Significance of Philadelphia Chromosome in Chronic Myeloid Leukemia Patients of Anmol Hospital, Lahore, Pakistan

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Abstract:
Chronic myelogenous Leukemia is (Clonal stem cell disorder) cancer which starts in bone marrow a soft tissue inside bones that aids to form blood cells. It is first form of cancer that was firstly recognize to associate strongly with the chromosomal abnormality and the chromosome [t (9; 22) translocation] called Philadelphia chromosome.

Objective:
Philadelphia chromosome is a characteristic chromosomal marker that is associated with chronic myelogenous leukemia.

Methods:
More than one hundred patients of either sex were selected for the experiment. RNA was isolated from whole blood of patients so can use exclusively in RT-PCR.

Results:
Philadelphia chromosome in blood samples of patients with suspected diagnosis of CML was detected in 63% of patients. During our experimental studies on CML patients we do not encounter any complex translocation involving chromosome 8, 9 and 22.

Conclusions:
Philadelphia chromosome is a precise cytogenetic marker the detection of which is significant for differential diagnosis and clinical organization of patients with clinical diagnosis of CML. It is of significant that Ph chromosome occurs in pre-leukemic stage and has great diagnostic significance.

Key words:
Leukemia, Philadelphia chromosome (Ph), chronic myelogenous Leukemia (CML), translocation, cytogenetic marker.

Introduction:
Cancer is recognized psychologically as a malignant neoplasm [1] is an exclusive group of numerous diseases [2] all include unregulated cell tumor or the failure of regulation of tissue growth [3]. The may also feast to more reserved parts of the body finished by the blood stream or the lymphatic system [4]. Typically changes in many genes are required to transform a normal cell into a cancer cell. To instruct each cell how to copy its genes correctly and how to split and grow in an organized manner a number of different genes act together [5]. Cancer can be known in a number of ways including the presence of certain signs and symptoms screening tests or medical imaging [6]. When a possible cancer is well known it is identified by microscopic examination of a tissue sample. Cancer is usually cured with chemotherapy [7] radiation therapy and surgery [8]. Chronic myelogenous leukemia (CML) is categorized by the Philadelphia (Ph) chromosome
created by the reciprocal translocation t (9:22) (q34; q11) [9] resulting in the chimeric gene breakpoint cluster region (BCR)-Abelson (ABL)10. Variant Ph chromosome translocations involving chromosomes other than 9 and 22 occur in 5-10% of CML cases[11]. Chronic myeloid leukemia (CML) accounts for 15% of adult leukemias. The median age of disease onset is 67 year [12]. In 2018, an estimated 8,430 people will be diagnosed with CML in the United States, and 1,090 people will die of the disease [13]. Another fusion protein, p190, is also produced, usually in the setting of Ph-positive acute lymphoblastic leukemia. p190 is detected only in 1% of patients with CML [14]. Gene expression profiling has shown a close correlation of gene expression between accelerated phase CML (AP-CML) and blast phase CML (BP-CML) [15]. The activation of beta-catenin signaling pathway in CML granulocyte-macrophage progenitors (which enhances the self-renewal activity and leukemic potential of these cells) may also be a key pathobiology event in the evolution to BP-CML [16].

Philadelphia chromosome is a characteristic chromosomal marker that is associated with chronic myelogenous leukemia.

**Materials and Methods:**
After the ethical approval from Anmol Hospital and University of Veterinary and Animal Sciences (UVAS), Lahore a structured questionnaire was used for personal record containing demographic data (age, sex and residency), clinical manifestations, diseased history and family history of patients.

**Sample Collection and RNA Extraction:**
More than one hundred patients of either sex were selected from Anmol Hospital. The peripheral blood was drawn and kept for thirty minutes for serum to get separated and centrifuged at 500rpm for ten minutes for the serum to be separated. The specific commercial enzymatic kits of the Cenix Diagnostic GmbH (Germany) was used to determine blood AST, ALP, ALT in CML patients by using spectrophotometer. The data collected was subjected to statistical analysis according to (steel and torrie., 1982) <0.05 was consider as level of significance. The RNA was extracted from all the blood samples. RNA extraction was done by organic method. Due the modest content of RNA in leukocytes, it is recommended to use the isolated RNA exclusively in RT-PCR. Erythrocytes are lysed by hypotonic lysis. We recommended the application of red blood cell lysis buffer. RNA template concentration and purification are the critical factors in polymerase chain reaction (PCR). The components of the Light Cycler t (9; 22) Quantification kit and their corresponding storage conditions were followed.

**Amplification of the BCR-ABL Gene and Sequencing:**
The hexamer-primed cDNA synthesis was performed in a normal thermal cycler with heated lid. For every sample at least one cDNA reaction is performed. Additionally, standards and controls were reverse transcribed, duplication of the three G6PDH RNA standards I-III, the t (9; 22) positive control and negative control (non-template control=water). Subsequently, the BCR-ABL and G6PDH PCR reactions were performed by using this cDNA as a template. Existing cDNA can also be used for BCR-ABL and G6PDH-specific PCR. The sensitivity of Light Cycler t (9; 22) quantification kit depends on the purity of mRNA or cDNA.

**Number Of cDNA Synthesis Reactions:**
Each individual run on the Light cycler instrument consist of six G6PDH RNA standards (G6PDH RNA Standards I-III; vial 9a-c in duplicates) for creating the standard curve one positive control reaction for control of the complete BCR-ABL RT-PCR t (9; 22) positive control; vial 10) one positive control reaction for control of the complete G6PDH RT-PCR [t (9; 22) positive control; vial 10] one negative control reaction for control of the BCR-ABL PCR master mix for contamination[H2O, PCR grade, vial 11] one negative control reaction for control of the G6PDH PCR master mix for contamination[H2O, PCR grade, vial 11] a variable
number of RNA samples to be analyzed for relative BCR-ABL expression [BCR-ABL- and G6PDH PCR in to capillaries; cDNA obtained from a single reaction]. Therefore a typical experiment consists of 10 capillaries needed for the RNA standards and controls and [2n] capillaries needed for the samples where [n] indicates the number of samples. The number of cDNA synthesis reactions were performed. The BCR-ABL PCR and the G6PDH PCR for positive control, the negative control and the samples are each performed from a single cDNA synthesis reaction. cDNA master mix was prepared by multiplying the amount in the “volume” column by the calculated number of reactions to be performed, plus 3 additional reactions described above. 20 µl standard reaction of master mix and the reaction n was run. The Florescence Parameters light cycler software 3.5 was use to assess the results.

Results:
Total 100 patients with a clinical diagnosis of CML were recruited from Anmol Hospital, Lahore. The selection criteria were based on clinical symptoms of CML in male and female patients. Control individuals were healthy normal volunteers. The mean age of the patients was 47.6 years. All of the patients were tested for their LFT’s. The results indicate a mean AST of 356.3 mg/dl, ALT of 251.1mg/dl, ALP of 60.2mg/dl. Table 1 summarizes the results of LFT’s of the study participants. The genomic RNA was extracted from all 100 blood samples and was detected through florescence Parameters light cycler software 3.5 was use to assess the results (Figure 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>47.6 ± 12.2</td>
</tr>
<tr>
<td>AST (mg/dl)</td>
<td>356.3 ± 96.8</td>
</tr>
<tr>
<td>ALT (mg/dl)</td>
<td>251.1 ± 85.9</td>
</tr>
<tr>
<td>ALP (mg/dl)</td>
<td>60.2 ± 47.2</td>
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Table 1: Liver Functioning test of selected blood samples
In which the blue area show the patients having the Philadelphia chromosome. The blue area is 63% which signify that most CML patients have Philadelphia chromosome and it can be used as diagnostic marker at the time of diagnosis. While the part of graph show the patients of CML without Philadelphia chromosome which is 37%.

Discussion:
Chronic myeloid leukemia is a clonal stem cell disease produced by a developed somatic mutation [17] that fuses through chromosomal translocation of the ABL/BCR genes on chromosome 9 and 22, respectively [18]. The Philadelphia chromosome resulting from the balance translocation of t(9; 22) is diagnostic hallmark of chronic myeloid leukemia. CML was first disease associated with consistent cytogenetic abnormality the Philadelphia chromosome [19] which is shorten chromosome 22 and represents a reciprocal translocation between chromosomes 9 and 22 t(9;22)(q34; q11). The t(9; 22) fuses the c-ABL gene on chromosome 9 with BCR gene on chromosome 22 resulting in the production of chimeric oncoproteins [20]. The results show the significance of presence of Philadelphia chromosome in CML patients as out of 100 blood samples in 63 (63%) samples the Philadelphia chromosome appear but it’s not necessary that all patients with cml show the presence of Philadelphia chromosome appear but it’s not necessary that all patients with cml show the presence of Philadelphia chromosome as 37 (37%) samples have not sufficient levels of mRNA encode by Philadelphia chromosome. The patients with the presence of Philadelphia chromosome signify different BCR-ABL and
G6PDH ratios, 40/63 signify ratios with power $E^{-2}$, 20/63 signify ratios with power $E^{-3}$, 1/63 signify ratios with power $E^{-4}$ and 2/63 signify ratios with $E^{-5}$.

**Conclusions:**
Chronic myeloid leukemia signifies a unique model to comprehend the molecular mechanisms fundamental to the onset and development of leukemic process. Philadelphia chromosome is a precise cytogenetic marker the detection of which is significant for differential diagnosis and clinical organization of patients with clinical diagnosis of CML. It is of significant that Ph chromosome occurs in pre-leukemic stage and has great diagnostic significance. Blood karyotyping and PCR is useful for precise identification of the cytogenetic outline. Standard cytogenetics for CML is designated at the time of diagnosis and hematologic relapse and it is sensible to deliberate it through the follow up of the whole blood examination for any indication. The conventional cytogenetic analysis left over the standard method for the purposes of diagnosis and minimal residual disease in patients with CML.

**References:**


