Bcl-2 gene rearrangement in Jordanian follicular and diffuse large B-cell lymphomas

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ABSTRACT

Objective: Follicular lymphoma (FL), a common subtype of non-Hodgkin's lymphoma (NHL) in the West, represents a rare subtype in Jordan. Bcl-2 gene rearrangement plays a crucial role in the biology of the vast majority of FL and a substantial number of diffuse large B-cell lymphoma (DLBCL) in the West; but its presence has not been studied in Jordan. Our aims are to document if bcl-2 gene rearrangement exists in Jordanian FL and DLBCL, and if present to determine whether its frequency among these lymphomas is different from the West and therefore may be responsible for some of the epidemiological differences seen between Jordan and the West.

Methods: The study was conducted in the year 2001 using polymerase chain reaction (PCR), to detect bcl-2 gene rearrangement in paraffin sections in 5 FL and 23 DLBCL cases diagnosed at the Department of Pathology at Jordan University of Science and Technology, Irbid, Jordan. Two sets of primers including the major breakpoint region (MBR) and the minor cluster region (MCR) were used.

Results: Amplifiable DNA was extracted from all cases. Bcl-2 gene rearrangement was seen among 4 (80%) of 5 FL cases, and 8 (35%) of 23 DLBCL cases. The majority of the rearrangements involved the MBR; however, one fourth of cases (one of 4 FL; 2 of 8 DLBCL) with bcl-2 rearrangement involved the MCR.

Conclusion: Bcl-2 gene rearrangement was seen in the vast majority of Jordanian FL cases and approximately one third of all DLBCL cases. These figures are similar to those reported in the West, and therefore bcl-2 gene rearrangement does not help in explaining the epidemiological differences of NHL between Jordan and the West. The presence of bcl-2 gene rearrangement in DLBCL may define a subset of lymphomas that may be biologically and clinically unique and different from the rest of DLBCL.

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The t(14;18) translocation is characterized by juxtaposition of the bcl-2 gene on chromosome 18 with the immunoglobulin H gene on chromosome 14.[1-3] This results in over expression of the Bcl-2 protein that promotes cell survival and prevents apoptosis,[4] and thus may be responsible for malignant transformation in follicular lymphoma (FL) and a significant number of diffuse large B-cell lymphoma (DLBCL). Most of the breaks on chromosome 18 are located in the 3` untranslated region of exon 3 of the bcl-2 gene, and are tightly clustered in 2 regions referred to as a major breakpoint cluster region (MBR) and minor breakpoint cluster region (MCR).[2,5] Translocations involving MBR and MCR are responsible for 60% and 20% of all FL cases.[6,7] Because of the tight clustering of the MBR and MCR breakpoints, it is possible to detect the t(14;18) by polymerase chain reaction (PCR). Malignant lymphoma (ML) in Jordan appears to have certain features that distinguish it from ML in the West.[8,9] In particular FL, one of the most common ML in the West, is

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very rare in Jordan, whereas DLBCL is by far the most common lymphoma in Jordan.[8,9] This epidemiological difference may be a reflection of biologic differences between ML in different parts of the world. Because t(14;18) represents the most common translocation encountered in ML, and because it can be detected in formalin fixed tissue by PCR, we attempted to evaluate its presence in Jordanian FL and DLBCL. As such this will be the first study to assess the presence of t(14;18) in Jordanian ML, and it will try to shed some light on the biology of ML in Jordan, and specifically identify differences or similarities between t(14;18) among Jordanian and Western ML.

Methods. Samples were collected from the Department of Pathology, Jordan University of Science and Technology, Irbid, Jordan to which all pathology specimens from North Jordan were referred. All cases diagnosed as follicular lymphoma or diffuse large B-cell lymphoma were re-examined, and diagnosis was confirmed by one of us (Almasri N). Twenty-eight cases with available paraffin blocks containing adequate tissue were included in this study. These cases included 5 FL and 23 DLBCLs. The FL cases included 3 males and 2 females with an age range from 39-71 years (average, 52.4). The DLBCL cases included 10 males and 13 females with an age range from 5-94 years (average, 47.9).

Deoxyribonucleic acid extraction. We used the method described by Tbakhi et al[10] with slight modifications. Eight to 10 mm thick sections from paraffin embedded sections were collected in 1.5 ml Eppendorf tube containing 800 ml xylene. Sections were incubated for 15 minutes, followed by spinning and discard of the supernatant. This last step was repeated 3 times. Xylene was removed by 3 times rinsing of the samples in absolute ethanol followed by centrifugation. Samples were allowed to dry in an oven at 80°C for 15 minutes. This was followed by incubation at 55°C for 3-6 hours or overnight in 300 ml of digestion buffer (50 mM Tris pH 8.5, 1 mM EDTA, 0.5% Tween-20) containing proteinase K at a concentration of 200 mg/ml. Proteinase K was inactivated by heating at 95°C for 10 minutes. Centrifugation was carried out and the supernatant was transferred into a new tube.

Table 1 - Primers used.

Set	Gene	Sequence
1	ß-globin	Sense 5'- CAA CTT CAT CCA CGT TCA CC -3' Anti-sense 5'- GAA GAG CCA AGG ACA GGT AC -3'
2	MBR	Sense 5'-GAG TTG CTT TAC GTG GCC TG-3' Anti-sense 5'-ACC TGA GGA GAC GGT GAC C - 3'
3	MCR	Sense 5'-GAC TCC TTT ACG TGC TGG TAC C- 3' Anti-sense 5'- ACC TGA GGA GAC GGT GAC C -3'

MBR - major breakpoint region, MCR - minor cluster region

Oligonucleotide primers. All primers used in PCR amplification were purchased from PROMIGA (2800 Woods Hollow Road, Madison, USA). The sequences of the primers used in this study are listed in **Table 1**.

Polymerase chain reaction amplification. Four ml of each sample was put in a thin PCR tube containing a reaction mixture composed of 10 mM Tris, pH 8.3; 50 mM KCl; 3mM MgCl2; 2.5 units Taq polymerase (PROMIGA, Woods Hollow Road, Madison, USA); 0.5 mM of each primer; 0.4 mM of each deoxyribonucleotides (dNTPs) adjusted to a final volume of 20 ml. Polymerase chain reaction was performed by using a programmable thermal cycler (PROMIGA Mastercycler Personal). The mixture was subjected for 40 cycles of amplification. Each cycle consisted of one minute denaturation at 95°C; one minute annealing at 55°C ß-globin, 50°C MBR, 54°C MCR; and 1.5 minute elongation at 72°C. The last cycle was followed by a 10 minute-elongation step at 72°C.

Analysis of amplified products. Ten ml of each PCR amplified product was electrophoresed on a 1.5% agarose gel containing 5 mg/ml ethidium bromide. Appropriate known positive and negative controls were run with each gel. The DNA bands were detected using ultraviolet light illuminator, and documented by taking pictures using a Polaroid camera. For the ß-globin, MBR and the MCR genes, a PCR reaction was considered positive if an appropriate size band (100-500 bp for MBR and MCR) was seen in the lane.

Results. Amplifiable DNA was successfully isolated from all cases as ß-globin gene was amplified in all of our 28 cases. **Figures 1 and 2** exemplify examples of our cases representing positive and negative bcl-2 gene rearrangement using primers for MBR and MCR. Bcl-2 gene rearrangement, defined by positive reaction by either MBR or MCR, was seen in 80% (4 out of 5) of the FL cases, and 35% (8 out of 23) of the DLBCL cases. Nine of the 12 positive cases were detected using the MBR primer, whereas the remaining 3 positive cases were detected using the MCR primer. Among the 4 FL cases with bcl-2 gene rearrangement, 3 cases were detected using the MBR primer, and only one case was detected by using the MCR primer. Similarly 6 out of the 8 cases of DLBCL with bcl-2 gene rearrangement were detected by using the MBR primer, and only 2 cases were detected by using the MCR primer. No

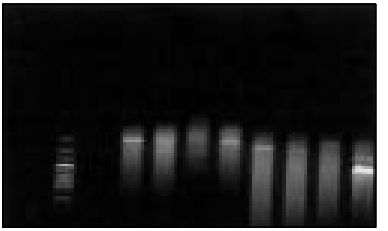

Figure 1 - Analysis of the polymerase chain reaction (PCR) products of the BCL-2 rearrangement at the major breakpoint cluster region (MBR). The PCR was performed with DNA extracted from paraffin embedded tissues. The primer used were JH/MBR primers. The reaction products were electrophoresed on 1.5% agarose gel and stained with ethidium bromide. Lane 1 represents 100 base pair ladder marker; lane 2 negative control; lane 3 positive control; lanes 4 and 5 represent negative cases; lanes 6-10 represent samples that are positive for BCL-2 rearrangement at the MBR region.

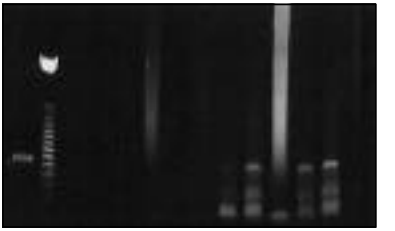

Figure 2 - Analysis of the polymerase chain reaction (PCR) products of the bcl-2 gene rearrangement at the minor breakpoint cluster region (MCR) region. The PCR was performed with DNA extracted from paraffin embedded tissues. The primers used were JH/MCR primers. The reaction products were electrophoresed on 1.5% agarose gel and stained with ethidium bromide. lane 1 represents 50 base pair ladder marker; lane 2 negative control; lanes 3, 4, 5, 6, 7, 10 negative samples; lanes 8, 9, 11 positive samples; and lane 12 positive control.

association of MBR and MCR rearrangements with age or gender was seen.

Discussion. The patterns and subtypes of ML show a wide geographic variation. B-cell non Hodgkin's lymphomas (NHL) predominate in the West, whereas T-cell NHL appear to be more frequent in East Asia.[11] Even among B-cell NHL, wide geographic variations are documented. In particular, follicular lymphomas appear to be a very common lymphoma in the West accounting for up to one third of all NHL in the USA, Canada and Britain.[12,13] On the other hand, FL account for less than 10% of NHL in Asia.[14-19] Non Hodgkin's lymphomas in the Middle East appear to have unique features that distinguish them from Western and Asian NHL. Unlike East Asia, T-cell lymphomas are rare in the Middle East; and yet unlike the West, FL appears to be rare in the Middle East accounting for less than 10% of all NHL.[9] These observations may reflect differences in the biological aspects of lymphomas in different parts of the world. Indeed the most common genetic abnormality seen in FL, namely bcl-2 gene rearrangement has shown similar geographic variations to those of FL. The frequency of bcl-2 gene rearrangement is the highest in the USA where it is seen in approximately 80% of FL.[20-22] This is in contrast to figures between 20-45% in Japan.[23-25] In this study, we documented the presence of bcl-2 gene rearrangement in 80% of all of our FLs. This figure is much higher than those reported from East Asia and appears to be similar to those reported in the USA. In neighboring countries, we are aware only of one study addressing bcl-2 gene rearrangement in FL.[26] In that study, Khalil et al[26] found bcl-2 in 36% of their FL, a frequency that appears to be lower than what we are reporting in this study. It should be pointed out that only 5 cases of FL were evaluated in our report, therefore solid conclusions cannot be drawn with certainty. However, we feel that our findings do not support the notion that biological differences exist between FL in Jordan and the West. Segel et al[27] reached similar conclusion in a review of the world literature on this topic. The frequency of bcl-2 gene rearrangement or its cytogenetic equivalent t(14;18) in DLBCL has been found to range from 10-40%.[3,28-43] Different methodologies and case selection may be responsible for some of the variation in frequency. Although not conclusive, some studies have found that bcl-2 gene rearrangement is associated with poor clinical outcome;[33,35,37] or disseminated disease,[44] other studies failed to confirm this association.[28,36,39] Yet others found that bcl-2 protein expression is more related to worse prognosis among DLBCL cases.[28,39,43,45,46] Regardless of the effect of bcl-2 rearrangement on the prognosis of DLBCL, it appears that this molecular abnormality helps in defining a biologically different subset of DLBCL. Indeed Haung et al[47] found that t(14;18) can define a subgroup of DLBCL which corresponds to a germinal center B-cell expression profile as defined by micro-array gene expression profiling. In the current study, we found evidence of bcl-2 gene rearrangement in 8 (35%) of 23 DLBCL. This figure is within the 10-40% range reported by Hill et al[28] in their review of the literature. Therefore, it would be plausible to conclude that bcl-2 gene rearrangement plays an important role in the development of DLBCL in Jordan, as it is seen in a

frequency similar to if not higher than that reported in the West. It is important to note that in our study we have used primers for both the MBR and the MCR regions of the bcl-2 gene, a fact that lead to increasing the frequency of bcl-2 gene rearrangement from 26% had only probes for the MBR region were used. These results for the first time indicate that one third of Jordanian DLBCL have evidence of bcl-2 gene rearrangement and therefore may be biologically different from the rest of DLBCL and may require further follow up to document if they have different clinical behavior.

In summary, we were able to confirm the presence of bcl-2 gene rearrangement in the vast majority of FL, and in one third of DLBCL, a fact that warrants more research in this field to confirm these findings and to see if they have any clinical implications. Despite the major epidemiological differences in NHL between Jordan and the West, our data do not indicate a significant role for bcl-2 gene rearrangement in these differences.

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