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Immunohistochemical expression of BCL-2 oncogene in chronic myeloid leukemia in Iraqi patients

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Abstract:

BACKGROUND: Chronic myeloid leukemia (CML) occurs when a pluripotent stem cell undergoes malignant transformation and clonal myeloproliferation, leading to a striking overproduction of immature granulocytes. It accounted for about 15% of all adult leukemias and can strike at any age but rare in childhood. Many studies revealed a significant role of antiapoptotic B-cell lymphoma-2 oncogene (Bcl-2) in disease progression.

OBJECTIVES: The aim of this study was to assess Bcl-2 expression across CML phases and its diagnostic value through immunohistochemistry compared to conventional morphology.

MATERIALS AND METHODS: A cross-sectional retrospective study of Bcl-2 expression in histological sections of bone marrow trephine biopsies of randomly selected 60 patients diagnosed with CML with age ranged between 14 and 81 years and presented with different clinical phases (30 with chronic phase, 15 with accelerated phase and other 15 with blastic transformation) which stained immunohistochemically for BCL-2 protein (Dakopatts Corporation) and evaluated by a hemopathologist.

RESULTS: The expression of the antiapoptotic protein Bcl-2 differs significantly with different clinical phases of CML and increases with more advanced stages. Bcl-2 oncoprotein expression increases significantly with high bone marrow blast percentage.

CONCLUSIONS: Immunohistochemistry is a useful method for the detection of Bcl-2 oncoprotein. There is a relatively great relationship between Bcl-2 oncoprotein expression with high bone marrow blast count and advanced stages of the disease.

Keywords:

B cell lymphoma-2 oncogene, chronic myeloid leukemia, immunohistochemistry

Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative neoplasm featured with uncontrolled proliferation of myeloid cells.[1] It accounts for approximately 15% of newly diagnosed cases of leukemia in adults[2] and typically affects older adults and rarely occurs in children, though it can occur at any age.[3] CML was the first neoplastic disease found to be associated with a well-defined genotypic anomaly.[4] It is characterized by a balanced genetic translocation, t(9;22) (q34; q11.2), involving a fusion of the ABL1 gene from chromosome 9q34 with the BCR gene on chromosome 22q11.2. This rearrangement is known as the Philadelphia chromosome. The molecular consequence of this translocation is the generation of a BCR-ABL1 fusion oncogene, which in turn translates into a BCR-ABL1 oncoprotein.[2] In many cases, CML is diagnosed accidentally in routine blood tests with leukocytosis and no other symptoms.[5] CML progresses into stages termed chronic phase (CP-CML), accelerated phase (AP-CML), and blast

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phase (BP-CML).[6] The global standard therapy for CML is tyrosine kinase inhibitors (TKIs). One of the causes of therapeutic resistance to some TKIs corresponds to point mutations in the breakpoint cluster region - abelson murine leukaemia viral oncongene homolog 1 (BCR-ABL1) fusion gene. Allogeneic hematopoietic cell transplantation is a treatment option for high-risk CML, including TKI resistance.[7] Apoptosis, programmed cell death, has a central role in developmental biology and in maintaining the equilibrium of renewing tissues.[8] The B-cell lymphoma 2 (BCL-2) protein family critically controls apoptosis by regulating the release of cytochrome c from mitochondria.[9] Through a variety of ways, overexpression of BCL-2 is a frequent occurrence that protects tumor cells against apoptosis.[10] BCL-2 is a key survival factor for CML stem/progenitor cells and that combined inhibition of BCL-2 and BCR-ABL tyrosine kinase has the potential to significantly improve depth of response and cure rates of CML.[11] The study aims to investigate Bcl-2 expression covering different phases of CML and to evaluate the value of immunohistochemical expression of the Bcl-2 oncoprotein in CML and compare that with the conventional morphological study.

Materials and Methods

A cross-sectional retrospective study of immunohistochemical staining for Bcl-2 expression in histological sections of bone marrow trephine biopsies of 60 patients diagnosed with CML. The study was conducted at the laboratory department, Hematology and bone marrow transplantation center, Medical City Complex; over a period between November 2022 and May 2023.

Patients were selected according to the followings:

Inclusion criteria

(1) All the cases that had bone marrow study in the period from the January 1, 2019, to the December 31, 2021, and have been diagnosed as CML by senior hematopathologist were collected. (2) From a total of (78) cases with CML in chronic phase, (30) cases have been chosen randomly, all the cases that were diagnosed as an accelerated phase (15 cases) and all the cases with blastic phase (15 cases) have been taken to form a total of 60 patients with CML to be included in this study.

Exclusion criteria

(1) All the cases with repeated (8 cases) or inadequate bone marrow trephine biopsies (5 cases) or deficient in clinical data (7 cases) were excluded. (2) All the cases with deficient clinical data (7 cases) were not included.

Another ten bone marrow trephine biopsies obtained from 10 subjects that do not have malignant conditions but have iron deficiency and megaloblastic anemia were used as normal controls (to obtain cut off value). Bone marrow trephine biopsies with chronic lymphocytic leukemia were used as positive controls for Bcl-2 oncogene while negative controls were achieved by omitting the primary antibodies for both markers, and those were used with each run. The patients' clinical information (age, sex, disease phase, and clinical features) with hematological studies (full blood count, peripheral blood smear findings, bone marrow aspiration findings, and bone marrow trephine biopsy findings) were carefully documented from the patients' laboratory records. All the previously stained slides with (H and E) stains were re-examined, and the diagnoses of these cases were confirmed by the histological examination of them before further processing for immunohistochemical staining.

B cell lymphoma 2 immunohistochemical staining

The procedure steps done according to the manufacture list supply with noting not letting the slides dry: Tissue sections of 5 μm in thickness were mounted onto charged slides, deparraffinized, rehydrated then antigen retrieval was done, primary antibody and 3,3'-Diaminobenzidine (DAB) (as chromogen), and Mayer's hematoxylin were added in sequence to be examined under light microscope later in different magnification powers. Only cells exhibiting a red–brown cytoplasmic staining for Bcl-2 oncoprotein were counted as positively stained cells. The intensity of staining of the red–brown coloration was considered negative if there is no staining at all even with magnification power of 100, weakly stained with score 1 if only can be detected at magnification power of 40, moderately stained and scored 2 if it was detected with difficulty at magnification power of 10 and it is considered high if it was detected very clearly at magnification power of 10 and scored 3. The extent of Bcl-2 positivity was interpreted as negative with either complete absence of staining or in cases with staining in <10% of nonerythroid cells (the cut off value that obtained from normal controls) on oil immersion, it interpreted as low expression with score 1 if seen in 10% up to 25%, moderately expressed with score 2 for staining in 25% up to 50%, highly expressed with score 3 for staining in 50% up to 75%, and a very high expression with score 4 when more than 75% of nonerythroid cells showed reddish cytoplasmic staining on oil immersion. Total score (TS) of expression equals the summation of the intensity score and the percentage score. TS ranged from 0 to 7, with TS of 3 or more is considered positive. This study was ethically approved by the scientific committee of the Hematology and bone marrow transplantation center, Medical City Complex, under the approval no. 27 on October 19, 2022.

Statistical analysis

All results were tabulated and calculated using the Statistical Package for the Social Sciences (SPSS) 24.0

software (IBM Corp., Armonk, N.Y., USA). Statistics were analyzed by means of Chi-square test, median, mean, standard deviation (SD), range, frequencies, and percentages. $P < 0.05$ were considered statistically significant.

Results

Among sixty patients with CML, there were 31 (51.7%) males and 29 (48.3%) females with a median age of 40 years, the age at diagnosis ranged between 14 and 81 years; the mean age was 41.6 years, and SD of ± 16.8 years. Packed cell volume (PCV) % ranged from 10% to 43% and had a mean of 27.0%. In contrast to the WBC count, it ranged from 0.200 to 1000.000 × 10^9/L, with a mean of 144.898 × 10^9/L. Platelet count ranged from 10.000 to 1000.000 × 10^9/L and showed a mean of 281.181 × 10^9/L. In the chronic phase, positive Bcl-2 staining was shown in only 9 out of 30 cases (30%). In the accelerated phase, positive Bcl-2 staining was shown in 9 out of 15 cases (60%), while all cases with blastic phase (100%) showed positive Bcl-2 staining. These results have a high statistically significance, $P < 0.001$ [Table 1 and Figure 1].

Positive Bcl-2 staining was reported in all cases with bone marrow aspirate blasts >20%, whereas negative Bcl-2 staining was seen in 21 out of 30 cases (70%) with bone marrow aspirate blasts <10% carries a high statistically significance [Table 2].

Discussion

Our sixty patients had a presenting age ranged between 14 and 81 years with a median age of 40 years, and a mean age of 41.6 years. This was to some extent similar with the findings of another study in 2021,[3] with a median age of 38.5 years which is expected as the CML is a disease of middle age and early elderly. Our patients had a mean PCV% of 27.0% and a mean WBC of 144.898 × 10^9/L, this was similar to another study in 2021,[12] where the mean PCV % and WBC were 30% and 143.100 × 10^9/L, respectively, whereas, the WBC of our patients ranged from (0.200 to 1000.000 × 10^9/L) unlike the study done in 2024,[13] with a range reported from (1.300 to 175.000 × 10^9/L). The PCV % of our patients ranged from (10% to 43%), and this carries a little difference from the same previous study,[13] where the range was from (12% to 51%). Platelet count mean in the present study was 281.181 × 10^9/L with a range from (10.000 to 1000.000 × 10^9/L), and this is much lower than what was documented in a study published in 2019,[14] where the mean was 438.000 × 10^9/L and the range was from (118.000 to 1090.000 × 10^9/L). The peripheral blood blasts percentage mean of our patients was 11% with a range from (0% to 94%) in the all three phases, and this is much higher than reported in another study done in 2016,[15] with a peripheral blast percentage mean of 1.4% and another study[13] showed a peripheral blast percentage mean of 3.5% and a range from (0% to 26%). All these results can be explained by the late presentation and diagnosis of our patients, with more advanced stages also could be due to the selection method of our patients. In the current study, we

Table 1: Relationship between disease phase and B-cell lymphoma-2 oncogene staining results

Phase	Bcl-2 positive, *n* (%)	Bcl-2 negative, *n* (%)	Total
Chronic	9 (30)	21 (70)	30 (100)
Accelerated	9 (60)	6 (40)	15 (100)
Blastic	15 (100)	0	15 (100)

Bcl-2=B cell lymphoma-2 oncogene

Table 2: Relationship between bone marrow aspirate blast percentage with B B-cell lymphoma-2 oncogene staining

Bone marrow blasts (%)	Bcl-2 positive, *n* (%)	Bcl-2 negative, *n* (%)	Total, *n* (%)
<10 (CP)	9 (30)	21 (70)	30 (100)
10–19 (AP)	9 (60)	6 (40)	15 (100)
≥20 (BP)	15 (100)	0	15 (100)

P<0.001. Bcl-2=B-cell lymphoma-2 oncogene, CP=Chronic phase, AP=Accelerated phase, BP=Blast phase

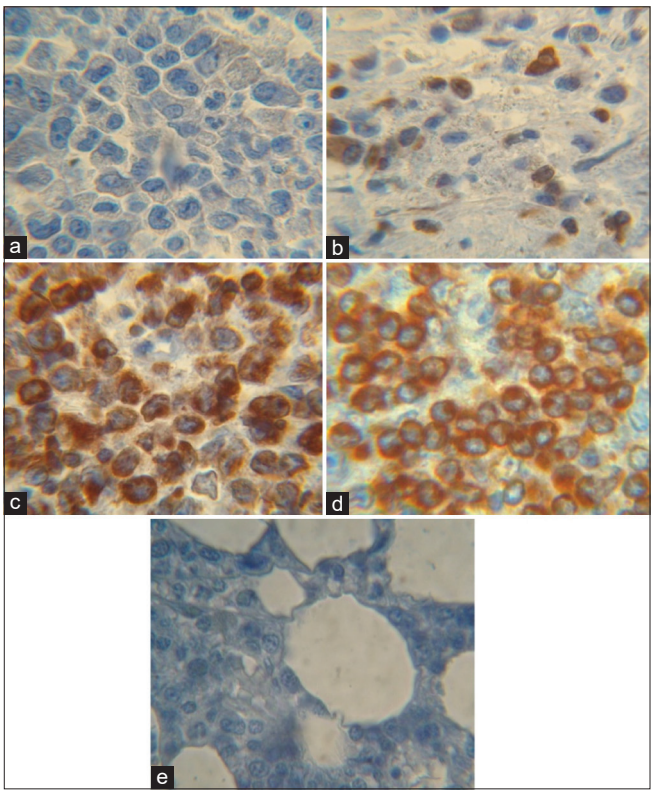

Figure 1: Bone marrow biopsies, immunohistochemical staining for B cell lymphoma-2 (BCL-2), power of ×100. (a) Chronic phase of chronic myeloid leukemia (CML); negative for BCL-2. (b) An accelerated phase of CML; positive for BCL-2. (c) Blastic phase of CML; positive for BCL-2. (d) Positive control (chronic lymphocytic leukemia). (e) Negative control

observed that the expression of the anti-apoptotic Bcl-2 oncoprotein had a positive relationship with advanced stages and differed significantly with different clinical phases of CML ($P < 0.001$), and this is parallel to findings of a previous study,[15] also with a high statistically significance ($P = 0.005$). The correlation of the expression of Bcl-2 and blast percentage in bone marrow at the same time carried a remarkable statistically significance in our study with a P value of 0.001 which is slightly close to what was documented in a study in 2011[16] with $P = 0.0172$. This goes with the suggestion that the expression of the antiapoptotic Bcl-2 oncoprotein increases with the evolution of the disease.

Conclusions

Immunohistochemistry is a valuable tool for detecting Bcl-2 oncoprotein expression, which shows a strong correlation with elevated bone marrow blast counts and advanced stages of CML. Incorporating immunohistochemical analysis into routine diagnostic protocols is recommended for accurate disease classification and prognostic assessment. The high prevalence of Bcl-2 expression among Iraqi CML patients highlights the need for further studies to explore underlying genetic, environmental, and etiological factors. In addition, these findings support the development of targeted therapies against Bcl-2 and the consideration of more intensive treatment approaches for patients exhibiting abnormal expression levels.

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Conflicts of interest
There are no conflicts of interest.

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