ORIGINAL RESEARCH ARTICLE

Molecular Genetic Characterization of Philadelphia Chromosome in Patients with Chronic Myeloid Leukemia in the North-West of Iran

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Abstract

Background The Philadelphia (Ph) chromosome, characterized by the translocation t(9;22)(q34;q11), is found in 90–95% of individuals diagnosed with Chronic Myeloid Leukemia (CML) and in 0.1 - 3% of those with Acute Myeloid Leukemia (AML). The detection of chromosomal abnormalities plays a crucial role in the diagnosis and management of hematological disorders. The present study aimed to perform molecular genetic characterization of Ph in patients with CML in the north-west of Iran.

Methods A total of 45 new cases of adult patients with CML were studied at the Department of Internal Medicine, Imam Khomeini Hospital. The Ph chromosome was detected using simple multiplex RT-PCR methods.

Results We studied 45 patients with a mean age of 55.03 ± 21.56, including 23 (51.1%) males and 22 (48.9%) females. The frequencies of b2a2, b3a3, b3a3/b3a2, b3a2, and e1a2 transcripts were 46.71%, 24.4%, 6.67%, 2.22%, 2.22%, respectively. BCR–ABL typical transcripts were not found in the rest of our patients.

Conclusion Our findings revealed that the b2a2 transcript was the most common among CML patients, while patients carrying the b3a2 transcript demonstrated better molecular responses to standard-dose imatinib. The identification of transcript types not only supports diagnosis but also guides targeted therapy and long-term monitoring. Furthermore, emerging strategies combining TKIs with novel therapeutic agents may offer promising alternatives for resistant CML cases.

Keywords BCR–ABL, CML, Multiplex RT-PCR, Philadelphia chromosome

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1 Introduction

Cancer is characterized as the leading cause of morbidity and mortality globally, particularly in economically developed nations. There are three primary categories of blood cancer: leukemia, lymphoma, and myeloma, as well as disorders related to plasma cells. Leukemia arises from the incomplete development and dysfunctional proliferation of white blood cells and their precursors in the blood and bone marrow. Leukemia represents different types of blood cancer that can impact the bone marrow and blood cells, which is often associated with a wide range of symptoms, including weight loss, persistent fatigue, anemia, and the enlargement of both the liver and spleen.[1] Leukemia is categorized according to cell lineage into two types: lymphoid and myeloid, which influence lymphocytes and myeloid cells, respectively. Leukemia refers to the classification encompassing the four primary types of this disease as follows: Acute Lymphoblastic Leukemia (ALL), Chronic Lymphocytic Leukemia (CLL), Chronic Myeloid Leukemia (CML), and Acute Myeloid Leukemia (AML).[2]

It has been shown that the global distribution of leukemia exhibits considerable variation. The predominant types of leukemia were CLL, which represented 35% of all newly diagnosed cases, and AML, accounting for 33% of new cases. CML comprised 15% of the new leukemia cases, while ALL constituted 9% of the total.[3] Globally, the incidence of CML ranges from 1.0 to 2 cases per 100,000 individuals. In 2017, the estimated prevalence of CML in the United States was around 80,000 to over 100,000 cases.[4] CML affects all age demographics; however, the incidence of CML tends to rise with advancing age. CML occurs more frequently in males compared to females, exhibiting a male-to-female ratio ranging from 1.2 to 1.7. The median age in developed nations ranges from 57 to 60 years, while in developing countries, it has been reported to be between 32 and 44 years.[5] The majority of patients with CML receive their diagnosis during the chronic phase, where they typically exhibit mild symptoms. Nevertheless, the condition has the potential to advance to the accelerated phases and blastic crisis, which are marked by an increase in the number of blast cells present in both the bloodstream and the bone marrow.[5]

Blood abnormalities are heterogeneous in biological and clinical contexts. Molecular studies of chromosomal aberrations have revealed the mechanisms of carcinogenesis. The function and activity of many proto-oncogenes and tumor suppressor genes that are located in or near the chromosomal breakpoint are changed under the influence of genomic fusion encoding proteins. The Philadelphia (Ph) chromosome, t(9;22)(q34;q11.2) BCR-ABL1, is a hallmark of the myeloid neoplasms and acute leukemias.[6] The Ph chromosome is observed in 90–98% of patients with CML,[7] 0.1–3% of patients with AML,[6] in about 3–5% of pediatric ALL, and 25% of adult ALL.[8] Various fusions of the Ph chromosome may be identified in myeloid neoplasms and acute leukemias. The 3' region of the c-abl proto-oncogene, located on chromosome 9, is adjacent to the 5' region of the bcr gene, which is found on chromosome 22. The break occurs in the first intron of the c-abl gene. Breaks in BCR typically take place in one of three distinct areas: M-bcr, m-bcr, and μ-bcr. Chromosome breakpoints and rearrangement within the M-bcr, m-bcr and μ-bcr areas, leading to the production of abnormal fusions of chromosomes that are in association with hematological malignancies such as e14a2 (b3a2), e13a2 (b2a2), e13a3 (b2a3), e14a3 (b3a3), e12a3 (b1a3), e1a2, e1a3. The transcripts result in the production of chimeric proteins with a molecular weight of 210 (p210), 190 (p190), 203 (p203), and 230 (p230) kDa.[8] BCR-ABL1 b3a2 and b2a2 transcripts represent the predominant types found in cases of CML. Individuals who express the e14a2 (b3a2) transcript attain a significant molecular response more swiftly compared to those with the e13a2 (b2a2) transcript.[9] BCR-ABL1 triggers the activation of various signaling pathways, including the RAS/RAF/MAPK, PI3K/AKT/mTOR, and JAK/STAT pathways, which are crucial for cellular proliferation and anti-apoptotic signaling. Figure 1 shows a schematic illustration of oncogenic signaling activated by bcr-abl transcripts and its inhibition through tyrosine kinase inhibitors (TKIs) in CML.

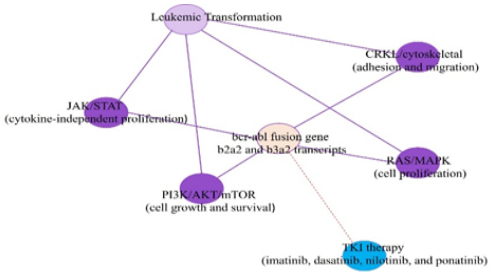


Figure 1 A schematic illustration of the BCR-ABL1 tyrosine kinase-dependent signaling cascade and its inhibition through tyrosine kinase inhibitors (TKIs) in CML

Several investigations have formulated important hypotheses and proposed potential strategies to improve the efficacy of combined therapies in patients with CML. In this context, the activation of STAT3 has been identified as a crucial survival mechanism in CML.[10] Given the pivotal role of BCR-ABL1 transcripts in influencing both prognosis and therapeutic response, the present study was designed to characterize BCR-ABL1 transcripts in CML patients at the molecular level.

2 Methods

This observational retrospective study was conducted in Urmia University of Medical Sciences (Urmia, Iran). The CML is diagnosed based on anamnesis, physical examination, and medical laboratory records, including cytogenetic and molecular tests. The samples included 45 newly diagnosed patients. Chronic Phase (CP)- CML diagnosis was based on European Leukemia Net's (ELN) CML diagnostic criteria.[11] Inclusion and exclusion criteria are summarized in Table 1. The diagnosis and classification of the patients were confirmed according to the morphologies described and specific criteria by a skilled oncologist. Informed consent was obtained from all subjects and/or their legal guardian(s).

Table 1 List of the criteria for the definition of CML, based on European Leukemia Net's (ELN) CML diagnostic criteria

Inclusion criteria:
Adult patients (Age more than 18 years)
Confirmed diagnosis of patients with the Ph chromosome
Prior administration of imatinib for a minimum duration of three months
Increasing white blood cell (WBC) count (more than 20,000/μL)
Patient not qualified for stem cell transplantation
Exclusion criteria:
Lymphoid blast phase of CML
Neurological/psychiatric disorder
Pregnant/lactating women
Patient with active systemic infection
Elevated bilirubin more than two times the upper normal limit
ALT is more than three times the upper normal limit
Patients with another severe disease

The inpatient ward nurse took peripheral blood (2 - 3 ml). From the obtained samples, RNA was extracted using the RNX Plus Solution Kit (SinaClon, Iran) (Catalog Number: RN7713C). The quality of RNA was evaluated and confirmed by the NanoDrop device. All samples were stored in a -20 freezer up use. The cDNA Synthesis kit (Thermo Scientific #K1622) was used to convert RNA to cDNA. The 4 μL cDNA samples were amplified using the Taq DNA Polymerase Master Mix RED (Ampliqon A/S) according to the manufacturer's recommendations. BCR–ABL transcripts were detected using a single-round multiplex RT-PCR with primer sequences as reported in Table 2.[12] The PCR reactions was carried out in a Bio-Rad T100 Thermal Cycler (United States). Then, the PCR products were separated in a 2.5% agarose gel in TBE buffer. The transcripts and expected PCR products were as following: e14a2 (b3a2): 418 bp, e13a2 (b2a2): 343 bp; e1a2: 474 bp; e19a2 (c3a2): 234; e6a2: 270 bp; e1a3: 300 bp; e13a3 (b2a3): 169 bp and e14a3 (b3a3): 244 bp.[12] Each atypical BCR–ABL transcript should be identified via direct sequencing.

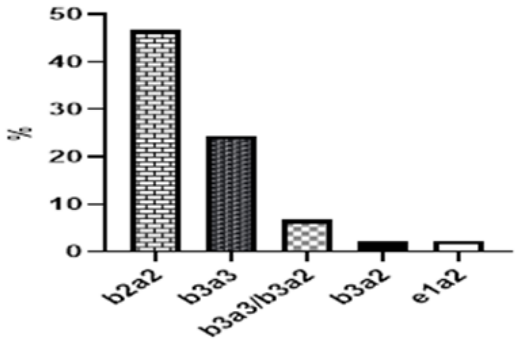
Table 2 Sequence of primers of multiplex RT-PCR for BCR-ABL transcripts in this study

Primer Name	Sequence (5'→3')	Location	PCR program	Ref
ABL-3	ccattgtgattatagccta-agacccggag	ABL exon 3	15 min at 95 °C; 95 °C for 10 sec, 58 °C for 20 sec, 72 °C for 30 sec, (40 cycles)	12
BCR-1	ctccagcgaggag-gacttctcct	BCR exon 1		
BCR-6	cctgagagccagaag-caacaaagatgcc	BCR exon 6		
BCR-12	agaacatccgggag-cagcagaagaa	BCR exon 12		
BCR-19	actgaaggcagccttc-gacgtc	BCR exon 19		
BCR-R	atgtccgtggccacac-cggacac	BCR exon 19		

3 Results

Forty-five CML patients were tested in this investigation. We studied 45 patients with a mean age of 55.03 ± 21.56, including 23 (51.1%) males and 22 (48.9%) females. The b2a2 (p210), b3a3 (p203), b3a3/b3a2 (p203/p210), b3a2 (p210), e1a2 (p190), transcripts were found in this study. In our patients, the frequencies of b2a2, b3a3, b3a3/b3a2, b3a2, and e1a2 transcripts were 46.71%, 24.4%, 6.67%, 2.22%, 2.22%, respectively (Figure 2). Typical BCR–ABL transcripts were observed in 82.22% of the studied cases (37 out of 45 cases).

Figure 2 The frequency of BCR-ABL1 transcripts in tested CML patients



4 Discussion

CML is characterized by the presence of the Philadelphia chromosome, which results from a reciprocal translocation, specifically t(9;22)(q34;q11). This translocation leads to the formation of the bcr-abl fusion gene, producing a constitutively active tyrosine kinase that drives uncontrolled proliferation of myeloid

cells.[7] The abnormal kinase activity activates several downstream pathways, including the RAS/MAPK pathway, which promotes proliferation and survival; the PI3K/AKT/mTOR pathway, which enhances metabolism, cell growth, and apoptosis resistance; the JAK/STAT pathway, which enables cytokine-independent proliferation and impaired differentiation; and the CRKL/cytoskeletal pathways, which contribute to altered adhesion and migration of leukemic cells.[10] Although the type of transcript does not alter the kinase domain, it may influence biological behavior. Certain studies have suggested that patients expressing b3a2 show more favorable molecular responses to TKI therapy compared to those with b2a2.

Targeted therapy with tyrosine kinase inhibitors (TKIs), such as imatinib, dasatinib, nilotinib, and ponatinib, blocks the ATP-binding site of the bcr-abl kinase. This inhibition suppresses downstream signaling and restores normal hematopoiesis.[10] The diagnosis of CML is established through blood counts and the identification of chromosomal abnormalities by quantitative RT-PCR. Approximately 90–98% of CML patients harbor the Ph chromosome,[7] which is also detected in 25–30% of adult ALL,[13] 3–5% of pediatric ALL,[14] and 0.5–3% of AML.[15]

In the present study, we investigated CML patients for the presence of the Ph chromosome and associated BCR-ABL1 transcripts. Our findings indicated that the b2a2 transcript was the most prevalent (46.71%). These results are consistent with previous reports by Bagheri et al.[16], Yaghmaie et al.[17], Yaghmaie et al.[7], Romero-Morelos et al.[18], and Ayatollahi et al.[19]. Co-expression of p210/p190 transcripts was not observed. The BCR-ABL fusions generate the oncoprotein p210, which plays a central role in leukemogenesis by persistently activating signaling pathways, including JAK/STAT, RAF, MYC, RAS/MEK, and PI3K/AKT. As a result, hematopoietic stem cells undergo malignant transformation into leukemia stem cells.[20] Geographic variation also appears to influence the incidence of these translocations among different ethnic groups.[19,20]

In this study, BCR-ABL transcripts were detected using a single-round multiplex RT-PCR, a simple and accurate approach for confirming specific gene fusions and identifying the Ph chromosome in leukemia. This method provides a comprehensive view of the genetic landscape and contributes to the personalization of treatment, with some findings guiding the use of targeted therapies. Moreover, several reports have highlighted improved outcomes with combined use of TKIs and non-TKI agents (e.g., PPAR-γ inhibitors), suggesting a potential therapeutic strategy for resistant CML.

Our study had several limitations. First, there was insufficient data to evaluate clinical parameters, such as spleen size, hematological indices, treatment responses, and patient histories. Second, some patients discontinued therapy at this center, resulting in incomplete follow-up information. Finally, the sample size was limited despite the institution being a referral center. Larger, multicenter studies with comprehensive clinical data are warranted to validate these findings.

5 Conclusion

The results of this study show that the b2a2 transcript was more common among CML patients. Improved outcomes have been observed in patients harboring the e14a2 (b3a2) transcript in comparison to those possessing the e13a2 (b2a2) transcripts following treatment with standard-dose imatinib. Consequently, the identification of either b2a2 or b3a2 not only confirms CML but also directs targeted therapy and serves as a dependable marker for long-term molecular monitoring. Although the type of transcript may have minor biological or prognostic significance, both variants are essential instruments in the diagnosis and management of the disease. The novel therapeutic strategies for treating CML present an alternative to address potential issues associated with traditional therapies. Despite the limited number of studies in this field, the concurrent application of TKIs alongside these innovative therapeutic options has been associated with improvements in patients' clinical conditions.

Declarations

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Authors' Contributions
Rahim Asghari participated in the medical evaluation and clinical characterization of patients. Salar Mahmoudi-Nejad and Sara Vazifeshenas have given substantial contributions to the conception or the design of the manuscript. Isa Abdirad and Morteza Bagheri supervised the experiments and determined the title. Morteza Bagheri analyzed the data and wrote the article. All authors read and approved the final manuscript.

Availability of Data and Materials
The data that support the findings of this study are available on request from the corresponding author.

Conflict of Interest
The authors declare that they have no conflicts of interest.

Consent for Publication
All authors have read and approved the final manuscript and have provided their consent for publication.

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Ethical Considerations

The Ethics Committee of Urmia University of Medical Sciences approved all stages of this study with the Code of Ethics IR.UMSU.REC.1398.428.

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