Polymorphism of Exon 1,3 Bcl-10 Gene in Paraffin Blocks with Non Hodgkin’s Lymphoma Diagnosis and Blood Sample of ALL in Northwest of Iran

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Abstract: Background and Aims: Bcl10 is a gene that recently identified in B-cell lymphomas of mucosa-associated lymphoid tissues. It has been suggested as a target for mutation in multiple types of tumours. The recently described Bcl10 gene has been suggested to be a major target gene for inactivation in a variety of human cancers. Methods: EDTA blood from 20 patient with ALL, 20 paraffin block with non hodgkin’s lymphoma diagnosis and 45 blood sample as controls were collected and DNA was extracted. The gene was amplified with PCR and product for exon 1,3 of BCL-10 gene were sequenced. Results:after sequencing of PCR product of exon 1,3 there is n’t any meaningful mutation in BCL-10 that could be caused diaease. Conclusion:mutation in BCL-10 could n’t be caused of malignancy and extracted DNA of paraffinic block could be used as a source of cellular genomic matter in molecular studies.

Key words: acute lymphoblastic leukemia (ALL) sequencing, paraffin block.

INTRODUCTION

The function of BCL10 is naturally performed as proapoptotic protein through second caspase recruitment domain (CARD) in amino terminal and activation of NF- kappa B pathway. This activity requires second oligomerization of CARD and reaction between BCL10 and other proteins having second CARD, including CARD14, CARD11, CARD10, and CARD9. BCL10 is known as a regulator molecule of apoptosis, which is directly effective in lymphoma related to mucosal association membrane (MALT) (Willis, 1999; Zhang, 1999). This gene encoded a protein with 233 amino- acids and it has a terminal recruiting domain and the second CARD, as well as carboxylic terminal 132 of amino acid which has not been identified particular functional units (Srinivasula, 1999; Belawski, 2001). BCL10 is within area of 1p22 chromosome which in most cases, the malignancies is eliminated completely. These cases include B-cell non- Hodgking's lymphoma (Srinivasula, 1999) and mesothelium lymphoma (Costanzo, 1999). Deficiency in BCL10 function is usually seen MALT lymphoma, which includes involvement of gaster (nearly in %4) and lung (nearly in %9 of cases). It is seen uncommonly in other lymphoma of other positions.

BCL10 mutations are also reported in malignancies such as follicular lymphoma, Sezary syndrome, malignant mesothelium, germ cell tumor and colon cancer (Tilly, 1994; Lee, 1996). Prevelance of lymphoma disease, especially its non- Hodgking's type in the societies is such that it prevents the possibility of case studies and availability of a sample for a more wide- spread investigation is unlikely. Thus, representation of non-invasive methods with a minimum mental and physical damage on the one hand, and representing an economical method for doing it, on the other hand, which is presented for investigation of meaningful mutations of the structure of effective genes in incidence of hematopoietic malignancies will be considered as a main objective of this study. It allows more practical studies on paraffin blocks by introducing the studying method of mutations of DNA structure in sequences of more than 400 base- pairs.

MATERIALS AND METHODS

In the present experimental study, the blood samples of 20 patients with acute lymphocytic leukemia not regarding the kind and degree of disease, and also 20 of lymph node of paraffin block of patients with non-Hodgking's lymphoma diagnosis (12 cases of high-grade and 8 cases of low-grade), not regarding the kind of disease, and 45 normal blood samples were investigated. DNA extraction from blood was performed by method of chloroform and extraction from paraffin blocks using extraction Kit (QIAGEN, cat. No 56404, QIAamp DNA FFPE Tissue). To validate test of extracted DNA quality and doing quality control test of extracted product, the measurement of concentration, purity degree (OD 260/OD 280) of DNA and electrophoresis on agarose gel was performed to evaluate β-actin gene, and PCR test was performed with primers of β-actin gene.
This study aims to investigate gene mutations in the area of exon 1,3 BCL10 gene. To do this, primers of this exon have been designed by fast-PCR software and they were used after offering. The utilized Reverse and Forward primers are presented in Table 1.

Table 1: The Forward and Reverse primers.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward:exon1</td>
<td>5′-GGA CCC GGA AGA AGC GCC ATC TCC-3′</td>
</tr>
<tr>
<td>Reverse:exon1</td>
<td>5′-GAT CCT CCT TGT CCT CGG ACT C-3′</td>
</tr>
<tr>
<td>Forward:exon3</td>
<td>5′-TTA ACA AGT CAC AAG ATG GAC AGT G-3′</td>
</tr>
<tr>
<td>Reverse:exon3</td>
<td>5′-CAT TAA AAA TTA AAA GGC AAT AAA GTG-3′</td>
</tr>
</tbody>
</table>

In this research, PCR-sequencing method was used and the requirement compounds in the reaction include: Taq: 0.05 unit; dNTP: 0.4 mM, MgCl₂: 3mM, primer (5 pmol), 2.4 μl was reported in final volume of 50 μl. The PCR Program which was used is presented in Table 2.

Table 2: The PCR program.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- First Denaturation</td>
<td>2 min/95°C</td>
</tr>
<tr>
<td>2- Denaturation</td>
<td>95°C/30 sec</td>
</tr>
<tr>
<td>3- Annealing</td>
<td>25 sec/67°C</td>
</tr>
<tr>
<td>4- Extension</td>
<td>40 sec/72°C</td>
</tr>
<tr>
<td>5- Final Extension</td>
<td>5 min/72°C</td>
</tr>
</tbody>
</table>

The 2 through 4 stages of the above program is repeated 35 times. The stages of polymerase chain reaction of resulted products is then investigated to determine the studied sequences and to investigate the presence or absence of mutation in gene area of exon 1,3.

Results:

The following results obtained after investigations of all samples and paraffin blocks with non- Hodgking's lymphoma Diagnosis:

After performing PCR on exon 1,3, we can see band of these exons with 186 base-pairs and 444 base-pairs in figure 1 and 2, respectively.

Fig. 1: exon 1 with 186 base-pairs.

Fig. 2: exon 3 with 444 base-pairs.

Sequencing of these samples identified 2 different nucleic acid changes, 3 in the coding sequence of codon 8 CTC ⇒CTG, and codon 213 GGA ⇒GAA. The polymorphism at codon 213 caused a GLY⇒GLU substitution. The polymorphisms at codon 8 and codon 213 were identical and occurred at a frequency comparable to those reported previously (Apostolou, 1999; Fakrddin, 1999). DNA samples from 20 lymphomas (12 cases of high-grade and 8 cases of low-grade), and 20 sample of ALL, were screened for BCL10 mutation by PCR-Sequencing; this was followed by sequencing analysis of specimens.

At first, the range of mutations, nucleotide being changed, and the curve that part of mutations is studied to investigate the presence or absence of the mentioned mutation. For exon 1 mutations, it was just mutation in codon 8 that changed CTC ⇒CTG and shifted amino-acid L [Leu] ⇒ L [Leu], 3 (2.2 %) case in normal, 6 (30%) in lymphoma 4 (20%) in high grade and 2 (10%) in low grad, and 3(15%) in ALL.
Homozygous mutation in case 4 of lymphoma Normal curve

For exon 3, mutation only in codon 213, 1 case (5%) in normal, 2 (10%) in lymphoma and 2 (10%) in ALL is observed that changing GCA → GAA and converting amino-acid G[Gly] → E[Glu], and this did not indicate any change in occurrence of other mutations.

Heterozygous mutation in case 18 of lymphoma Normal curve

Table 3: Summary of BCL10 mutations.

<table>
<thead>
<tr>
<th>Cases</th>
<th>Number of alteration CTC ⇒ CTG</th>
<th>Number of alteration GCA ⇒ GAA</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphom</td>
<td>6 (30%)</td>
<td>2 (10%)</td>
<td>20</td>
</tr>
<tr>
<td>ALL</td>
<td>3 (15%)</td>
<td>2 (10%)</td>
<td>20</td>
</tr>
<tr>
<td>normal</td>
<td>3 (2.2 %)</td>
<td>1 (5%)</td>
<td>45</td>
</tr>
</tbody>
</table>

Discussion:

To study lymphoma samples in this research, samples of paraffin blocks is tested with lymphoma diagnosis, which in most cases particularly in retrospective studies, only fixed and paraffinized samples are available for molecular studies. However, %4 formalin is an appropriate complex for typical histopathological studies, which can not be ignored, through this complex is considered to be an effective factor in molecular studies (Ben-Ezra, 1991; Belawski, 2001; Srinivasan, 2002). Fixation of tissues with formalin is basically obtained by establishing cross links by formaldehyde and as a result of degradation and precipitation of cellular matrix proteins, nucleotid acids and other bio-molecules. Metz et al. in a research studying the influence of formaldehyde-induced modifications in proteins structure indicated that it includes several physiochemical changes, which can cause different damages on structure of nucleic acids. These changes include elimination of base, tearing of DNA, and non-exclusive interaction of protein-DNA, and so on. On the basis of this DNA data, derived from fixed paraffinic tissue by formalin (FFPET), it is expected that is would not have a quality and totality of DNA tissue, but this resulted DNA can be used as a prototype of molecular studies, which aims sequences less than 200 to 300 base-pairs (Metz, 2004). But with respect to the represented study on exon 1,3, BCL10 gene is shown with a length of 187 and 444 base-pairs respectively, in which extracted DNA of paraffinic block could be used as a source of cellular genomic matter in molecular studies. The present study reviews exon 1,3 polymorphisms of BCL-10 gene. aims of this project are represented as: studying common mutations in BCL-10 gene in non-Hodgkin's lymphoma There was a tendency for the mutation frequency to be higher in high-grade (20%) than low-grade (10%) lymphomas. and acute lymphoblastic Leukemia and showing prevalence of these mutations in the above-mentioned cases in order to indicate presence or absence of meaningful relationship. The prioritized method for studying presence of mutation for cost-effectiveness and possibility of more study of one mutation is, PCR-Sequencing technique. Ming-Quing Du and colleagues studying lymphoma mutations in MALT lymphoma and control group have also reported the above change in codon 8 exon 1 (Ming, 2000). S. Luminari et al. have also reported CTC ⇒ CTG changes in codon 8 exon 1 in studying mutation of axon 1-3 BCL10 gene in lymphoedid malignancies (Luminari, 2000). Yuille and et al indicated also CTC ⇒ CTG change in studying BCL10 gene in T-PLL and CLL (Yuille, 1999). Also, Lambers and colleagues in a similar study of BCL10 showed change in codon 8 (Lambers, 1999). Bullinger et al. also conducted an study in MCL suggested that
elimination of 1p22 area does not inactivate BCL10 gene in MCL. They reported CTC \(\rightarrow\) CTG change in this case, and verified results of represented study (Bullinger, 2000). Studying BCL10 gene in prostate carcinoma, small-cell lung cancer, head and neck tumours, renal carcinoma and sarcomas, Gill and colleagues also indicated CTC \(\rightarrow\) CTG change in codon 8 (Gill, 1999). Ming-Quing Du and colleagues indicated amino acid G[Gly] \(\rightarrow\) E [Glu] change in codon 213 in studying of lymphoma mutation in MALT lymphoma and evidence group (Ming, 2000). In studying mutation of axon 1,3 BCL10 gene, S. Luminar: reported polymorphisms lymphoideic malignancies in third base codon 213 as base chaging G/A, which is related to this change in axon 3 (Luminar, 2000). Studying BCL-10 gene in prostate carcinoma, small-cell lung cancer, head and neck tumours, renal carcinoma and sarcomas. Gill and Colleagues also reported change in third base codon 231 as CGA (gly) \(\rightarrow\) GAA (glu) (Gill, 1999).

Tadokora, et al., in their study on prevalence of mutations of BCL10 gene in type B non-Hodgking's lymphoma, have reported replacement of G/A nucleotide in codon 213 which leads to conversions of amino acid Gly to Glu (Tadokoro, 2001). In another study, Grimwade et al evaluating the mutations of BCL10 gene in Leukemia, reported polymorphism G/A researched in codon 213 axon 3 like other studies which results in conversion of amino acid Gly to Glu (Grimwade, 2000). Besides the polymorphisms mentioned above no other sequence alterations were found in cases. In agreement with our data, several groups failed to detect any abnormality of BCL10 in a wide variety of solid tumors (Van Schothorst, 1999; Stone, 1999; Gill, 1999).

ACKNOWLEDGMENTS

We are grateful to pathology laboratory of dr.esmaeili and mrs moradi for assistance with participant.

REFERENCES


