin K., Moslem B., Foroozan M., *et al.* IHC Detection of P53, bcl-2 and Retinoblastoma Proteins in Primary Mediastinal Large B Cell Lymphoma,2002; Page 29.
15. McNeil C. Non-Hodgkin's lymphoma trials in elderly lookbeyond CHOP. J Natl Cancer Inst 1998; 90:266–7.
16. Al-Obaidi AH. Molecular study of Epstein Barr Viruses, P53 tumor suppressor gene, bcl2 oncogene in patients with Non Hodgkin Lymphoma.MSc., thesis (2012).
17. Almasri N., Mahmud A., Hassan S., Khalidi. Types and patterns of 111 cases classified According to the WHO classification of Hematological Malignancies; 2004, 25(5): 609-614.
18. Jon L., Odqvist L., Diaz-Perez JA., *et al.* EBV-positive diffuse large B-cell lymphoma of the elderly is an aggressive post-germinal center B-cell neoplasm characterized by prominent nuclear factor-kB activation. Mod Pathol 2012, 25, 968-82.
19. Bouman A., Heineman MJ, Faas MM. Sex hormones and the immune response in humans. Hum Reprod Update 2005; 11: 411–423.
20. Lal A, Bhurgr Y, Vazir I, *et al.* Extranotal Non-Hodgkin's lymphomas- A Retrospective .Review of clinic-Pathologic Futures and Outcomes in Comparison with Nodal Non- Hodgkin's lymphoma.2008;9:453-458.
21. Randy D. Gascoyne, Sheryle A. Adomat, Stanislaw Krajewski, *et al*. PrognosticSignificance of Bcl-2 Protein Expression and Bcl-2 Gene Rearrangement in Diffuse Aggressive Non-Hodgkin's Lymphoma ;1997: 90, 244-246.
22. Castella A, Sandhya J, Tove R, Neil M. Pattern of malignant lymphoma in United Arab Emirates. ActaOncologica 2001; 40(5):660-664.
23. Al-Humari E.K. Overexpression of P53 & CD20 in relation to histopathological parameters in lymphomas, Iraq, 2009; ch. 4, P. 45.
24. Dragosky M, Alcaraz S, Anneta I, *et al.*: Presentation of 2375 of oncohematological pathologies from two oncologic centers in Buenos Aries, Argentina: Marie Curei Hospital and Henry Moore Institute, 1997-2007.

25. Leoncini L. Vecchio MTD, Megha T, *et al.* Correlations between apoptotic and proliferative indices in malignant non-Hodgkin's lymphomas. Am J Path01 1993; 3: 754- 63.
26. Mahmoud R. Hussein, Tarek M. Al-Sabae, and Marcelle N. Georgis. Analysis of Bcl-2 and P53 protein expression in the Lymphoproliphrative lesion in the upper Egypt, Cancer Biology and Therapy 2005. V.4; I.3:324-328.
27. Myung-JuAhn, Hawk, In-Soon Kim *et al*. P53Protien Expression and it's Prognostic Importance in Patients with Nodal non-Hodgkin Lymphoma, J Koreen Med Sci 2000;15:59-64 ISSN 1011-8934.
28. Charalambous GK., Gomatos IP., Konstadoulakis MM., *et al.* Protein expression of bax, bcl-2, and p53 in patients with non-Hodgkin's gastric lymphoma: prognostic significance. World J Surg 2000; 24: 608–614.
29. Kramer M.H.H., HermansJ., Parker J.,*et al.* Clinical Significance of bc12 and p53 Protein Expression in Diffuse Large B-Cell Lymphoma, Journal of Clinical Oncology1996, Vol 14, No 7: pp 2131-2138.
30. kucukzeybek BB., Bener S., Calli A.O, *et al.*Prognostic Significant of Bcl-2 and P53 protein Expressions and Ki67 Proliferation Index in Diffuse Large B-cell Lymphoma, 2013,Turk J Hematol 30:275-282
31. Kiberu S W, Pringle J H, Sobolewski S, *et al.* Correlation between apoptosis, proliferation and bcl-2 expression in malignant non-Hodgkin's lymphoma, 8Clin Pathol: MolPathol 49;1996:M268-M272.