Clinical Significance Of Genomic Deep Sequencing In Egyptian Patients With Diffuse Large B-Cell Lymphoma

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Abstract: **Background:** Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma. It has heterogeneous clinical features and varies markedly in response to treatment and prognosis. Distinctive molecular and genetic abnormalities have been identified in DLBCL. Next-generation sequencing (NGS) has detailed the genomic characterization of DLBCL by identifying recurrent somatic mutations. This study aimed to identify genetic alterations and rearrangements in DLBCL and to examine their association with clinical features, response to therapy and final outcome. **Objective:** The aim of the present work was to identify genetic alterations and rearrangements in diffuse large B-cell lymphoma and to examine their association with clinical features, response to therapy and final outcome. **Materials and Methods:** This study included 40 subjects; 30 patients with diffuse large B-cell lymphoma and 10 normal subjects as a control group. The Ann Arbor classification system was used to determine the stage of the patients. The patients were evaluated according to age, sex, stage, B symptoms, lactate dehydrogenase level, response to treatment and overall survival. Target sequencing was performed on lymph node or bone marrow biopsy samples from patients with DLBCL and on peripheral blood samples from healthy subjects for detection of some genetic mutations, including *BCL 10, GNA13, MEF2B, PRDM1, BCL6, BCL2, CARD11, PIM1* and *TBX21*. **Results:** This study revealed that only 4 genetic mutations were detected (*BCL2, CARD11, PRDM1* and *TBX21)* in 12 out of 30 patients. 11 patients had *BCL2* mutation with 34 variants, 2 patients had *CARD11* mutation with 3 variants, one patient had *PRDM1* mutation with 2 variants and 4 patients had *TBX21* mutation with 6 variants. *BCL2* was the most frequent gene affected representing 75.6% with statistically significant increased frequency as compared to other genes ($p < 0.001$). Moreover, *C-T* was the most frequent variant affected representing 62.2% with statistically significant increased frequency as compared to other variants ($p < 0.001$). There was statistically significant difference between the stage of the disease and genetic variants ($P = 0.048$), however, no significant difference was observed between the response to treatment and genetic variants. Patientswho didn't have identified variants had slight superiority in overall survival than those who had identified variants, with no statistically significant difference between both patient groups. No any genetic mutation was detected in control group. **Conclusions:** *BCL2* gene mutation can be regarded as a potential genetic risk factor in Egyptian patients with DLBCL, however it has no significant impact on the clinical outcome. The prognostic significance of genetic alterations and rearrangements in DLBCL should be evaluated in the context of molecular subtypes in future studies. Clearly, further well-designed prospective studies to clarify the exact role of genetic mutations as prognostic factors, predictors of outcome and emerging therapeutic targets in DLBCL would be warranted.

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1. Introduction

The lymphoid neoplasms represent a diverse range of tumors characterized by variable stages of maturation ranging from pro T or B cells in acute lymphoblastic leukaemia to cells representive of the lymph node in the non-Hodgkin lymphomas to mature plasma cells in myeloma and related disorders. (1)

Non-Hodgkin lymphoma has several types. Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma worldwide. It represents a diverse spectrum of disease from morphological and prognostic point of view. (2)

The classification of diffuse large B-cell lymphoma has been significantly refined as a result of novel insights into the biology of lymphoid tumors. Although it has been known for some time that DLBCL is a clinically and biologically diverse disease, new diagnostic technologies such as gene

expression profiling have defined a new molecular classification for DLBCL and led to the identification of different mutations and potentially therapeutic targets. DLBCL can now be divided into at least three molecular subtypes that correspond to distinct stages of B-cell differentiation. It is critical to understand and consider these pathologic distinctions in the context of novel targets and strategies in DLBCL. (3)

The most recent World Health Organization classification of tumors of hematopoietic and lymphoid tissues divides DLBCL according to clinicopathologic and molecular characteristics into three subtypes on the basis of gene expression profiling: 1- activated B-cell like (ABC) arise from post-germinal centre B-cells that are blocked during plasmacytic differentiation, 2- germinal centre B-cell like (GCB) arise from germinal centre B-cell, 3- primary mediastinal B-cell like (PMBL) arise from a thymic B-cell. (4,5)

Next-generation sequencing is applied to genome sequencing, genome resequencing, transcriptome profiling, DNA-protein interactions and epigenome characterization. (6) The high demand for low-cost sequencing has driven the development of high-throughput sequencing (next-generation sequencing) technologies that parallelize the sequencing process, producing thousands or millions of sequences concurrently. (7-11)

The advent of next-generation sequencing technologies has revolutionized our approach to perform structural and functional genomics studies, (12,13) with the aim to provide a comprehensive map of human genetic variants. (14)

Next-generation sequencing has the potential to achieve a higher level of sensitivity compared to PCR-based methods, it enables the analysis of genetic diversity and clonal heterogeneity which may contribute to our current understanding of DLBCL biology and relapse kinetics. (15,16) Also, it has identified potential new markers for diagnosis, risk stratification, and therapeutic intervention. (17)

In the present study we performed genetic sequencing to identify genetic alterations and rearrangements in diffuse large B-cell lymphoma and to examine their association with clinical features, response to therapy and final outcome.

2. Patients and Methods

Our study was carried out on 40 subjects of matched age and sex divided into two groups:

Group (1): included 30 patients with diffuse large B-cell lymphoma. They were 17 males and 13 females with age ranging from 22 to 70 years.

Group (2): included 10 normal subjects as a control group. They were 5 males and 5 females with age ranging from 42 to 63 years.

The diagnosis of diffuse large B-cell lymphoma was based on histopathological examination of lymph node and bone marrow biopsies.

The patients were further subdivided according to the Ann Arbor staging system (50) into stages (I, II, III and IV (.

An informed consent was obtained from all participants in the research.

This study was performed in the Hematology and Oncology Unit of Internal Medicine Department at Tanta University Hospital, Genomic Signature Cancer Centre, Next Generation Sequencer Unit, Global Educational Hospital at Tanta University, National Cancer Institute and International Medical Centre. The duration of the study was from October 2014 to April 2017.

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1-Inclusion criteria: Patients >18 years old with diffuse large B-cell lymphoma.

2-Exclusion criteria: All patients < 18 years old. All patients with other types of lymphoma. All patients with other malignancies.

A-Sample collection:

1-FFPE lymph node were collected by the hematologist from patients and the samples were preserved at room temperature till the time of DNA extraction.

2-EDTA blood was collected from healthy controls and preserved at -80 °c till the extraction time.

B-DNA extraction, quantification:

1-DNA was extracted from FFPE LN using Qiagen amp mini spin column kit (Germany, cat no. 56404) and from the whole blood of controls using Qiagen blood mini kit (Germany, cat no 51104) according to manufacture instructions.

2-The DNA quality and quantity were determined using Qubit™DsDNAHs assay kit

(USA, cat no Q32851).

C-Target amplification and quantitation:

DNA targets for the selected genes were amplified by PCR using Ion AmpliSeq™ kit (USA, cat no 4475345). After adaptor ligation the target library was purified using Ageno Court ™ AMPure™ XP KIT (USA, cat no #000130) following manufacture instructions. Then, the eluted library was quantified by ion library Taqman ® quantification kit (USA, cat no 4468802).

D-Sequencing of the library:

The library was diluted to 8 PM and checked for clonally amplified DNA using 250 ™ kit OT2 (USA, cat no A27751). Then the template positive ion sphere particle was enriched with one touch ™ ES (Thermofisher Scientific, USA), then loaded onto 520

TM chip. Finally the loaded chip was sequenced using Ion S5 TM Sequencer (Thermofisher Scientific, USA).

3. Results

Basic demographic data obtained for patients involved in this study are outlined in Table (1) and Table (2)

Table (1): Distribution of age in patient groups and control.

	Patients						Control (n = 10)		Test of sig.	p₀
	Total (n = 30)		Stage (I+II) (n =16)		Stage (III+IV) (n =14)					
	No.	%	No.	%	No.	%	No.	%		
Age (years)										
Range	22.0 – 70.0		40.0 – 70.0		22.0 – 70.0		42.0 – 63.0			
Mean ± SD	52.43 ± 10.47		54.75 ± 8.60		49.79 ± 12.05		54.50 ± 6.98		F=1.164	0.323
Median	53.0		56.0		49.0		55.50			
p₁	0.565									

Table (2): Sex distribution in patient groups and control

	Patients						Control (n = 10)		Test of sig.	p₀
	Total (n = 30)		Stage (I+II) (n =16)		Stage(III+IV) (n =14)					
	No.	%	No.	%	No.	%	No.	%		
Sex										
Male	17	56.7	9	56.3	8	57.1	5	50.0	χ^2 = 0.137	0.934
Female	13	43.3	7	43.8	6	42.9	5	50.0		
p₁	0.714									

Clinical findings in the patient groups are outlined in table (3)

In patients with stage (I + II): 2 patients had pallor (12.5%), 2 patients had fatigue (12.5%) and all patients had lymphadenopathy (100%).

In patients *with stage (III+ IV):* 6 patients had weight loss (42.9%), 7 patients had fever (50%), 5 patients had pallor (35.7%), 5 patients had fatigue (35.7%), 6 patients had anorexia (42.9%), night sweats was present in one patient (7.1%), one patient had abdominal pain (7.1%), all patients had lymphadenopathy (100%). By clinical examination, there were 4 patients presented by enlarged liver (28.6%) and 11 patients presented by enlarged spleen (78.6%) and B symptoms were present in 11 patients (78.6%).

Serum LDH level ranged from 128 to 2300 U/L in total patients while in control group, it ranged from 130 to 290 U/L (mean: 206.20 ± 53.82), there was statistically significant increase in serum LDH level in patients with stage (III+IV) (mean: 729.5 ± 631.45) as compared to those with stage (I+II) (mean: 288.8 ± 227.47) (p = 0.021), also LDH level was significantly higher in patients with stage (III+IV) than control group (p = 0.028).

Table (3): Clinical findings in the studied patients

Findings	Patients (no=30)			
	Stage (I+II) (no=16)		Stage (III+IV) (no=14)	
	NO.	%	NO.	%
Weight loss	0	0	6	42.9
Fever	0	0	7	50
Pallor	2	12.5	5	35.7
Fatigue	2	12.5	5	35.7
Anorexia	0	0	6	42.9
Night sweats	0	0	1	7.1
Abdominal pain	0	0	1	7.1
Lymphadenopathy	16	100	14	100
Hepatomegaly	0	0	4	28.6
Splenomegaly	0	0	11	78.6
B symptoms	0	0	11	78.6

Figure (1) shows comparison between the studied groups regarding LDH

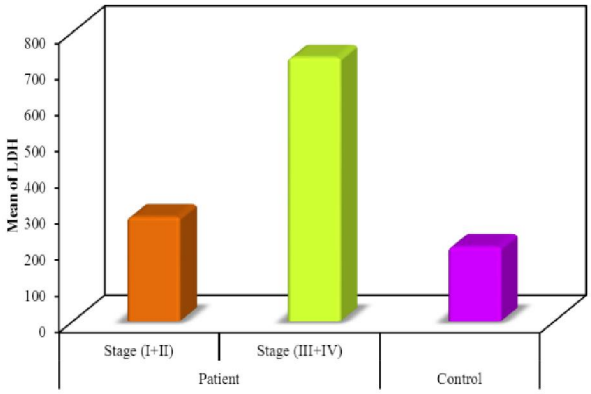

Figure (1): Comparison between the studied groups according to LDH

Figure (2) shows comparison between the studied groups regarding β2microglobulin.

There was statistically significant increase in serum β2 microglobulin in patient groups as compared to control group, while no significant difference was observed between patients with stage (III+IV) and those with stage (I+II).

Table (4) shows distribution of the studied patients according to bone marrow biopsy findings.

There were 23 patients with normal bone marrow; 9 of them in stage I, 7 in stage II, 5 in stage III and 2 patients in stage IV. One patient in stage IV had hypocellular bone marrow, while another one patient in stage IV had hyper-cellular bone marrow. It was found that 5 patients had bone marrow infiltration by lymphocytes and all of them in stage IV. There was statistically significant difference between the different groups.

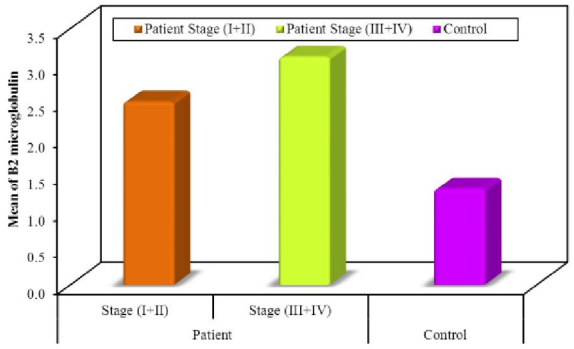

Figure (2): Comparison between the studied groups according to β_2 microglobulin

Table (4): Distribution of the studied patients according to bone marrow biopsy findings

Findings	Patients						χ^2	MCp
	Total (n = 30)		Stage (I+II) (n =16)		Stage (III+IV) (n =14)			
	No.	%	No.	%	No.	%		
Bone marrow								
Normal	23	76.7	16	100.0	7	50.0	10.086*	0.002*
Hypocellular	1	7.1	0	0.0	1	7.1		
Hypercellular	1	7.1	0	0.0	1	7.1		
Infiltrated by lymphocytes	5	16.7	0	0.0	5	35.7		

Table (5): Distribution of the studied patients according to response to treatment and follow up

	Patients						Test of sig.	p_0
	Total (n = 30)		Stage (I+II) (n =16)		Stage (III+IV) (n =14)			
	No.	%	No.	%	No.	%		
Response to TTT								
CR	22	73.3	15	93.8	7	50.0	χ^2= 7.075*	MCp= 0.015*
PR	7	23.3	1	6.3	6	42.9		
Refractory	1	3.3	0	0.0	1	7.1		
Follow up								
Alive	22	73.3	14	87.5	8	57.1	χ^2= 3.519	FEp= 0.101
Died	8	26.7	2	12.5	6	42.9		

Table (5) shows distribution of the studied patients regarding response to treatment and follow up.

There were 22 patients achieved complete remission after the R-CHOP regimen; 15 of them in early stage of the disease (stage I+II) and 7 in late stage (stage III+IV), while 7 patients achieved partial remission; one of them was in stage (I+II) and 6 in stage (III+IV), however one patient in stage (III+IV) was refractory to treatment. There was statistically significant difference between these groups ($p = 0.015$).

22 patients were alive; 14 of them in the early stage of the disease and 8 in the late stage, while 8 patients died; 2 of them in early stage and 6 in late stage with no statistically significant difference between these groups.

Kaplan Meier analysis for estimation of the overall survival of both patient groups revealed that patients with stage (I + II) had slight superiority in overall survival than those with stage (III + IV), with no statistically significant difference between both patient groups (p value=0.056).

Figure (3) show comparison between the studied patients according to overall survival by Kaplan Meier survival curve

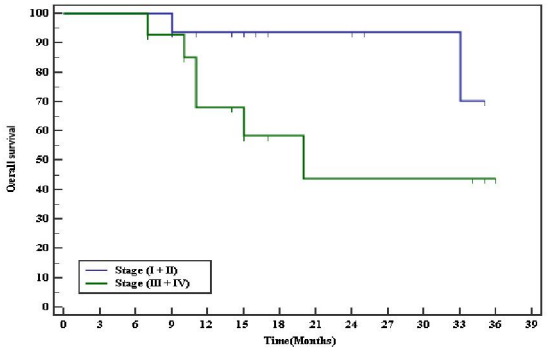

Figure (3): Overall survival of both patient groups by Kaplan Meier survival curve

Table (6): Relation between response to treatment and some laboratory parameters

Serum LDH:

Serum LDH level was significantly higher in patients who achieved partial remission (mean: 895.86 ± 716.23) than those who achieved complete remission (mean: 312.55 ± 224.01) ($p = 0.039$).

Serum β2 microglobulin:

There was no statistically significant difference in serum β2 microglobulin between patients who achieved partial remission and those who achieved complete remission.

Table (6): Relation between response to TTT and some laboratory parameters

	Response to TTT			Test of sig.	p
	CR (n= 22)	PR (n= 7)	Refractory (n= 1*)		
LDH(U/L)					
Range	128.0 – 890.0	156.0 – 2300.0			
Mean ± SD	312.55 ± 224.01	895.86 ± 716.23	1687.0	U=40.0	0.039*
Median	197.0	850.0			
β2 microglobulin (µg/ml)					
Range	0.60 – 6.0	0.50 – 7.0			
Mean ± SD	2.52 ± 1.35	3.30 ± 2.23	5.0	U=61.0	0.414
Median	2.45	3.10			

Table (7) shows Correlation between overall survival and some parameters in DLBCL patients

There was non-significant positive correlation between overall survival and age ($r_s = 0.170$, $p = 0.371$).

There was non-significant negative correlation between overall survival and serum LDH (r_s = - 0.195, p = 0.303).

There was non-significant positive correlation between overall survival and serum β2 microglobulin (r_s = 0.079, p = 0.679).

Collectively, there was no statistically significant effect of age, serum LDH and serum β2 microglobulin on overall survival.

Table (7): Correlation between overall survival and some parameters in DLBCL patients

Parameters	Overall survival	
	r_s	p
Age (years)	0.170	0.371
LDH (U/L)	-0.195	0.303
β2microglobulin (µg/ml)	0.079	0.679

Distribution of the studied DLBCL patients according to genetic mutations:

In the present study, target sequencing was performed on 25 lymph node biopsy samples and 5 bone marrow biopsy samples from patients with DLBCL for detection of some genetic mutations, including ***BCL 10, GNA13, MEF2B, PRDM1, BCL6, BCL2, CARD11, PIM1*** and ***TBX21.*** Only 4 of these genetic mutations were detected (***BCL2, CARD11, PRDM1*** and ***TBX21***). It was found that 12 out of 30 patients had genetic mutations while the remaining 18 patients did not have any genetic mutation; 11 out of 12 patients had BCL2 mutation with 34 variants, 2 patients had CARD11 mutation with 3 variants, one patient had PRDM1 mutation with 2 variants and 4 patients had TBX21 mutation with 6 variants (Table7). Therefore, most variants were detected in BCL2 gene (34 variants). Regarding control group in this study, target sequencing was performed on 10 peripheral blood samples from healthy subjects for detection of the same genetic mutations and no any genetic mutation was detected.

Table (8): Distribution of the studied DLBCL patients according to genetic mutations

Relation between the affected genes and exon numbers in DLBCL patients:

In the current study, exon 2 was the most frequent affected exon, representing 77.8% of the total affected exons.

As regards ***BCL2*** gene, exon 2 was the most frequent affected exon representing 85.3%, while exon 1 constituted 8.9%, exon 6 (6.7%), exon 15 (4.4%) and exon 18 (2.2%).

Regarding ***CARD11*** gene, the affected exon was exon 2 representing 66.7% and exon 1 representing 33.3%.

In ***PRDM1*** gene, the only exon affected was exon 1 representing 100%.

As regards ***TBX21*** gene, the affected exon was exon 2 representing 66.6%, while exon 1 constituted 16.7% and also exon 6 representing 16.7%.

There was statistically significant difference between these groups (p = 0.015) (Table 9).

Patients	BCL2	CARD11	PRDM1	TBX21
1	6	0	0	3
3	4	0	0	0
5	3	0	2	0
7	1	0	0	0
10	5	0	0	0
11	4	0	0	0
13	1	0	0	0
14	0	2	0	0
15	1	0	0	0
18	1	1	0	1
19	4	0	0	1
29	4	0	0	1
Total	34	3	2	6

Table (9): Relation between the affected genes and exon numbers in DLBCL patients

	Genes										χ^2	^{MC}p
	Total (n= 45)		BCL2 (n= 34)		CARD11 (n= 3)		PRDM1 (n= 2)		TBX21 (n= 6)			
	No.	%	No.	%	No.	%	No.	%	No.	%		
Exon No.												
Exon 1	4	8.9	0	0.0	1	33.3	2	100.0	1	16.7		
Exon 2	35	77.8	29	85.3	2	66.7	0	0.0	4	66.6		
Exon 6	3	6.7	2	5.9	0	0.0	0	0.0	1	16.7	23.407*	0.015*
Exon 15	2	4.4	2	5.9	0	0.0	0	0.0	0	0.0		
Exon 18	1	2.2	1	2.9	0	0.0	0	0.0	0	0.0		
p_1			0.006*		0.540		0.026*		0.450			

Table (10): Relation between the affected genes and genetic variants in DLBCL patients

	Genes										χ^2	^{MC}p
	Total (n= 45)		BCL2 (n= 34)		CARD11 (n= 3)		PRDM1 (n= 2)		TBX21 (n= 6)			
	No.	%	No.	%	No.	%	No.	%	No.	%		
C-T	28	62.2	22	64.7	1	33.3	0	0.0	5	83.33		
G-A	12	26.7	7	20.5	2	66.66	2	100.0	1	16.66	10.257	0.015*
A-G	2	4.44	2	5.82	0	0.0	0	0.0	0	0.0		
T-C	3	6.67	3	8.82	0	0.0	0	0.0	0	0.0		

Relation between the affected genes and genetic variants in DLBCL patients:

In the current study, C-T was the most frequent genetic variant, representing 62.2% of the total affected variants.

As regards ***BCL2*** gene, C-T was the most frequent genetic variant, representing 64.7%, while G-A constituted 20.5 %, T-C (8.82%) and A-G (5.82%).

Regarding ***CARD11*** gene, the affected genetic variant was G-A representing 66.66% and C-T representing 33.3%.

In ***PRDM1*** gene, the only genetic variant was G-A representing 100%.

As regards ***TBX21*** gene, the affected genetic variant was C-T representing 83.33%, while G-A constituted 16.66%.

There was statistically significant difference between these groups (p = 0.015) (Table 10).

Distribution of genes, genetic variants and exon numbers in the affected patients:

In the present study, 12 DLBCL patients had genetic mutations in ***BCL2***, ***TBX21***, ***PRDM1*** and ***CARD11*** genes. ***BCL2*** was the most frequent gene affected representing 75.6% with statistically significant increased frequency as compared to other genes (p <0.001), followed by ***TBX21*** which constituted 13.3%, ***CARD11*** representing 6.7% and ***PRDMI*** representing 4.4% (Table 11).

Table (11): Distribution of genes, genetic variants and exon numbers in the affected patients

	No.	%	p
Genes			
BCL2	34	75.6	<0.001*
CARD11	3	6.7	
PRDM1	2	4.4	
TBX21	6	13.3	
Genetic variants			
A-G	2	4.4	<0.001*
C-T	28	62.2	
G-A	12	26.7	
T-C	3	6.7	
Exon numbers			
Exon 1	4	8.9	<0.001*
Exon 2	35	77.8	
Exon 6	3	6.7	
Exon 15	2	4.4	
Exon 18	1	2.2	

As regards genetic variants, C-T was the most frequent variant affected representing 62.2% with statistically significant increased frequency as compared to other variants (p < 0.001), followed by G-A representing 26.7% then T-C constituted 6.7% and A-G representing 4.4% (Table 11).

Regarding exon numbers, exon 2 was the most frequent exon affected representing 77.8% with statistically significant increased frequency as compared to other exons (p < 0.001), followed by exon 1 (8.9%), exon 6 (6.7%), exon 15 (4.4%) and exon 18 (2.2%) (Table 11).

Effects of genetic mutations on amino acid substitutions in the affected patients:

Table (12) demonstrated amino acid substitution in each affected chromsome and prediction of the functional effect of this substitution whether it was damaging, neutral or tolerated using Sift and Provean predictors.

Regarding amino acid substitution in ***BCL2*** gene, it was mostly damaging according to Sift and Provean predictors.

Regarding amino acid substitution in ***TBX21*** gene, it was damaging according to Sift predictor and neutral according to Provean predictor.

As regards amino acid substitution in ***CARD11*** gene, it was mostly damaging according to Sift and Provean predictors.

As regards amino acid substitution in ***PRMD1*** gene, it was neutral or tolerated according to Sift and Provean predictors.

Table (12): Effects of genetic mutations on amino acid substitutions in the affected patients

Case NO.	Genes	Chromosome	Amino acid substitution	SIFT	Provean	Genetic variants			
						A-G	C-T	G-A	T-C
29,19	TBX21	Chr17:45822665	p.Pro514Leu	Damaging	Damaging	0	2	0	0
18	TBX21	Chr17:45822673	p.Ala517Thr	Damaging	Neutral	0	0	1	0
1	TBX21	Chr17:45822676	p.Pro518Ser	Tolerated	Neutral	0	1	0	0
1	TBX21	Chr17:45822682	p.Pro520Ser	Damaging	Neutral	0	1	0	0
1	TBX21	Chr17:45822725	p.Pro534Leu	Damaging	Neutral	0	1	0	0
3,10,11	BCL2	Chr18:60985440	p.Gly154Ser	Damaging	Damaging	0	3	0	0
1	BCL2	Chr18:60985454	p.Ala149Val	Damaging	Damaging	0	0	1	0
1	BCL2	Chr18:60985455	p.Ala149Thr	Damaging	Damaging	0	1	0	0
1,3,11	BCL2	Chr18:60985458	p.Val148Met	Damaging	Damaging	0	3	0	0
10	BCL2	Chr18:60985461	p.Ile147Val	Tolerated	Neutral	0	0	0	1
19,29	BCL2	Chr18:60985463	p.Arg146Lys	Damaging	Damaging	0	2	0	0
1,10	BCL2	Chr18:60985467	p.Gly145Arg	Damaging	Damaging	0	2	0	0
5	BCL2	Chr18:60985479	p.Gly141Arg	Damaging	Damaging	0	1	0	0
3,11	BCL2	Chr18:60985490	p.Leu137Pro	Damaging	Damaging	2	0	0	0
10	BCL2	Chr18:60985491	p.Leu137Phe	Damaging	Damaging	0	0	1	0
5	BCL2	Chr18:60985497	p.Glu135Lys	Damaging	Neutral	0	1	0	0
5	BCL2	Chr18:60985503	p.Val133Met	Damaging	Damaging	0	1	0	0
1	BCL2	Chr18:60985505	p.Thr132Met	Damaging	Damaging	0	0	1	0
7,10,13	BCL2	chr18:60985509	p.Ala131Thr	Tolerated	Neutral	0	3	0	0
19,29	BCL2	Chr18:60985518	p.Gly128Arg	Tolerated	Neutral	0	2	0	0
3,11	BCL2	Chr18:60985532	p.Pro123Leu	Damaging	Damaging	0	0	2	0
19,29	BCL2	Chr18:60985536	p.Thr122Ala	Damaging	Damaging	0	0	0	2
19,29	BCL2	Chr18:60985550	p.Ser117Asn	Tolerated	Neutral	0	2	0	0
1,18	BCL2	Chr18:60985893	p.His3Tyr	Damaging	Neutral	0	0	2	0
5	PRDM1	Chr6:106534442	p.Cys5Tyr	Tolerated	Neutral	0	0	1	0
5	PRDM1	Chr6:106534459	p.Gly11Ser	Damaging	Neutral	0	0	1	0
14	CARD11	Chr7:2949732	p.Pro1071Leu	Damaging	Damaging	0	0	1	0
14	CARD11	Chr7:2949733	p.Pro1071Ser	Damaging	Damaging	0	0	1	0
18	CARD11	Chr7:2963867	p.Arg647Lys	Damaging	Neutral	0	1	0	0

Relation between genetic variants and clinical characteristics in DLBCL patients (Table 13)

As regard stage of the disease, 18 out of 30 patients didn't have identified variant; 5 patients of them were stage I, 2 patients were stage II, 3 patients were stage III and 8 patients were stage IV. The remaining 12 patients had identified variant; 4 patients of them were stage I, 5 patients were stage II, 2 patients were stage III and 1 patient was stage IV. There was statistically significant difference between the stage of the disease and genetic variants (P = 0.048).

Regarding the response to treatment, 22 out of 30 patients achieved complete remission; 13 patients of them didn't have identified variant and 9 had identified variant. Moreover, 7 out of 30 patients achieved partial remission; 4 patients of them didn't have identified variant and 3 had identified variant, while 1 patient was refractory to therapy and didn't have identified variant. There was no statistically significant difference between the response to treatment and genetic variants.

As regard follow up, 22 out of 30 patients were alive, 15 patients of them didn't have identified variant and 7 had identified variant. Unfortunately, 8 patients died; 3 of them didn't have identified variant and 5 had identified variant, with no statistically significant difference between follow up and presence of genetic variants.

Regarding sex, 17 out of 30 patients were males, 9 patients of them didn't have identified variant and 8 had identified variant, while 13 out of 30 patients were females, 9 patients of them didn't have identified variant and 4 had identified variant, with no statistically significant difference between sex and presence of genetic variants.

As regard B-symptoms, 11 out of 30 patients complaint of B-symptoms, 8 patients of them didn't have identified variant and 3 had identified variant, while 19 out of 30 patients didn't complaint of B-symptoms, 10 patients of them didn't have identified variant and 9 had identified variant, with no statistically significant difference between B-symptoms and presence of genetic variants.

Regarding extra-nodal involvement, 10 out of 30 patients had extra-nodal involvement, 7 patients of them didn't have identified variant and 3 had identified variant, while 20 out of 30 patients didn't have extra-nodal involvement, 11 patients of them didn't have identified variant and 9 had identified variant, with no statistically significant difference between extra-nodal involvement and genetic variants.

Regarding the age, there was statistically significant difference between patients who had and those who didn't have identified variant (p = 0.023).

As regard serum LDH, it was significantly higher in patients who didn't have identified variant than in those who have identified variant (p = 0.016).

Table (13): Relation between genetic variants and clinical characteristics in DLBCL patients

Characteristics	Variants: Patients didn't have identified variant (n= 18) No.	%	Patients had identified variant (n= 12) No.	%	Test of sig.	p
Stage						
I + II	7	38.9	9	75.0	χ^2=3.772	0.048*
III +IV	11	61.1	3	25.0		
Response to TTT						
CR	13	72.2	9	75.0	χ^2=0.749	MCp= 1.000
PR	4	22.2	3	25.0		
Refractory	1	5.6	0	0.0		
Follow up						
Alive	15	83.3	7	58.3	χ^2=2.301	FEp= 0.210
Died	3	16.7	5	41.7		
Sex						
Male	9	52.9	8	47.1	χ^2=0.814	0.367
Female	9	69.2	4	30.8		
B-symptoms						
+ ve	8	72.7	3	27.3	χ^2=1.172	0.279
- ve	10	52.6	9	47.4		
Extra-nodal involvement						
+ ve	7	70	3	30	χ^2=0.625	0.426
- ve	11	55	9	45		
Age (years)						
Range	22.0 – 67.0		45.0 – 70.0		t=2.414	0.023*
Mean ± SD	48.94 ± 10.06		57.67 ± 9.11			
Median	49.0		57.0			
LDH (U/L)						
Range	160.0 – 2300.0		128.0 – 850.0		U=151.0	0.016*
Mean ± SD	636.22 ± 587.58		281.83 ± 240.50			

Table (14) & Figure (4) show comparison between patients who had and those who didn't have identified variants according to overall survival by Kaplan Meier survival curve

Kaplan Meier analysis for estimation of the overall survival of both patient groups revealed that patients who didn't have identified variants had slight superiority in overall survival than those who had identified variants, with no statistically significant difference between both patient groups (p value=0.224).

Table (14): Effect of genetic variants on overall survival in DLBCL patients

Overall survival	Mean	95% CI of mean (LL-UL)	%	Log rank	
				χ^2	p
Patients who didn't have identified variants	31.241	26.354– 36.127	70.3	1.476	0.224
Patients who had identified variants	26.117	19.676 – 32.558	43.8		

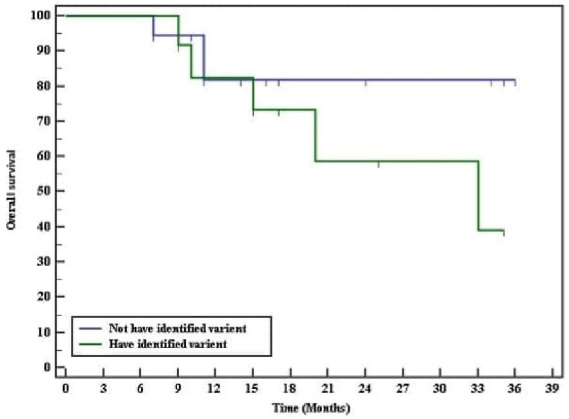

Figure (4): Effect of genetic variants in DLBCL patients on overall survival by Kaplan Meier survival curve

Table (15): Effect of genetic mutations on overall survival in DLBCL patients

Overall survival	Mean	95% CI of mean (LL-UL)	%	Log rank	
				χ^2	p
BCL2	25.976	19.458– 32.493	38.8	1.474	0.688
CARD11	20.000	20.0 – 20.0	0.0		
PRDM1	33.000	33.0 – 33.0	0.0		
TBX21	17.500	12.022 – 22.978	25.0		

Table (15) & Figure (5) show effect of genetic mutations on overall survival by Kaplan Meier survival curve

Kaplan Meier analysis for estimation of the overall survival of DLBCL patients who had genetic mutations in ***BCL2***, ***CARD11***, ***PRDM1*** and ***TBX21*** genes revealed that patients who had ***BCL2*** gene mutation had lower overall survival than those who had ***CARD11*** or ***PRDM1*** mutation, with no statistically significant difference between patient groups (p value=0.688).

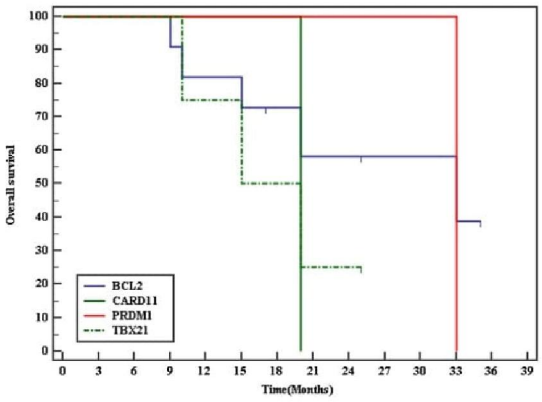

Figure (15): Effect of genetic mutations in DLBCL patients on overall survival by Kaplan Meier survival curve

4. Discussion

Non-Hodgkin's lymphoma (NHL) represents a heterogeneous group of neoplasms of different biology and prognosis. It includes both B-cell and T-cell neoplasms, of which 90% are B-cell. [18]

Diffuse large B-cell lymphoma (DLBCL) is the most common B-cell non-Hodgkin lymphoma (NHL) throughout the world, comprising 30–35% of all NHLs. [18] In Egypt, it represents about 49% of NHL presenting to the National Cancer Institute (NCI), Cairo University. [19] DLBCL is biologically aggressive, but can be cured in >50% of cases, even in advanced stages. [20]

Despite major advances in treatment, approximately one third of the patients experience refractory disease or early relapse, suggesting the existence of additional oncogenic events. In this respect, recent studies focused on the evaluation of molecular and genetic markers associated with survival. [21]

Next generation sequencing (NGS), or deep sequencing are related terms that describe a DNA sequencing technology which has revolutionized genomic research. Using NGS, an entire human genome can be sequenced within a single day. Next-generation sequencing (NGS) has detailed the genomic characterization of DLBCL by identifying recurrent somatic mutations. [22]

Gene expression profiling (GEP) has made steps in decoding the molecular heterogeneity of DLBCL, enabling the entity's subdivision into three main

molecular subtypes: germinal center B-cell like (GCB), activated B-cell like (ABC), and primary mediastinal B-cell lymphoma (PMBL). [23] The GCB subtype is characterized by t (14;18) (q32;q21) translocations [24] and loss of PTEN, [25] while the ABC subtype is characterized by t (3;14) (q27;q32) translocations, deletion of the INK4A-ARF locus, [26] and BCL2 amplification. [27] PMBL displays strong molecular similarities to classical Hodgkin lymphoma (cHL), exhibiting frequent amplifications of JAK2 and deletions of SOCS1. [28,29]

This study was conducted to identify genetic alterations and rearrangements in diffuse large B-cell lymphoma and to examine their association with clinical features, response to therapy and final outcome.

In the present study, there was significant decrease in Hb level in patient groups as compared to control group, also Hb level was significantly lower in patients with stage (III+IV) than those with stage (I+II) ($p = 0.025$). Moreover, platelet count was significantly lower in patients with stage (III+IV) than those with stage (I+II) ($p = 0.035$). These findings are similar to those reported by ***Lim et al. (2005)***. [30]

Regarding serum LDH and β2 microglobulin in our study, LDH was significantly higher in late stage (stage III+IV) than in early stage (stage I+II) of the disease ($p = 0.021$). Serum β2 microglobulin was significantly elevated in total patients as compared to control group with no significant difference between patients with stage (III+IV) and those with stage (I+II).

These findings are in agreement with ***Shamoon et al. (2010),*** [31] who found that there was a significant correlation between serum LDH activity and the extent of the lymphoma represented by stage of the disease. Furthermore, ***Dumontet et al. (1999),*** [189] studied profiles and prognostic values of LDH isoenzymes in patients with non-Hodgkin's lymphoma and found that high absolute values of isoenzyme 3 were correlated with an altered performance status and advanced tumor stage. Increased serum LDH is generally attributed to tumor aggressiveness or a high tumor burden.

Recently, ***Mazher et al. (2017),*** [32] found that serum levels of β2 microglobulin and lactate dehydrogenase 2 isoenzyme were significantly raised in NHL patients compared with controls. There was also significant difference when the values were compared between the patients of NHL with and without bone marrow infiltration.

In the current study, the stage of the disease was evaluated as a prognostic factor for response to treatment and overall survival. It was observed that the number of DLBCL patients who achieved complete remission was significantly higher in early stage of the disease (stage I+II) than in late stage (stage III+ IV). On the other hand, the number of DLBCL patients who achieved partial response to treatment was significantly higher in late stage than in early stage. Reportedly, there was no significant difference in overall survival of patients over 36 months between early and late stages of the disease.

In agreement with our study**, *Abdelhamid et al. (2011),*** [26] evaluated the clinical prognostic factors of diffuse large B cell non-Hodgkin's lymphoma and found that the CR of cases with stages I + II was 93.8% versus 68.5% for those with stages III + IV ($p < 0.001$).

Recently, ***Valencia et al. (2017),*** [33] studied the response rate, survival, and prognostic factors in patients with DLBCL treated with R - CHOP and observed that the response rates and survival were lower than those reported in other studies probably because most patients had disseminated disease.

In addition, ***Yang et al. (2009),*** [34] evaluated the prognostic factors of international prognostic index (IPI) in DLBCL patients in an era of R-CHOP in Chinese population and they found that only early clinical stages and absence of bulky disease were statistically significantly associated with the better CR rate.

Interestingly, ***Cho et al. (2017),*** [35] investigated the treatment strategy, prognostic factors, and risk factors of early death in elderly DLBCL patients (age ≥ 65 years) in the rituximab era. They identified independent prognostic factors included high-risk age adjusted international prognostic index (aaIPI) score, very old age and bone marrow involvement.

In our study, target sequencing was performed on 25 lymph node biopsy samples and 5 bone marrow biopsy samples from patients with DLBCL for detection of some genetic mutations, including ***BCL 10, GNA13, MEF2B, PRDM1, BCL6, BCL2, CARD11, PIM1*** and ***TBX21.*** Only 4 of these genetic mutations were detected (***BCL2, CARD11, PRDM1*** and ***TBX21)***. 12 out of 30 patients had genetic mutations while the remaining 18 patients did not have any genetic mutation; 11 out of 12 patients had BCL2 mutation with 34 variants, 2 patients had CARD11 mutation with 3 variants, one patient had PRDM1 mutation with 2 variants and 4 patients had TBX21 mutation with 6 variants. Moreover, target sequencing was performed on 10 peripheral blood samples from healthy subjects for detection of the same genetic mutations and no any genetic mutation was detected.

BCL2; B-cell lymphoma 2, is an anti-apoptotic protein belongs to a large family of proteins involved in the regulation of programmed cell death. It is important in normal B-cell development and

differentiation, and plays a major role in the response of malignant cells to a variety of stresses that may lead to apoptosis, including chemotherapy. [36-38] ***BCL2*** was the most frequent gene affected in our patients representing 75.6% with statistically significant increased frequency as compared to other genes (p <0.001). This finding was supported by ***Schuetz et al. (2012),*** [39] who sequenced BCL2 in primary DLBCL biopsies, additional non-Hodgkin's lymphoma biopsies, DLBCL cell lines and germline DNAs. They found frequent BCL2 mutations in follicular lymphoma and germinal center B-cell (GCB) DLBCL, but low levels of BCL2 mutations in activated B-cell DLBCL, mantle cell lymphoma, small lymphocytic leukemia, and peripheral T-cell lymphoma and no BCL2 mutations in GC centroblasts. Considering these observations, BCL2 seems to be the most highly mutated gene in germinal center B-cell (GCB) DLBCL.

Additionally, ***Arif et al. (2009),*** [40] investigated the frequency of BCL2 gene rearrangement in B-cell non-Hodgkin's lymphomas (follicular lymphoma, diffuse large B-cell lymphoma and T-cell rich B-cell lymphoma) and reported that BCL2 gene rearrangement is quite frequent in follicular lymphoma, followed by diffuse large B-cell lymphoma.

Meanwhile, ***Almasri et al. (2005),*** [41] studied BCL2 gene rearrangement in Jordanian follicular and diffuse large B-cell lymphomas and observed that BCL2 gene rearrangement was present in the vast majority of Jordanian follicular lymphoma and approximately one third of DLBCL cases.

As regards the genetic variants in our study, C-T was the most frequent variant representing 62.2% with statistically significant increased frequency as compared to other variants (p < 0.001). There was statistically significant difference between the stage of the disease and genetic variants (P = 0.048), however, no significant difference was observed between the response to treatment and genetic variants. Patientswho didn't have identified variants had slight superiority in overall survival than those who had identified variants, with no statistically significant difference between both patient groups (p value=0.224).

The association of BCL2 expression with survival in patients with DLBCL treated with CHOP or CHOP-like regimens had conflicting results. It is worth noting that ***Wei et al. (2015),*** [42] studied expression and prognostic value of BCL-2 protein in DLBCL and found that BCL-2 expression was positive in 64.8%, and negative in 35.2% of cases and that BCL-2 expression level had no significant impact on overall survival in all DLBCL patients. Similar findings were also presented by ***Hans et al. (2004),*** [43] ***Vitolo et al. (1998),*** [44] and ***Küçükzeybek et al. (2013).*** [45]

On the contrary, ***Kawasaki et al. (2001),*** [46]detected structural alterations of BCL1, BCL2, BCL6, and c-MYC in DLBCL and concluded that rearrangements of BCL1, BCL2, BCL6, and c-MYC genes correlated with the clinical outcome and may serve as prognostic markers in DLBCL patients. Also, the results of ***Song et al. (2009),*** [47] showed that the BCL2 negative GC patients had the most favorable prognosis among patients with DLBCL that received R-CHOP. Furthermore, ***Yunis et al. (1989),*** [48] studied the prognosis of BCL2 and other genomic alterations in large-cell lymphoma and concluded that BCL2 rearrangement is associated with a relatively poor prognosis. In addition, ***Colomo et al. (2003),*** [49] found that the expression of BCL2 was associated with advanced stage, high or high-intermediate IPI, and poor overall survival.

Notably, BCL2 over-expression has been reported in approximately 40%-60% of patients with DLBCL, and has been associated with poorer survival. However, no correlation with survival was seen in patients receiving chemotherapy and rituximab, implying that the addition of rituximab had eliminated the negative impact of the BCL2 over-expression. [208]

Recently, the prognostic significance of BCL2 expression was evaluated within the context of DLBCL molecular subtypes in patients treated with R-CHOP. [50] BCL2 expression was predictive of poorer outcome within the germinal center B-cell (GCB) but not the activated B-cell (ABC) subtype of DLBCL, which is the opposite of what had been noted in patients treated with CHOP alone. [51]This finding may be explained by the differential mechanism by which BCL2 expression occurs within the molecular subtypes and the mode of action of rituximab. [52]

TBX21 (T-bet); a T-box transcription factor, is expressed in CD4+ T lymphocytes committed to Th1 T-cell development and may participate in immunoglobulin class switching in B lymphocytes. T-bet is also expressed in a subset of T-cell lymphomas, particularly those that express other markers of Th1 T-cell differentiation, and in a subset of B-cell non-Hodgkin's lymphomas. [53] ***TBX21*** gene mutation representing 13.3% in the current study.

Conversely, ***Dorfman et al. (2004),*** [54] studied T-bet expression in a subset of B-cell lymphoproliferative disorders and found that T-bet was expressed consistently in precursor B-cell lymphoblastic leukemia /lymphoblastic lymphoma and that T-bet was expressed in memory B cell-derived neoplasms (chronic lymphocytic leukemia, marginal zone lymphoma, hairy cell leukemia), but not in cases of mantle cell, follicular, and large cell

lymphomas. Moreover, ***Dorfman et al. (2005),*** [53] found that almost all cases of DLBCL and most cases of anaplastic large cell lymphoma, neoplasms that may be confused with Hodgkin's lymphoma, are negative for T-bet.

CARD11; caspase recruitment domain-containing protein 11, provides instructions for making a protein involved in the function of immune system cells (lymphocytes). In normal B cells, antigen receptor-induced NF-kappa B activation requires CARD11. [55] ***CARD11*** gene mutation representing 6.7% in the current study.

In agreement with our study, ***Bu et al. (2012),*** [215]studied the role of nuclear factor-κB regulators TNFAIP3 and CARD11 in Middle Eastern diffuse large B-cell lymphoma and found that the incidence of CARD11 was 10.7%. Interestingly, CARD11 amplification was seen in a significant proportion of cases of DLBCL (23%) and was linked to NF-κB activation (p=0.01). Immunohistochemical analysis of DLBCL samples showed CARD11 over-expression. [56]

Of interest, ***Lenz et al. (2008),*** [55] studied CARD11 gene mutations in human diffuse large B cell lymphoma and demonstrated that CARD11 is a bona fide oncogene in DLBCL, providing a genetic rationale for the development of pharmacological inhibitors of the CARD11 pathway for DLBCL therapy.

Recently, ***Zhao et al. (2016),*** [57] studied the expression and prognostic value of CARD11 in diffuse large B cell lymphoma and observed that the positive rate of high CARD11 expression in DLBCL was 65.33% and that CARD11 expression was associated with an inferior event free survival.

On the other hand, ***Thompson et al. (2011),*** [58]described for the first time a molecular link between increased NF-κB activity and reduced expression of CD10, both of which are poor prognostic markers for B-cell lymphoma, high nuclear NF-κB activity which induced by expression of a lymphoma-derived mutant CARD11 protein is associated with the ABC subtype of DLBCL, which also carries a worse clinical outcome than GCB subtype DLBCL, which has lower NF-κB activity.

Positive regulatory domain zinc finger protein 1(PRDM1)/B lymphocyte-induced maturation protein 1 (BLIMP1) is a transcriptional repressor expressed in a subset of GCB and in all plasma cells and required for terminal B cell differentiation. The BLIMP1 locus lies on chromosome 6q21-q22.1, a region frequently deleted in B cell lymphomas, suggesting that it may harbor a tumor suppressor gene. [59] ***PRDM*** gene mutation representing 4.4% in our study.

Pasqualucci et al. (2006), [59] reported that the BLIMP1 gene is inactivated by structural alterations in 24% activated B cell–like diffuse large cell lymphoma (ABC-DLBCL), but not in GCB or unclassified DLBCL. These findings pointed to a role for BLIMP1 as a tumor suppressor gene, whose inactivation may contribute to lymphomagenesis by blocking post–GC differentiation of B cells toward plasma cells.

Also, ***Tate et al. (2007),*** [60]studied BLIMP1/PRDM1 gene mutations in B-cell lymphoma and found that in DLBCL a single base substitution in exon 6 results in a somatic nonsense mutation. These findings indicate that mutational analysis of the BLIMP1 gene may be useful for characterizing the molecular basis of B-cell lymphoma.

On the other hand, **Song et al. (2010),** [61] studied the effects of BLIMP1 on the prognosis of diffuse large B-cell lymphoma and found that BLIMP1 was detected in 30.0% of patients, and was associated with a significantly shorter overall survival.

Recently, ***Xia et al. (2017),*** [62] studied the effect of loss of PRDM1/BLIMP1 function on poor prognosis of activated B-cell-like diffuse large B-cell lymphoma and suggested that loss of PRDM1/BLIMP1 function contributes to the overall poor prognosis of ABC-DLBCL patients.

Regarding ***BCL10*** gene, no any genetic mutation could be detected in our study. Our results are similar to those obtained by ***Takahashi et al. (1999),*** [63] who suggested that somatic mutations of BCL10, if they occur at all, are rare in B-cell NHLs and do not commonly contribute to their molecular pathogenesis. Also, ***Achuthan et al. (2002),*** [64] concluded that rearrangements of the BCL10 gene are uncommon in lymphoma and may be limited to the MALT subtype of non-Hodgkin's lymphomas.

In contrast to our study, ***Ohshima et al. (2001),*** [65] studied the role of BCL10 expression in DLBCL and found that BCL10 expression was associated with extra-nodal DLBCL, but not with prognosis.

GNA13; guanine nucleotide-binding protein subunit alpha-13, is the gene encoding G protein Gα13 in Burkitt's lymphoma and DLBCL and functions as critical signal transduction molecule that regulates cell survival, proliferation, motility and differentiation. The aberrant expression and/or function of this molecule has been linked to the growth, progression and metastasis of various cancers. [66] No any genetic mutation in ***GNA13*** could be detected in our study. In contrast to our results, ***Dubois et al. (2016),*** [67] found that TNFAIP3 and GNA13 mutations in ABC patients treated with R-CHOP were associated with significantly less favorable prognosis.

MEF2B; myocyte enhancer binding factor 2B, is the gene encodes a transcriptional activator and is mutated in 11% of DLBCL and 12% of follicular

lymphoma. It directly activated the transcription of the proto-oncogene BCL6 in normal germinal-center (GC) B cells and was required for DLBCL proliferation. [68] Mutation in this ***MEF2B*** gene could not be detected in any patient included in our study.

On the other hand, ***Lohr et al. (2011),*** [69] studied somatic mutation in DLBCL by whole exome sequencing, and found that MEF2B mutations were observed in 18% of DLBCL patients.

BCL6 is a transcription factor that has essential role in normal antibody response. It is involved in chromosomal translocations in DLBCL and nodular lymphocyte predominant Hodgkin's lymphoma, and is expressed in follicular lymphoma and Burkitt's lymphoma. [70] In our study, ***BCL6*** genetic mutation could not be detected in any patient, similar to the finding of ***Jovanovic et al. (2015).*** [71]

In contrast to our study, ***Barrans et al. (2002),*** [72] studied rearrangement of the BCL6 locus at 3q27 as an independent poor prognostic factor in nodal diffuse large B-cell lymphoma. They concluded that rearrangement of 3q27, BCL2 expression and the absence of a GC phenotype were associated with a poor prognosis. These factors can be used in conjunction with the IPI to improve risk stratification in nodal DLBCL.

PIM1; proto-oncogene serine/threonine protein kinase, is a member of a class of serine /threonine kinases with distinct molecular and biochemical features that regulate various oncogenic pathways including B- cell development. Its inhibition suppressed cell proliferation and migration, induced apoptotic cell death and synergized with other chemotherapeutic agents. [73] ***PIM1*** could not be detected in any patient included in our study.

On the other hand, ***Schatz et al. (2010),*** [74]studied PIM as a common and adverse prognostic marker in lymphoma, and observed the common expression of PIM1, PIM2, or both proteins in DLBCL (65.5%), follicular lymphoma (58%), small lymphocytic lymphoma (76.5%) and mantle cell lymphoma (89.7%). Importantly, Kaplan-Meier survival analysis of clinical data linked to patients with DLBCL showed a strong trend toward a worse overall survival when PIM expression is present in diagnostic tumor samples compared to PIM-negative tumors. They concluded that PIM kinase activity can be used as a major mediator of oncogenesis in multiple NHL subtypes.

Also **Brault et al. (2012),** [75] studied the role of PIM kinases as progression markers and emerging therapeutic targets in DLBCL, evaluated the correlation of nuclear PIM1 expression with disease stage and the modest response to small-molecule inhibitors and suggested that PIM kinases are progression markers rather than primary therapeutic targets in DLBCL.

The conflicting reports about the association of genetic alterations and rearrangements in diffuse large B-cell lymphoma with the clinical outcome in the literature can partly be attributed to the following: (i) heterogeneity of the DLBCL cases studied with different proportion of GCB and ABC- DLBCL cases, (ii) patient population with different risk factors other than DLBCL subtype distinction, (iii) variables in management, (iv) technical factors affecting immunostaining, and (v) experience and subjectivity of the pathologist scoring the cases.

Conclusion

- **From this work, it could be concluded that:**
- *BCL2* gene mutation can be regarded as a potential genetic risk factor in Egyptian patients with DLBCL, however it has no significant impact on the clinical outcome.
- The prognostic significance of genetic alterations and rearrangements in DLBCL should be evaluated in the context of molecular subtypes in future studies.
- Clinical prognostic factors and pathological markers are mandatory to individualize treatment in patients with DLBCL.
- Mutational analysis of the BCL2 gene could provide new insights into the molecular pathogenesis of DLBCL.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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