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CYTOGENETIC RESPONSE OF IMATINIB MESYLATE IN NEPALESE CHRONIC MYELOID LEUKEMIA PATIENTS IN CHRONIC PHASE

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ABSTRACT

Background: The cause of chronic myeloid leukemia (CML) is the Philadelphia (Ph) chromosome formed by a reciprocal translocation between chromosomes 9 and 22 giving rise to a constitutively active BCR-ABL tyrosine kinase. Imatinib Mesylate inhibits this kinase in newly diagnosed chronic phase chronic myeloid leukemia. The aim of this study was to evaluate the cytogenetic response of Imatinib Mesylate in Nepalese chronic myeloid leukemia patients in chronic phase.

Methods: Fifty clinically diagnosed and hematologically proven chronic myeloid leukemia patients in chronic phase were selected for cytogenetic analysis at the time of diagnosis. Philadelphia chromosome positive patients received Imatinib Mesylate 400 mg orally daily. The follow up cytogenetic analysis was done to monitor the size and progression of the Philadelphia positive clone.

Results: After median follow up of 13 months of Imatinib therapy 3 patients (10%) had complete (0% Philadelphia positive metaphases), 18 patients (60%) had partial (1% to 35% Philadelphia positive metaphases), and 7 patients (23%) had minor (36% to 65% Philadelphia positive metaphases) cytogenetic responses while 2 patients (7%) showed no cytogenetic response (more than 95% Philadelphia positive metaphases at all points and increase in Philadelphia positive metaphases).

Conclusion: Major cytogenetic response of Imatinib Mesylate was found in 70% of chronic myeloid leukemia patients in chronic phase; complete cytogenetic response only in 10%. The important causes for these suboptimal results of Imatinib therapy would be unavailability of patients at regular follow up due to financial constraints, unavailability of the diagnostic facility in the country and drug dose adjustment.

Keywords: Chronic Myeloid Leukemia, Philadelphia Chromosome, Imatinib mesylate.

1. INTRODUCTION

Chronic Myeloid Leukemia (CML) is a malignant haematopoietic stem cell disorder which accounts for 15-20% of all leukemia cases.[1,2] CML is characterized by the Philadelphia (Ph) chromosome which is a shortened chromosome 22 formed by the balanced reciprocal translocation between chromosomes 9 and 22 [t(9;22)].[3,4] This translocation gives rise to a chimeric BCR-ABL fusion gene which plays a pivotal role in the pathogenesis of CML.[5] CML has three distinct clinical phases: chronic phase (CP), accelerated phase (AP) and blast crisis phase (BP).[6]

The treatment of CML with busulfan and hydroxyurea lead to hematological responses but did not provide survival advantage, so the disease progressed to advanced phases.[7,8] Interferon-alpha produced cytogenetic responses in 20-60 percent of

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patients.[9,10,11] CML is also curable with allogenic stem cell transplantation, but less than 30 percent of patients have suitably matched donors.[1,4,6,8]

Imatinib mesylate (IM) (Glivec®, Gleevec™, formerly called STI571) is a tyrosine kinase inhibitor used in targeted treatment of CML.[12] In preclinical studies, imatinib showed 92-98% decrease in the number of BCR-ABL positive colony formation. This observation suggested the potential utility of Imatinib in the treatment of BCR-ABL positive leukemias.[13] Therefore in the present study we carried out chromosomal analysis of Nepalese CML-CP patients at the time of diagnosis and at follow up patients under Imatinib therapy to evaluate the cytogenetic response of IM.

2. MATERIALS AND METHODS

2.1 Patients

This was a laboratory based intervention study conducted at B. P. Koirala Institute of Health Sciences, Dharan, Nepal during a period of two years. Fifty clinically diagnosed and hematologically proven CML-CP patients were selected randomly from those attending the Laboratory for cytogenetic analysis of bone marrow samples. Informed consent was taken from the participating patients.

2.2 Methods

Cytogenetic analysis was performed by the conventional cytogenetic method. Bone marrow aspirates were examined on direct or short term (24-hour) cultures using Rosevill Park Medical Institute-1640 (RPMI-1640) media, fetal calf serum and Phytohemagglutinin. Metaphase cell were arrested by using colcimid, harvested by potassium chloride solution and fixed with fixative (methanol and acetic acid in 3:1). The slides were prepared and stained with 5% Giemsa. The preparations were screened under Zeiss light microscope and at least 20 well spread metaphases were analysed. Analysis was limited to less than 20 metaphases in a very few samples due to inadequacy of good dividing cells. Karyotype was analysed from the photographs of metaphase spreads. The homologous pairs were arranged according to International System for Human Cytogenetic Nomenclature 1995 on a predesigned format (ISCN 1995).[14]

All the Ph+ve CML-CP patients received IM 400 mg orally daily. Cytogenetic analysis at follow up was done. Cytogenetic responses (CRs) were determined by the percentage of metaphase cells that were negative for the Ph chromosome (Table 1).

Table 1: Cytogenetic responses [15]

Cytogenetic Response Criteria	Philadelphia chromosome percentage
Major cytogenetic response	<35%
Complete cytogenetic response	0%
Partial cytogenetic response	1% to 35%
Minor cytogenetic response	36% to 65%
No cytogenetic response	>95%

3. RESULTS

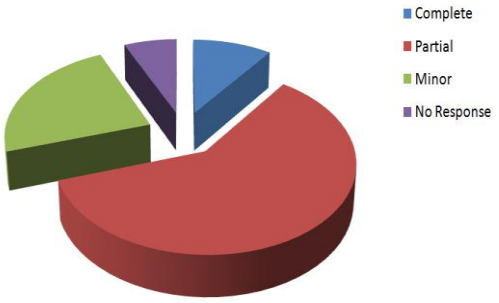
The present study contained 36 (72%) male and 14 (28%) female CML-CP patients. The median age of the patients was 39.5 yrs. Forty eight (96%) patients were Ph positive and 2 (4%) were Ph negative at the time of diagnosis. (Table 2)

Table 2. Patients' demographics and cytogenetic findings

Age (years)	No. of Patients		Total	Cytogenetic findings	
	Male	Female		Ph+ve	Ph-ve
Less than 10	0	0	0 (0%)	0	0
10 – 20	5	1	6 (12%)	6 (12%)	0
20– 30	9	6	15 (30%)	13 (26%)	2 (4%)
30– 40	8	3	11 (22%)	11 (22%)	0
40– 50	7	2	9 (18%)	9 (18%)	0
50 – 60	3	0	3 (6%)	3 (6%)	0
60 – 70	4	2	6 (12%)	6 (12%)	0
Total	**36 (72%)**	**14 (28%)**	**50 (100%)**	**48 (96%)**	**2 (4%)**

Out of 48 Ph positive CML-CP patients who were under Imatinib therapy, 30 patients were available at follow up for cytogenetic analysis of bone marrow samples to monitor the cytogenetic effect of IM. CRs in this study of the CML-CP patients were extremely variable. Three patients (10%) had CCR within 11 to 19 months of starting IM. Eighteen additional patients (60%) had partial CRs. Seven patients (23%) showed minor CRs. Two patients (7%) showed no CR. (Table 3, Fig. 1)

Fig. 1. Bar diagram showing cytogenetic response.



Although the numbers are small and the findings are only preliminary, some differences were noted among the patients groups showing different CRs. Patients showing CCR to IM tend to be younger (mean, 28.33 years; range, 22-34 years) than patients showing no CR (mean, 43.5 years; range, 25-62 years). (Table 3)

4. DISCUSSION

IM is a 2-phenylaminopyridine derivative and a BCR-ABL tyrosine kinase signal transduction inhibitor 571 (STI-571). It acts as an inhibitor of the ATP binding site on the protein and prevents its phosphorylation and thus its activity.[12] With IM, a MCR can be achieved in more than 80 percent of the patients who are treated in the early chronic phase.[16]

In the present study, 48 CML patients who were Ph+ received Imatinib 400 mg daily in an early chronic phase. After a median follow up of 13 months, 30 patients were available for cytogenetic analysis of bone marrow samples for evaluation of Imatinib therapy. Out of 30 patients, 21 (70%) patients had MCR; partial response in 18 (60%) and complete response in 3 (10%) patients. 7 (23%) patients had minor CR. In a study by Kantarjian et al in 2003,[17] 90% patients achieved a MCR, complete in 72% patients at a dose of 400 mg daily and after a median follow up of 9 months. Similarly, Rajappa et al in 2008[18] had shown CCR in 56%, partial response in 23%, minor response in 17% and no response in 4% patients after a median follow up of 29.5 months.

Table 3. Cytogenetic analysis

S.N.	Age/ Sex	Ph% metaphases at diagnosis	Ph% metapha-ses at follow up	Duration observed (months)
1.	25M	10	20	20
2.	17M	85	65	6
3.	62M	80	95	6
4.	40M	85	20	7
5.	60M	100	25	10
6.	58M	70	10	12
7.	21F	100	40	10
8.	39M	100	30	20
9.	16F	100	45	8
10.	23M	100	30	20
11.	27F	100	50	13
12.	21M	80	20	11
13.	29F	80	Ph-	11
14.	27M	95	50	21
15.	22F	90	Ph-	18
16.	47M	95	60	10
17.	60F	40	20	7
18.	36M	80	30	22
19.	23M	25	10	12
21.	42M	70	50	13
23.	28F	85	30	6
28.	34M	90	20	12
29.	32M	80	10	20
33.	32M	90	30	9
36.	34M	80	Ph-	19
38.	20M	70	20	9
39.	28M	90	10	21
48.	35M	100	30	12
49.	47M	80	30	20
50.	14M	80	30	7

This study showed remarkable difference in MCR mainly in CCR which may be due to either small sample size or short duration of Imatinib therapy or low dose of Imatinib (400 mg daily). Recent data suggest that higher dose of 800 mg daily improves overall responses as well as rapidity of response. Researchers from MD Anderson Cancer Center have reported that increasing the dose of Gleevec to 400

mg twice daily improves the response rate as measured by cytogenetics analysis and PCR in patients with CML in chronic phase.[19] Therefore, higher dose of Imatinib would have done better responses in the patients. Low cytogenetic responses shown in this study may also be due to unavailability of patients at regular follow up of 6 months of interval or less due to financial constraints. Patients with partial or absent CRs may be at risk for clonal evolution, which occurs late in IM therapy. So, optimal monitoring of patients with partial or absent CR will require periodic metaphase cytogenetic analysis.

While the majority of CML patients had an excellent response to Imatinib treatment, there are still a proportion of patients who did not achieve optimal or, any kind of response, which implies to treatment resistance. Resistance is more common in patients with long disease history and advanced phase disease, as initial responses are usually poorer and the achieved responses are prone to be only transient in advanced phases of CML.[20] Our study showed Imatinib resistance in 2 patients (7%) after a median follow up of 13 months. Study conducted by Rajappa, et al in 2008[18] also shows no imatinib response in 4% patients after a median follow up of 29.5 months.

Our study shows suboptimal cytogenetic response of IM therapy in Nepalese CML patients in chronic phase. Some of the important causes behind these suboptimal results would be the financial constraints of the patients, unavailability of the diagnostic facility in the country and lack of drug dose adjustment. Eventhough IM has become the first-line treatment for Nepalese CML patients.

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