

# Cytogenetic Response Assessment to Imatinib Mesylate Therapy for Chronic Myeloid Leukemia in Iraqi Patients

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## Abstract

**Background:** Chronic Myeloid Leukemia (CML) is a disorder in myeloproliferative pluripotent hematopoietic primogonitor cells distinguished by huge reproduction of myeloid cells. CML cytogenetic distinguished by reciprocal translocation between the long arms of chromosomes 9 and 22 – to produce hallmark Philadelphia chromosome.

**Objective:** The main goal of the current study was to identify the secondary chromosomal aberrations associated with the CML in Iraqi patients have positive Ph- chromosome using Imatinib therapy, at different phases of the disease.

**Patients and Methods:** Practical part of this study was conducted from April/2018 to October/ 2019 in the Al-Kadumyha Teaching Hospital and Tissue Culture and Immunochemistry Lab/ University of Technology, for  $\leq 18$  years age for 75 CML eligible patients. Karyotypes of Philadelphia chromosome-positive bone marrow samples were examined for CML patients which were used Imatinib every twelve weeks. Secondary chromosomal abnormalities associated with development of disease were detected and calculated.

**Results:** The average age of patients was  $42 \pm 8.7$  years, while male to female ratio was (1.7:1), males number were 48 with percentage about 64%, while females number were 27 with percentage 36%, only 89.3% of CML patients showed a typical shape of Ph- chromosome, t(9:22)(q34;q11.2), while 27 of 75 showed real cytogenetic response to Imatinib therapy.

**Conclusion:** CML patients with high secondary chromosomal aberrations revealed low cytogenetic and molecular response to imatinib therapy, especially in blast phase.

**Keywords:** Chronic Myeloid Leukemia, BCR/ALB gene, Imatinib, Chromosomal Aberrations, FISH.

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## Introduction

Chronic myeloid leukemia (CML) is infrequent type of neoplasia with incidence of 1-2 patients per 100,000 people. CML is

described by clonal development of hematopoietic progenitor cells, producing increased circulating cells of granulocytic

progenitors. The typical symptoms of this disorder are chronic weakness, fever, weight loss, bleeding, and; whereas signs are anemia, splenomegaly, granulocytosis, immature granulocytes, the appearance of basophils, and thrombocytosis [1]. CML distinguished by rearrangement of the long arms of both chromosomes 9 and 22, lead to formation of Philadelphia (Ph) chromosome, as a result of this reunion produce, BCR/ABL1 tyrosine kinase [2]. Cytogenetic is very important tool, for the diagnosis, prediction and therapy of neoplasms like CML [3, 4]. The BCR/ABL translates to a protein of Mr 210,000, the BCR/ABL oncoprotein is located in the cytoplasm and causes activated tyrosine kinase [5]. Like to other type of kinases, the main function BCR/ABL is to bind with ATP and transfers phosphate from ATP to residues of tyrosine [6]. This product causes excessive proliferation of cells, turning off apoptosis, and inhibition of cellular adhesion [7]. It is obvious that the CML pathogenesis is associated with the enhanced tyrosine kinase of BCR/ABL protein [8]. Clinically, CML passed through three featured periods the first one is chronic phase it is easy to suppress the disease in this stage followed by unsettled accelerated phase, finally the neoplastic phase [9-11]. The most of CML patients display the Ph- chromosome as the unique alteration during the chronic phase [12]. In advanced phases, neoplastic cells show new chromosomal abnormalities, it may be a modified version of the Ph- chromosome i(17q), and trisomy of 19 and may be 8[13].

The growing of genetic instability of the leukemic cells accelerates to appear of subclones of highly oncogenic phenotypes, but observing cytogenetic and molecular responses to therapy developed as a good indicator to deal with a long-term disease. The CCR is determined as the loss of Ph+ cells in at least 20 bone marrow cells examined in the metaphases stage. Patients with active cytogenetic or molecular remission and CCR have affirmative prognosis; more than 70% of them remain alive after 10 years. A karyotype is important in post-remission treatment choice and the molecular indicator will define treatment in individuals with normal karyotype. Extreme monitoring can have a high economic cost, but treatment failure may result in the quickening of the disease or death of the patient [14]. The present study was conducted from April/2018 to October/ 2019 aims to identify the secondary chromosomal aberrations associated with the CML in Iraqi patients have positive Ph- chromosome at different phases of the disease.

### Patients and Methods

This study which was continued from April /2018 to October/ 2019, included 75 CML Iraqi patients, conducted in Al-Kadumyha Teaching Hospital and Tissue Culture and Immunochemistry Lab/ University of Technology. Briefly, the eligible patients were male or female  $\leq 18$  years of old and had confirmative diagnosis of CML with positive assay for Ph- chromosome t(9:22)(q34;q11.2). Also patients with BCR/ABL positive. The first chronic stage

was distinguished by presence of 30% blasts with promyelocytes within blood and marrow, 20% basophil and 15% blasts, while counting of platelet over than 100000/mm<sup>3</sup>. Accelerated phase was detected by >30% blasts with promyelocytes in bone marrow and peripheral blood, ≤30% blasts and 15% basophils in blood while platelet count still ≤100000/mm<sup>3</sup>. The current investigation was carried out according to the Declaration of Helsinki, with our concern to obtain written approvals from the study patients according to institutional guidelines. The rules were evaluated and approved by an Institutional Review Ethical Committee at each contributing center.

### Cytogenetic analyses

Chromosomal analyses were conducted at metaphase stage for bone marrow derived cells, by using short- term technique. Briefly, 10µg/mL of colcemide was added to culture media (RPMI-1640) and left in 37°C in CO<sub>2</sub>

incubator for one hour, after that bone marrow cells were incubated for 20 minutes at hypotonic solution (0.075 M KCL), then, cells were fixed with fixative solution ( 1:3 glacial acetic acid to absolute methanol). The remain pellet was dropped on clean cold slide and left to dry at room temperature. All slides were stained with G- banding technique. The chromosomal abnormalities were recorded by karyotypes according to human cytogenetic nomenclature system for pretreated patients and every twelve weeks beyond beginning of therapy [15, 16].

### Results

The experimental group contains 75 patients with main age 42± 8.7 years, male to female ratio was (1.7:1), males number were 48 with percentage about 64%, while females number were 27 with percentage about 36%, the characteristics of patients were summarized in Table (1).

**Table (1) :** Characteristics and Disease Feature for CML Patients

Variables	Value
Total patients	75
Sex n%	Male= 48, 64% female =27, 36%
Age	Mean= 42, Standard deviation = 8.7
Phase	CP=35 AP=27 BP=13
WBC count	125,000±22,000 cell/mm <sup>3</sup>
Platelet count	670,000±450,000

Chromosomal analyses of the 75 CML cases showed the existence of the ideal Ph chromosome, t (9: 22) (q34;q11.2), in 67

patients (89.3%). In 8 cases, the Ph-chromosome founded by multiple alterations of translocations involving chromosomes 22

and 9, eight cases were absence the Ph-chromosome, but FISH assay showed BCR/ABL gene fusion localized on the 22q. Beside of the Ph-chromosome, all 75 of the cases revealed additional derivative Structural and/or numerical aberrations. These aberrations demonstrated particularly in the Ph-clone in most of the cases, as a sign of clonal progression. Most of the patients revealed the main pathways of CML karyotypic development involving the hallmark Ph-chromosome in 67 patients, trisomy 19 in 5 cases, trisomy 8 in 15 cases, trisomy 21 in two cases in addition to isochromosome 17q in 7 cases, while less frequent aberrations noted were losing of 17p in 6 patients loses or rearrangement of ch- 7 in 3 patients, rearrangement of chromosome 13 (frequently 13p) in 8 cases, and 11p in 3 cases. Through blastic phase transformation of one case had an inversion of chromosome 16, inv. 16(p13q22), other cases revealed translocation (15: 17) (q22: q25) as

secondary abnormalities. From FISH assay duple-dye pml/rara DNA probes, this means pml gene translocate to ch-17 without fusion with rara gene, not as found in acute myelocytic leukemia (AML) t(not as found in 15: 17) is real fusion between pml/rara genes, all primary and secondary chromosomal alterations were summarized in Table (2), 55 patients demonstrated one or more minor abnormalities with multiple combinations at deferent recurrence. Cytogenetic response (CR) to the therapy was noted in 27 of 75 CML patients (36%), 15 of 27 (55.5%) at chronic phase, 8of 27 (29.6%) in the accelerated phase, and 4 of 27 (14.8%) at the blastic phase. 12 of those 27 CR cases obtained a complete cytogenetic response one of the 5 patients with advanced disease. Fifteen remain cases divided as the following: 9 with major cytogenetic response and 6 with minor cytogenetic response as shown in Table (2).

**Table(2):** Cytogenetics and BCR/ABL FISH Analyses for 27 CML Patients with Cytogenetic Responses

Case No.	Phase	Ph- Ch status	Pre-therapy	3m	6m	9m	Cytogenetic response
1.	CP	t(9:22)+Ph	t(9:22),t(9:11)	12:20	4:20	1:20	MCR
2.	CP	t(9:22)+Ph	t(9:22),inv(11)	8:20	3:20	0:20	CCR
3.	CP	t(9:22)+Ph	t(9:22), +11q	16:20	7:20	2:20	MCR
4.	CP	t(9:22)+Ph	t(9:22), 8	6:20	2:20	0:20	CCR
5.	CP	t(9:22)+Ph	t(9:22), in (17)	BCR:ABL+	BCR:ABL-	BCR:ABL-	CCR
6.	CP	t(9:22)+Ph	t(9:15:22),8	4:20	4:20	0:20	CCR
7.	CP	t(9:22)+Ph	t(9:22)+8+11	8:20	6:20	1:20	MCR
8.	CP	t(9:22)+Ph	t(9:22)+8-6q	18:20	11:20	1:20	MCR
9.	CP	t(9:22)+Ph	t(9:22),17p	17:20	12:20	0:20	CCR
10.	CP	t(9:22)+Ph	t(9:22),t(9:11)	15:20	10:20	4:20	mCR
11.	CP	t(9:22)+Ph	t(9:22),14q	12:20	8:20	1:20	MCR
12.	CP	t(9:22)+Ph	t(9:22),17p,6q	9:20	6:20	0:20	CCR
13.	CP	t(9:22)+Ph	t(9:15:22),8	11:20	7:20	2:20	MCR
14.	CP	t(9:22)+Ph	t(9:22),+21	17:20	14:20	0:20	CCR
15.	CP	t(9:22)+Ph	t(9:22),t(9:11)	15:20	11:20	2:20	MCR
16.	AP	t(9:22)+Ph	t(9:22)+8-8q	7:20	2:20	2:20	MCR
17.	AP	BCR/ABL gene fusion	i(17)q+8	8:20	6:20	3:20	mCR
18.	AP	t(9:22)+Ph	t(9:22), i(Ph)	BCR:ABL+	BCR:ABL-	BCR:ABL-	CCR
19.	AP	t(9:22)+Ph	t(9:22), i13q	11:20	7:20	4:20	mCR
20.	AP	t(9:22)+Ph	t(9:22), +14q	7:20	5:20	3:20	mCR
21.	AP	t(9:22)+Ph	t(9:22), +8q,	10:20	4:20	0:20	CCR
22.	AP	t(9:22)+Ph	t(9:22), +17q	20:20	11:20	3:20	mCR
23.	AP	t(9:22)+Ph	t(9:15:22),8	5:20	5:20	2:20	mCR
24.	BP	t(9:22)+Ph	t(9:22), t(9:11)	18:20	12:20	6:20	mCR
25.	BP	t(9:22)+Ph	t(9:22), +11	BCR:ABL-	BCR:ABL-	BCR:ABL-	CCR
26.	BP	t(9:22)+Ph	t(9:22),8p-	12:20	7:20	3:20	mCR
27.	BP	t(9:22)+Ph	t(9:22),13q-	16:20	9:20	6:20	mCR

\* Rate ph: standard based on an examination of 20 mitotic cells. BCR:ABL, BCR:ABL+ fusion-positive; BCR:ABL-, BCR:ABL fusion-negative; AP, accelerated phase; BP, blastic phase; CP, chronic phase; CR, cytogenetic response; CCR, complete CR; MCR, major CR; mCR, minor CR

## Discussion

Practically, the Ph-chromosome in the chronic phase of CML considered a basic genetic phenomenon in most cases and plays a vital role in the evolution and pathogenesis of CML [17]. The BCR/ABL gene fusion is adequate to begin CML, so any therapy can prevent this genetic fault like Imatinib can

cause hematological remission in most newly diagnosed CML [18]. Delayed-phase CML, the Ph- clone can obtain more molecular and/or cytogenetic abnormalities, causes more aggressive phenotypes [19]. Thus, CCR was noted in only 25% of patients in the accelerated phase and 25% in the blastic

phase [20]. The MCR was found in 12.5% of accelerated phase, it was not seen in the blastic phase, and the current findings refer to a small percentage of patients whose Ph leukemic cells were a response to imatinib therapy, twenty-seven of the 75 (36%) patients in this study acquired CRs. Other results have recorded similar CRs in accelerated and blastic phases, through the patient community which has a high repetition of secondary chromosomal aberrations [21, 22]. CRs were showed even in patients who have an extra copy of the Ph-chromosome also noted in some cases with complex abnormalities [23, 24].

Finally, the implications of additional genetic aberrations on clinical response to Imatinib therapy in CML have considerable biological and clinical impacts. The present study recommends that in CML patients with additional chromosomal abnormalities, Imatinib therapy was able to enucleate the Ph- clone in a small portion of patients [25]. However, in many patients, Imatinib failed to stimulate cytogenetic response suggesting that the Ph- clone genetically developed and got rid of the dependency on BCR/ABL kinase for proliferation. The limited activity of Imatinib on CML patients with secondary genetic alterations elevates the necessity for conjugation Imatinib with other therapeutic agents to induce treatment outcomes. Nowadays, in vitro studies have shown the synergetic effects of many agents used in joining with Imatinib, and several Phase linked therapy clinical tests have been suggested [24, 25].

## Conclusions

From present findings, can be concluded many CML patients with secondary abnormalities have a weak cytogenetic and molecular response to imatinib therapy so this raises the demand to replace imatinib with other therapeutic agents to enhance treatment outcomes.

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## Conflicts of Interest

There are no conflicts of interest

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