The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89 (2), Page 6239- 6246

Relationship between C-MYC, BCL-2 and BCL-6 Genetic Abnormalities and Bone Marrow Biopsy in B-Non-Hodgkin Lymphoma

Hanan M.Bedair, Olfat .M Hendy, Mona Wahba, Yasmin Mohsen, Soha Abuelela
Department Clinical and Chemical Pathology, National Liver Institute, Menoufia University, Egypt
***Corresponding author:** Yasmin Mohsen, **E-Mail:** yasooo250@gmail.com, **Mobile:** (+20) 01005047478

ABSTRACT

Background: C-MYC, BCL2 and BCL6 genes are the most commonly involved oncogenes detected in B-non-Hodgkin's lymphomas (B-NHLs). Histopathology is the best method used to diagnose B-NHLs. However, the cytological analysis of bone marrow smears and imprints could provide a chance for a precise diagnosis of these disorders.

Objective: This study aimed to evaluate bone marrow findings as an integral part of the staging workup in B-NHLs and to study possible relationship to C-MYC, BCL-2, and BCL-6 gene abnormalities.

Patients and methods: This study was conducted as a cross-sectional study that included 51 adult B-NHL patients. Each patient underwent careful history assessment, clinical examination, laboratory tests (e.g. complete blood count, BM smears, BM biopsy, lymph node biopsy, immunophenotyping on bone marrow aspirates by flowcytometry and interphase FISH dual color break-apart probes of C-MYC, BCL2 and BCL6 on bone marrow aspirates).

Results: Results of lymph node biopsy histopathological examination revealed that, FL, DLBCL, and MCL were diagnosed in 43.1%, 39.2% and 17.6% of them respectively. Among 51 B-NHL cases, there were 35.29%, 31.37% and 23.53% positive cases for C-MYC, BCL2 and BCL6 respectively. Concurrent genetic aberrations showed double expression. of C-MYC and BCL2 constituted 17.6% and triple expression of C-MYC, BCL2 and BCL6 that constituted 7.8% of the cases. However, the FISH positive cases were more frequent in MCL than FL and DLBCL. The BCL2 was more significantly associated with FL subtype and BCL6 with DLBCL subtype.

Conclusion: C-MYC, BCL2 and BCL6 gene aberrations frequently occurred in B-NHLs and could be considered as independent prognostic factors that carry different impacts on BM marrow biopsy finding and BM infiltration which affect clinical outcome of patients.

Keywords: BCL2, BCL6, C-MYC, B-Non-Hodgkin Lymphoma, FISH, Bone marrow.

INTRODUCTION

B-NHLs are a diverse group of immune system malignancies that can affect any organ in the body. They have a wide range of presentations that range from mild to extremely aggressive clinical behavior [(1)]. Histopathology is the best method used to diagnose B-NHLs. However, the cytological analysis of bone marrow smears and imprints could provide a chance for precise diagnosis of these disorders [(2)].

Bone marrow (BM) aspirate along with BM trephine biopsy specimens are usually complementary to each other, but bone marrow biopsy is critical and more diagnostic in hematological disorders with focal infiltration as in lymphomas and in bone marrow metastasis. Many patterns of bone marrow infiltration in B-NHLs are documented and occur in any one or a combination of five different patterns: focal random, focal para trabecular, interstitial, diffuse, and intrasinusoidal [(3)].

C-MYC functions as a transcriptional factor in normal cell behavior to control cell cycle progression from G1 to S phase beside the terminal differentiation inhibition. The C-MYC translocation t (8;14) (q24;q32) lead to MYC expression abnormalities. C-MYC over-expression in healthy cells makes them more susceptible to different apoptotic triggers, which makes them resistant to cell death and causes cancer[(4)].

A crucial part of the cell apoptosis cycle is played by BCL2, a protein belongs to the BCL family. BCL2 functions as a pro-survival protein by inhibiting apoptotic cell death and protecting cells from a wide variety of cytotoxic stimuli, including UV radiation and cytokine deficiency. BCL2 translocation, t (14;18) (q32;q21), results in its overexpression, which make the cell resists dying [(5)].

When it comes to the growth of B-cell germinal centers, BCL6 is essential. In the regulation of cell cycle, proliferation and differentiation, apoptosis, and DNA damage response, it serves as a transcriptional repressor. Loss of the normal controls leads to over BCL6 expression that results in a lymphoproliferative condition that resembles diffuse large B cell lymphoma (DLBCL) [(6)].

Over the past 30 years, improvements in cytogenetic methods have increased the sensitivity of chromosome abnormalities' identification. A revolution in cytogenetic analysis and significant advancements in the diagnosis and study of haematological malignancies were particularly brought about by the development of FISH in the late 1980s [(7)].

The goal of FISH is to locate specific nucleotide sequences in tissues, metaphase spreads, or cell nuclei. FISH works by annealing tagged probes into complementary sequences in situ on histological specimens as well as samples ready for karyotyping [(8)].

The aim of this study was to evaluate bone marrow findings as an integral part of staging workup

Received: 14/06/2022
Accepted: 22/08/2022

in B-NHL and to study possible relationship to C-MYC, BCL-2, and BCL-6 gene abnormalities.

PATIENTS AND METHODS

The present study was conducted as a cross-sectional study that included 51 adult B-NHL patients presented to out-patient clinic and in-patient unit of internal medicine (hematology/oncology) department ,Ain shams Hospitals and National Liver Institute in the duration from May 2020 to the end of September 2021.

Inclusion criteria: Age more than 18 years and cases diagnosed as B–NHLs types by BM/or LN biopsy and/or IPT.

Exclusion criteria: Age less than 18 years, cases of Hodgkin lymphoma and cases of T cell and natural killer (NK) neoplasms.

All patients were subjected to the following:
Careful history assessment, clinical examination, chest X-ray, abdominal ultrasound and/or CT scan for detection of organomegaly or abdominal lymph node involvement.

Laboratory methods: Two ml of venous blood were aseptically collected from all patients into K-EDTA vacutainer tube for CBC on Sysmex xn1000 cell counter (Sysmex Corp, USA) and differential counting by Leishman stain. Two ml of BM aspirate were collected, one mL was put on K-EDTA for multicolor direct flow cytometry using Beckman Navios flowcytometer (Coulter Electronics, Hialeah, FL, USA). The remaining one mL of BM aspirate was placed in a sterile, lithium heparin-coated vacutainer tube devoid of preservatives so that it could be utilised for culture, karyotyping, and FISH. BM trephine biopsy was performed for all patients, a core of about 1-2 cm was drawn and dispensed into 2 mL fixative solution for histo-pathological evaluation of paraffin-embedded core biopsy.

Cytogenetic analysis by fluorescent in situ hybridization (FISH). Analysis was done using fluorophore-labeled locus specific identifier (LSI). Break-apart rearrangements probe for C MYC (8q24), BCL2 (18q21) and BCL6 (3q27) was applied to BM aspirate samples (Vysis, Inc., Downers Grove, USA). Slide examination of cytogenetic analysis where every case used a fluorescence microscope with an oil immersion objective to scan the majority of accessible metaphases and at least 100 interphases for the identification of C-Myc, BCL2 and BCL6 break-apart rearrangement.

Procedure of FISH technique: Preparation of culture media: (RPMI 1640 (100 mL bottle) + 20 mL FCS + 400 μL penicillin + 200 μL streptomycin + 200 μL amphotrecin) for each case, a sterile culture tube was set up under laminar air flow to avoid any contamination, containing: • 5 mL of the previous mixture. • 250 μL of BM sample. • 200 μL PHA. Briefly, immediately after heparinized sample withdrawal, for each case, a sterile culture tube was set up under laminar air flow to avoid any contamination, containing 5 mL of the previous mixture + 250 μL of BM sample + 200 μL PHA), then the tube was then covered tightly, mixed and incubated at 37°C for 72-120 hours.

Harvesting:

50 μL of colcemid were added to the tube at the end of the incubation time then the tube was mixed and incubated at 37 °C for 1 hour. After that the tube was then centrifuged at 800-1000 rpm for 10 minutes. After centrifugation most of the supernatant was discarded leaving only 0.5 mL to resuspend the cell pellet in hypotonic solution treatment was done by adding pre-warmed 5-8 mL of 0.56% KCl solution, the tube was then incubated at 37°C for 20 minutes. This was followed by centrifugation at 800-1000 rpm for 10 minutes and the supernatant was discarded. And 10 mL of freshly prepared chilled fixative were added to the cell pellet with gentle mixing. Finally, the tube was centrifuged at 800-1000 rpm for 10 minutes and the supernatant was discarded, the previous 2 steps were repeated until the supernatant became clear and the yielded cell pellet was suspended in 5 mL fresh chilled fixative then stored at -20 °C till the step of slide preparation.

Slide preparation:

The glass slides were washed with water and detergent, soaked in methanol, then placed in DW and kept in the refrigerator ready to use (clean, cold, wet slides). The tube containing the cell pellet was removed from -20 °C, tilted gently and centrifuged at 800-1000 rpm for 10 minutes. The supernatant was discarded and 0.5 mL of freshly prepared chilled fixative was added to resuspend the cell pellet then 1 or 2 drops of cell suspension were added and the slides were then tilted away from their ends to allow proper spreading of cells. At least 2 slides were prepared for each tube then were left to dry in air. Finally, they were evaluated under the light microscope for the presence or absence of metaphases. And then kept in a dry, clean closed slide box to protect from dust and to age for at least 48 hours at room temperature.

Hybridization with probe:

After probe preparation the probe mixture was applied on each slide at the marked area and immediately covered with a glass coverslip to avoid dryness of the probe and the perimeter of the glass coverslip was sealed to the slide by a thick layer of rubber cement. The sample DNA and the probe were co-denatured by leaving the slides in hot air oven at 73

°C for 5 minutes and the slides were then incubated at 37 °C in a pre-warmed humidified chamber for overnight to allow probe hybridization. NB: Two healthy subjects (1 male & 1 female) with normal karyotyping were used as a control group for each probe used .After that slides were washed and counter staining was done to be examined.

Slides examination: Most of available metaphases and at least 100 interphases were scanned in every case under the fluorescence microscope using oil immersion objective for the detection of c-Myc, BCL2 and BCL6 break-apart rearrangement.

In negative cases: Two red/green (yellow) fusion signals were seen representing the two copies of gene (Figure 1).

In positive cases : One of the fusion signals was splitted apart resulting in one red and one green signals, in addition to 1 red/green (yellow) fusion signal (Figure 2)

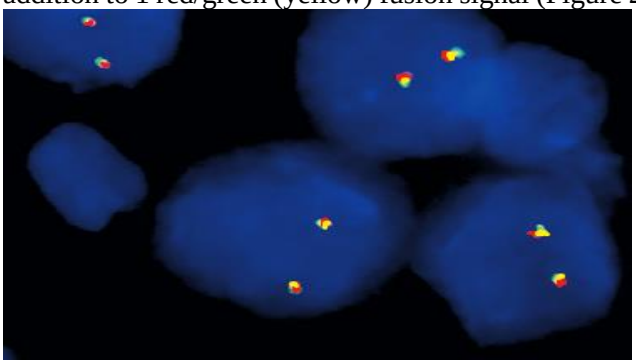

Figure (1): Interphase FISH analysis showing negative result for BCL2 rearrangement, 2 yellow signals (Juxtaposed red and green signals) using Vysis LSI BCL2 Dual Color Break Apart Rearrangement Probe.

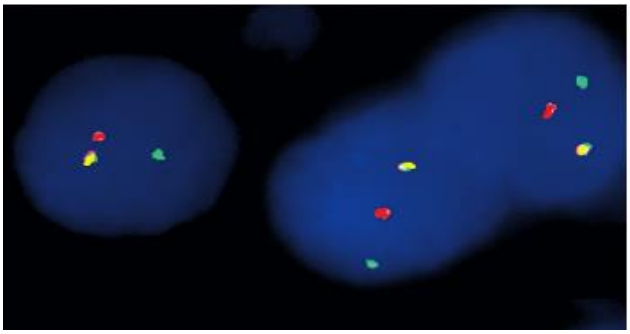

Figure (2): Interphase FISH analysis showing positive result for BCL2 rearrangement, 1 yellow signal (Juxtaposed red and green signals), 1 red and 1 green signals using Vysis LSI BCL2 Dual Color Break Apart Rearrangement Probe.

At the end of induction therapy, the effectiveness of the treatment was evaluated. Complete remission (CR) defined as the absence of all physical and radiographic signs of lymphoma for at least 4 weeks following systemic chemotherapy and/or radiation [(9)]. Additionally, in order to be classified as complete responders, individuals with initial bone marrow involvement had to have clearance (as determined by repeat aspiration and biopsy). Patients in partial remission are defined as no new lesions developing and a 50% or more reduction in the size of detectable lesions. Resistant cases are defined as those who did not respond to the chemotherapy regimen used, they were considered non-responders [(10)]. Relapse was determined as the recurrence of lymphoma in patients who had been in CR for at least 4 weeks [(9)].

Ethical consent:

An approval of the study was obtained from Menoufia University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 20 for Windows® (IBM SPSS Inc., Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test (χ^2) was used to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± Standard deviation. Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). P value < 0.05 was considered significant.

RESULTS

The studied patients included 37 (72.5%) males and (14) 27.4% females with male to female ratio 2.6:1. Their ages ranged from 20 to 77 years with a mean age of 45.9 ± 15.8 years. There were 22 of follicular lymphoma (FL) cases (43.1%), 20 of diffuse large B cell lymphoma (DLBCL) cases (39.2%), and 9 Mantel cell lymphoma (MCL) cases (17.6%). Bone marrow involvement was seen in 39 (76.5%) cases. Most common pattern of involvement was diffuse 17 (33.3%) cases, followed by nodular 10 (19.6%) cases, interstitial 5 (9.8%) and patchy 4 (7.8%) cases (Table 1).

Table (1): Demographic and clinical characteristics of B-NHL cases

Demographic and clinical characteristics	B-NHL (n=51)
Age (years)	
Mean ± SD	45.9 ± 15.8
Range (min-max)	20 - 77
Gender [n (%)]	
Male	37 (72.5)
Female	14 (27)
LN biopsy [n (%)]	
DLBCL	20 (39.2)
Follicular	22 (43.1)
Mantle	9 (17.6)
BM biopsy (Infiltration pattern) [n (%)]	
1. Infiltrative	*39 (76.5)*
a) Diffuse	17 (33.3)
b) Other patterns:	22 (43.1)
Nodular	10 (19.6)
Interstitial	5 (9.8)
Patchy	4 (7.8)
Paratrabecular	2 (3.9)
Mixed	1 (1.9)
2. Non-infiltrative	*12 (23.5)*

Among 51 B-NHL cases, there were 25 (49%) cases positive for routine FISH probes as follows: 14 (27.45%) cases positive for t (14:18), 9 (17.65%) cases positive for t (11:14) and only one case (1.96%) was positive for each of del17p and 14q rearrangement. C-MYC positive cases constituted 35.2% of cases, while 31.3% and 23.5% of cases were positive for BCL2 and BCL6 respectively. There were some of the cases found to have concurrent genetic aberrations through FISH analysis. The cases that showed double expression (C-MYC+/BCL2+) constituted 17.6% and those showed triple expression (C-MYC+/BCL2+/BCL6+) constituted 7.8% of the cases (Table 2).

Table (2): Expression of BCL2, BCL6 and C-MYC genetic aberrations

Routine FISH findings [n (%)]	
Positive	***25 (49)***
t(14'18)	14 (27.45)
t(11'14)	9 (17.65)
del 17p	1 (1.96)
14q rearrangement	1 (1.96)
Negative	***26 (51)***
Genetic alterations	**B-NHL (n=51)**
C-MYC [n (%)]	
Positive	18 (35.2)
Negative	33 (64.7)
BCL2 [n (%)]	
Positive	16 (31.3)
Negative	35 (68.6)
BCL6 [n (%)]	
Positive	12 (23.5)
Negative	39 (76.4)

The FISH positive cases were more significantly higher in MCL than in FL and DLBCL, which were found in 100% of MCL cases. Regarding BCL2 and BCL6 gene rearrangement, BCL2 was more significantly frequent in FL subtype than other subtypes (DLBCL and MCL). However, BCL6 was significantly frequent in DLBCL subtype. But, C-MYC was not significantly different among three subtypes **(**Table 3).

Table (3): The frequency of C-MYC, BCL2 and BCL6 gene aberration among B-NHL subtypes

Genetic alterations	DLBCL (n= 20)	FL (n= 22)	MCL (n= 9)	Significance test	P-value
Routine FISH findings [n (%)]				χ^2= 25.19 [b]	**<0.001**
Positive	2 (10.0)	14 (63.6)	9 (100.0)		
Negative	18 (90.0)	8 (36.4)	0 (0.0)		
C-MYC [n (%)]				χ^2= 0.98 [a]	0.635
Positive	7 (35.0)	9 (40.9)	2 (22.2)		
Negative	13 (65.0)	13 (59.1)	7 (77.8)		
BCL2 [n (%)]				χ^2= 10.80 [a]	**0.004**
Positive	4 (20.0)	12 (54.5)	0 (0.0)		
Negative	16 (80.0)	10 (45.5)	9 (100.0)		
BCL6 [n (%)]				χ^2= 8.24 [b]	**0.013**
Positive	9 (45.0)	3 (13.6)	0 (0.0)		
Negative	11 (55.0)	19 (86.4)	9 (100.0)		

[S]: Significant at P-value <0.05 a: Pearson Chi-square test [NS]: Non-significant at P-value ≥ 0.05 b: Fisher's Exact test [HS]: Highly significant at P-value <0.01

Regarding response to therapy, complete remission (CR) was significantly higher in cases with no BM infiltration pattern (p value <0.001). Moreover**,** the diffuse pattern of BM infiltration was significantly associated with the presence of C-MYC, BCL2 and BCL6 (P-value 0.002, 0.22 and <0.001 respectively) (Table 4**)**.

Table (4): Comparison of pattern of BM infiltration with genetic alterations and response to therapy

Data	Diffuse (n= 17)	Other patterns (n= 22)	No infiltration (n= 12)	Significance test	p-value
Response to therapy [n (%)]				χ^2= 17.40 [a]	**0.001**
PD	8 (47.1)	8 (36.4)	0 (0.0)		
PR	8 (47.1)	9 (40.9)	3 (25.0)		
CR	1 (5.9)	5 (22.7)	9 (75.0)		
C-MYC [n (%)]				χ^2= 10.68 [a]	**0.002**
Positive	10 (58.8)	8 (36.4)	0 (0.0)		
Negative	7 (41.2)	14 (63.6)	12 (100.0)		
BCL2 [n (%)]				χ^2= 7.17 [a]	**0.022**
Positive	7 (41.2)	9 (40.9)	0 (0.0)		
Negative	10 (58.8)	13 (59.1)	12 (100.0)		
BCL6 [n (%)]				χ^2= 16.22 [b]	**<0.001**
Positive	10 (58.8)	2 (9.1)	0 (0.0)		
Negative	7 (41.2)	20 (90.9)	12 (100.0)		

[S]: Significant at P-value <0.05; a: Fisher's Exact test; PD: progression of disease [HS]: Highly significant at P-value < 0.01 b: Chi--square test PR: partial response; CR: complete response.

There was no statistical significant association between either co-expression of C-MYC+ and BCL2+ and each of age, gender, LN pathology, pattern of BM infiltration or response to therapy. Regarding triple expression there were 4 cases harbouring rtiple expression of C-MYC,BCL2 and BCL6 ,but not mentioned in tables due low sample size of these cases (Table 5).

Table (5): Comparison between the double expression (C-MYC, BCL2) and various studied parameters

Characteristics	Double expression (C-MYC+/BCL2+)		P-value and Significance
	Positive (n= 9)	Negative (n= 42)	
Age (years)			0.570
Mean ± SD	48.67 ± 13.65	45.32 ± 16.33	
Range (min-max)	31.00 - 75.00	9.50 - 77.00	
Gender [n (%)]			0.692
Male	6 (66.7)	31 (73.8)	
Female	3 (33.3)	11 (26.2)	
LN biopsy [n (%)]			0.079
DLBCL	2 (22.2)	18 (42.9)	
Follicular	7 (77.8)	15 (35.7)	
Mantle	0 (0.0)	9 (21.4)	
Pattern of infiltration [n (%)]			0.122
Diffuse	5 (55.6)	12 (28.6)	
Other patterns	4 (44.4)	18 (42.9)	
No infiltration	0 (0.0)	12 (28.6)	
Response to therapy [n (%)]			0.240
PD	5 (55.6)	11 (26.2)	
PR	3 (33.3)	17 (40.5)	
CR	1 (11.1)	14 (33.3)	

NS: Non-significant at P-value ≥0.05

DISCUSSION

B-NHLs are a diverse group of immune system malignancies that can affect any organ in the body. They have a wide range of presentations that range from mild to extremely aggressive clinical behavior [(1)]. This cross-sectional study that included 51 adult B-NHL patients aimed to evaluate bone marrow findings as an integral part of staging workup in B-NHLs and to study possible relations to C-MYC, BCL-2, and BCL-6 gene abnormalities

In this study, 37 (72.5%) were males and 14 (27.4%) were females with male to female ratio 2.6:1. In the same way a previous study found that the incidence of B-NHL was higher among men with a male to female ratio of 1.27:1 [(10)]. The age in this study ranged from 20 to 77 years with a mean age of 45.9 ± 15.8 years. A previous Egyptian study included 100 patients with B-NHL with ages ranging between 28 and 83 years the median age of the included patients was 57 years [(12)].

In the present study, lymph node biopsy revealed that there were 22 follicular lymphoma (FL) cases (43.1%), 20 diffuse large B cell lymphoma (DLBCL) cases (39.2%), and 9 Mantel cell lymphoma (MCL) cases (17.6%). In accordance to our findings a previous study reported that DLBCL account for 42.5% of all cases [(13)]. Also, the prevalence of FL frequency is of up to 30% [(14)]. This is in contrast with what has been published in a previous study as it was found that, DLBCL occurred in 63.9% of all diagnosed cases of NHL [(15)].

The BM biopsy showed that infiltration was evident in 76.5% of the studied patients, 33.33% showed diffuse infiltration and 43.14% of them showed other patterns of infiltration (nodular, interstitial, patchy, paratrabecular and mixed). A previous study found that the incidence of bone marrow infiltration was 40.8% [(16)]. In another work it was reported that 55.1% of NHL cases showed infiltration of marrow [(17)].

Regarding C-MYC, BCL2 and BCL6 gene rearrangement, in this study C-MYC was detected in 35%, 40.9% and 22.2% in DLBCL, FL and MCL, respectively with no significant difference among the three subtypes. In a previous Egyptian study which included 30 cases of patients diagnosed as DLBCL positivity for MYC was determined in 3.7% of cases [(18)]. BCL2 translocation was detected in 74% of all cases, which were 54.5% of FL cases and 20% DLBCL cases. A previous Egyptian study included fifty patients of FL showed that BCL-2 translocation was detected in 86% and 14% showed no translocation [(19)]. Also, BCL6 was positive in 58% of total cases, 45% and 13.6% of DLBCL and Follicular, respectively. In a previous Egyptian study included 30 cases of patients diagnosed as DLBCL, positivity for BCL6 was determined in 7.4 % [(18)].

In the current study FL, DLBCL and MCL showed bone marrow infiltration in 46.2 %, 33.3% and 20.5% of cases respectively. **Ishtiaq and his coworkers** [(20)] discovered that the frequency of bone marrow infiltration in DLBCL was 47.6%, followed by follicular with 50%. A previous study showed that DLBCL has a low frequency of BM infiltration and the infiltration patterns in DLBCL are highly diverse, ranging from mild, isolated infiltrates to a packed

marrow with complete replacement of hematopoiesis [21]. Diffuse infiltration was the most frequent pattern of infiltration in the current study. Our findings are consistent with a prior study that found that the diffuse type of bone marrow infiltration was the most prevalent, followed by the interstitial and paratrabecular patterns of invasion [22].

In this study the routine FISH positive cases were more significantly higher in MCL than in FL and DLBCL which were found in 100% of MCL cases. Regarding BCL2 and BCL6 gene rearrangement, BCL2 was more significantly frequent in FL subtype than other subtypes (DLBCL and MCL). However, BCL6 was significantly frequent in DLBCL subtype. But, C-MYC was not significantly different among three subtypes. In terms of the relationship between the pattern of bone marrow infiltration and genetic changes, the presence of C-MYC, BCL2, and BCL6 was significantly associated with the diffuse pattern of BM infiltration. In the individuals with BM involvement, a prior investigation discovered several genetic abnormalities including MYC, TP53, BCL2 and BCL6 [23].

With regards to double hit and triple hit lymphoma among the three genes, our analysis revealed that BCL6 +/C-MYC + was discovered in 2 cases of DLBCL and 7 cases of FL. Additionally, BCL2 +/BCL6 +/C-MYC + was discovered in 2 cases of FL and 2 cases of DLBCL. In the same manner, a prior study done in Egypt found 3 cases of DHL out of 30 cases of DLBCL (10% with MYC and BCL2 translocations) [18].

DHL is relatively uncommon, occurring in 5% to 7% of patients with DLBCL, according to a prior study [24]. In a prior Malaysian DLBCL study, the prevalence of DHL was fairly uncommon (1.6%) [25]. This is most likely because DLBCL patients in Malaysia are younger than those in western countries (where the median age of diagnosis is 70.6 years), with a median age of diagnosis of 54.1 years [26].

In the present study we noted that patients with triple hit had older age. A previous investigation of 40 cases of THL revealed that the majority of patients are older (median age 61 years) and predominately male [27].

Regarding response to therapy, complete remission (CR) was significantly higher in cases with no BM infiltration pattern, only one case with double expression showed complete remission and cases with triple expression did not show complete remission. According to reports, the prognosis for MYC/BCL2 DHL patient's had very poor prognosis [28].

In this study ,there was no statistical significant association between either double hit cases (C-MYC+ and BCL2+) or triple positivity of BCL2, BCL6 and C-MYC and each of age, gender, LN pathology, pattern of BM infiltration or response to therapy.

CONCLUSION

C-MYC, BCL2 and BCL6 gene rearrangements occured frequently in B-NHL and more associated with diffuse pathological pattern.

RECOMMENDATION

According to our findings in BM biopsy, pattern of infiltration and fate of therapy, the C-MYC, BCL2 and BCL6 could be considered independent prognostic markers which affect clinical patient's outcome. Patients with B-NHLs should be stratified according to the presented clinical, laboratory and cytogenetic features including C-MYC, BCL2 and BCL6 gene status. The clinical outcome of further studies including larger number of B-NHL patients with positive C-MYC, BCL2 and BCL6 rearrangements and to be correlated with the bone marrow biopsy findings.

Financial support and sponsorship: Nil.
Conflict of interest: Nil.

REFERENCES

1. **Sawalha Y, Maddocks K (2022):** Novel treatments in B cell non-Hodgkin's lymphomas. BMJ., 377: e063439. doi: https://doi.org/10.1136/bmj-2020-063439
2. **Naghmana M, Seema M, Zafar N (2011):** Frequency of Bone Marrow Infiltration in NHL Patients; Unilateral Versus Bilateral Bone Marrow Examination Ann. Pak. Inst. Med. Sci., 7 (2): 72-74.
3. **Mokhtar A, Esh S, Khorshed A *et al.* (2017):** Diagnostic and prognostic significance of bone marrow biopsy in non Hodgkins lymphoma. Zagazig University Medical Journal, 23 (3): 1-10.
4. **Salam D, Thit E, Teoh S *et al.* (2020):** MYC, BCL2 and BCL6 Translocation in B-cell Non-Hodgkin Lymphoma Cases. J Cancer, 11 (1): 190–198.
5. **Scarfò L, Ghia P (2013):** Reprogramming cell death: BCL2 family inhibition in hematological malignancies. Immuno Lett., 155 (1): 36-9.
6. **Aukema S, Siebert R, Schuuring E *et al.* (2011):** Double-hit B-cell lymphomas. Blood, 117 (8): 2319-31.
7. **Zwirglmaier K, Fichtl K, Ludwig W (2005):** In situ functional gene analysis: recognition of individual genes by fluorescence in situ hybridization. Methods Enzymol., 397: 338-51.
8. **Lomov N, Zerkalenkova E, Lebedeva S *et al.* (2021):** Cytogenetic and molecular genetic methods for chromosomal translocations detection with reference to the KMT2A/MLL gene. Crit Rev Clin Lab Sci., 58 (3): 180-206.
9. **Elis A, Blickstein D, Klein O *et al.* (2002):** Detection of relapse in non-Hodjkin's lymphoma: Role of routine follow-up studies. Am J Hematol., 69: 41-44.

10. **Cheson B, Pfistner B, Juweid M *et al.* (2007):** Revised response criteria for malignant lymphoma. J Clin Oncol., 25 (5): 579-586.
11. **Alyahya N, Adiga B, Alwadei A *et al.* (2019):** The clinico-pathological profile of non-Hodgkin's lymphoma in Aseer region of Saudi Arabia. BMC Res Notes, 12 (1): 1-7.
12. **Azzazi M, Mohamed M, Mousa M *et al.* (2017):** Multicentre study of hepatitis C virus status in Egyptian patients with B-cell non-Hodgkin's lymphoma with assessment of patients' immunological state. Egyptian Journal of Haematology, 42 (1): 19-30.
13. **Perry A, Diebold J, Nathwani B *et al.* (2016):** Non-Hodgkin lymphoma in the developing world: review of 4539 cases from the International Non-Hodgkin Lymphoma Classification Project. Haematologica, 101 (10): 1244-1250.
14. **Ambinder A, Shenoy P, Malik N *et al.* (2012):** Exploring risk factors for follicular lymphoma. Adv Hematol., 12: 626035. https://doi.org/10.1155/2012/626035
15. **Hernandez-Ruiz E, Alvarado-Ibarra M, Lien-Chang L *et al.* (2021):** Epidemiology and Clinical Characteristics of Non-Hodgkin Lymphoma in Mexico. World J Oncol., 12 (1): 28-33.
16. **EL Bolkeney T, Wael S, Mokhtar N *et al.* (2008):** Evaluation of Bone Marrow in 143 Lymphomas:The Relative Frequency and Pattern of Involvement, Secondary Myelopathies, Pitfalls and Diagnostic Validity. Journal of the Egyptian Nat Cancer Inst., 20 (3): 17-30.
17. **Kumar S, Rau A, Naik R *et al.* (2009):** Bone marrow biopsy in NHL: A morphological study. Indian Journal of Pathology and Microbiology, 52 (3): 332-338.
18. **Gouda M, Amer M, Abd ELmonem M (2019):** Prognostic Significance of Concurrent MYC, BCL2 and/or BCL6 Expression in Diffuse Large B-Cell Lymphoma. Med J Cairo Univ., 87 (6): 3581-3588.
19. **Ibrahiem A, Ghorab D, Azab N *et al.* (2016):** Evaluation of t(14;18)(q32;q21) and BCL2 protein and their prognostic role in follicular lymphoma. Bull Egypt Soc Physiol Sci., 36 (2): 68-84.
20. **Ishtiaq J, Jehanzeb K, Ali N *et al.* (2017):** bone marrow infiltration and patterns in previously diagnosed patients of non hodgkin lymphoma (a single centre study). Pak Armed Forces Med J., 67 (2): 249-52.
21. **Fend F, Kremer M (2007):** Diagnosis and classification of malignant lymphoma and related entities in the bone marrow trephine biopsy. Pathobiology, 74: 133–143.
22. **Sultan S, Baloch N, Ahmed Z *et al.* (2018):** Pattern of bone marrow involvement in non Hodgkin's lymphoma classified according to WHO classification: Report of a developing country Pakistan. J Lab Physicians, 10 (1): 17-20.
23. **Alonso-Álvarez S, Alcoceba M, García-Álvarez M *et al.* (2020):** Biological Features and Prognostic Impact of Bone Marrow Infiltration in Patients with Diffuse Large B-Cell Lymphoma. Cancers (Basel), 12 (2): 474. doi: 10.3390/cancers12020474.
24. **Landsburg D, Petrich A, Abramson J *et al.* (2016):** Impact of oncogene rearrangement patterns on outcomes in patients with double-hit non-Hodgkin lymphoma. Cancer, 122 (4): 559-64.
25. **Ting C, Chang K, Kuan J *et al.* (2019):** Clinical Significance of BCL2, C-MYC, and BCL6 Genetic Abnormalities, Epstein-Barr Virus Infection, CD5 Protein Expression, Germinal Center B Cell/Non-Germinal Center B-Cell Subtypes, Co-expression of MYC/BCL2 Proteins and Co-expression of MYC/BCL2/BCL6 Proteins in Diffuse Large B-Cell Lymphoma: A Clinical and Pathological Correlation Study of 120 Patients. Int J Med Sci., 16 (4): 556-566.
26. **Smith A, Howell D, Patmore R *et al.* (2011):** Incidence of haematological malignancy by sub-type: a report from the Haematological Malignancy Research Network. British Journal of Cancer, 105 (11): 1684
27. **Huang W, Medeiros L, Lin P *et al.* (2018):** MYC/BCL2/BCL6 triple hit lymphoma: a study of 40 patients with a comparison to MYC/BCL2 and MYC/BCL6 double hit lymphomas. Mod Pathol., 31 (9): 1470-1478.
28. **Testo N, Olson L, Subramaniyam S *et al.* (2016):** Primary Cutaneous Diffuse Large B-Cell Lymphoma With a MYC-IGH Rearrangement and Gain of BCL2: Expanding the Spectrum of MYC/BCL2 Double-Hit Lymphomas. Am J Dermatopathol., 38 (10): 769-74.