RESEARCH COMMUNICATION

Frequency of bcl-2 Gene Rearrangement in B-Cell Non Hodgkin's Lymphoma

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Abstract

Objective: The objective of the study was to determine the frequency of bcl-2 gene rearrangement in B-cell Non-Hodgkin's lymphoma (NHL) and identify different breakpoints of bcl-2 gene. Methods: Thirty cases of B-cell lymphoma (including 8 cases of follicular lymphoma, 19 cases of diffuse large B-cell lymphoma and 3 cases of T-cell rich B-cell lymphoma) were included in the study. Good quality of DNA was extracted in 4 cases from formalin fixed paraffin embedded tissue and in 26 cases from fine needle aspirate. The polymerase chain reaction was done for major break point region (mbr), minor cluster region (mcr) and intermediate cluster region (icr) of bcl-2 gene. Results: The bcl-2 gene rearrangement was identified in 23.3% of B-cell lymphoma, 50% of follicular lymphoma, 15% of diffuse large B-cell lymphoma and no bcl-2 rearrangement was identified in any of the T-cell rich B-cell lymphomas. Further analysis showed, icr breakpoint in 16.7% of B-cell lymphoma, 37.5% of follicular lymphoma and 10.5% of diffuse large B-cell lymphoma. Involvement of mbr breakpoint was found in 6.7% of B-cell lymphoma, 12.5% of follicular lymphoma, 5.3% of diffuse large B-cell lymphoma. Involvement of mcr breakpoint was not seen in any of the case. Conclusion: The bcl-2 gene rearrangement is quite frequent in follicular lymphoma, followed by diffuse large B-cell lymphoma. The commonest breakpoint in present series is icr followed by mbr. This indicates that primers for bcl-2 gene must include icr primer, whenever bcl-2 gene is being evaluated for B-cell NHL in this part of the world and this might reduce the variability of frequency of bcl-2 gene rearrangement within and between different regions.

Key Words: bcl-2, Non-Hodgkin /follicular/diffuse large B-cell lymphoma - PCR

Asian Pacific J Cancer Prev, **10**, 237-240

Introduction

In the West, among B-cell non-Hodgkin lymphoma (NHL), follicular lymphoma (FL) is a common variety constituting 35-40% of all NHL (Harris et al., 2001) but in our country the proportion varies from less than 5% to 6.48% (Ahmed et al., 1993; Naresh et al., 2004; Mushtaq et al., 2008). Recent developments in the understanding of lymphomas by introduction of new diagnostic tools have enhanced the precise disease definition and recognition of factors predicting prognosis and response to treatment. Genetic abnormalities occur during the gene rearrangements and mutation of genes that characterize normal B cell differentiation (Harris et al., 2001).

The most common chromosomal abnormality associated with B-cell NHL is t(14;18)(q32;q21), resulting in bcl-2 gene rearrangement. This is found in 80-90% of follicular lymphomas and about 20% of diffuse large B-cell lymphoma (DLBCL) (Harris et al., 1994; 2001). The bcl-2 gene rearrangement is diagnostic of follicular lymphoma and poor prognostic marker for DLBCL (Armitage, 1993; Jack & Barrans., 2004). This translocation results in the juxtaposition of the bcl-2, apoptotsis inhibitor proto-oncogene, from chromosome band 18q21 to the heavy-chain IGH enhancer (Eμ) region 14q32, resulting in over expression of bcl-2 thus prolong the life span of B-cells (Ngan et al, 1998; Harris et al., 2001; Chao & Korsmeyer., 1998; Cline., 1994). Breaks of bcl-2 gene commonly occur at major cluster region (mbr) and minor cluster region (mcr). The mbr is located within 3' non coding part of exon 3 while mcr is located 25 kb downstream 3' of exon 3 of bcl-2 (Weiss et al., 1987).

Apart from mbr and mcr, some degree of clustering has been observed 5.3 kb and 6.2 kb downstream of the mbr locus. A significant percentage of translocation t(14;18) can also be detected in a 200-bp-long sequence portion, now called as the "intermediate cluster region (icr)" (Albinger-Hegyi et al., 2002). The present study was planned to find out the frequency of bcl-2 gene in various types of B-cell NHL using the primers for mbr, mcr and icr.

Materials and Methods

The study was carried out at Armed Forces Institute

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of Pathology, Rawalpindi, Pakistan. Thirty cases of B-cell NHL including 8 cases of FL, 19 cases of DLBCL and 3 case of T-cell rich B-cell lymphoma were included in the study. Formalin fixed paraffin embedded (FFPE) blocks of 20 cases of diagnosed FL were subjected to DNA extraction using EX-WAX Paraffin-embedded DNA Extraction Kit, S4530 Chemicon. Good quality of DNA was extracted in only 4 cases.

In rest of 26 cases the source of DNA was fine needle aspirate. Fine needle aspirates were obtained from the cases which were referred for fine needle aspiration with the suspicion of lymphoma. DNA was obtained by using an in-house method for extraction of DNA. Twenty six cases were included in the study, which were typed according to WHO after immunohistochemistry on subsequent biopsy. Quality of DNA was assessed after running on agarose gel. Polymerase chain reaction (PCR) was performed for the detection of bcl-2 gene rearrangement using the primer to consensus JH region and the primers homologus to sequence in the mbr, mcr and icr breakpoints of bcl-2, as already described (Baststone et al., 2005).

A 4 ml PCR mix was prepared: by adding 10x PCR Buffer, 500 µl: dNTPs i.e. dTTP, dATP, dGTP, dCTP, 10 µl x 4: 1M Spermidine, 4 µl: distilled water, 3.5 ml). For 5 ml of 10x PCR buffer: 2M KCL, 1.25 ml: 1M Tris (pH8.3), 0.5 ml: 1M MgCl2, 75 µl: Gelatin (300 bloom), 5 mg, distilled water, 3.2 ml was added). Each reaction volume for PCR contained 10 µl of PCR mix, 1 µl of JH primer, 1 µl of one of the three primers for the sequence regions for bcl-2 and 2µl of the DNA.

Initial denaturation was done for five minutes at 98˚C, followed by 30 cycles, of denaturation at 94°C for one minute, annealing and extension at 60°C for 1.5 minutes, followed by a final extension step at 72°C for seven minutes (Baststone et al., 2005). Positive and negative controls were run with each reaction. The amplified product was detected by polyacrylamide gel electrophoresis followed by staining with 0.1% silver stain. DNA ladder (Helena Biosciences) was run with the amplified product and controls.

Results

Follicular lymphoma, DLBCL and T-cell rich B-cell lymphoma were the types of B cell NHL included in the present study. Out of 30 cases of B-cell NHL, 22 (73.3%) were male patients and eight (26.7%) were female patients. The rearrangement of bcl-2 gene and the involvement of icr, mbr and mcr in different B-cell NHL, is shown in Table-1. Mean age for cases showing bcl-2 rearrangement was 63.7 years with a standard deviation of + 6.18. Out of 22 male patients 6 (27.27%) showed the bcl-2 rearrangement. Out of 8 female patients, one (12.5%) case was found to have bcl-2 rearrangement.

Table 1. Total bcl2 Gene Rearrangement and Involvement of icr, mbr and mcr among CD-20 Positive B-cell Non-Hodgkin Lymphomas

Type	No	bcl-2	icr	mbr	mcr
Follicular	8	4 (50.0)	3 (37.5)	1 (12.5)	0 (0)
Diffuse large B-cell	19	3 (15.7)	2 (10.5)	1 (5.30)	0 (0)
T-cell rich B-cell	3	0 (0)	0 (0)	0 (0)	0 (0)

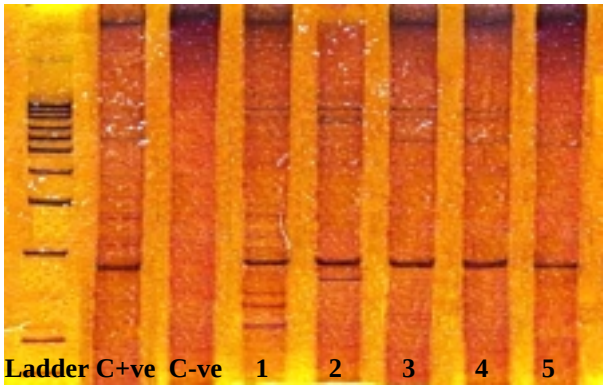

Figure 1. Silver Nitrate Stained Polyacrylamide Gel Electrophoresis Strip Showing icr Region Involvement in Five Cases. 100 bps DNA ladder, Positive control, C+ve; negative control, C-ve

The product size for icr was around 200 bps (100 bp DNA ladder, Helena Biosciences) and is shown in Figure-1. The product size for mbr was around 100 bps (100 bp DNA ladder, Helena Biosciences). Minor cluster region involvement was not seen in any of the B-cell NHL.

Discussion

In theWest, most of the work regarding bcl-2 gene rearrangement has been done on FL as compared to DLBCL, probably owing to the larger percentage of FL and its diagnostic utility in FL. The prevalence of bcl-2 gene rearrangement is believed to be higher in the Western populations as compared with Asian populations. Only a small number of studies are available from our region of the world and Middle East. Various studies from different parts of the world have demonstrated the frequency of bcl-2 gene rearrangement in FL and DLBCL. These frequencies are variable not only in different regions but also within the same region. This variation of prevalence among different studies may be attributed to technical and/or geographical factors (Barrans et al., 2003; Belaud-Rotureau et al., 2007).

Technical factors causing the variation in the results are the source of DNA, method of amplification and detection like, conventional cytogenetics, FISH, southern blotting or PCR (Macintyre et al., 2000; Albinger-Hegyi et al., 2002; Barrans et al., 2003; Gomez et al., 2005; Belaud-Rotureau et al., 2007).

The frequency of bcl-2 gene rearrangement in FL ranges from 45-69% in Middle East (Sayhan et al., 2000; Mahfouz et al., 2006), 0-78% in Latin America (Leone et al., 2002; Noriega et al., 2004), 58-73% in Europe (Buchonnet et al., 2000; Batstone et al., 2005), 31-81% in Asia (Chuang et al., 2006; Au et al., 2005) and 50% in United States of America (Lopez-Guillermo et al., 1999). A relatively less number of studies have been carried out for the detection of bcl-2 gene rearrangement in DLBCL as compared to FL. The reported frequency of bcl-2 gene rearrangement in DLBCL is found to be 14-20% in United States (Jacobson et al., 1993; Gascoyne et al., 1997): 10-

18% in Europe (Lee et al., 1993; Volpe et al., 1996); 13% in South America (Noriega et al., 2004), and almost same was observed in the present study. The mbr comprises of a major portion of bcl-2 gene rearrangement in both FL and DLBCL in these studies. This is followed by the involvement of mcr as shown by different studies in different regions of the world. In present study mbr involvement was seen in 12.5% cases of FL and in 5% cases of DLBCL. Minor cluster region was not seen in any of the case in the present series, which is different from the previous reports from other regions.

These frequencies are variable within the same region and also between different regions. Asian countries have a lower frequency of bcl-2 gene rearrangement as compared to Western countries. However more recent study from Far East has reported higher bcl-2 gene rearrangement incidence rates, suggesting that improved detection methods might decrease the importance previously given to geographical differences in explaining the variation in incidence of bcl-2 gene rearrangement (Ismail et al., 2007). In present series, the conventional PCR is used as a first step in the detection of bcl-2 gene rearrangement and it was found to be 50% in FL and that of DLBCL was found to be 15% which is slightly lower than that reported in the West particularly that in the follicular lymphoma.

Better detection rates are seen in studies that had utilized high quality DNA from fresh or frozen samples, as it was difficult to gain such high integrity DNA from formalin-fixed and paraffin-embedded tissue (Leone et al., 2002; Mahfouz et al., 2006; Ismail et al., 2007). Same was observed in the present study where good quality DNA was extracted from 4 cases from FFPE tissue was the source. The source of the DNA extraction was fine needle aspirate in rest of the cases. Similarly the studies using the long distant PCR or nested PCR have a better detection rate as compared to conventional PCR, especially when samples are FFPE, which is known to badly affect the quality of extracted DNA (Au et al., 2005; Mahfouz et al., 2006; Ismail et al., 2007).

A part of inconsistency of bcl-2 gene rearrangement can be explained by the fact that most of the published reports do not include primers for the recently identified icr breakpoint region, which had accounted for up to 10% of the bcl-2 gene rearrangements (Albinger-Hegyi et al., 2002). In the present study, icr involvement was seen in 37.5% of FL and 10.5% of DLBCL. Breaks in icr have also been detected in other parts of the world in FL; 5-10% in Europe (Albinger-Hegyi et al., 2002; Batstone et al., 2005); 10% in Middle East, (Ismail et al., 2007); 4% in Asia, (D'Haese et al., 2005). Studies using the icr in cases of DLBCL were not found in literature.

The difference in frequencies of bcl-2 gene rearrangement is supportive of the fact that the different breakpoints of bcl-2 gene occur on chromosome 18 with different frequencies in different population. Such clustering in different regions might be the reason of variable frequencies of bcl-2 gene rearrangement in different populations. The addition of icr primer sets to bcl-2 gene rearrangement PCR assays thus increase detection rates in the routine laboratory settings for FL as well as for DLBCL.

In conclusion, present study adds an input towards the better understanding of international picture of B-cell NHL. The icr is the commonest breakpoint of bcl-2 followed by mbr in this part of the world, both for FL and DLBCL. Involvement of mcr was not found in any of the case of present series. A different pathogenetic mechanism with the involvement of icr might be the underlying reason for the low occurrence of FL in this part of the world. None of the T-cell rich B-cell lymphoma has shown bcl-2 gene rearrangement. However further larger scale studies are required to substantiate these findings.

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