BCL6 Immunohistochemical Expression In Diffuse Large B Cell Lymphoma

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ABSTRACT

Background: Diffuse large B cell lymphoma (DLBCL) is an aggressive Non-Hodgkin lymphoma subtype.It is the most prevalant and responsible for thirty to forty percent of cases across various geographical locations with variable clinical presentations and underlying genetic diversity. B cell lymphoma 6 (BCL6) is a Proto-oncogenic transcriptional factor which is highly expressed in many lymphomas including diffuse large B-cell lymphoma.

Objectives: To assess BCL6 immunohistochemical expression in lymphomas of diffuse large B cell and its association with some clinical parameters (age, gender & presentation).

Material and Methods: During a period of ten months extending from November (2022) through August (2023), 61 diffuse large B cell lymphoma cases were included in this retro and prospective case series study, obtained from histopathological departments of governmental and private laboratories. Immunohistochemistry investigation using BCL6 was performed, and data of the positive BCL6 expression were evaluated.

Results: Out of 61 cases of lymphoma of diffuse large B cell, the mean age was 55 years, and the median was 61 years, with male predominance (55.7 %) vs (44.3 %) females. The nodal presentation is more frequent than extra nodal site (72.1% vs 27.9 %).41% of DLBCL cases was BCL6 positive (of which 56% were associated with age <60 years and 76% was nodal) with significant association between BCL6 expression and female gender (p value= 0.39).

Conclusion: BCL6 expression in diffuse large B cell lymphoma patients is more frequent in females and younger age (less than 60 years old).

Keywords: BCL6, Diffuse large B cell lymphoma, Immunohistochemistry.

التعبير المناعي الكيميائي BCL6 في سرطان الغدد الليمفاوية ذو الخلايا البائية الكبيرة المنتشرة

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الخلاصة

الخلفية: سرطان الغدد الليمفاوية ذو الخلايا البائية الكبيرة المنتشرة (DLBCL) هو النوع الأكثر انتشارًا وعدوانية من ليمفوما اللاهودجكين، وهو ما يمثل ٣٠ إلى ٤٠ بالمائة من الحالات عبر مواقع جغرافية مختلفة مع عروض سريرية متغيرة وتنوع جيني أساسي. سرطان الغدد الليمفاوية للخلايا البائية ٦ (BCL6) هو عامل نسخي بروتو أنكجنيك والذي يتم التعبير عنه بشكل كبير في العديد من الأورام اللمفاوية بما في ذلك سرطان الغدد الليمفاوية للخلايا البائية الكبيرة المنتشرة.

الأهداف: تقييم التعبير عن عامل ال BCL6 في سرطان الغدد الليمفاوية في الخلايا البائية الكبيرة المنتشرة وارتباطه مع بعض المعايير السريرية (العمر والجنس وموقع ظهور المرض).

مواد و طرق البحث: خلال فترة عشرة أشهر تمتد من نوفمبر (٢٠٢٢) حتى أغسطس (٢٠٢٣)، تم تضمين ٦١ حالة سرطان الغدد الليمفاوية ذات الخلايا البائية الكبيرة المنتشرة في دراسة سلسلة الحالات الرجعية والمستقبلية هذه، والتي تم الحصول عليها من أقسام التشريح المرضي في المختبرات الحكومية والخاصة. تم إجراء فحص الكيمياء المناعية باستخدام عامل ال BCL6، وتم تقييم بيانات التعبير الإيجابي لعامل ال BCL6.

النتائج: من بين ٦١ حالة من حالات سرطان الغدد الليمفاوية ذات الخلايا البائية الكبيرة المنتشرة، كان متوسط العمر ٥٥ عامًا، وكان الوسيط ٦١ عامًا، مع غلبة الذكور (٥٥.٧٪) مقابل (٤٤.٣٪) الإناث. كان ظهور المرض في العقد اللمفاوية أكثر تكرارًا من ظهوره خارج العقد اللمفاوية (٧٢.١% مقابل ٢٧.٩%).٤١٪ من حالات سرطان الغدد الليمفاوية في الخلايا البائية الكبيرة المنتشرة كانت إيجابية لـعامل ال BCL6 (56%) منها ارتبطت بالعمر أقل من ٦٠ عامًا و ٧٦٪ كانت عقدية) مع وجود ارتباط كبير بين تعبير عامل الBCL6 والجنس الأنثوي (قيمة p = 0.39).
الاستنتاج: يكون تعبير عامل ال BCL6 أكثر شيوعًا عند الإناث والمرضى الأصغر سنًا (أقل من ٦٠ عامًا) المصابين بسرطان الغدد الليمفاوية في الخلايا البائية الكبيرة المنتشرة.

الكلمات المفتاحية : BCL6، سرطان الغدد الليمفاوية في الخلايا البائية الكبيرة المنتشرة، الكيمياء المناعية.

INTRODUCTION

Diffuse large B cell lymphoma (DLBCL),an aggressive subtype of non-Hodgkin lymphoma, is the most prevalent globally, responsible for between thirty and forty percent of cases across various geographical locations. It is an aggressive subset of B-cell lymphomas with varying clinical presentations and underlying genetic diversity, with curable rate ~60%. Patients typically present with one or more rapidly developing nodal or extra nodal tumor masses [1,2]. Two unique molecular subtypes of DLBCL have been identified by gene expression profiling, activated B-cell-like (ABC) subtype and germinal center B-cell-like (GCB) subtype; in addition to 10 to 15% of cases are not classifiable [3]. Based on distinct oncogenic mechanisms, these subtypes are thought to originate from distinct stages of lymphoid development (the cell of origin), with the ABC subtype exhibiting a worse prognosis [4]. B cell lymphoma 6 (BCL6) is a Proto-oncogenic transcription factor needed for establishing the germinal center B cells phenotype and follicular helper T cell differentiation, it is located in 3q27 chromosome [5,6]. BCL6, as key oncogene, is an important regulator of humoral mediated immunity and lymphoma survival [7,8]. There are three conserved domains in the BCL6 protein that are critical to its function: the central RD2 area which interacts with CTBP, NuRD, MTA2, and HDAC2, the N-terminal BTB/POZ domain which recruits corepressors like BCOR, NCOR1, and NCOR2, and the C-terminal zinc finger domain which interacts with specific DNA sequences [9]. By repressing the genes involved in the cell cycle,death of cells, plasma cell maturation and differentiation, and DNA injury response, BCL6 promotes and preserves the GC phenotype [10]. Normal tonsils, lymph nodes, and spleen germinal center B cells all express the BCL6 protein. Also, it has been expressed in both normal skin squamous cells and their malignant counterparts [11,12]. BCL6 is highly expressed in DLBCL, Hodgkin lymphoma of nodular lymphocyte predominant subtype, Burkitt and follicular lymphomas [13,14].

Additionally, A subset of B-lymphoblastic leukemias may express it, particularly those with the (1:19) translocation [15]. This study aim is to determine the expression of BCL6 in diffuse large B cell lymphoma in addition to its association with some clinicopathological parameters (age, gender & site).

PATIENTS AND METHODS

All patients identified as DLBCL at governmental hospitals and those referred from some private laboratories in Nineveh province in northern Iraq were registered in this case series study, which ran over a ten-month period from November (2022) to August (2023). There are 61 DLBCL cases in the research. Clinicopathological information (age, gender, and site) was reviewed in all histopathology reports. The stained sections with H&E were reviewed for diagnosis according to WHO Classification at 2017 [16], these sections were already stained and positive for CD20. From each case one section was chosen for the immunohistochemistry analysis.

Immunohistochemistry:

A block of formalin-fixed paraffin-embedded tissue cases was obtained, 4 micron-thick sections were rehydrated after being deparaffinized in xylene. The immunohistochemistry analysis was done in compliance with the guidelines given by the manufacturer. Techniques for heat-induced antigen retrieval were applied. After reaching operational temperature and pressure, the sections were placed in a pressure cooker containing 10 mmol/L Tris buffer, 1 mmol/L EDTA, and pH 9.0 for three minutes. Monoclonal Mouse Anti-Human BCL6 Protein (Dako USA) (code M7211) at a dilution range of (1:10-1:20) was used, incubated with the section for half an hour at room temperature. Next, 3% H2O2 was used to block the activity of endogenous peroxidase enzyme. Prior to applying the secondary antibody, the section was incubated with DAKO Mouse Linker again for a duration of 20 minutes.

Horseradish peroxidase (HRP) polymer solution (DAKO Real Envision Detection System) was used to detect the primary antibody. Tonsils exhibiting reactive lymphoid hyperplasia employed as an external control tissue.

Interpretation of BCL6 Expression:

Thirty percent of non-necrotic malignant B-lymphoid cells stained for BCL6 were considered a positive result; while less than thirty percent of non-necrotic tissue stained for BCL6 was regarded as a negative result [17]. The collected data were evaluated using the computer program (SPSS) version 26. Descriptive statistics were used to summarize demographic variables. The relationships between some clinicopathological parameters and expression of BCL6 in tumor cells were examined using the Chi-square tests. A P-value was considered statistically significant when it was equal to or less than 0.05.

RESULTS

Sixty-one DLBCL cases were comprised in the study sample (figure 1). The mean age (SD) of the studied sample was 55 (21.5) years and the median was (61 years). The patients' ages varied from (5 to 93 years) old. The most common age group was 60-69 years in 15 cases (24.59 %) (table 1). Gender distribution of the study sample revealed that 27 cases (44.3%) were female, and 34 cases (55.7%) were male. The ratio of M:F was 1.25:1. Of the cases, 44 (72.1%) had nodal sites, while 17 (27.3%) had extra nodal locations. BCL6 positive staining cases were 25 (41 %), while 36 (59 %) cases had negative staining (figures 2,3) (table 2). Table (3) shows the comparison of the study parameters with respect to BCL6. There were no statistically significant differences regarding age or presentations but it did show a statistically significant difference regarding gender (p value= 0.039).

Table (1): Distribution of the study sample according to age groups.

Average age	Frequency	Percentage
< 10	2	3.27 %
10-19	2	3.27 %
20-29	7	11.47 %
30-39	4	6.55 %
40-49	7	11.47 %
50-59	6	9.83 %
60-69	15	24.59 %
70-79	14	22.95 %
80-89	3	4.91 %
90-99	1	1.63 %

Table (2): The frequency of BCL6 expression.

IHC Marker	Expression	Total	
		No.	%
BCL6	Positive	25	41
	Negative	36	59

Table (3): Comparison of the study parameters in relation to BCL6.

		BCL6				p-value*
		Positive (n=25)		Negative (n=36)		
		No.	%	No.	%	
Age	< 60 years	14	56.0	14	38.9	0.187*
	≥ 60 years	11	44.0	22	61.1	
Gender	Males	10	40.0	24	66.7	0.039*
	Females	15	60.0	12	33.3	
Presentation	Nodal	19	76.0	25	69.4	0.574*
	Extra nodal	6	24.0	11	30.6	

*Chi square test has been used

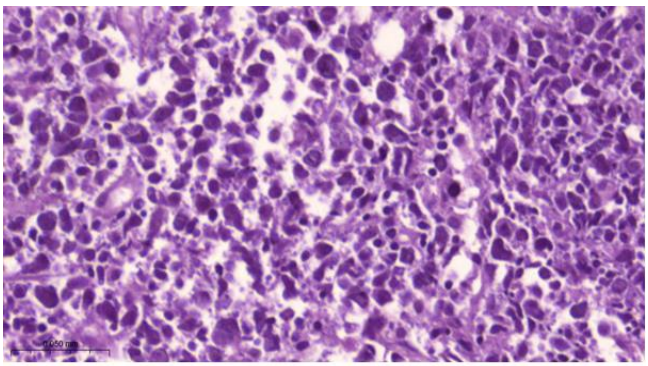

Figure 1: Diffuse large B cell lymphoma (H & E X 400).

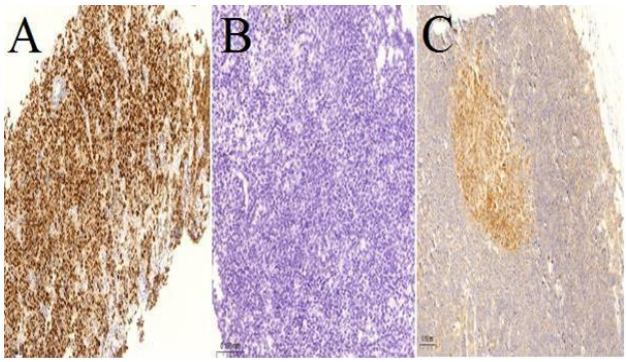

Figure 2: (A) DLBCL with positive BCL6 (nuclear stain) (IHC X 100). (B) DLBCL with negative BCL6 (IHC X 100). (C) BCL6 positive control (nuclear stain) (IHC X 100).

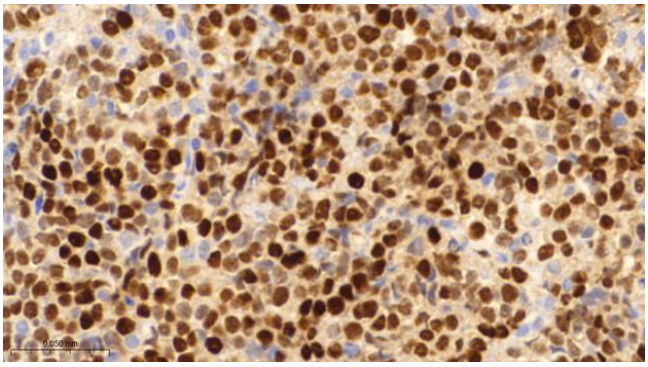

Figure 3: DLBCL with positive BCL6 (nuclear stain) (IHC X 400).

DISCUSSION

The most prevalent non-Hodgkin lymphoma subtype in adults is diffuse large B-cell lymphoma, exhibiting a wide range of histological and clinical characteristics, that mainly affects older patients [18,19]. Of the 61 patients with the condition submitted in the current study,a median age of (61y) was revealed, this finding corresponded to the study done in Slovenia by Boltežar et al., in which the median age was 62 years [20]. Furthermore "Diffuse large B-cell lymphoma is the most common lymphoma subtype, with a median age at diagnosis of 66 years" according to the National Cancer Institute of US (NCI, 2023) [21]. The gender distribution of the study sample revealed that male made up 55% of the sample, while female made up 44%., which is near the results observed in the study by Abu Sabaa et al in Sweden, where 56.6% of the patients were males and the median age was 64.6 years [22]. In contrary, Frauenfeld et al. Research in Western countries, found that the males constituted 40% of the study sample, while females constituted 60 % [23]. In terms of the presenting site, 72.1% of the patient sample had nodal presentations while extra-nodal presentations were present in 27.9% of the patient included in this study. In concordance with Huang et al. study in China , who found that out of 204 cases, 161 were present with nodal presentation [24]. Conversely, a study by Frauenfeld et al. in Western countries, revealed that 58% of cases indicated extra nodal presentation [23]. In the present study, BCL6 marker was assessed, it had been found to be expressed in 41% of the study sample, which is approximate to the results reported by Bajwa et al. in Pakistan , and Davies et al. in UK, who found that BCL6 was expressed in 37.5 % and 38 % respectively [25,26]. In the current study, BCL6 expression was associated with patients younger than 60 years with no statistically significant differences, these findings were run in parallel to findings of another study in Egypt showing BCL6 was significantly associated with patients below 45 years [27]. In the current study BCL6 expression is more frequent in females with diffuse large B cell lymphoma, with a significant association between BCL6 expression and female gender (p value= 0.39). The association of BCL6 with gender was reported by a study in Egypt conducted by Ahmed et al., in which BCL6 positive immunohisochemical stain was associated with female gender with (p=0.067) [28]. While the study conducted by Ting et al. in Malaysia, reported that rearrangement of BCL6 gene was more prevalant in female gender (70.5%, P value = 0.033) [29].

New studies from different regions show BCL6 has an important role in trophoblastic cells [30-32] and is upregulated in pre-eclamptic placenta [30,33,34], as well as in endometriotic lesions [35,36]. These results suggest BCL6 may have in the placenta and the endometrium, important physiological and pathological duties and this may explain the significant association between BCL6 and female gender in our study. The nodal presentation was the main site for the presentation of BCL6 positive in the current study and this agrees with a study in Egypt reported by Ahmed et al., [28].

CONCLUSION

BCL6 in patients with diffuse large B cell lymphoma revealed more frequent expression in females and it is associated with younger age less than 60 years. Nodal or extra nodal presentation sites of DLBCL show no association with BCL6 expression.

Conflect of Interest

No

Ethical Approval

The study was approved by the Research Committe of Nineveh Health Directorate with a research protocol (2023019) by Decision number 19 in 4/1/2023.

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