Molecular Detection of BCR/ABL Fusion Gene in Saudi Acute Lymphoblastic Leukemia Patients

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ABSTRACT

Background: Molecular cytogenetics is becoming one of the most useful tools targeting some genes which are generally considered to lead to leukemic transformation (as well as for numerical abnormalities). A fraction of acute lymphoblastic leukemia (ALL) cases carry the translocation t(9;22) (q34;q11.2) which juxtaposes the ABL proto-oncogene to the BCR gene generating a chimeric gene, BCR/ABL. This aberration is more frequent in adult ALL (20%-40%) than in pediatric ALL (<5%), and predicts poor clinical outcome.

Aim of our Work: Is to study BCR/ABL fusion gene in ALL cases using fluorescent in situ hybridization.

Patients and Methods: Twenty newly diagnosed ALL patients, 16 adult and 4 paediatric cases, were included in the study, 11 cases (55%) were of precursor B phenotype, 8 cases (40%) belonged to T lineage, while one case was biphenotypic expressing mainly precursor B cell markers tether with CD13, CD33, CD117. Detection of BCR/ABL fusion gene was done using interphase FISH technique and was confirmed molecularly using the RT-PCR technique.

Results: BCR/ABL fusion gene was negative in all the examined cases, yet abnormality involving 9q34, ABL gene, either by addition or deletion was detected in three cases (15%). Two of these cases were associated with BCR gene extra copies (three and four copies, respectively).

Conclusion: This may reflect the frequency of association of ABL gene and BCR gene abnormality in our cases, and that absence of fusion gene BCR/ABL does not exclude their role in the leukemogenic process, yet a larger study is required to confirm and detect the prevalence of these gene disturbances in ALL and their association.

Key Words: Acute lymphoblastic leukaemia - BCR/ABL fusion gene - FISH - RT-PCR.

INTRODUCTION

In acute leukemias, recurring cytogenetic abnormalities with prognostic implications have been well documented. Karyotype is an independent prognostic indicator in leukemias [1,2]. Ploidy distribution whether hyperdiploidy, hypodiploidy, pseudodiploidy and normal karyotype were reported to be highly associated with disease outcome, yet in acute lymphoblastic leukemia (ALL), cytogenetic analysis has high failure rates due to fewer number and poor analyzable metaphases and overgrowth of normal cells. Sometimes, prognostically important structural as well as numerical aberrations may not be detected with conventional cytogenetic studies [3]. Molecular cytogenetics is becoming one of the most useful tools targeting some genes which are generally considered to lead to leukemic transformation as well as for numerical abnormalities; also concurrent interphase fluorescence in situ hybridization (FISH) allows screening of more cells [3]. Nordgren et al. [4] reported that up to 20% more cases were detected with chromosomal abnormalities using molecular cytogenetics.

Current therapeutic strategies for leukemia are based on prognostic factors that allow stratification of therapy. The typical risk factors identified over the years include age, white blood cell count, immunophenotype and gene rearrangement most commonly secondary to chromosomal translocations [5-8].

Translocation (9;22) (q34;q11.2) which juxtaposes the ABL proto-oncogene to the BCR gene generating a chimeric gene BCR/ABL, is the hallmark of chronic myeloid leukemia [9]. It is also well established that a fraction of ALL carry this chromosomal translocation which is more frequent in adult ALL (20%-40%) than in pediatric ALL (<5%) [7]. The presence of
BCR/ABL fusion gene in ALL is associated with poor prognosis and is an indication for stem cell transplantation in certain treatment protocols [10,11]. Three different proteins are produced depending on the breakpoint p210, p190, and rarely p230kd [12]. Although the vast majority of CML cases express p210, yet sporadic cases of CML expressing p190 were reported. Furthermore CML patients at diagnosis may co-express very low amounts of p190 by alternative splicing events [13]. On the other hand, ALL blast cells typically express p190 but p210 expression has been observed in some cases especially in adults [14].

**Aim of the work:**

Our objective is to study the frequency BCR/ABL fusion gene in Saudi acute lymphoblastic leukemia cases using the FISH technique.

**PATIENTS AND METHODS**

Twenty newly diagnosed ALL patients were included in this study, 16 adult and 4 paediatric cases. All patients were subjected to:

A- Complete blood picture.

B- Bone marrow examination, the interpretation of which was done according to the WHO classification [15]. Acute leukemia was diagnosed if blasts in bone marrow constituted ≥20% of the total count.

C- Flowcytometric analysis: The Coulter Q-PREP EPICS system (Coulter corporation, Hialeah, FL, USA) and a reagent system (Coulter Diagnostics, USA) were used to prepare bone marrow or whole blood samples for immunophenotypic analysis by flowcytometry. The panel of monoclonal antibodies used included: B cell panel (CD19, CD20, CD22, CD24, CD10, CD9, CD79a, surface and cytoplasmic immunoglobulin); T cell panel (CD2, CD5, CD7, CD3, CD4, CD8); myeloid panel (myeloperoxidase, CD13, CD33, CD14, CD15, CD11b, CD11c, CD117, CD65); miscellaneous (CD34, CD45, HLA-DR, TdT, CD56).

D- Cytogenetic analysis including preparation, banding and karyotyping technique of 24, 48, and 72 hours cultured cells according to the basic techniques of Moorhead et al. [16]. Chromosomal analysis and karyotyping: The chosen metaphases were photographed and analyzed using a computer image analyzer (Vysis Quips XL=Genetics work station) according to Paris conference recommendations [17] and the International System of Human Cytogenetic Nomenclature (ISCN) [18] recommendations. For each case, 20 metaphases were analyzed to detect any chromosomal aberrations.

E- Interphase fluorescent in situ hybridization (FISH): Using LSI BCR/ABL (9;22) dual color (orange, green) DNA probe (ABOTT, VYSIS probes).

Specimen slides were prepared, the hybridization area was marked with a diamond tipped scribe. The slides were dehydrated for one minute in 70% ethanol (ETOH) in a Coplin jar, followed by one minute in 85% ETOH and one minute in 100% ETOH. Slides were kept in 100% ETOH until the probe mixture was applied. For each slide the following reagent mixture was prepared in a microcentrifuge tube at room temperature [7µl hybridization buffer (Vysis, USA), 1µl probe and 2µl purified water]. The micro-centrifuge tube was vortexed for 1-3 seconds for proper mixing of the probe with hybridization mixture. After removal from 100% ETOH, 10µl of the probe mixture were dropped on the target area and slides were placed in the Hy Brite apparatus (Vysis, USA), at 73°C for one minute which automatically abruptly dropped to 37°C (hybridization temperature). The slides were air-dried using an electric air blower, 10µl of DAPI-II counterstain were added to the target area of the slide and a cover slip was applied. All slides were examined using a fluorescence microscope (Olympus BX40) and a computerized image analysis system (Vysis Quips XL Genetics workstation).

F- Reverse transcriptase-polymerase chain reaction (RT-PCR): Total RNA was isolated according to the standard guanidium thiocyanate procedure [19]. cDNA was synthesized by using AMV reverse transcriptase, and the cDNA was subjected to nested PCR amplification using primers for BCR and ABL genes [20]. Detection of BCR/ABL fusion gene was done using interphase FISH technique and was confirmed molecularly using the RT-PCR technique.

**RESULTS**

Twenty newly diagnosed ALL patients were included in the study, they were 18 male and 2 females with a mean age of 16.8 years. Twelve
cases (60%) were precursor B cell ALL, 7 cases (35%) were T cell ALL, while one case was biphenotypic expressing mainly precursor B cell surface markers together with the myeloid markers CD13, CD33, CD117. The immunophenotypes and karyotypes of all studied cases are shown in Table (1).

Using the FISH and RT-PCR techniques, the BCR/ABL fusion gene was negative in all the examined cases, yet abnormalities involving 9q34 (ABL gene), either by addition or deletion, were detected in three cases (15%), abnormalities in the BCR gene (22q11.2) were detected in two cases (10%) (Table 2).

In two of the three patients (case 5 & 6), cytogenetic analysis revealed normal karyotype, yet interphase FISH for BCR/ABL fusion gene revealed the presence of three copies of ABL gene (Table 2). The abnormality was detected as a sole abnormality in 84% of the examined cells for case 5 and was associated with trisomy signal for BCR gene in 87% of the examined cells in case 6, which is strongly suggestive of a clonal abnormality (Figs. 1,2).

In case 10, cytogenetic analysis revealed a complex karyotype involving chromosome 2, 9 and 10 (Fig. 3) (This was confirmed by metaphase FISH using whole chromosome painting for those chromosomes). A single ABL gene signal was detected in 62% of the examined cells while 29% showed four copies of BCR signal on chromosome 22 (Table 2) (Fig. 4).

The three discussed cases were of precursor B phenotype, two of which (cases 6 & 10) were co-expressing myeloid markers CD33, CD13 and CD15, respectively (Table 2).

Table (1): Immunophenotyping and karyotyping of all study cases.

<table>
<thead>
<tr>
<th>Case</th>
<th>Cell surface marker expression</th>
<th>Coexpression</th>
<th>Immunophenotype</th>
<th>Karyotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CD45, CD34, HLA-DR, cCD3, CD5, CD7</td>
<td>CD33, CD9</td>
<td>T cell</td>
<td>46XY; t(10;11) (p13;q21)</td>
</tr>
<tr>
<td>2</td>
<td>CD45, CD34, HLADR, CD10, CD19, CD22, CD23, CD9, TdT</td>
<td>–</td>
<td>Pre B</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>CD45, CD34, HLADR, CD10, CD19, CD22, CD79a, cIGM, CD9</td>
<td>CD15, CD65</td>
<td>Pre B</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>CD45, CD34, cCD3, CD7, CD5, CD2, CD56</td>
<td>CD13, CD33</td>
<td>T cell</td>
<td>50-52XY, +4 marker</td>
</tr>
<tr>
<td>5</td>
<td>CD45, CD34, HLADR, CD10, CD9, CD19, CD22, cIGM, TdT</td>
<td>–</td>
<td>Pre B</td>
<td>46XX</td>
</tr>
<tr>
<td>6</td>
<td>CD45, CD34, HLADR, CD10, CD19, CD20, CD9, CD22</td>
<td>CD33</td>
<td>Pre B</td>
<td>46XX</td>
</tr>
<tr>
<td>7</td>
<td>CD45, CD34, CD3, CD5, CD7, TdT</td>
<td>–</td>
<td>T cell</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>CD45, CD34, HLADR, CD10, CD19, CD20, CD9</td>
<td>CD11b</td>
<td>Pre B</td>
<td>50XY, +X,+14,+21,+21</td>
</tr>
<tr>
<td>9</td>
<td>CD45, CD34, HLADR, CD10, CD19, CD22, CD9, TdT</td>
<td>CD11b</td>
<td>Pre B</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>CD45, CD34, HLADR, CD10, CD19, CD22, cIGM</td>
<td>CD13, CD15, CD2</td>
<td>Pre B</td>
<td>44XY; dic (2;9) (q21;p24) add (10) (q25), -9 del (17) (p11.2)</td>
</tr>
<tr>
<td>11</td>
<td>CD45, CD34, HLADR, CD10, CD19, CD22, CD9, TdT</td>
<td>–</td>
<td>Pre B</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>CD45, CD34, CD3, CD7, CD5, CD8, TdT</td>
<td>CD117</td>
<td>T cell</td>
<td>–</td>
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<tr>
<td>13</td>
<td>CD45, CD34, cCD3, CD5, CD7, CD2, CD8</td>
<td>CD13, CD33</td>
<td>T cell</td>
<td>46XY</td>
</tr>
<tr>
<td>14</td>
<td>CD45, CD34, HLADR, CD10, CD19, CD22, CD79a, CD20</td>
<td>–</td>
<td>Pre B</td>
<td>–</td>
</tr>
<tr>
<td>15</td>
<td>CD45, CD34, CD5, cCD3, CD2, CD7, TdT</td>
<td>CD33</td>
<td>T cell</td>
<td>46XY,t(4;16) (q24;p12) del9 (p12)</td>
</tr>
<tr>
<td>16</td>
<td>CD45, CD34, cCD3, CD5, CD7, CD4, CD8, TdT, CD2</td>
<td>–</td>
<td>T cell</td>
<td>46XY, (9;12) (p13;q13)</td>
</tr>
<tr>
<td>17</td>
<td>CD45, CD34, HLADR, CD10, CD19, CD20, CD22, CD24, CD79a, cIGM, CD56</td>
<td>CD13, CD33, CD117, CD2</td>
<td>Biphenotypic</td>
<td>46XY</td>
</tr>
<tr>
<td>18</td>
<td>CD45, CD34, HLADR, CD10, CD19, CD20, CD22, CD24, CD79a, TdT</td>
<td>CD33, CD13</td>
<td>Pre B</td>
<td>46XY t(12;21) (p12;q22)</td>
</tr>
<tr>
<td>19</td>
<td>CD45, CD34, HLADR, CD10, CD19, CD20, CD9</td>
<td>CD5</td>
<td>Pre B</td>
<td>46XY</td>
</tr>
<tr>
<td>20</td>
<td>CD45, CD34, HLADR, CD10, CD19, CD20, CD22</td>
<td>–</td>
<td>Pre B</td>
<td>–</td>
</tr>
</tbody>
</table>
Table (2): Age, sex, bone marrow blast%, phenotype, surface marker co-expression, karyotyping and FISH results for cases with ABL and BCR gene abnormalities.

<table>
<thead>
<tr>
<th></th>
<th>Case 5</th>
<th>Case 6</th>
<th>Case 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>3 years</td>
<td>15 years</td>
<td>14 years</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Bone marrow blast %</td>
<td>87%</td>
<td>90%</td>
<td>60%</td>
</tr>
<tr>
<td>Phenotype</td>
<td>Pre B</td>
<td>Pre B</td>
<td>Pre B</td>
</tr>
<tr>
<td>Co expression of myeloid markers</td>
<td>Negative</td>
<td>CD33</td>
<td>CD13, CD15</td>
</tr>
<tr>
<td>Co expression of T cell markers</td>
<td>Negative</td>
<td>Negative</td>
<td>CD2</td>
</tr>
<tr>
<td>Karyotype</td>
<td>46XX</td>
<td>46XX</td>
<td>44XY; dic (2;9) (q21;24)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>add (10) (q25), -9 del (17) (p11.2)</td>
</tr>
<tr>
<td>FISH for BCR/ABL</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>ABL gene abnormality</td>
<td>3 copies of ABL gene (84%)</td>
<td>3 copies of ABL gene (87%)</td>
<td>1 copy of ABL gene (62%)</td>
</tr>
<tr>
<td>BCR gene abnormality</td>
<td>–</td>
<td>3 copies of BCR gene (87%)</td>
<td>4 copies of BCR gene (29%)</td>
</tr>
</tbody>
</table>

Fig. (1): Case No. 5: 3 copies for ABL gene.

Fig. (2): Case No. 6, 3 copies each ABL gene and BCR gene.

Fig. (3): Case No. 10, 1 copy for ABL gene, 4 copies for BCR gene.
**DISCUSSION**

BCR/ABL fusion gene is considered a bad prognostic factor in acute lymphoblastic leukemia and was reported to be present in 20%–40% of adult ALL and less than 5% of pediatric cases [7]. BCR/ABL generates multiple chimeric transcripts which translate to proteins of molecular weight 210, 190 and rarely 230kd according to the breakpoint. CML typically carries 210 BCR/ABL while ALL is most often associated with p190 [12]. In this study, FISH technique was performed to detect BCR/ABL fusion gene resulting from t(9;22) (q34;q11). RT-PCR was used to detect of p190 and p210 transcripts in 20 newly diagnosed ALL cases.

None of our cases was positive for the ABL/BCR fusion gene. In contrast, other studies reported BCR/ABL positivity in varying frequencies ranging from 5.7% to 33% (21-25). Abla et al. [26], reported a T-cell ALL case with BCR/ABL fusion transcript coding for p210 protein.

In our study, abnormalities involving the targeted breakpoint 9q34 (ABL gene) and 22q11.2 (BCR gene) were detected in three (of pre-B phenotype) out of 20 ALL cases either by addition of the gene or deletion. Similar results were reported by Bhavana et al. [3]. They studied 25 newly diagnosed ALL cases, all of which were negative for BCR/ABL fusion gene yet three cases (12%) showed translocation of 9q34 (ABL gene) to either chromosome 2 and 3, and this finding was confirmed by whole chromosome painting metaphase FISH.

Many investigators (3,27-29) reported karyotypes of ALL patients involving 9q34 abnormalities either by addition, deletion or ring chromosome, (mostly as add 9) as a part of complex translocations or unbalanced translocations involving 9q34 without the formation of t(9;22) (BCR/ABL fusion gene), raising a question about the frequency and implications of 9q34 (ABL gene rearrangements) in ALL and that any derangement of the ABL gene either by addition, deletion or translocation can be a part of the leukemogenic process. Kim et al. [30] reported a T-cell ALL case with ABL gene amplification and they speculated that this has been implicated in the leukemogenic process.
None of the studies reported an associated BCR gene abnormality, yet in our study 2 cases were associated with BCR gene extra copies (gain of three and four copies, respectively). This may reflect the frequent association of ABL gene and BCR gene abnormality in our ALL cases, and that absence of the fusion gene BCR/ABL does not exclude its role in the leukemogenic process, yet a larger study is required to confirm and detect the prevalence of these gene disturbances in acute lymphoblastic leukaemia and their association.

Regarding the cytogenetic pattern, two of these three cases showed normal karyotype (case 5,6), while the third one (case 10) showed a complex karyotype involving three chromosomes 2,9,10 confirmed by metaphase FISH. Similar results were reported by Myaer-Monard et al. [31], based on 189 leukemia patients screening using conventional karyotyping and molecular techniques. They reported discrepancy in genetic risk assignment obtained from molecular screening and karyotype as molecular techniques can detect hidden and cryptic rearrangements, addition or deletion of the targeted points that cannot be detected by conventional cytogenetics, yet in 18% of their cases, cytogenetics revealed structural and numerical chromosomal abnormalities that could define risk assignment not detected by molecular screening, concluding that molecular screening and cytogenetics are complementary in the diagnosis and genetic risk assignment of acute leukemia.

The immunophenotypic analysis for all our study cases showed no specific surface marker pattern characteristic for BCR/ABL negative ALL. Wang et al. [32] and Li et al. [33] reported that BCR/ABL was detected mainly in CD34, CD10 precursor B-cell ALL, in contrast to our results, where all the reported precursor B phenotype cases were CD10, CD34 positive yet negative for BCR/ABL fusion gene.

Concerning cytogenetic analysis of all the study cases, karyotyping was successful in 55% of cases only due to insufficient sample or insufficient or unanalyzable metaphases, yet results available showed no characteristic pattern or association with the phenotypic pattern. Our results are in agreement with He et al. [34] reporting that among 62 ALL cases there was no characteristic cytogenetic or molecular pattern associated with different phenotypes, in contrast to Pais et al. [35] reporting significant association of MLL gene re-rearrangement and precursor B-cell ALL.

From our study, we conclude that the frequency of Philadelphia chromosome is very low in Saudi ALL patients compared to literature and that routine molecular screening by FISH may improve cytogenetic risk assignment in leukemic patients, as it can provide more precise information on specific genomic imbalances and their prevalence. It may also be helpful in monitoring minimal residual disease.

ABL gene duplication, amplification, deletion or translocation may by itself or associated with BCR derangement, truly contribute to the leukemogenic process, or may represent an underlying genomic instability implicated in this process.

A larger study is warranted to clarify any abnormality involving the ABL and BCR genes, frequency, pathogenic role and treatment outcome.

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