RESEARCH COMMUNICATION

Cytogenetic Investigation in Chronic Myeloid Leukemia: Study from an Indian Population

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Abstract

Chronic myeloid leukemia (CML) is a malignant neoplasm of hematopoietic cells characterized by abnormal proliferation of myeloid precursors, decreased rates of self destruction and an arrest in cellular differentiation. The bone marrow and peripheral blood accumulates all forms of mature and immature granulocytes, primarily blast cells. It is the most common type of leukemia seen in India, accounting for 30% of all leukemias. Cytogenetic analysis plays a vital and important role in the diagnosis of CML patients. The present study consists of cytogenetic evaluation of 175 CML cases from the Indian population with ages ranging from 6 – 86 years (mean of 42.8). The study population included 115 males (65.72%) and 60 females (34.28%) with a Male: Female ratio 1.9:1. Out of the175 cases, 164 (93.7%) were successfully karyotyped while culture failure was observed for 11 (6.3%). Among the 164 reported cases, 53 (32.3%) showed a normal karyotype while within the 111 (67.7%) abnormal cases, 96 cases (86.5%) showed the presence of Philadelphia (Ph') chromosome with standard translocation t(9;22); Ph'+ve along with secondary aberrations was detected in 9 (8.1%) cases. Variants of Ph' chromosome were detected in only one case (0.9%). Ph'-ve CML with other chromosomal aberrations were detected in 5 (4.5%) cases, including +8, del 20q, del 11q and marker chromosome. Furthermore, we believe that availability of more advanced molecular techniques can be used as a supportive tool in CML diagnosis even though it cannot fully replace cytogenetics, which remains the backbone for laboratory investigation of the disease.

Key Words: Chronic myeloid leukemia - cytogenetics - Philadelphia chromosome - India

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Introduction

Chronic myeloid leukemia (CML) is a hematopoietic stem cell disorder that comprise a heterogeneous group of acquired clonal neoplastic disorders. It is usually characterized by morphological abnormalities with evidence of leukocytosis and accumulation of all forms of mature and immature granulocytes. CML accounts for about 7-15% of all leukemias in adults with approximately 1-1.5 cases per 100,000 population worldwide (Morrison, 1994), while in India it is the commonest type of leukemia accounting for 30% of all leukemia cases.

The cytogenetic hallmark of CML is Philadelphia (Ph') chromosome, which is found in around 91-96% of CML cases, results from a balanced reciprocal translocation between long arm of chromosome 9 and 22, t(9;22) (q34;q11) (Rowley, 1973). At the molecular level, breaks occur in the ABL and BCR genes on chromosome 9 and 22 respectively, giving a hybrid BCR-ABL gene which finally gets transcribed and translated into a fusion protein of varying size- p210, p-190 (Saglio et al., 2004). The BCR-ABL p210 protein is known to augment tyrosine kinase activity and is also thought to be important in the pathogenesis of CML (Brusa et al., 2006).

Although the Ph' chromosome is the sole genetic abnormality in most of the CML cases, variant Ph' involving a third or rarely fourth chromosomes have been observed previously (Kadam et al., 1990), however as the disease progresses from chronic phase to blast phase, additional genetic abnormalities are also found in about 80% cases. (Jobanputra et al., 1999).

Most of the work regarding cytogenetic profile in CML and their significance in terms of diagnosis and prognosis has been reported from countries other than India (Mitelman, 1993; Chase et al., 2001; Johansson et al., 2002), while cytogenetic abnormalities in CML patients from Asia are limited (Kadam et al., 1991; El-Assaad et al., 1998; Koo et al., 1998; Jacob et al., 2002). Thus, the present study was aimed to detect various chromosomal aberrations in Indian population using conventional cytogenetic analysis and to find their prevalence as well as frequency within the Indian scenario.

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Table 1. Age and Sex Distribution of the 111 CML Patients Showing Different Chromosomal Anomalies

CML Type	No. of Patients (%)	Age in Years *				Sex*	
		0-25 (%)	26-50 (%)	51-75 (%)	>75 (%)	Male (%)	Female (%)
Ph' +ve	96 (86.5)	11 (11.5)	63 (65.6)	21 (21.9)	1 (1.0)	61 (63.5)	35 (36.5)
Ph' +ve with Secondary Aberrations	9 (8.1)	--	6 (66.7)	3 (33.3)	--	6 (66.7)	3 (33.3)
Ph'–ve with Other Aberrations	5 (4.1)	--	3 (60.0)	1 (20.0)	1 (20.0)	2 (40.0)	3 (60)
Variant Ph'	1 (0.9)	--	1 (100)	--	--	--	1 (100)
Total	111	11 (9.9)	73 (65.8)	25 (22.5)	2 (1.8)	69 (62.2)	42 (37.8)

*Percentages calculated on the number of patients with the various types of CML

Table 2. Cytogenetic Findings for the 164 CML Cases

Karyotype	No. of Cases	Frequency %
Normal	53	32.31[a]
Abnormal	111	67.69[a]
t(9;22)(q34;q12)	96	86.49[b]
t(9;22), +8	3	2.70[b]
t(9;22), i(17q)	1	0.90[b]
2 x t(9;22)	2	1.80[b]
t(9;22), +Y	1	0.90[b]
t(9;22), del 11q	1	0.90[b]
t(9;22), del Xq	1	0.90[b]
t(9;11;22) (q34;q13;q11)	1	0.90[b]
+8 (Sole)	2	1.80[b]
del 20q	1	0.90[b]
del 11q	1	0.90[b]
Marker	1	0.90[b]

[a,b]Percentages calculated from the totals of 164 reported and 111 abnormal reported cases, respectively

Patients and Methods

The present study was conducted at Cytogenetic Department of SRL Ranbaxy (Clinical Reference Laboratory), Mumbai. A total of 175 cases diagnosed with CML were investigated for cytogenetic analysis from different hospitals and laboratories in India during the period September 2003 – March 2006.

Cytogenetic analysis was performed using heparinzed bone marrow or whole blood cells for 0 hr, 3 hrs, 24 hrs and 48 hrs as per standard protocol with some modification (Henagariu et al., 2001). Briefly, the samples was first washed in RPMI-1640 media prior to setting the culture and about 1.0 ml of washed bone marrow was inoculated in 7.5 ml of RPMI media supplemented with 20% FBS. The culture was incubated for respective time interval in 5% CO2 incubator, the cells were harvested: hypotonic solutions treatment followed by chilled fixative treatment to fix the cells for study. Fixed cells were dropped on slides, stained for Giemsa-Trypsin-Giemsa (GTG) banding at about 400-band level according to ISCN (1995). Atleast 20 metaphase plates were analyzed from each sample and 3 to 4 well spread plates were photographed and karyotyped using Ikaros Metasystem Software (Gmbh, Germany).

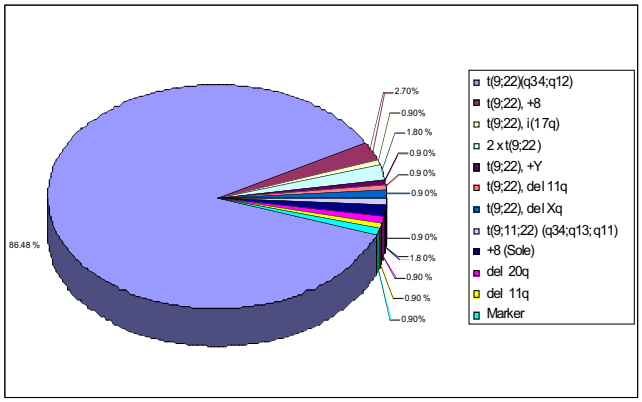

Figure 1. Overall Distribution of Various Cytogenetic Findings in 164 CML Cases

Results

In this study, we describe chromosomal alterations in CML by conventional cytogenetic analysis. The study population included 175 hematologically confirmed CML cases, of which 115 were males (65.72%) and 60 were females (34.28%) with Male: Female ratio 1.9:1. Among 175 cases, 164 (93.71%) were successfully karyotyped while in 11 (6.28%) cases karyotyping could not be obtained because of inadequate metaphases and culture failure. Of the 164 reported cases, 53 (32.31%) cases showed normal karyotype while within 111(67.69%) abnormal cases, 96 (86.49%) showed Ph' chromosome.; Ph'+ve along with secondary aberrations was detected in 9 (8.1%) cases. Variants of Ph' chromosome was detected in only one case (0.9%). Ph'-ve CML with other chromosomal aberrations were detected in 5 (4.5%) cases which includes +8, del 20q, del 11q and marker chromosome.The frequency of age and sex distribution as well as the various cytogenetic findings are depicted in Tables 1 and 2, respectively. Figure 1 shows the distribution pattern for the various aberrations observed in the study.

Discussion

Cytogenetic analysis plays a vital role in the diagnosis of CML patients and as a prognostic indicator for monitoring therapy in these patients. Over the last decades the frequency of CML has been increasingly recognized through out the world. The present study is an attempt to detect various cytogenetic abnormalities in CML as well as their incidence in Indian population.

We analyzed 175 CML cases with their age ranging between 6 – 86 years and mean age 42.76 years. The most

consistent cytogenetic abnormality showing translocation between chromosome 9 and 22 yielding Philadelphia chromosome was observed in 96 cases (86.49%). In our study we observed that the incidence of CML with Ph' chromosome was predominant in the age group of 26 – 50 years (65.62%). The frequency of Ph'+ve CML was more preponderant in males (n=61, 63.548%) than females (n=35, 36.46%). The mean age for males with Ph+ve CML was found to be approximately 41.6 years while that of females was approximately 39.6 years. Identification of patients as Ph'+ve or Ph'-ve have been found to be clinically significant because Ph'+ve CML patients has a better prognosis than did patients who do not have this rearrangement (Kantarjian et al., 1985).

Variants of Ph' chromosome was observed in one case (0.9%) of our study. Variants of Ph' has been reported earlier where apart from chromosome 9 and 22, break occurs on a third, rarely on fourth chromosome which lead to the formation of either standard Ph', a derivative Ph', marked Ph' or derivative 9 (Kadam et al., 1990). In our case we detected, a complex type variant in which there was a physical rearrangement among chromosomes 9, 11 and 22 resulting in the karyotype t (9,11,22) (q34;q13;q11). Similarly, Zaccaria et al (1989) reported variants of Ph' translocation showing t (9; 11; 22) (q34; q13; q11) and concluded that these variants of Ph' translocation may be associated with a typical molecular break points which ultimately leads to leukimogenesis in patients. Nevertheless, Johansson et al (2002) reported that despite of the genetically complex nature of variants of Ph', available data indicates that variant rearrangements do not confer any specific phenotypic or prognostic impact as compared to CML with a standard Ph' Chromosome.

Secondary chromosomal abnormalities in addition to Ph' in CML patient have now been reported in large number of cases (Wang et al., 2004). In our study we observed 9 such cases (8.1%) in which secondary aberrations were found along with Ph' chromosome; these anomalies were +8 (2.7%), i(17q) (0.9%), extra Ph' (1.8%), +Y (0.9%), del 11q (0.9%), del Xq (0.9%). Chromosomes 8, 17 and 22 are by far the ones most often involved in the karyotypic evolution and are indicative of the disease progression. These secondary changes sometimes precede the hematologic and clinical manifestations of malignant disease by several months and thus may serve as valuable prognostic indicators. Loss of Y chromosome as a secondary anomaly has been reported in earlier study (Mitelman, 1993). However, in our study we observed vary rare secondary aberration involving gain of an extra Y, deletion in the long arm of chromosomes 11 and X. This type of karyotype may have an over all unfavorable prognosis. We believe that further unraveling the biology of CML with such type of aberrant karyotype by molecular studies may provide deeper insights into the pathogenesis as well as reason for chromosome resistance in this type of CML.

Although the hallmark of CML is Ph translocation, but there are cases with the absence of Ph' chromosome i.e. Ph'–ve CML that shows other chromosomal aberrations. In our study we detected five such types of cases (4.5%) which were Ph'-ve but had other chromosomal abnormalities such as +8 (2.7%), del 20q (0.9%) and marker chromosome (0.9%). Gain of an extra chromosome 8 is the most common aberration found in Ph'-ve CML (Aurich et al., 1998), while deletion in the long arm of chromosome 20 (del 20q) is the most common recurring abnormality in malignant myeloid disease)Aatola et al., 1992). In our case we observed one such case which was Ph'-ve but showed the loss of chromosomal material from the long arm of chromosome 20. Nevertheless, from prognostic point of view, the presence of del 20q does not appear to adversely affect survival (Alistair et al., 2003). Moreover in order to correctly define the Ph'+ve or Ph'-ve CML, molecular cytogenetic studies like Fluorescent-In-Situ-Hybridization (FISH) must be done to obtain direct visualization of the rearrangement of the BCR-ABL gene.

Further, we believe that several advanced molecular methods are available to detect the BCR-ABL gene fusion; each one having its own advantages and limitations, Conventional Cytogenetic Analysis remains the first choice and backbone for laboratory investigation in cancer research. Its usefulness in initial diagnosis as well as in monitoring the therapy cannot be overlooked. Thus, more ongoing cytogenetic analysis along with molecular cytogenetic will allow better evaluation of the genomic aberrations involved in CML, and will offer a new directions for its further molecular investigation. This will further facilitate our understanding of the neoplastic process more precisely for the better prognostification of the patient.

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