Evaluation of frequency IGH /BCL-2 gene rearrangements in non-Hodgkin lymphoma patients using BIOMED-2 primers

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ABSTRACT

The t(14;18)(q32;q21) translocation is the most common translocation in B cell malignancies being found in follicular lymphomas (FL) and diffuse large B cell lymphomas (DLBCL). We were Detected IGH/BCL-2 translocation in 50 sample of DLBCL patients. The Polymerized chain reaction (PCR) analysis using standardized PCR primers and protocols of BIOMED-2 Concerted Action BMH4-CT98-3936 for detection of B cell clonality in lymphoproliferative disorders. By multiplex PCR, a total of 2 (4%) positive cases showed IGH-BCL-2 gene rearrangements in major breakpoint region (MBR). Thus, DLBCL patients in this study had low frequency of MBR breakpoints cluster, even though many other studies reported a higher MBR incidence. These results indicate an increase in the prevalence of t(14;18) translocation in adults depending on age. In conclusion, t(14;18) detection in DLBCL patients by Multiplex PCR can be achieved at significantly higher rates by including BIOMED-2 primers for MBR breakpoint regions and performing multiplex amplification.

INTRODUCTION

Malignant lymphomas are neoplasms that arise from B or T cells during various stages in normal lymphocyte development. The most frequent nonrandom chromosome rearrangement in NHL of B-cell type is t(14;18)(q32;q21), which is found in 50 – 85% of FL, 15 – 30% of DLBCL, and occasionally in other histological subtypes of NHL[1]. Molecular studies of this translocation have disclosed a juxtaposition of BCL2 gene rearrangements in major breakpoint regions and performing multiplex amplification.

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MATERIALS AND METHODS

For this investigation, we have analyzed a total of 50 cases DLBCL between November 2009 and August 2011. We used the formalin-fixed, paraffin-embedded (FFPE) blocks that were collected from the archives of the two sources (Department of Pathology, Cancer Institute of Imam Reza Hospital, Tabriz, Iran). The pathological board re-evaluated all samples and confirmed the diagnosis based on WHO classification criteria. In addition, Ethics and Human Rights Committee in both centers approved the present study.

DNA Extraction:

Three or four 5 μm sections were obtained from each FFPE tissue, and transferred into sterile containers and stored at room temperature until used for DNA extraction. DNA was extracted from the FFPE specimens using by FFPE DNA Extraction Kite as described by manufacturer’s protocol (Quick Extract FFPE DNA Extraction Epic enter). In order to evaluate the quality and quantity of extracted DNA [15] was amplified by PCR and UV spectrophotometry at 260/280 nm. The average DNA samples included had DNA concentration and OD260/280 ratios 156 ng/μl (ranging between 122-560 ng/μl) and 1.85.

PCR was performed using three set of primers from of BIOMED-2 protocol to detect the MBR(Table1). A negative control, in which the DNA solution was replaced by de-ionized distilled water, was used in every PCR run. Positive control for breakpoint regions was extracted from the CA46 cell line (provided from of the Nanoziostarryeh office, Pastur Institute of Iran).

IGH /BCL2 Amplification:

DNA successfully isolated from paraffin-embedded tissue and amplified by the primers directed against the cluster regions. For determination of breakpoints, we included in each run, DNA sample, negative control and positive control. The PCR reactions were carried out in a final volume of 25μl that consists of 155 ng genomic DNA, 1 pmol of each primer, and 14 μl master mix red with 0.1 Units/μl Ampliqon Taq DNA polymerase [Ampliqon A/S, Stenhuggervej 22, Denmark]. Amplification conditions followed PCR thermocycler program, initiated with pre-incubation at 95°C for 10 min, followed by 34 cycles of 30 s at 95°C, 30 s at 64° C, and 60 s at 72°C. After the final cycle, an extension step was performed for 10 min at 72°C. Subsequently, multiplex PCR products were directly loaded onto the agarose gel (1%) and stained with ethidium bromide.

Result:

The translocation of the IGH / BCL2 were analyzed in non-Hodgkin patients using BIOMED-protocol and defined multiple primers. To evaluate the translocation, the 3 primer as a forward [MBR (MBR1, MBR2)] and reverse primers (JH)used(Table1).

PCR conditions were optimized to fit of primers for reproducing the translocation. The PCR conditions optimized were applied to detect translocation in all of the samples. A total of 2 cases (4%) were identified as positive samples for BCL-2 translocations based on multiplex PCR analysis. (table 2). The results showed an increase in the incidence of t(14;18) with age among adults.

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Diagnostic</th>
</tr>
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<tr>
<td>DLBCL</td>
<td>50</td>
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Discussion:

Malignant lymphomas are neoplasms that arise from lymphoid cells of either B-cell or T-cell lineage[16]. B-cell lymphoid malignancies comprise about 90 % – 95 % of all lymphoproliferative disorders [17]. The majority of them can be accurately diagnosed based on histomorphology or cytomorphology supplemented with immunohistochemistry [17,18,19]. In such cases, molecular gene rearrangement studies have proven useful as an additional diagnostic tool [20]. Gene rearrangement analysis can be performed by PCR-based techniques [21]. In a European BIOMED-2 collaborative study, multiplex PCR assays have successfully been standardized for the detection of chromosome aberrations t(14;18). This strategy is capable of amplifying across the breakpoint region in the majority of cases of suspicious lymphoma with a cytogenetically defined translocation [22]. In this study, we have analyzed a total of 50 cases lymphoma malignancies using BIOMED-2 multiplex PCR and showed a total of 4% positive translocation.
The frequency of IgH/BCL-2 gene rearrangements from our patients was compared with data from different geographic regions. In order to optimize the analysis of data, we have limited the comparison of PCR results to those reports in which MBR loci were examined. Simultaneously, data from previous studies have also suggested geographic variation in the frequencies of IgH/BCL-2 gene rearrangements in different geographical regions [23]. Thus, results indicate frequency of BCL-2 gene rearrangement 9% among South America, 12% United States, 16% Europe and 4% among the our patients series.

PCR results were reported of frequency of breakpoint regions in IgH/BCL-2 gene rearrangements and our results showed that our patients in MBR breakpoints (4%) had least percentage. This support the notion that the concentration of breakpoints along chromosome 18, may be different in cases of NHL [24].

The increase of the incidence t(14;18) translocation with age in adults has also been shown by studies [25,26], our findings confirm the importance of the increase of the incidence t(14;18) translocation with age in adults.

In conclusion, our findings confirm the importance of an accurate detection of t(14;18) translocation by BIOMED-2 Multiplex PCR and determine MBR breakpoint regions.

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REFERENCES


