RESEARCH ARTICLE

Evaluation of BCL6 and MUM1 Expression in Patients with Diffuse Large B cell Lymphoma and their Correlations with Staging and Prognosis in Iran

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Abstract

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common form of non-Hodgkins lymphoma (NHL), accounting for approximately 25% of NHL cases. The aim of this study was to evaluate the association between the BCL6 and MUM1 gene expression and patient prognosis and stage. Materials and Methods: After ethical approval, in a cross-sectional study, tissue samples of 80 patients with diffuse large B-cell lymphoma were analyzed for BCL6 and MUM1 gene expression. Immunohistochemical staining was performed with division into categories of 0-5%, 5-25%, 26-50%, 51-75% and more than 75%. Other clinical and histological information such as lymph node involvement, T-stage, B symptoms and patient outcome were also recorded. Data were analyzed with SPSS version 16 and a P-value less than 0.05 was considered significant. Results: The patient mean age was 46.9±10.5 years (47.6±10.7 and 46.1±9.6 for males and females, respectively). A significant association was seen between lymphoma stage and BCL6 (p=0.045) but not MUM1 expression (p=0.09). However, the latter was associated with mortality (p=0.006) as was also the BCL6 level (p=0.006). Conclusions: : Overexpression of MUM1 and BCL6 is associated with poor prognosis in patients with diffuse large B-cell lymphoma.

Keywords: Diffuse large B-cell lymphoma - MUM1 - BCL6 - prognosis - Iran

Asian Pac J Cancer Prev, **16 (1)**, 83-86

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL) which includes about 25% of the cases. Patients with DLBCL have a rapidly growing mass that is located in the neck or abdominal region (Gatter et al., 2001). B systematic symptoms (fever, weight loss, extreme night sweats) are observed in about 30% of patients, and serum LDH increases in more than 50% of patients. Approximately in 60% of patients' disease progresses into advanced stage lymphoma (usually stage III or IV) whereas 40% have localized lymphoma. Bone marrow is involved in 30% of cases (Vaque et al., 2014). Extranodular forms happen in 40% of cases (Kolokotronis et al., 2005; Muller et al., 2005; Sehn et al., 2005; Cook, 2007; Davies et al., 2007; Anderson et al., 2009; Gascoyne, 2014). The most common extranodular location of non-Hodgkin lymphoma is gastrointestinal system. But the disease can originate from any tissue such as skin, lung, bone, thyroid, mediastinal, central nervous system, breast, and testis. Normal cells transform into cancerous cells throughout the course of several stages as they mutate to pre-cancer and finally cancer (Anderson et al., 2009). The existence of thousands of chromosomal changes in cancer and pre-cancer cells mutate multiple times causing cancer. Transgenic laboratory mice confirm the role of multiple designs in the cause of cancer and express a high rate of BCL2. Principles of tumor immunology include studying antigens on tumor cells and immune response to antigens. Two types of tumor antigens on tumor cells were recognized: tumor-specific transplantation antigens (TSTAs) and tumor associated transplantation antigens (TATAs) (Habara et al., 2012).

MUM1 has an important role in lymphoid cell differentiation. MUM1/IRF4 is as oncogene related to myeloma and translocation (p25; q32), t (Cook, 2007; Zhang et al., 2012) and leads to a change in the heavy chain of immunoglobulin (IgH). BCL6 exists in approximately 100% of follicular cell cancer cases, 100% of Burkitt lymphoma, 80% of large B-cell lymphoma, and in more than 80% of nodular lymphocytic Hodgkin lymphoma cases. BCL6 is related to cancers with a high proliferation cell index. In some studies BCL6 is expressed as a prognostic factor in LBCL patients (De Mello et al., 2011).

Determining the gene profile using an array-based

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technology provided the assessment of thousands of genes. This technology leads to the possibility of identifying patterns in gene incidence with diagnostic importance, prognosis, and establishment of new treatment goals. Therefore this study deals with the relation between BCL6 expression and MUM1 with staging and prognosis in patients with diffuse large B-cell lymphoma.

Materials and Methods

In this cross-sectional descriptive analytical study, BCL6 and MUM1 expressions were reviewed in biopsies of patients diagnosed with DLBCL in Ghaem Hospital in 1388-1391. This study was approved by the ethical committee of Mashhad University of Medical Sciences.

Patients

The non-probability sampling method (purposive sampling) was used for data selection in our study. Regard to Khaldoun and colleagues study which reported 78% BCL6, sample size was estimated 60 cases. Patients who were diagnosed with DLBCL between 1388 and 1391 in Ghaem Hospital were included in the study, and those without adequate immunohistochemical tissue samples, complete records, or a definite diagnosis were excluded from the study.

Staining and tissue processing

In this study, paraffin blocks of lymph node biopsy samples of patients diagnosed with DLBCL between 1387-1391were selected, and their biopsies were cut at 5 microns thickness. After cutting, they were placed at 60 degrees for one hour, and by covering a slide with a poly-L-lysine, a glass slide was made. Sections removed from paraffin, were cleared in xylene for 10 minutes, re-hydrated, and placed in ethanol for a further 3 minutes. The slides were then placed in a salt buffer. 3% H2O2 at room temperature for 10 minutes was used for enzyme blocks, and citrate was used for active biotin blocks. After blocking protein, MUM1 and BCL6 antibodies were added to samples that were reviewed for CD10. Samples were incubated for 60 minutes. After that, the EnVision polymer was poured on the LAMs for 30-45 minutes and 3 rinsing phases with a Tris buffer were performed. Samples were stained with DAB solution 10 minutes after having been rinsed with distilled water. The sections were finally stained with non-alcoholic hematoxylin. First, sections with a few microns were separated from lymph node paraffin blocks and placed in 20% alcohol. Then wrinkled boils were opened with bain-marie temperature and placed on active LAMs to be fixed at environmental temperature in an 8-24 hour period of time. After that, each sample was placed in a xylene dish for 5 minutes until it was deparaffinized. After that, four dishes with 70, 80, 90, and 100 degrees of alcohol were dehydrated in four stages of 2-3 minutes. Then samples were rinsed with distilled water. Retroval antigens were added to Tris-EDTA solution and placed in a bain-marie at 95 degrees for 30 minutes. After being taken out of the bain-marie, they were rinsed with distilled water again. A Dako Pen was used to draw a line on the LAM around the tissue. The samples were placed in a solution of 3% H2O2 for 10 minutes and then rinsed in a TRIS buffer solution for

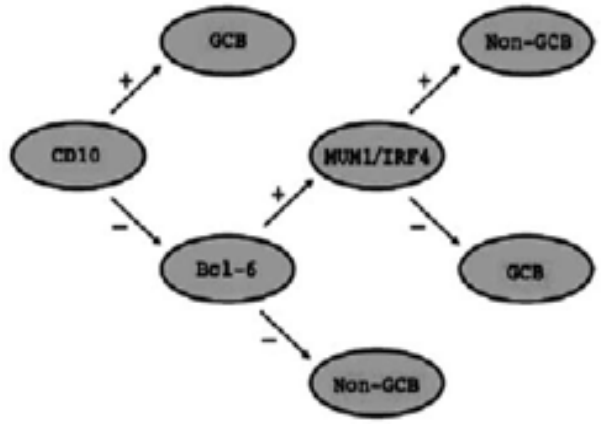

Figure 1. The Classification of Lymphoma Based on GCB and Non-GCB is Shown

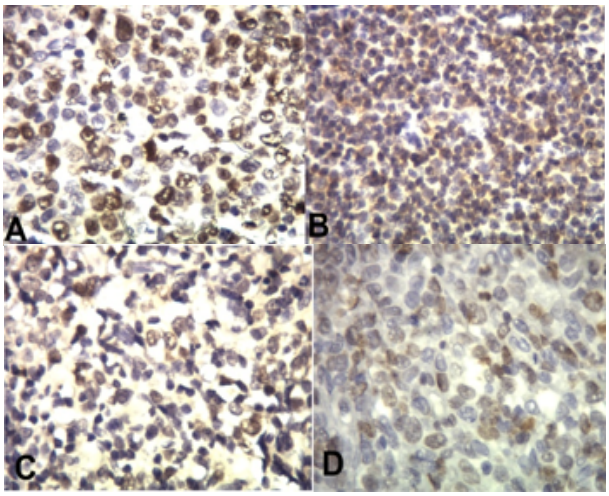

Figure 2. Immunohistochemical Staining of Nuclei for MUM1. A) Staining is observed in 80% of cells. **B)** Staining is seen in 60% of cells. **C)** Staining is observed in 10% of cells. **D)** Staining is observed in 20% of cells

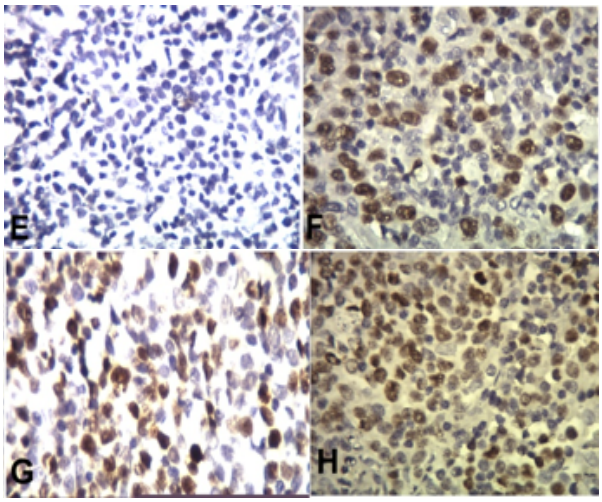

Figure 3. Immunohistochemical staining of nuclei for BCL6. E) Lack of staining is observed in the cells. **F)** Staining is observed in 35% of cells. **G)** Staining is observed in 50% of cells. **H)** Staining is observed in 60% of cells

Table 1. The Relationship between BCL6 and MUM1 Staining with a Cutoff Level of 25% and Disease Stage

		Stage				p value
		1	2	3	4	
BCL6 Staining	Less than 25%	0	17 (68)	8 (32)	0	0/001
	More than 25%	4 (7/3)	8 (14/5)	33(60)	10 (18/2)	
MUM1 Staining	Less than 25%	4 (13/3)	13 (43/3)	13 (43/3)	0	0/001
	More than 25%	0	12 (24)	28 (65)	10 (20)	

Table 2. The Relationship between MUM1 and BCL6 and Patient Mortality

		-	+	p value
MUM1 Staining	0-5%	9 (52/9)	8 (47/1)	0/006
	5-25%	0	8 (100)	
	26-50%	4 (18/2)	18 (81/8)	
	51-75%	9 (37/5)	15 (62/5)	
	75%<	0	9 (100)	
BCL6 Staining	0-5%	9 (52/9)	8 (47/1)	0/006
	5-25%	0	8 (100)	
	26-50%	4 (18/2)	18 (81/8)	
	51-75%	9 (37/5)	15 (62/5)	
	75%<	0	9 (100)	

Table 3. The Average Life Expectancy of Patients Based on MUM1 and BCL6

		Mean±Standard deviation (months)	p value
MUM1 Staining	25%>	9/3±2/9	0/463
	25%<	8/6±4/7	
BCL6 Staining	25%>	8/5±3/3	0/598
	25%<	9±4/4	

Table 4. Mean Age and Average Life Expectancy of GCB and non-GCB Patients

		Mean±Standard deviation	p value
Age (years)	GCB	49/2±8/7	0/005
	NON-GCB	44/6±12	
Life expectancy	GCB	9±3/2	0/151
(months)	NON-GCB	8/7±5	

5 minutes. BCL6 antibody with a dilution of 1/5% was poured on the samples overnight, and MUM1 antibody with a dilution of 1/100 was poured on the samples for a period of 1/5 of an hour. CD10 did not need to be prepared and was placed in proximity to the samples overnight. (Figure-2 and Figure-3)

After that, the samples were once again rinsed with a TRIS buffer solution for 5 minutes and then the secondary antibody from the Abcam Company was placed in proximity to the samples for 30 minutes. The next stage involved placing the samples in a Chromogen buffer for 10 minutes and then again using the TRIS buffer solution for 5 minutes. The samples were then immersed in an ammonia dip and again rinsed with distilled water. Hydration of the samples with the use of alcohol with a dilution of 100, 90, 80, and 70 degrees was performed.

In a similar study conducted by Anderson and colleagues in 2009, samples with a count of 1000 cells in 10 fields were investigated for percentage of cell staining for MUM1, and were MUM1 positive if there was staining of more than 40% of cells and MUM1 negative if there was less than 40% staining.

Likewise, in the investigation of BCL6, cells providing a stain greater than 20% positive and less than that negative were considered.

The relationship between the expression level BCL6 and MUM1 variables in the study were investigated. In Image 2, the various stains for MUM1 cells are shown. In Image 3, the various stains for BCL6 cells are shown.

Statistical analysis

The statistical analysis of this study was conducted using SPSS version 16. A chi-square test was used for nominal variables and, as necessary, a Fischer's exact test. For quantitative variables, the Kolmogorov-Smirnov test was used to determine the parametric and non-parametric data. For dependent comparative data, a paired T-test (parametric) was used, and in cases of non-parametric data, a Wilkinson's test was used. For independent comparative data, an independent T-test (parametric) was used and in case of non-parametric data, a Mann-Whitney test was used. 0.05 was considered significant for this study.

Results

The patients' mean age was 46.9±10.5 years. The mean age of men was 47.6±10.7 years, and the mean age of women was 46.1±9.6. 61 patients (76.2%) were male, and 19 patients (23.7%) were female. 4 patients (5%) had stage I of the disease; 10 patients had stage IV. The frequency of stages III and IV were 31.2% and 51.2%, respectively.

Finally, 58 patients (72.5%) died, and 22 (27.5%) survived. Stains for MUM1 in 38.8% of patients were between 51-75%, and 11.2% of patients had a MUM1 stain greater than 75%. In 27.5% of patients, the MUM1 stain was between 0-5%. The stain for BCL6 in 30% of patients was between 51-75%, between 26-50% in 27.5% of patients, 0-5% in 21.2% of patients, greater than 75% in 11.2% of patients, and between 5-25% in 10% of patients.

A cutoff level of 25% for BCL6 and MUM1 staining was considered, and based on that, the relationship between disease stage and two antigens was investigated. (Table 1)

In Table 2, the staining relationship between MUM1 and BCL6 and patient mortality is shown.

In Table 3, the patients' average of life expectancy based on MUM1 and BCL6 is given.

In Table 4, the mean age and average life expectancy of GCB and non-GCB patients is compared.

There was no significant difference in the mortality of GCB and non-GCB patients (p=0.458)

Discussion

Determining the gene profile using an array-based technology provided the assessment of thousands of genes. This technology leads to the possibility of identifying patterns in gene incidence with diagnostic importance, prognosis, and establishment of new treatment goals. The goal of this study was the identification of new prognostic factors in diffuse large B-cell lymphoma. MUM1/IRF4 is made by means of interferon and related to cell proliferation, survival, and resistance to viral infection. Evidence shows that MUM1 in the last stage the plasma cell's change to B lymphoma plays an important role. MUM1 is expressed in 50%-70% of DLBCL cases (12). BCL6 is one of the regulators in a multi-functional cell cycle and plays a role in many of the various processes like differentiation of lymphocytes and immune response.

In most studies, a cutoff level of the MUM1 and BCL6 gene expression equal to 75% was considered, but our study showed that a cutoff level of 25% is linked with the stage of disease. A MUM1 gene expression more than 25% is linked with patient mortality. However, these two genes with a cutoff level of 25% play a role in determining the survival of patients.

Our study also showed that MUM1 and BCL6 gene expression in affected patients regardless of classification of germinal center are factors in determining patient mortality which can be used as a prognostic factor in both GCB and non-GCB.

On the other hand, many studies of BCL6 gene expression were accompanied by better prognosis, but in the present study, for reasons of consistency and positive correlation between BCL6 and MUM1, more expression of these two genes was attributed to a higher mortality.

In one study, the existence of MUM1 and CD10 did not have an effect on the survival of patients (Bodoon et al., 2012). In Zhang's study, the patient's life expectancy with positive BCL6 was significantly better than patients with negative BCL6 (Zhang et al., 2012). Mello introduced BCL6 as the strongest factor in predicting prognosis and following it, CD1 and MUM1 (De Mello et al., 2011). The results of this study were not consistent with our study which may be due to the low mean age of our patients.

The results of our study showed that MUM1 gene expression is the most important factor in determining patient mortality. MUM1 gene expression with a cutoff level of 25% is an important factor in predicting disease stage and mortality in patients affected with diffuse large B-cell lymphoma. MUM1 gene expression is not an effective factor in estimating the life expectancy of patients. BCL6 gene expression with a cutoff level of 75% is a worse predictive prognosis for patients.

One of the most important limitations of our study was that part of the information was collected retrospectively from incomplete patient records which made obtaining some information impossible.

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