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Response to Imatinib Mesylate in Patients with Early Chronic Phase Chronic Myeloid Leukemia and Derivative Chromosome 9 Deletion or Clonal Evolution

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Abstract

Objectives: The significance of clonal evolution and derivative chromosome 9 in Philadelphia-positive CML is not fully characterized and studies have yielded conflicting results. After working on emergence of clonal evolution from our region, we continued to find out the response of Imatinib Mesylate on such cases of CML treated in our center.

Materials and methods: We conducted a cross sectional, prospective analysis on response of Imatinib Mesylate on patients with Philadelphia positive chronic myeloid leukemia with clonal evolution treated from period of September 2007 till 2010. Patients were grouped on basis of cytogenetic analysis performed by conventional cytogenetic and fluorescence in situ hybridization (FISH) techniques and followed for three years to see the response rate of imatinib mesylate.

Results: We reported here the response rate in one hundred and two previously untreated cases of chronic myeloid leukemia (Philadelphia positive). Twelve patients (11.7%) exhibit derivative chromosome 9, three had trisomy 8, one with addition 15 and one had deletion 16. At follow-up of 30 months 78 cases were evaluable and 45% and 61% showed complete and major cytogenetic response respectively. There is no significant association of derivative chromosome 9 with the response of imatinib mesylate in our group.

Conclusion: Imatinib mesylate is the first line therapy in chronic phase of CML but the role in patients with clonal evolution need to be established by larger group of patients.

Keywords: CML; Cytogenetics; Imatinib mesylate

Introduction

Chronic Myeloid Leukemia (CML) is a clonal hematologic malignancy that arises in the stem cell compartment [1-3]. Its molecular hallmark is the BCR-ABL fusion gene, [4,5] which usually occurs as the result of the Philadelphia (Ph) translocation involving the long arms of chromosomes 9 and 22 [6].

The development of Fluorescence In Situ Hybridization (FISH) techniques has allowed identification of unexpected deletions of the reciprocal translocation product, the derivative chromosome 9, in 10% to 15% of patients with CML [4,5]. These deletions are large, and occur at the same time as the Ph translocation. Such deletions therefore give rise to unsuspected molecular heterogeneity from the very beginning of this disease. Several studies have demonstrated that CML patients who carry derivative chromosome 9 deletions exhibit a more rapid progression to blast crisis and a shorter survival [7]. Deletion status is independent and more powerful than the Sokal and Hasford/European prognostic scoring systems [7].

On the other hand, the emergence of non-random chromosomal abnormalities or aberrations in addition to the Ph chromosome is a well-recognized occurrence in CML and is referred to Clonal Evolution (CE). CE may be a marker of disease progression in CML and is thought to reflect the genetic instability of the highly proliferative CML progenitors.

Regardless of the underlying mechanisms, the net result of clonal evolution is the potential for a more malignant phenotype and, possibly, less dependence on BCR-ABL for proliferation and survival [8,9]. Given its association with disease progression, CE is considered a feature that defines accelerated-phase CML. Clonal evolution that seen frequently in CML include extra Ph chromosome, trisomy 8, trisomy 19, and isochromosome 17q (with loss of p53) or 20q deletion [10,11]. Among them, isochromosome 17q abnormality carries the worst prognosis [7]. Trisomy 8 is commonly occurring abnormality; in 25% of CML cases with clonal evolution has significance in blast transformation of disease [12].

Imatinib mesylate, STI571, an oral, specific inhibitor of the BCR-ABL tyrosine kinase, was well tolerated and had substantial activity against CML [12] but the role in cases with clonal evolution needs to be established.

This study will be helpful to determine the prevalence and prognostic significance of clonal evolution abnormalities in our cohort of patients. The detection of these chromosomal abnormalities is important; as on this basis we would be able to stratify the patients into good or bad prognostic groups and will be able to offer them suitable treatment option in our setting.

The aims and objectives of our study were to observe the occurrence of non-random chromosomal abnormalities, clonal evolution; other than or in addition to Philadelphia chromosome 9, chromosome 22 (q34; q11) in Chronic Myeloid Leukemia (CML) at time of presentation by using conventional cytogenetic and FISH (Fluorescence In Situ Hybridization) and to identify new cytogenetic aberration or clonal evolution in patients receiving treatment.

In addition; we tried to correlate the prognostic significance of these clonal evolutions and their association or impact on response of imatinib mesylate treatment.

Materials and Methods

This was a prospective cross sectional analysis extending from September 2007 till September 2010. The study population was comprised of all patients with Ph and/or BCR/ABL positive CML (chronic phase according to WHO criteria [12], of all age groups and both sex treated with imatinib mesylate at Aga Khan University Hospital (supported by MAX foundation) after obtaining informed consent.

The MAX foundation is a USA based NGO who provides Imatinib mesylate to needy patients in developing countries. Aga Khan University Hospital is registered with Max foundation and in this study all of our patients were supported by this foundation.

The patients who had Philadelphia chromosome negative, or BCR/ABL negative myeloproliferative disorder or who were diagnosed as in accelerated or blast phase of the disease were not included in this study.

The diagnosis of CML was based on characteristic peripheral blood smear and bone marrow examination findings after reviewing the slides and was confirmed by the presence of Philadelphia chromosome on bone marrow cytogenetic analysis by conventional cytogenetic or detection of BCR/ABL translocation by Fluorescence In Situ Hybridization (FISH). Patients were grouped on basis of clonal evolution identified and were followed for three years to assess the response of imatinib mesylate.

Fluorescence In-situ Hybridization (FISH), this technique allows the visualization of specific nucleic acid sequence within a cellular preparation. FISH involves the precise annealing of single stranded fluorescent labeled DNA probe to complementary target sequences. The hybridization of probes (Vysis, Abott Laboratories) with the cellular DNA site is visible by direct detection by using fluorescence microscopy.

In this study, FISH analysis was performed in all of our patients for detection of trisomy 8 and 19, Iso-chromosome 17 abnormalities with loss of p53 gene and 20q deletions at time of diagnosis and follow-up. Cases were also screened for derivative chromosome 9 only once at time of diagnosis by using FISH.

The laboratory analyses were performed in Department of Pathology & Microbiology; Section of Hematology and Section of Molecular Pathology of the Aga Khan University Hospital, Karachi, Pakistan.

The response of imatinib mesylate was analyzed. All patients had bone marrow cytogenetic and FISH analysis at time of diagnosis and afterwards at six months of starting Imatinib mesylate therapy. If they achieved complete cytogenetic response then FISH analysis of described probes was performed on yearly basis till end of study. If the patients were in no or partial cytogenetic response group then both cytogenetic study and FISH analysis were done every 6 monthly till they achieve complete cytogenetic response.

Response of Imatinib mesylate was assessed according to standard defined criteria i.e. Complete Hematological Response (CHR) is defined as normalization of the bone marrow (blast cells less than or equal to 5%) for at least four weeks and the peripheral leucocytes count <10×109/L and platelets <450×109/L, without peripheral blasts, promyelocytes and myelocytes), in addition to the disappearance of all signs and symptoms of CML [13,14].

Cytogenetic response was based on the proportion of the Ph-positive metaphases among at least 20 metaphases, and defined as complete cytogenetic response; CCR (0% Ph-positive metaphases), partial cytogenetic response; PCR (Ph-positive 1-35%) [14], and rest of the other responses were merged in a single category; no cytogenetic response; NCR (>35% Ph positive metaphases).

Major cytogenetic response characterized as combination of both complete and partial cytogenetic responses (CCR+PCR). Clonal Evolution (CE); defined as the appearance of additional chromosomal aberration in at least two metaphases.

Statistical Analysis

All statistical analysis was computed with SPSS statistical software (version 18.0.1). Data is presented as mean or median values; and percentages. The prognostic value of various clonal evolutions on the achievement of hematological and cytogenetic response by imatinib mesylate was evaluated by the chi-square test.

Results

A total of 102 previously untreated patients were registered over a period of 36 months. The median age of presentation was 35 years (range, 15-65 years) among these 75 males and 27 were females (M:F is 2.7:1).

Median follow-up was 26 months (range, 3-36 months).

Derivative 9q34 was the commonest chromosomal aberration in this group; found in twelve cases by FISH at time of diagnosis. Other detected chromosomal abnormalities were, trisomy 8 in three patients, one patient had deletion 16 and one with addition 15. No other tested probes identified any other chromosomal aberrations.

Cases with derivative 9q34, the median age is 35 years. Among this group eleven were males and one was female.

Response Rate

At the end of study, out of one hundred and two registered cases only 78 completed at least six months of treatment and are evaluable for cytogenetic response.

Overall response rate of imatinib mesylate in chronic phase is shown in Table1.

	6 months	12 months	24 months	30 months
Response	N=60	N=78	N=78	N=78
Complete hematologic response	57	75	75	75
	-95%	-96%	-96%	-96%
Cytogenetic Response				
Complete cytogenetic response	31	29	28	35
	-52%	-37%	-36%	-45%
Major cytogenetic response (CCR +PCR)	41	52	53	48
	-68%	-67%	-68%	-61%
No cytogenetic response (NCR)	19	26	25	30
	-32%	-33%	-32%	-39%

Table 1: Response rate of imatinib mesylate in chronic phase of chronic myeloid leukemia

In first six months of study period, 60 cases were registered and cytogenetic analysis revealed major cytogenetic response in 41 cases (31 patients achieved complete cytogenetic response) and 19 showed no cytogenetic response.

At the completion of 36 months, out of 78 patients; 35 had complete cytogenetic response, (48 achieved major cytogenetic response) and 18 had no cytogenetic response.

Twelve cases that had der 9q34 in addition to t (9; 22) at time of presentation; only two of them achieved complete cytogenetic response and 4 had no cytogenetic response and rests were in evaluable due to loss to follow-up in subsequent visits. There was no difference in cytogenetic response among patients with der9 chromosome and clonal evolution.

Three cases with trisomy 8, one with deletion 16 and addition 15 failed to achieve cytogenetic response with imatinib mesylate. In our study, no patients acquire additional chromosomal aberration on treatment.

Discussion

In the chronic myeloid leukemia, acquisition of chromosomal aberrations (clonal evolution) is a well-known phenomenon and appears to interfere the response of imatinib mesylate, the tyrosine kinase inhibitor which has changed the current approach to the management of chronic myeloid leukemia. Deletion around the breakpoints on derivative chromosome 9 including 5'ABL and 3' BCR sequences occur in 10-15% of Ph-positive chronic myeloid leukemia patients and are thought to have prognostic significance [14].

We examined the outcome of newly diagnosed cases of chronic myeloid leukemia treated with imatinib mesylate and in addition, identify the chromosomal aberration that is present at diagnosis or occur subsequently and can affect the response of the therapy.

In this study, derivative chromosome 9 was seen in twelve cases (11.7%), which is similar to the previous reports [14]. Trisomy 8 was identified in only three cases (2.9%) which are on contrary to prior reports [15,16], including the one we reported earlier on patients with CML treated with interferon prior to starting imatinib mesylate [17]. No other significant chromosomal aberrations were identified in our group of patients either at time of enrollment in the study or on follow-up cytogenetic analysis. This fact might be explained by that in our study all cases were newly diagnosed CML, never received interferon or any other drugs, as occurrence of clonal evolution in interferon treated patients is a well-known phenomenon [18]. However, small subset of patients in this study and limited number of probes for karyotypic analysis by FISH cannot be ignored.

In this series we reported major cytogenetic response rate in 68% cases at 6 months follow-up and in 61% patients at completion of study period. The fall in the response rate is due to loss of follow-up of some cases which seen often in our setting. The rates of cytogenetic responses in this study are in concordance to other reports [19,20], and in fact more encouraging than our earlier publication [21], this might be because in this group all cases are newly diagnosed CML without any prior treatment and were in low risk group in contrast to our previous report in which substantial number of cases were in intermediate and high risk category according to Sokal prognostic score [21].

Only two cases with derivative chromosome 9 achieved complete cytogenetic response on long term follow-up, four were non-responder and six were lost to follow-up. Similar to other reports [22,23] it remain controversial whether patients with derivative chromosome 9 have a different outcome if treated with imatinib mesylate. Thus, derivative chromosome 9 was not yet an independent significant factor for achieving cytogenetic response in our group of patients as well [24,25].

Conclusion

Imatinib mesylate is effective first line therapy of chronic phase of chronic myeloid leukemia especially in absence of clonal evolution. Derivative chromosome 9 showed no prognostic significance in our group; however, long term follow-up in larger cohorts of patients with focus on specific chromosomal aberration including derivative chromosome 9 are required in assessing the uncertain importance of clonal evolution.

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