DETECTION OF BCR-ABL FUSION SIGNAL IN PERIPHERAL BLOOD CELLS OF APPARENTLY HEALTHY INDIVIDUALS BY USING FISH TECHNIQUE

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ABSTRACT

The Philadelphia (Ph) chromosome which results from translocation t(9;22), it is acquired cytogenetic abnormality that characterizes all leukaemic cells in CML. The (9;22) translocation generates the break cluster region –c-abl oncogene 1 (BCR-ABL ) fusion gene on the Ph chromosome and also a reciprocal fusion gene, designated ABL1 – BCR on the derivative 9q+ chromosome. Measurement of gene fusions in peripheral blood cells of normal individuals may be used as a sensitive assay for the detection of genomic instability, and may contribute to risk estimation for development of lymphoid malignancies. The present study aimed to investigate the presence of bcr-abl fusion gene in the peripheral blood of healthy individuals by using Fluorescence in situ hybridization (FISH) technique. Detection of BCR-ABL fusion signal in apparently healthy Iraqi population is reported for the first time in the present study. BCR-ABL fusion signal was detected in(10/18 ,55%) of study sample and in(18/20,90%) of CML patients. There was a significant difference between CML cases and sample study regarding signal positive score (P<0.0001). All sample had low score. (33.3%) of CML cases had high score, (27.8%) had intermediate score, (38.8%) had low score. Detecting BCR-ABL fusion signal in peripheral cells of healthy individuals reflect long term exposure to carcinogens consequently they may aid in leading to tumor formation.

INTRODUCTION

The Philadelphia (Ph) chromosome is an acquired cytogenetics abnormality that characterize all leukaemic cell in chronic myeloid leukemia. It represent the diagnostic molecular hallmark, it is detected in virtually all chronic myeloid leukemia (CML) patients, it also found...
in approximately one-third of cases of acute lymphoblastic leukemia (ALL) and 1% of acute myelogenous leukemia AMLs.\cite{1} The (9;22) translocation generates the BCR – ABL1 fusion gene on the Ph chromosome and also a reciprocal fusion gene designated ABL1 – BCR on the derivative 9q+ chromosome. The BCR-ABL fusion gene that expresses an ancoprotein p210\text{BCR-ABL}, that has central role in generation the chronic phase of CML. It has a constitutively activated tyrosine kinase which ultimately leads to a deregulated proliferation and decreased cell adherence.\cite{2}

Numerous gene fusion including BCR-ABL thought to be solely associated with leukemias and lymphomas, though have been detected in healthy individuals with negative history of hematologic disordered.\cite{3,4} As of now the biological explanation of this observation remain largely unexplained. These and other findings suggest that measurement of fusion in peripheral blood cells within study group may be used as sensitive assay for detection of genomic instability, and may contribute to risk estimation for development of malignancies.\cite{5}

One hundred sixty three apparently healthy individuals from Baghdad were involved in a previous study,\cite{6} to assessment genomic damage induce in vivo by using cytokinesis block micronucleus assay assuming that they are exposed to high level of pollutant. Surprisingly, the mean count of micronuclei in sample study was substantially higher than the reference value for the general population which is considered early marker genomic instability. In the present study we selected eighteen individuals with high mean value of micronuclei from previous study to investigate the presence BCR-ABL fusion gene in the peripheral blood cell by using Fluorescence \textit{in situ} hybridization (FISH) technique.

**SUBJECTS, MATERIALS AND METHODS**

Eighteen healthy participant aged (15-33) years were recruited in the present study as well as 20 cases of newly diagnosed and untreated chronic myeloid leukemia patients (CML) as a positive control group. This CML patients group included 14 males and 6 females with an age ranges between 22-67 years. They were recruited at the hematology clinic of the medical city teaching hospital. Smears of EDTA stored blood were made on ordinary slides for each individual for the purpose of FISH technique. The samples were brought to the laboratory in a well-insulated ice box. Information on age, marital status, smoking habit, alcohol consumption, diagnostic X rays, chemical exposure during occupation, family history of cancer, medical and residential history were collected by questionnaire. All analyses were carried out on anonymous, coded samples.
Fluorescence *in situ* hybridization (FISH) technique

Fluorescence *in situ* hybridization (FISH) procedure was conducted according to kit leaflet.[7] FISH for BCR/ABL t(9;22) was carried out using the Poseidon repeat free translocation, dual-fusion probe (Kreatech) according to manufacturer's instructions. 1000 interphase nuclei were scored for each sample to determine the degree of positivity for the fusion and interphase signal patterns of different cell populations. According to the protocol of the manufacturer (Kreatech), the expected signal pattern for normal cell nucleus is two green and two red signals (for BCR and ABL genes). The BCR-ABL translocation is detected in interphase nuclei by the presence of fusion signals (yellow), along with the BCR gene (green) and the ABL gene (red).

Scoring of smears according to the Percentage of positive fusion signals was calculated assigning cases to the following scores:-

- Low Score = 1-5 %
- Intermediate score = 6-10 %
- High score = > 10 %

Statistical analysis

All statistical analysis was conducted using an SPSS (version 11). Significant difference in the distribution of fusion signal of BCR-ABL fusion signal score between positive control and study sample was analyzed by X² test. P value regarded significant at P < 0.05.

RESULTS

The BCR-ABL translocation is detected in interphase nuclei by the presence of fusion signals (yellow), along with the BCR gene (green) and the ABL gene (red), normal cell with two copies of BCR and ABL genes (2 green, 2 red signals).

The screening of structural aberration (BCR-ABL translocation) in 18 individuals compared to 20 early diagnosed non treated CML patients (positive control) is shown in table (1). BCR-ABL fusion signal was detected in 55% (10) of the sample study compared to 90% (18) of CML patients. The difference was statistically significant (P < 0.05).
Table 1: The detection of BCR-ABL fusion signal in the CML patients (positive control) and the study sample

<table>
<thead>
<tr>
<th>BCR-ABL Translocation</th>
<th>Study Sample (18)</th>
<th>CML patients (20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive N (%)</td>
<td>10(55%)</td>
<td>18(90%)</td>
</tr>
<tr>
<td>Negative N (%)</td>
<td>8(45%)</td>
<td>2(10%)</td>
</tr>
</tbody>
</table>

Chi-square test; P < 0.05

Low score of BCR-ABL signals (1-5%) was seen in 10(55%) of the sample study and 2 (10%) of CML patients. Intermediate and high scores were seen in 5 (27.77%) and 6 (33.33%) of CML patients respectively. Intermediate and high scores were not detected in the study sample. The difference of percentage score between CML patients and study sample was statistically significant (P<0.05) table(2).

Table 2: Frequency distribution of BCR-ABL fusion signal score in CML patients and study sample

<table>
<thead>
<tr>
<th></th>
<th>CML patient (20)</th>
<th>Study Sample (18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low score</td>
<td>7 (38.8%)</td>
<td>10 (61.1%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>6 (33.3%)</td>
<td>0</td>
</tr>
<tr>
<td>High score</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

DISCUSSION

Detection of BCR-ABL fusion signal in apparently healthy Iraqi population is reported for the first time in the present study. BCR-ABL fusion signal was detected in (10/18, 55%) of study sample and in (18/20, 90%) of CML patients (table 1).

There was a significant difference between CML cases and sample study regarding signal positive score (P<0.0001). All sample had low score whereas the cutoff value of BCR-translocation was found to be (>1 %).[8] (33.3%) of CML cases had high score,(27.8%) had intermediate score, (38.8%) had low score( table 2). Individuals in study sample, although showing low scores, should be put under observation.

The present study is the first to report the presence of BCR-ABL fusion genes at a genomic level in peripheral blood cell of healthy adults using FISH technique. To date, all screenings of leukemia-associated rearrangements have been based on RT-PCR.

BCR-ABL translocation was primarily detected in peripheral blood lymphocyte from healthy adults and children by Biernaux et al.[9] Afterwards, Bose et al.[10] confirmed these data.

As indicated above, these findings suggests that the translocations t(9;22) do not immediately initiate hematopoietic disorders but depend on a latency period. Progression to malignancy in positive individuals seems to depend on additional factors such as secondary oncogenic alterations, the affected sub fraction, and the immunological control of altered cells. The relevance of secondary alterations is supported by experimental findings in mouse models.[12,13,14,15]

Leukemia-associated gene fusions are generally believed to occur in utero, before birth.[16] According to Greaves and Wiemels,[17] the Knudson model, in addition to the twin concordance data, indicates that for every child with a particular translocation-positive leukemia, there has to be a greater number of healthy individuals that harbor the same translocation in a silent pre-leukemic clone.

Other authors suggested that such rearrangement could be expressed in hematopoietic cells that have entered the apoptotic pathway and that might have already lost their significance.[10] In addition, the mere presence of the aberrations in hematopoietic cells is not sufficient for the development of clinical malignancy in affected persons. In order to produce a leukemic phenotype, these rearrangement should fulfill two conditions: the structure of gene fusion must allow the production of a functional protein and the translocation must occur in early precursors with self-renewal capacity.[10,18] Therefore it is possible that the gene fusion in normal individuals arise in already differentiated cells or in a mature one which may be eliminated by normal mechanisms of cell differentiation.[19]

As mention previously all healthy individuals included in present study had higher mean count of micronuclei in peripheral blood lymphocytes. Elevated frequency of micronuclei consider a useful prognostic marker to provide an index of the genomic instability, either because of inherited factors predisposing to genomic instability, nutritional inadequacies or environmental exposure.[20] This finding provide a strong evidence that increased cytogenetic damage reflects enhance cancer risk. Moreover increased mean count of BNMN in PBL of disease-free individuals is a good predictor of cancer death risk.[21]
Detecting BCR-ABL fusion signal in peripheral blood cells of healthy individuals reflects long-term exposure of individuals to carcinogens. Consequently, they may aid in leading to tumor formation. In this context, those individuals may harbor mutation in certain genes associated with genomic instability and elevated risk of malignancy.

The present study recommended for further studies be conducted, where larger numbers of healthy individuals are recruited, to reinforce the result of the current study.

REFERENCES


