IJHOSCR

Original Article

International Journal of Hematology-Oncology and Stem Cell Research

Immunocytochemical Detection of BCL-2 Protein in Chronic B-Cell Lymphoproliferative Disorders

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Received: 06, Mar, 2024
Accepted: 30, May, 2024

ABSTRACT

Background: The expression of anti-apoptotic B-cell lymphoma 2 (BCL-2) protein in B-cell chronic lymphoproliferative disorders (B-CLPDs) can provide valuable prognostic information and assist in assessing minimal bone marrow (BM) infiltration. This study aimed to detect BCL-2 expression in B-CLPDs and correlate the findings with various clinicobiologic factors.

Materials and Methods: Immunocytochemical staining was performed on mononuclear cell smears from 46 Sudanese patients, including 25 with B-cell chronic lymphocytic leukaemia (B-CLL) and 21 with B-cell non-Hodgkin's lymphomas (B-NHL), who were enrolled during their visit to the Radiation and Isotope Centre and Fedail Hospital, Khartoum. Diagnosis was based on clinical examination, morphology, and immunophenotyping.

Results: Among the 46 B-CLPD cases, BCL-2 expression was identified in 13 (28.2%), including 8/25 (32%) cases with B-CLL and 5/21 (23.8%) with B-NHL. No statistically significant associations were found between BCL-2 expression and age, sex, total white blood cell count, disease stage, and serum lactate dehydrogenase levels (all P>0.05). However, BM involvement was significantly associated with BCL-2 expression (P=0.02).

Conclusion: The immunocytochemical staining method effectively detects BCL-2 protein in B-CLPDs, even in cases with minimal BM infiltration, thereby facilitating the correlation of this protein's expression with morphological and other clinicobiologic features. By combining cytologic morphology with immunocytochemistry, this technique enables earlier and more accessible evaluation of BM involvement.

Keywords: BCL-2; Immunocytochemistry; B-CL; B-lymphoma

INTRODUCTION

B-cell lymphoma 2 (BCL-2) is an anti-apoptotic regulator protein involved in modulating cell death (apoptosis) by either inhibiting it (anti-apoptotic) or, in cases of related proteins, inducing it (pro-apoptotic)[1,2]. BCL-2 and its related proteins play a crucial role in apoptosis control, influencing prognosis and response to therapy in various tumours, including resistance to cancer treatments. In high-grade B-cell lymphomas and diffuse large B-cell lymphoma the co-expression of BCL-2 and p53 has been linked to a poor prognosis[3-12]. This co-expression typically results from an imbalance in the homeostatic regulation of cell division and growth. The characteristic resistance to cell death observed in cancer can result from the overexpression of anti-apoptotic genes and the underexpression of pro-apoptotic genes. Independently, overexpression of anti-apoptotic BCL-2 protein in lymphocytes is not sufficient to cause cancer. However, when co-expressed with proto-oncogene myc (myelocytomatosis), it can result in aggressive B-cell malignancies, such as lymphoma[13]. A common genetic abnormality

observed in follicular lymphoma is the chromosomal translocation t(14;18), which places the BCL-2 gene adjacent to the immunoglobulin heavy chain locus, resulting in dysregulated transcriptional overexpression of BCL-2[14]. This overexpression decreases the propensity of affected cells to undergo apoptosis. BCL-2 gene abnormalities have been implicated in several malignancies, including chronic lymphocytic leukaemia (CLL)[13]. Owing to the complexity of bone marrow (BM) and haematopoietic cell histology, conventional histopathological and immunohistochemical staining techniques used to assess non-Hodgkin's lymphoma (NHL) based on stage and BM involvement often fall short diagnostically. Microinvasive lesions, a major source of lymphoma recurrence, may not be adequately diagnosed by these methods. A total of 25–35% of NHL cases currently staged as II/III may be more correctly classified as stage IV.

Currently, BM involvement in lymphoma is evaluated through morphological assessment of BM smears, particularly when variations in lymphoma cell morphology hinder accurate identification. According to World Health Organisation criteria, BM infiltration is defined when ≥5% of BM cells are naïve lymphocytes, while ≤1% is considered normal. Minimal BM infiltration is increasingly being evaluated based on BCL-2 protein expression, which may also reflect response to therapy.

Therefore, this study aimed to identify potential associations between BCL-2 expression and other clinicobiologic features in B-CLPDs. Understanding these associations may enhance the detection of minimal BM infiltrates in low-grade lymphomas and other mature B-cell neoplasms, thereby improving diagnostic accuracy and therapeutic monitoring.

MATERIALS AND METHODS

A hospital-based cross-sectional study was conducted at the Radiation and Isotope Centre and Fedail Hospital, Khartoum. Newly diagnosed Sudanese patients with B-CLPDs were enrolled, including 46 cases in total (21 with B-cell non-Hodgkin's lymphoma [B-NHL] and 25 with B-chronic lymphocytic leukaemia [B-CLL]). Diagnosis was based on clinical examination, morphology, and immunophenotyping markers assessed from blood, BM, and/or lymph node aspirates. Laboratory investigations, including a full blood count, biochemical profile, and standard immunophenotyping, were conducted at the centre as part of the diagnostic workup. Demographic and clinicobiologic data were obtained from patients' medical records. Written informed consent was obtained from each patient before enrolment. From each enrolled patient, 2 mL of venous blood was collected into ethylenediaminetetraacetic acid tubes. Multiple thin blood films were immediately prepared, fixed, and stored for further immunocytochemical staining of BCL-2. Additionally, blood samples from 10 healthy individuals were collected and used as controls to determine the cut-off for BCL-2 staining positivity.

Procedure

BCL-2 protein was assessed using an immunocytochemical kit, following the manufacturer's instructions. [15, 16] To interpret the staining results, five samples from healthy individuals were assessed for BCL-2 expression. For each patient sample, 100 lymphoid cells were examined based on leucocyte count, and the percentage of BCL-2-positive cells was recorded. Only definite and unambiguous staining was considered positive. The mean and standard deviation (SD) of the percentage of BCL-2-positive cells from the control samples were calculated. A patient sample was considered positive for BCL-2 expression if the percentage of positive cells exceeded the control mean by more than three SDs (mean + 3SD). Data were analysed using the SPSS software. Fisher's exact test was used to assess associations between BCL-2 expression and clinicobiologic parameters of the study participants.

RESULT

Among the 46 patients with B-CLPDs, including 25 with B-CLL and 21 with B-NHL, assessed for BCL-2 protein expression, 28 (60.8%) were male and 18 (39.2%) were female (Table 1). Their mean age was 57 years, range: 28–77 years. BCL-2 expression was identified as definite unambiguous staining of lymphoid cells upon immunocytochemical staining.

Among the 46 B-CLPD cases, positive BCL-2 staining was observed in 13 (28.2%), including 8/25 cases (32%) with B-CLL and 5/21 (23.8%) with B-NHL. No significant associations were found between BCL-2 expression and sex, age, disease stage, total white blood cell count, or serum lactate dehydrogenase levels (P=0.1, 0.2, 1.0, 0.6, and 0.4, respectively) (Table 2). However, a significant association was observed between BCL-2 expression and BM involvement (P=0.02). Specifically, BM infiltration by <5% naïve lymphocytes was observed in 9 (47.4%) of 19 B-CLPD cases with BM involvement. Additionally, a significant association was found with BCL/IgH gene rearrangement (detected in a previously unpublished study using the same patient samples) (P=0.02), with BCL-2 expression present in 100% of cases exhibiting the BCL/IgH fusion.

Table 1: Frequency of BCL-2 expression in B-CLPDs patients (N=46)

B-CLPDs	Cases No.	BCL-2 +ve (N=13)		BCL-2 –ve (N=33)	
		Male	Female	Male	Female
B-CLL	25	8	0	10	7
B-NHL	21	3	2	9	7
Total	46	11	2	19	14

Table 2: Association of BCL-2 and other clinicobiologic parameters

Parameters		Bcl-2 +ve (N=13	Bcl-2 -ve (N=33)	P
Sex	Males	11	19	0.1
	Females	2	14	
Age/Year	60<	4	18	0.2
	60>	9	15	
Disease stage	1+2	11	28	1.0
	3+4	2	5	
TWBCs	5 x10^3cmm<	2	3	0.6
	5 x10^3cmm>	11	30	
Serum LDH	Increased	12	25	0.4
	Normal	1	8	
BM involvement	Yes	9	10	0.02
	No	4	23	
T(14;18)	Yes	3	0	0.02
	No	10	33	

DISCUSSION

In haematological malignancies, recurrent structural cytogenetic abnormalities—most of which are balanced translocations—have significant diagnostic and prognostic implications[17]. The t(14;18) translocation, which results in overproduction of the BCL-2 protein, is commonly observed in B-NHL and B-CLL and can be detected using FISH. Detection of this translocation is valuable for monitoring therapeutic response and assessing minimal residual disease in the BM[18]. However, in patients with relapsed or treatment-resistant disease, post-induction BCL-2/IgH status is not considered useful for guiding subsequent treatment decisions[19].

The study results revealed that BCL-2 protein was present in the peripheral lymphoid cells of 28.2% (13/46) of B-CLPD cases, as detected by immunocytochemistry. The expression level of the BCL-2/IgH fusion correlates with disease status, being low in patients in remission and high in newly diagnosed or relapsed cases. This study supports this pattern, as all included cases were newly diagnosed, and the majority of BCL-2-positive cases (84.6%, 11/13) were at early disease stages (I/II). These findings suggest that BM and peripheral blood are suitable sites for detecting BCL-2 expression. Patients with BM-infiltrating B-lymphoma cells showed a higher proportion of BCL-2-positive cells compared to those without BM involvement, aligning with a recently published study that used LightCycler Polymerase Chain Reaction[20]. BCL-2 protein is frequently detected during staging in both blood and BM, even in Stage 1 follicular lymphoma[21]. The t(14;18) translocation is more commonly observed in patients with histological grade 1 or 2 tumours compared to those without the translocation. Other studies have also shown that unlike BCL-2-negative disease, BCL-2-positive disease tends to occur in younger patients[22]. However, in the present study, no significant difference in age at diagnosis was found between BCL-2-positive and -negative cases.

In our B-CLPD cases, BCL-2 protein expression (assessed by immunocytochemistry) showed a significant correlation with the presence of the BCL-2/IgH rearrangement (previously assessed by FISH in the same patient cohort), as 100% of the cases with BCL-2/IgH rearrangement were positive for BCL-2. However, 23.2% (10/43) were positive for BCL-2 protein but negative for BCL-2/IgH rearrangement. This finding should be interpreted with caution because of the small sample size of the study.

However, we demonstrated that immunocytochemistry is a reliable technique for detecting BCL-2 expression and can be effectively applied to slides containing abnormal cells following morphological evaluation.

CONCLUSION

BCL-2 expression was detected in cases of B-CLPDs, with higher frequency in patients at early disease stages. A greater number of BCL-2-positive cells was observed in cases with BM infiltration. Therefore, BCL-2 expression may be valuable in assessing BM involvement and predicting BCL-2/IgH fusion status.

Financial disclosure statement

The authors received no financial support for this research.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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