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Research paper

# Genomic amplification of *BCR-ABL1* fusion gene and its impact on the disease progression mechanism in patients with chronic myelogenous leukemia

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ABSTRACT

Identification of *BCR-ABL1* fusion gene amplification status is critically important in the effective management of chronic myelogenous leukemia (CML) patients. Earlier reports suggested that overexpression of *BCR-ABL1* either through amplification of *BCR-ABL1* fusion gene or by the up regulation of *BCR-ABL1* transcript level might be an early phenomenon in the establishment of IM resistance and disease evolution in CML. In the current study, we performed dual color dual fusion locus specific *BCR/ABL1* FISH analysis along with karyotype analysis using GTG banding (G-banding using trypsin and Giemsa) technique in 489 patients with different clinical stages of CML at diagnosis or during the course of the disease to unravel the spectrum of *BCR-ABL1* fusion gene amplification status. Among the study group analyzed, it was found that prevalence of occurrence of *BCR-ABL1* fusion gene amplification was significantly higher in advanced stages of disease and in IM resistant CML-CP patients when compared to initial stage of disease, *de novo* CML-CP. Cytogenetic and metaphase FISH characterization on our study samples revealed that *BCR-ABL1* fusion gene amplification was occurred through the formation of extra copies Ph chromosomes and isoderived Ph chromosomes. Current study suggests that unrestrained activity of *BCR-ABL1* played a vital role in resistance to targeted therapy and disease evolution in CML. In our study population, patients in progressive stage CML and in IM resistant CP with multiple copies of *BCR-ABL1* fusion gene displayed a poor response to targeted treatment with IM. Hence, the early identification of *BCR-ABL1* fusion gene amplification using FISH technique will lead to improved interventions and outcome in future CML patients.

## 1. Introduction

Chronic myeloid leukemia (CML) is a hematopoietic stem cell malignancy leading to myeloproliferation, characterized by the presence of Philadelphia (Ph) chromosome resulting from translocation t(9;22)(q34.13;q11.23) lead to *BCR-ABL1* fusion gene, which plays a central role in the pathogenesis of CML (Melo and Barnes, 2007). CML gradually progresses from stable chronic phase (CP) to an intermediate

*Abbreviations:* ABL, Abelson murine leukemia viral oncogene homolog 1; AP, accelerated phase; BC, blast crisis; BCR, breakpoint cluster region; BM, bone marrow; C/EBPα, CCAAT/Enhancer-Binding Protein Alpha; CCyR, complete cytogenetic response; CHR, complete hematologic response; CML, chronic myelogenous leukemia; CP, chronic phase; CyR, cytogenetic response; DAPI, 4′,6-diamidino-2-phenylindole; FISH, fluorescent *in situ* hybridization; GTG, G banding using Trypsin and Giemsa; Ider (22), isoderivative chromosome 22; IM, Imatinib Mesylate; ISCN, International System for Human Cytogenomic Nomenclature; KD, kinase domain; LPC, leukemic progenitor stem cell; LSC, leukemic stem cell; LSI, locus specific identifier; MCyR, major cytogenetic response; mCyR, minimal cytogenetic response; PCyR, partial cytogenetic response; Ph, Philadelphia chromosome; PHR, partial hematologic response; PP2A, protein phosphatase 2 alpha; TKI, tyrosine kinase inhibitor

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accelerated phase (AP) and finally to terminal blastic phase (BC) of lymphoid or myeloid phenotype. Compared with advanced stages of CML, survival rates are exceptionally high in early phase of the disease, CP-CML. Imatinib Mesylate (IM), a tyrosine kinase inhibitor, selectively bind and inhibit the tyrosine kinase activity of BCR-ABL1 protein and prolonged the survival rate in CML-CP than AP or BC (Kantarjian et al., 2012). However, the blast crisis of CML is highly resistant to chemotherapy with a response rate of < 30% and the 5 year survival rate is only 6%. The blast crisis stage of CML is highly fatal with a median survival of about 2–3 months (Axdorph et al., 2002; Kantarjian et al., 2012; Jabbour et al., 2014). Advances in treatment options of patients with CML-AP and BC are obstructed in part by several factors such as the mechanisms responsible for progression of disease from the initial chronic phase into fatal blast crisis remain poorly understood and the array of cytogenetic, genetic and molecular defects involved in CML evolution, which underscores the complexity of the disease in its advanced stages. Since the treatment options for AP and BC are very much limited, it is extremely urgent to better understand the biology of disease progression and thereby illuminating the path of therapeutic strategies for effective management of patients with CML-AP/BC.

The remarkable functional changes associated with disease progression of CML are variations in proliferation, differentiation, apoptosis and cell adhesion, which lead to decreased treatment response in advanced phases of CML (Calabretta and Perrotti, 2004). Blastic transformation of CML is a multistep, time-dependent process initiated by DNA damage associated with impaired DNA repair process in the early phase of CML and it provides a selective advantage to one or more CML blast cell clone for further proliferation (Perrotti et al., 2010). The presence or accumulation or combination of different additional genetic lesions like numerical and structural chromosomal changes, gene deletions, gene insertions, point mutations and differential gene expression in this selective blast cell clone allow them to stimulate the malignancy towards a more aggressive stage (Roche-Lestienne et al., 2008). About 15–20% CML-CP patients who developed clinical resistance to tyrosine kinase Inhibitors (TKI) were classified as non-responders (Foroni et al., 2009). The disease biology of these TKI resistant non-responders is same as that of advanced phases of disease and therefore after the accumulation of genetic and epigenetic anomalies, these patients may eventually progress in to BC of CML.

The *BCR-ABL1* gene expression level has been considered to be associated with disease evolution in CML. *BCR-ABL1* gene expression level can be increased by upregulation of transcript level or by amplification of *BCR-ABL1* fusion gene or by increase in number of *BCR-ABL1* positive leukemic cells in the peripheral blood (PB) or bone marrow (BM) (Jabbour et al., 2014). Overexpression of the BCR-ABL1 protein due to amplification of *BCR-ABL1* fusion gene was first identified in IM resistant CML cell lines and suggested that the overexpression of *BCR-ABL1* might be an early phenomenon in the establishment of IM resistance and disease evolution in CML (Barnes et al., 2005; Mahon et al., 2000). Expression studies revealed that constitutive BCR-ABL1 activity, the level of which starts to elevate from CML AP, drastically changes the transcriptome level of CML, which in turn results in differential expression of many candidate genes and that might play an important role in disease evolution of CML (Radich et al., 2006). Immense and vigorous activity of BCR-ABL1 in advanced phases of CML results in alterations of several cellular, genetic and molecular events. It is also noted that, unrestrained activity of BCR-ABL1 in CML BC directly or indirectly involved in the silencing of main tumor suppressor genes such as p53 and p16 either by deletion/mutation or by gene rearrangements. However, data regarding the effect of *BCR-ABL1* fusion gene amplification on disease progression and increased resistance to IM therapy in CML patients are not conclusive.

Altogether, it supported the fact that, there is no well characterized genetic or molecular pathway that distinctly contributes to BC evolution in CML, which strengthens the complexity of the disease. Thus, it can speculate that proper identification of molecular mechanisms responsible for blastic transformation of CML which will help in the elucidation of novel therapeutic agents and will improve to alleviate therapeutic challenges associated with advanced phases of CML.

Therefore, determination of the incidence and patterns of *BCR-ABL1* fusion gene amplification in different stages of CML is extremely vital for effective treatment and management of CML patients. The present study was designed to perform dual color dual fusion locus specific *BCR/ABL1* fluorescence *in situ* hybridization (FISH) analysis in 489 patients during different stages of CML at diagnosis or throughout the course of the disease to unravel the spectrum of *BCR-ABL1* fusion gene amplification status.

## 2. Patients and methods

### 2.1. Study subjects

A total of 489 CML patients with Ph chromosome/*BCR-ABL1* fusion gene, who attended the Medical Oncology out-patient clinic, Regional Cancer Centre (RCC), Thiruvananthapuram, Kerala, India during the period from January 2013 to January 2016, formed the study subjects. At our center (RCC), CML accounts for 15–20% of all leukemia cases. The study population consists of 332 males and 157 females (M: F ratio = 2.1:1) with age ranging from 15 to 81 years (median age 43 years). Among the 489 CML cases, 313 patients (64%) were in *de novo* CP, 78 cases (15.96%) were in IM resistant CP, 38 cases (7.77%) were in AP and rest 60 patients (12.27%) were in BC phases of CML (Table 1). The data regarding *BCR-ABL1* fusion gene amplification status obtained in the current study were analyzed and compared between these selected study phases of CML. The study was approved by the Institution Review Board (IRB) and Human Ethics Committee of RCC (HEC Approval No: 6/2010). After getting written informed consent, ~2–3 ml of Bone marrow/Peripheral blood samples were collected aseptically in the heparinized vacutainer from the study subjects.

### 2.2. Conventional cytogenetic analysis

Bonemarrow aspirate collected from the patients was used for classical cytogenetic analysis. BM cells were then subjected to short-term culture using RPMI medium. Harvesting and chromosome analysis using GTG banding (G-banding using trypsin and giemsa) were performed according to standard protocol (Henegariu et al., 2001). A total of 30 well-spread metaphase cells were analyzed and G-banded karyotypes at the 550 band levels were constructed in each patient. Metaphases were karyotyped using cytogenetic software (Genasis, Applied Spectral Imaging, Migdal Ha'Emek, Israel and Cytovision, USA – Olympus Microscope BX53, Tokyo, Japan). Karyotypes were described according to the International System for Human Cytogenetic Nomenclature 2016 (ISCN, 2016).

### 2.3. Fluorescence in situ hybridization (FISH) analysis

FISH analysis using a locus specific identifier (LSI) *BCR/ABL1* dual-color dual-fusion translocation probe (Abbott Molecular/Vysis, Des Plaines, IL, USA) was performed according to the manufacturer's instructions (Al-Achkar et al., 2007). A total of 20 metaphase spreads and 200 interphase nuclei were analyzed using a fluorescence microscope

**Table 1**
Frequency distribution of study subjects in different clinical stages of CML.

| Disease phase | Number of patients (%) |
|---|---|
| *De novo* chronic phase | 313 (64%) |
| Accelerated phase | 38 (7.77%) |
| Blast crisis | 60 (12.27%) |
| IM resistant chronic phase | 78 (15.96%) |

(Olympus BX53, Tokyo, Japan) equipped with appropriate filter sets to discriminate between a maximum of 5 fluorochromes and the counterstain DAPI (4′,6-diamidino-2-phenylindole). The hybridization signals were acquired with a Spectra Cube SD200 spectral imaging system (Genasis, Applied Spectral Imaging, Migdal Ha'Emek, Israel and Cytovision, USA).

### 2.4. Treatment response analysis

The data regarding the hematological and cytogenetic analysis of CML patients were recorded initially at the time of diagnosis and these parameters were validated periodically at three months interval for the first year and at six months interval for the next two years after the commencement of IM therapy.

#### 2.4.1. Hematological response analysis

The Complete Hematologic Remission (CHR) was defined as the complete disappearance of immature cells and the normalization of WBC and platelet counts in PB in association with entire reversal of splenomegaly. Patients with CHR would show total leukocyte count $< 10 \times 10^9$/L and platelet count $< 450 \times 10^9$/L. CHR would also reveal the absence of peripheral blast, immature granulocytes such as promyelocytes or myelocytes and < 5% peripheral basophils. Criteria for partial hematologic response (PHR) is similar to that of CHR, except that there could be persistence of immature cells, or platelet count < 50% of the pre-treatment count but $> 450 \times 10^9$ cells/L, or persistent splenomegaly but > 50% of the pretreatment extent.

#### 2.4.2. Cytogenetic response analysis

Cytogenetic response (CyR) is defined by reduction in the percentage of Ph positive metaphase cells. The absence of any Ph positive cells is considered as complete cytogenetic response (CCyR), presence of 1–35% of Ph positive metaphase cell is called major cytogenetic response (MCyR), partial cytogenetic response (PCyR) is 36–65% of metaphase cells with Ph and minor or minimal cytogenetic response (mCyR) is the cells having 66–95% of Ph positive metaphase cells. Patients whose BM revealed > 95% of Ph positive metaphase cells were considered as IM non responders.

### 2.5. Statistical analysis

All calculations were performed using SPSS software version 21. Chi-square test and Fisher's exact test was used to compare the rate of incidence of *BCR-ABL1* fusion gene amplification status among the study groups. Student's *t*-test and Mann Whitney *U* test was used to find the difference in the distribution of covariates such as Hemoglobin (gm %), Total Count (cmm), Platelet (cmm), Lactate dehydrogenase or LDH (IU/L), PB Blast (%) and BM blast (%) with *BCR-ABL1* amplified FISH signal pattern. A *P* value < 0.05 was taken as statistically significant.

## 3. Results

*BCR-ABL1* fusion gene amplification or duplication has been found to be one of the prime factors leading to drug resistance and there by disease evolution in CML. Hence, quantification of *BCR-ABL1* fusion gene is of paramount important for the proper management of CML patients. Amplification status of *BCR-ABL1* fusion gene was detected by FISH analysis using *BCR/ABL1*dual color dual fusion locus specific probes. Typical translocation event between chromosome 9 and 22 results in the generation of single copy of *BCR-ABL1* fusion gene, which was represented by two fusion signals [*BCR-ABL1* fusion gene on der (22) and *ABL1-BCR* fusion gene on der (9)] by FISH analysis (Fig. 1). Acquisition of additional fusion signals in interphase FISH indicates either the amplification of *BCR-ABL1* or *ABL1-BCR* fusion gene [Fig. 2 (A)]. Therefore, *BCR-ABL1* fusion gene amplification status detected in interphase FISH analysis was further confirmed by cytogenetics and metaphase FISH characterization on those patients.

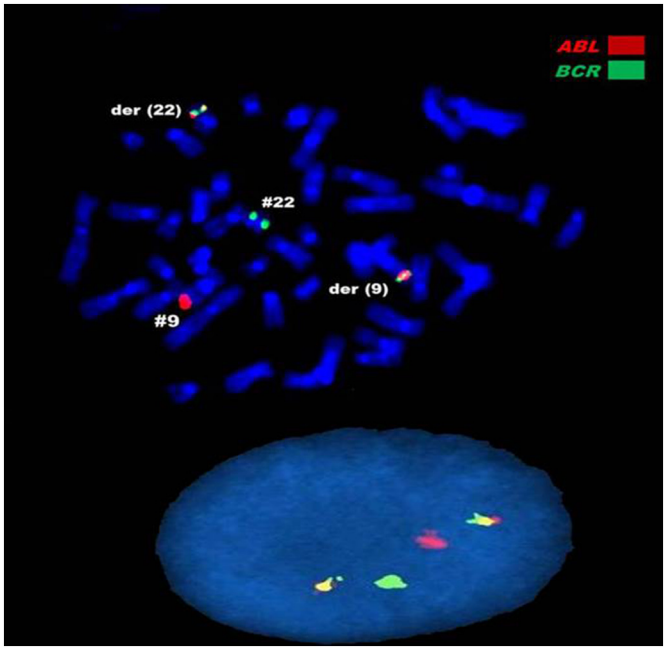


**Fig. 1.** FISH image of interphase & metaphase by using *BCR* (green)-*ABL1* (red) probe localized to chromosome 22q11.2 & chromosome 9q34 respectively showing presence of two red-yellow-green fusion signals for *BCR-ABL1*on der (22) & *ABL1-BCR* on der (9). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

### 3.1. BCR-ABL1 fusion gene amplification status among the study groups

Out of 313 *de novo* CP cases analyzed, only 5 patients showed the presence of additional fusion signals on interphase FISH analysis. In all these 5 cases, an extra Ph (double Ph) chromosome was observed on cytogenetic and metaphase FISH analysis [Fig. 2 (B)]. In total, *BCR-ABL1* gene amplification status was reported only in 1.60% of *de novo* CP patients. While in CML-AP patient group, 4 cases (10.53%) revealed the presence of *BCR-ABL1* fusion gene amplification. Likewise *de novo* CP, here also *BCR-ABL1* amplification was observed through double Ph chromosome on cytogenetic and metaphase FISH analysis [Fig. 2 (B)]. In CML BC, *BCR-ABL1* fusion gene amplification was reported in 13 cases (21.67%) out of 60 cases analyzed. Cytogenetic and metaphase FISH characterization on these *BCR-ABL1* amplified cases showed three copies of Ph chromosome along with hyperdiploid metaphase in one case and double Ph chromosome in rest of the cases. Apart from this, 6 cases (7.69%) among the IM resistant CP patients were shown to possess amplification of *BCR-ABL1* fusion gene. Further cytogenetic and FISH analysis on metaphase spreads from these amplified cases unmasked two rare cases of isoderivative chromosome 22 [ider (22)] positive patients. Analysis revealed one to three copies of ider (22) in one case and one to two copies of ider (22) along with two copies of Ph chromosome in second case resulting in two to six copies of *BCR-ABL1* fusion gene in each case [Fig. 3 (A & B)]. In the remaining 4 cases, fusion gene duplication took place through the doubling of Ph chromosome.

### 3.2. Comparison of incidence of BCR-ABL1 fusion gene amplification status between the study groups

Comparison of *BCR-ABL1* fusion gene amplification status between the study groups geared up the fact that unrestrained activity of *BCR-ABL1* played a vital role in resistance to targeted therapy and disease evolution in CML. That is, in the current study it was pinpointed that

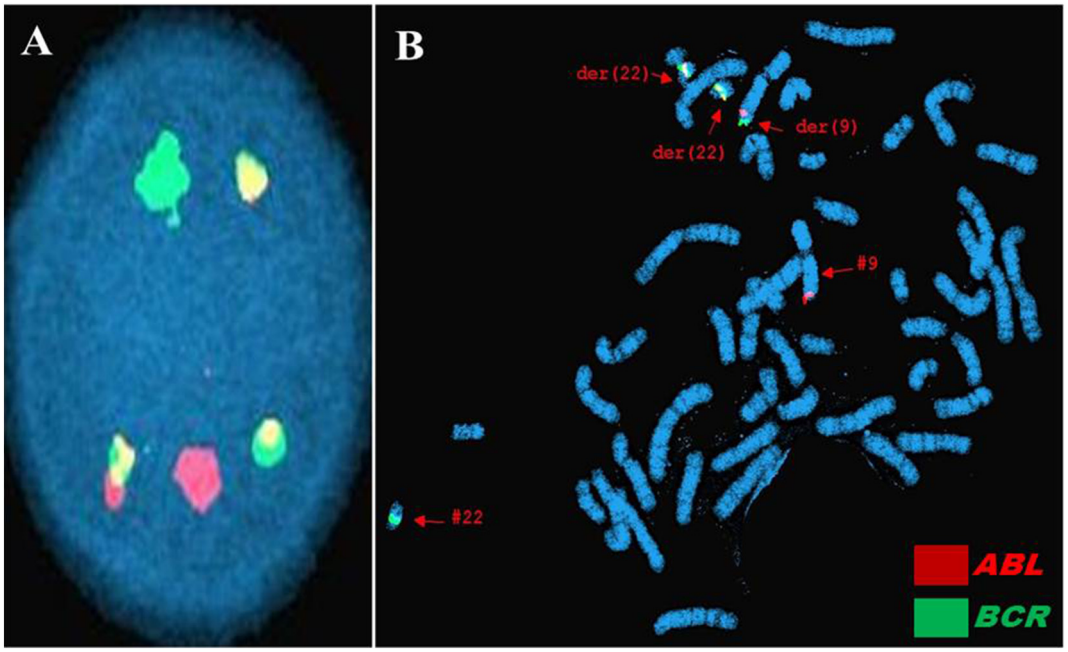


**Fig. 2.** FISH image of interphase (A) & metaphase (B) by using BCR (green) & ABL1 (red) probe localized to chromosome 22q11.2 & chromosome 9q34 respectively showing the presence of three red-yellow-green fusion signals representing two copies of *BCR-ABL1* & one copy of *ABL1-BCR*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the prevalence of occurrence of *BCR-ABL1* fusion gene amplification was higher in advanced stages of disease and IM resistant CP group when compared to initial stage of disease, CML-CP. The data analysis between BC and *de novo* CP showed that *BCR-ABL1* amplified cases were significantly higher in BC (21.67% *vs* 1.6%, $P < 0.0001$). Similarly, it was also significantly higher in AP (10.53% *vs* 1.6%, $P < 0.009$) and IM resistant CP groups (7.69% *vs* 1.6%, $P < 0.01$) when compared to *de novo* CP. Between BC and IM resistant CP group analyzed, it was revealed that the frequency of incidence of *BCR-ABL1* amplified cases were more in BC (21.67% *vs* 7.69%, $P < 0.02$). Even though patients in BC showed a higher rate of *BCR-ABL1* fusion gene amplification compared to AP patients (21.67% *vs* 10.53%), it was not statically significant ($P > 0.1$). Likely, there was not any significant relationship observed between AP and IM resistant CP group ($P > 0.4$) [Fig. 4].

### 3.3. Correlation of BCR-ABL1 fusion gene amplification status with hematological and laboratory parameters

A significant correlation was obtained between *BCR-ABL1* fusion gene amplification and LDH level in IM resistant CML-CP stage. In IM resistant CP, patients with *BCR-ABL1* fusion gene amplification displayed significantly higher white blood cell (WBC) and BM blast level in comparison with non-amplified cases ($P = 0.003$ and $P < 0.0001$ respectively). In addition, PB blast level was also high in IM resistant CP cases with BCR-ABL1 fusion gene amplification, but only a marginal significance was obtained ($P = 0.098$) depicted in Table 2.

## 4. Discussion

Chronic myelogenous leukemia (CML), a hematopoietic disorder of multipotential stem cells, exhibits excessive proliferation of immature

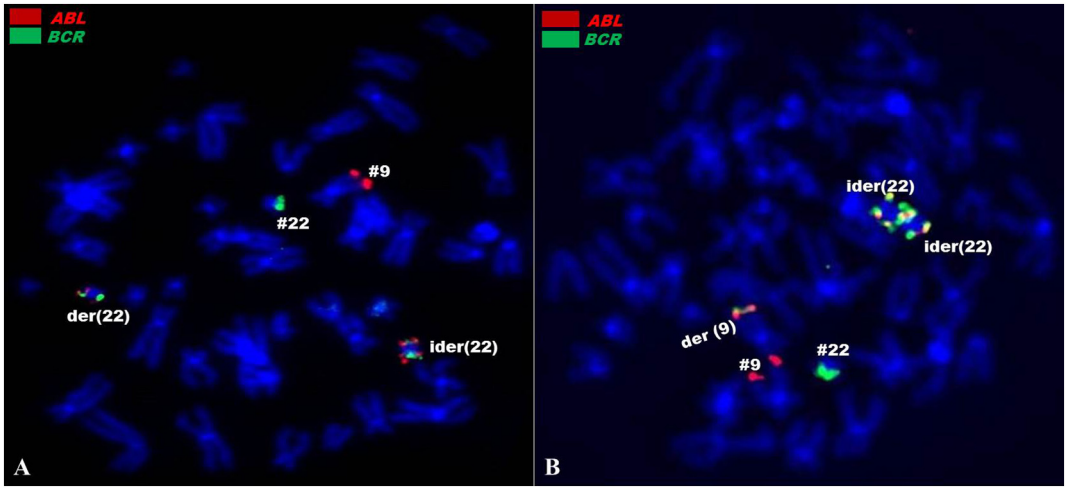


**Fig. 3.** Metaphase FISH analysis by using BCR (green) & ABL1 (red) probe localized to chromosome 22q11.2 & chromosome 9q34 confirming the presence of 3 copies of *BCR-ABL1* fusion gene (A) and four copies of *BCR-ABL1* fusion gene (B) through the formation of ider (22). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

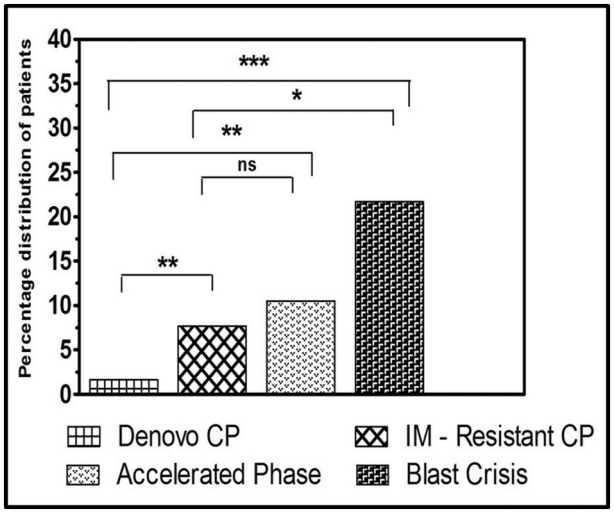


**Fig. 4.** Frequency of incidence of *BCR-ABL1* amplification status among the study groups (***; *P*-value < 0.001, **; P-value < 0.01, *; *P* value < 0.05).

and mature myeloid cells. The *BCR-ABL1* fusion gene derived from Philadelphia chromosome, resulting from a classical translocation event t(9;22)(q34.13;q11.23), which is responsible for pathogenesis of CML patients (Deininger et al., 2000; Ren, 2005; Perrotti et al., 2010). In CML, the identification of *BCR-ABL1* fusion gene amplification is of profound importance in clinical diagnosis, prognosis, and decision about treatment modality. *BCR-ABL1* fusion gene amplification status, one of the prime factors for drug resistance and disease evolution in CML, was evaluated using interphase FISH analysis by *BCR* and *ABL1* specific probes. Therefore, the formulation of a *BCR/ABL1* FISH signal pattern is extremely important for better management of CML patients. The *BCR/ABL1* dual color dual fusion probe comprises an *ABL1* probe fluorescently tagged with spectrum orange, which spans an area from centromeric region of *arginosuccinate synthetase* gene to the telomeric end of the *ABL1* gene, and a *BCR* probe directly tagged with spectrum green covering an area from within the region of immunoglobulin lambda light chain locus to the telomeric region of *BCR* gene. Therefore, a typical *BCR-ABL1* fusion gene-negative cell will show 2R2G and a positive cell will display 2F1R1G signal pattern (2F1R1G). Acquisition of additional fusion signals in interphase FISH indicates either the amplification of *BCR-ABL1* or *ABL1-BCR* fusion gene. Therefore, *BCR-ABL1* fusion gene amplification status detected in interphase FISH analysis was further confirmed by cytogenetics and metaphase FISH characterization on those patients.

Current study revealed a gradual increase in the *BCR-ABL1* fusion gene amplification status from initial chronic phase to terminal blast crisis stage. Significantly higher frequency of amplification status was observed in BC patients compared to CP patients at initial diagnosis (21.67% *vs* 1.6%, $P < 0.0001$) and CP patients with IM resistance (21.67% *vs* 7.69%, $P < 0.02$). Similar pattern was also observed in AP patients when compared to *de novo* CP (10.53% *vs* 1.6%, $P < 0.009$). Among the chronic phase patients compared, patients with IM resistance showed significantly a higher occurrence of *BCR-ABL1* fusion gene amplification compared to patients at the time of diagnosis (7.69% *vs* 1.6%, $P < 0.01$). Cytogenetic and metaphase FISH characterization on these *BCR-ABL1* amplified cases unraveled that amplification was occurred through the formation of double Ph chromosome in all, except in two IM resistant patients. Here in these two patients, we observed two to six copies of *BCR-ABL1* fusion genes through multiple copies of rare ider (22) chromosomes (Ramachandran et al., 2016). Also one patient in BC owned three copies of Ph chromosome. However, the presence of three or more copies of Ph through a non-dysjunction process is an unusual event (Mohammed et al., 2000).

Even if several mechanisms were coined for imatinib resistance, the genetic instability caused by the unrestrained activity of BCR-ABL1 might have played a pivotal role in chromosomal aberrations, mutations, and changes in gene expression that hallmarked resistance to targeted therapy and disease progression (Vaidya et al., 2011). It was reported that disease progression in CML was associated with overexpression of BCR-ABL1 in committed progenitors, leading to a multiplicity of genetic and epigenetic events (Perrotti et al., 2010), which was in accordance with our findings that *BCR-ABL1* amplification status was higher in progressive and therapy resistant stages of CML. So the early detection of the amount of *BCR-ABL1* is of paramount importance in the effective management of CML patients. The presence of multiple copies of the *BCR-ABL1* oncogene was considered as an indicator of poor prognosis and higher possibilities for resistance to drug treatment (Chua et al., 2010). Likely, in our study population, patients in progressive stage CML and in IM resistant CP with multiple copies of *BCR-ABL1* fusion gene displayed a poor response to targeted treatment with

**Table 2**
Comparison of clinical & laboratory parameters between *BCR-ABL1* amplified & non-amplified CML cases.

| Clinical parameters | BCR-ABL1 amplification status | *de novo* CP | | IM resistant CP | | Accelerated phase | | Blast crisis | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | *P* | Mean | *P* | Mean | *P* | Mean | *P* |
| Hb (gm%) | Amplification | 9.75 | 0.204 | 9.33 | 0.267 | 9.67 | 0.863 | 9.77 | 0.267 |
| | Non amplification | 11.02 | | 10.44 | | 9.49 | | 9.04 | |
| WBC ($\times 10^9$/L) | Amplification | 194.49 | 0.911 | 508.45 | 0.003 | 248.03 | 0.432 | 117.00 | 0.337 |
| | Non amplification | 268.72 | | 111.17 | | 152.34 | | 112.41 | |
| PLC ($\times 10^9$/L) | Amplification | 280.75 | 0.369 | 251.50 | 0.536 | 560.00 | 0.167 | 152.38 | 0.451 |
| | Non amplification | 369.69 | | 259.40 | | 333.82 | | 189.89 | |
| BM Blast (%) | Amplification | 5.50 | 0.345 | 5.50 | < 0.0001 | 13.33 | 0.936 | 47.62 | 0.402 |
| | Non amplification | 4.52 | | 2.46 | | 13.17 | | 54.51 | |
| PB Blast (%) | Amplification | 5.25 | 0.371 | 3.50 | 0.098 | 13.67 | 0.620 | 44.54 | 0.466 |
| | Non amplification | 4.27 | | 1.78 | | 12.80 | | 49.53 | |
| LDH (IU/L) | Amplification | 2393.75 | 0.965 | 2241.24 | 0.358 | 3347.50 | 0.313 | 3520.89 | 0.955 |
| | Non amplification | 2477.31 | | 2464.62 | | 2582.52 | | 3480.14 | |

Hb-Hemoglobin, WBC-White Blood Cell Count, PLC-Platelet Count, BM-Bone Marrow, PB- Peripheral Blood, LDH- Lactate dehydrogenase. Student's t-test and Mann Whitney U test was used to find the difference in the distribution of covariates such as Hb (gm%), TC (cmm), Platelet (cmm), LDH (IU/L), PB Blast (%) and BM blast (%) with *BCR-ABL1* amplified FISH signal pattern. A *P* value < 0.05 was taken as statistically significant.

IM. This finding was also supported by Morel et al. (2003) and they reported that, *BCR-ABL1* gene amplification was not only associated with disease progression but also resulted in resistance to IM therapy. In addition, consistent with our results it was reported that CML BC patients who relapsed after initial response to IM therapy displayed multiple copies of *BCR-ABL1* fusion gene by FISH analysis (Gorre et al., 2001). It was also reported that expression level of *BCR-ABL1*started to increase in CML-AP and its constitutive tyrosine kinase activity created major epigenetic changes responsible for disease progression in CML (Melo and Barnes, 2007). Furthermore, the accumulation of additional non-random cytogenetic aberrancies in the advanced phases of disease and in IM resistant CP of CML in our study is considered to be the reflection of genetic instability caused by the hyper activity of *BCR-ABL1* that characterizes disease evolution in CML. In agreement with previous reports, current study highlighted that additional cytogenetic abnormalities in AP, BC and IM resistant CP were allied with decreased cytogenetic response to IM, increased risk of hematological relapse and subsequent reduction in OS (Cortes et al., 2003; O'Dwyer et al., 2004).

According to Virgili and Nacheva (2010), the Ph chromosome does not possess a stable structure and was prone to further rearrangements during disease progression which might be one of the reasons for the intrachromosomal amplification of *BCR-ABL1* in the form of ider (22). Moreover, these isoderivative chromosomes could be heterogeneous in copy number, which led to amplification and duplication of the hybrid *BCR-ABL1* genes in the ider (22) chromosomes (Szych et al., 2007). It is well known that, gene amplification and genomic heterogeneity were associated with the drug resistance (Campbell et al., 2002; Hochhaus and Hughes, 2004). This amplification of genes activated in the Ph chromosome led to a growth advantage of leukemic cells, which in turn, resulted in an adverse clinical evolution with poor prognosis (Becher et al., 1984). The problem (gene amplification and genomic instability) may be overcome by administration with high dose of IM to patients who develop this subcategory of IM resistance. However, if IM is implicated in the etiology of the chromosomal breakpoints, inducing non-random breakpoints at the sub-telomere or telomere region of the Ph chromosomes, alternative therapies should be explored further. Elucidation of these specific disease mechanisms might help for additional therapies while co-administered with IM [Szych et al., 2007].

Moreover, expression studies revealed that, the virulent activity of *BCR-ABL1* drastically disturbed the overall CML transcriptome level (Yong and Melo, 2009), thereby altered the expression level of several genes and some of which might have a vital role in blastic transformation of CML (Oehler et al., 2009; Terragna et al., 2008). Furthermore, the pleiotropic effects of high tyrosine kinase activity of BCR-ABL1 end resulted in continuous activation of factors with marked mitogenic, anti-apoptotic, and anti-differentiation activity and blockade of main cellular processes like those regulated by the tumor suppressors p53, CCAAT/enhancer binding protein-α (C/EBPα), and protein phosphatase 2-α (PP2A) (Jamieson, 2008; Melo and Barnes, 2007). Thus, it could be inferred that, the constitutive activity of BCR-ABL1 might promote genetic, epigenetic and molecular alterations, thereby induce disease evolution in CML. This might happen at the leukemic stem cell (LSC) level or leukemic progenitor stem cell (LPC) level, which might associated with innate or acquired drug resistance (Jiang et al., 2007; Brazma et al., 2007).

In our study, we observed that IM resistant CP patients with *BCR-ABL1* amplification showed significant higher BM blast level ($P < 0.0001$) and WBC count ($P = 0.003$) compared to patients without *BCR-ABL1* amplification. In addition, PB blast level was also found to be high in IM resistant CP cases with *BCR-ABL1* fusion gene amplification, but only a marginal significance was obtained ($P = 0.098$). Similar to our findings, a recent study evidenced that, a positive correlation was found to exist between *BCR-ABL1* level and patient's WBC count in the blood and also reported that, the *BCR-ABL1* tumor load decreased to a minimal level, then the WBC count was also found to get normalized (Ting et al., 2017). However, the data regarding the correlation of *BCR-ABL1* level with BM or PB blast level was very limited and needs further investigation. Apart from this, no other clinical parameters were found to be significantly correlated with *BCR-ABL1* amplification status in any of the study group analyzed.

In conclusion, the present study strongly suggests *BCR-ABL1* fusion gene amplification portrayed a significant role in the disease progression and the emergence of drug resistance clone in CML. Therefore, establishment of *BCR-ABL1* fusion gene amplification FISH signal pattern in CML is of paramount important as it was associated with adverse clinical prognostic implications in advanced stage of the disease. Large cohort studies should be carried out prospectively regarding the pathogenic involvement of *BCR-ABL1* fusion gene amplification and its prognostic significance of amplification status will aid in the effective management of CML patients especially those cases with AP/BC. Undoubtedly, early identification of *BCR-ABL1* fusion gene amplification using FISH technique will definitely lead to improved interventions and develop curative strategies for CML patients.

## Disclosures

No conflict of interest to declare.

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