J. Anat. Sciences, 24(2): Dec. 2016, 17-22

Original Article

TRISOMY 8 AS THE COMMONEST ADDITIONAL CHROMOSOMAL ABNORMALITY IN PHILADELPHIA POSITIVE CHRONIC MYELOGENOUS LEUKEMIA

Fatima Bhopalwala Ali*, RK Verma*, Mustafa Ali, Navneet Kumar*,**

*Department of Anatomy, KGMU, Lucknow

**Department of Pathology, Vivekanand Polyclinic & Institute of Medical Sciences, Lucknow

ABSTRACT

Introduction - The aim of our study was to observe the presence of additional abnormalities and their relative frequencies in chronic myelogenous leukemia and correlate their clinical and prognostic importance in CML.

Methods - 66 cases of CML were included in the study. History taking, clinical examination and relevant laboratory test of all the cases were done. Bone marrow and peripheral blood sample were collected and culture of these samples using suitable medium was done. Karyotyping was done manually as well as with the help of cytovision software.

Results - Additional chromosomal abnormalities along with Philadelphia chromosome were observed in 7 cases (13.5%) out of 52 reported karyograms. Trisomy 8 was the most common additional abnormality observed (3 cases, 42.9%). It was seen as an isolated additional abnormality in 2 cases while in 1 case trisomy 8 was present in association with other chromosomal abnormalities. Chronic stage of CML was documented in 2 of the patients showing trisomy 8 while one patient had blastic phase of CML.

Discussion - The commonest additional chromosomal abnormality observed in CML is trisomy of chromosome 8. Trisomy 8 and other abnormalities affecting the 8q24 region are very important, because this includes the gene locus for the c-Myc gene. C-Myc is a key player in cell growth and differentiation and a correlation between high c-Myc expression and CML progression has been reported. Our results have re-emphasized the importance of trisomy 8 and its role in the pathogenesis of CML.

Key words: Chronic myelogenous leukaemia, Trisomy 8, Chromosomal abnormalities, Philadelphia chromosome.

INTRODUCTION

Chronic myelogenous leukemia (CML) is characterized by t(9;22)(q34;q11.2) translocation resulting in BCR-ABL1 fusion gene. [1] Chronic myelogenous leukemia (CML) is also defined at molecular level by BCR-ABL1 fusion gene generated from a translocation between chromosome 9q34 and 22q11.2, forming Philadelphia chromosome (Ph). [2] BCR-ABL1 is the only genetic abnormality in 90% of CML cases in chronic phase. As disease progresses, clonal evolution with additional chromosomal changes (ACAs) emerges. [3]

The chromosomal abnormalities present in CML, other than or along with Philadelphia chromosome are termed as Additional Chromosomal Abnormalities (ACAs). Although Ph chromosome may be the initial event in CML, the acquired additional cytogenetic abnormalities are responsible for progression of disease to more aggressive phase. [4] Additional chromosomal abnormalities occur in most patients in Chronic myelogenous leukemia superimposed over Philadelphia chromosome, especially in their accelerated phase and blast crisis and thus are known to reflect karyotypic evolution of malignant cells in vivo. [5,6] Trisomy 8 (+8) is a common clonal evolution marker for progression in chronic myelogenous leukemia. Trisomy of chromosome 8 is frequently reported in myeloid lineage disorders and also detected in lymphoid neoplasms as well as solid

Address for Correspondence :
Dr. **Fatima Bhopalwala Ali**
JR-III, 12-Orapura, Kamri Marg,
Ujjain, M.P. 456006
E-mail. id dr. fatimaali@ymail.com

tumors suggesting its role in neoplastic progression in general. It is likely to be a disease-modulating secondary event with underlying cryptic aberrations as it has been frequently reported in addition to known abnormalities contributing to clinical heterogeneity and modifying prognosis. [7]

Heim and Mitelman[8] proposed a hypothesis for karyotypic evolution in CML. They analyzed the additional chromosomal abnormalities other than Philadelphia chromosome and suggested that abnormalities such as trisomy 8, +Ph, or i(17q) are the main changes occurring after t(9;22), while trisomy 19 occurs later and in combination with +8 and +Ph. These are termed major route changes or major route of karyotypic evolution which include commonly observed anomalies. Minor route cytogenetic aberrations involve rarely occurring anomalies such as t(3;12), t(4;6) and t(1;21).

The aim of our study was to observe the presence of additional abnormalities and their relative frequencies in chronic myelogenous leukemia and correlate their clinical and prognostic importance in CML.

MATERIAL AND METHODS

Selection criteria and Collection of samples-

The study was conducted after obtaining the approval from the Ethical Committee of the King George's Medical University U.P., Lucknow. Screening of the patients was done in the Department of Clinical Hematology, and samples were collected in the hematology laboratory of the same department and also in the Department of Pathology, King George Medical University U.P., Lucknow. The consent was taken from each participant after explaining the purpose of the study. The diagnosed cases of CML (diagnosis confirmed on the basis of clinical and hematological evaluation) irrespective of age and sex were included in the study. Lack of confirmed diagnosis and/or consent from the patient served as exclusion criteria. Duration of disease and treatment were not included as selection criteria i.e., newly diagnosed cases as well as CML cases already diagnosed and undergoing treatment were included in the study.

Bone marrow aspirate and/or peripheral blood samples of the CML patients were collected. Detailed personal history, occupational history was taken and thorough clinical examination was done at time of sample collection.

Harvesting of sample & Preparation of Karyogram

Bone marrow aspirate and blood sample of the CML patients were collected in BD Vacutainer sodium heparin vial. The sample was taken in a test tube containing culture media (RPMI 1640) and incubated in CO2 incubator in slanting position. After incubation, Colchicine solution was added and test tube was again incubated for one hour and then centrifuged at 1000 rpm for 10 minutes. Supernatant was discarded by pipetting of media leaving as little medium as possible over the cell button at bottom of test tube. Cell button was suspended in hypotonic solution (Potassium chloride + Sodium citrate).

Slides were prepared by dropping method, and were treated with trypsin to obtain better banding. Adequately aged slides were stained with Giemsa stain. Karyotyping results were obtained by analyzing 20 metaphase fields for each case and in cases where abnormal karyotype was suspected, the observation was extended to a total of 30 fields. The karyotypes were reported as per International System for Human Cytogenetic Nomenclature guidelines. (ISCN, 2013) [9]

Statistical analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 15.0. Data has been represented as frequencies and percentages and mean and standard deviation. Chi-square test has been used for the purpose of analysis. The confidence level of the study was kept at 95%, hence a “p” value less than 0.05 indicated a significant association.

OBSERVATIONS

A total of 66 CML patients were enrolled out of which karyogram was obtained in 52/66 cases (78.8%). Age of patients ranged from 20 to 69 years with a mean age of 49.77±11.76 years. Majority of enrolled patients were males (n=36; 54.5%). Male to female ratio of enrolled patients was 1.2:1.

Chronic stage of CML was most common (86.4%) followed by accelerated (7.6%) and blastic (6.1%) phases. All the patients were BCR-ABL positive as

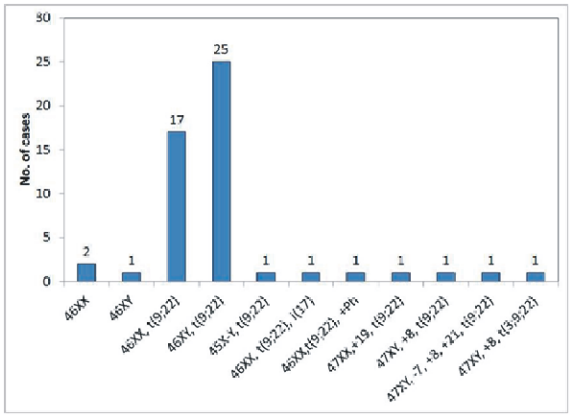

Figure1: Karyogram Profile of patients (n=52)

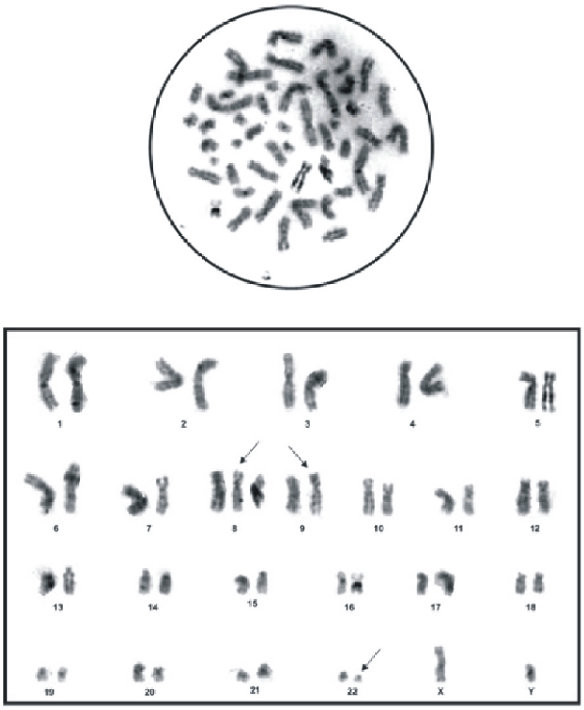

Figure 2: karyogram - 47XY, +8, t(9;22)

noted at the time of sample collection. The cytogenetic analysis revealed abnormal karyogram in 49/52 (94.2%) patients. All of the abnormal karyograms showed the presence of Philadelphia chromosome. Additional chromosomal aberrations were seen in 7/52 cases (13.5%) (Table-1). Trisomy 8 (Fig.2) was the most common additional abnormality observed in 3 out of 7 cases (42.9%) in our study subjects. It was noted as isolated additional abnormality in 2 cases and in combination with other aberrations in one case (Table 2). Thus the unique finding of this study was establishment of trisomy 8 as the most common additional chromosomal abnormality seen in Philadelphia chromosome positive CML cases.

The most common genotype was 46XY, t(9;22) (n=25; 48.08%) Ph+ve male followed by 46XX, t(9;22) (n=17; 32.69%) i.e.; Ph+ve female. Karyogram profile of Ph-ve normal female 46XX (3.85%) was present in 2 patients. There was 1 patient (1.92%) each with profile 46XY, 45X-Y,t(9;22); 46XX,t(9;22),i(17q); 46XX,t(9,22),+Ph; 47XX,+19,t(9;22); 47XY,+8,t(9;22), 47XY,+8,+21,-7,t(9;22) and 47XY,+8,t(9;x,22) (Fig.1).

Table-1: General Profile and Findings of Subjects Enrolled in the study.

S. N.	Outcome	Statistics	Gender	Stage of CML
1.	No alnormality	3 (5.8%)	1 Male, 2 Female	All chronic
2.	Philadelphia chromosome only	42 (80.8%)	17 Female 25 male	3 accelerated, 2 blastic, 37 chronic
3.	Philadelphia chromosome with other aberrations	7 (13.5)		3 accelerated, 2 blastic, 37 chronic
	Absent Y	1	Male	Accelerated
	Isochromosome 17	1	Female	Chronic
	Second Philadelphia	1	Female	Accelerated
	Trisomy 19	1	Female	Blastic
	Trisomy 8	1	Male	Chronic
	Trisomy 8, 21, Monosomy 7	1	Male	Blastic
	Trisomy 8, variant Ph	1	Male	Chronic

Table 2: Distribution of different additional chromosomal abnormalities (n=52)

S. N.	Characteristic	Statistic
1.	Total number of subjects enrolled	66
2.	Karyogram obtained	52/66 (%)
3.	Mean age±SD (Range) in years	49.77±11.76 (20-69)
4.	Gender	
	Male	36 (54.5%)
	Female	30 (45.5%)
	Male:female ratio	1.2:1
5.	Source of specimen	
	Peripheral blood	55 (83.3%)
	Bone marrow & blood	11 (16.7%)
6.	Mean Hb±SD (Range) in g/dl	7.69±1.58 (4-13)
7.	Mean TLC±SD (Range) (thousands/µL)	77.52±57.34 (11.1-250)
8.	Mean Blast cells±SD (Range) (%)	5.06±6.06 (1-29)
9.	Mean Basophils±SD (Range) (%)	7.53±2.34 (4-15)
10.	Mean platelet count±SD (Range) (lakhs/mm3)	5.90±2.06 (0.12-10)
11.	Stage/Phase	
	Chronic	57 (86.4%)
	Accelerated	5 (7.6%)
	Blastic	4 (6.1%)
12.	Palpable spleen	52 (78.8%)
13.	BCR-ABL Positivity	66 (100%)
14.	Philadelphia chromosome present	49/52 (%)
15.	Abnormal karyogram	49/52 (%)
16.	Additional chromosomal aberrations	7/52 (%)
17.	Type of additional abnormalities	
	Absent Y	1/7 (%)
	Isochromosome 17	1/7 (%)
	Second Philadelphia	1/7 (%)
	Trisomy 19	1/7 (%)
	Trisomy 8	1/7 (%)
	Trisomy 8, 21, Monosomy 7	1/7 (%)
	Trisomy 8, variant Ph	1/7 (%)

DISCUSSION

The cytogenetics of human neoplasms is intimately associated with their diagnosis and prognosis. Therefore, conventional cytogenetic analysis has been considered mandatory for all newly diagnosed cases of leukemias, because karyotyping plays a vital role in their diagnosis, classification and prognostification. [10] This holds true for chronic myelogenous leukemia as well. Accumulation of various chromosomal aberrations and mutations is believed to be responsible for the transition of a relatively benign chronic phase to aggressive blastic phase.

Our study consisted of 66 cases of CML who were hematologically confirmed. CML was found to be more common in men, with a male: female ratio of 1.2:1. Our findings were in resonance with the study conducted by Chavan et al[11] who found a male: female ratio 1.9:1 out of 175 hematologically confirmed CML cases. Similarly, Fabarius et al[12] had 60% male cases in their large scale study of 1151 CML patients in Germany. The CML cases in the present study comprised of adult patients with their ages ranging between 20- 69 years and the mean age was 49.77 years. Our results regarding age of patients were same as seen by Boronova et al[13] who analyzed 72 CML patients and found their age in the range of 19-74 years with median age of 46.4 years. The age pattern was also resonant with the observations of Fabarius et al[12] and Bozkurt et al[14].

In our study, we observed 7 cases (13.5%) in which additional chromosomal abnormalities were present along with Philadelphia chromosome. All the cases with additional chromosomal abnormalities were Philadelphia positive. Our finding is in agreement with earlier observations made by Mohamed et al[15] who found that secondary abnormalities appeared exclusively in the Ph+ clone in all of the patients, and Fabarius et al[12] where all of the 79 patients with ACAs had t(9;22) or variant translocation -t(v;22) in addition to ACA.

Out of the additional chromosomal abnormalities in this study, trisomy 8 were in 3 cases, one each of trisomy 19, trisomy 21, Monosomy 7, Absent Y chromosome, Isochromosome 17q (1 case), second Philadelphia and a variant Philadelphia. This finding is consistent with Fabarius et al[12] and Syed et al[16].

Trisomy 8 was the most common additional abnormality observed in 3 out of 7 cases (42.9%) in our study subjects. It was noted as isolated additional abnormality in 2 cases-47XY,+8,t(9;22) and 47XY + 8,t (9;x,22) (Fig.2) and in combination with other aberrations in one case-47XY,+8,+21, -7,t (9;22). This finding is consistent with Syed et al (16) who detected Trisomy 8 as an isolated abnormality in 9 cases, and in combination with other chromosomal aberrations in 4 cases. However, Luatti et al[17] studied 559 patients of CML and observed loss of Y chromosome in 43%, trisomy 8 in 14% cases and Fabarius et al[12] who also found Trisomy of chromosome 8 in 9 and lack of chromosome Y in 38 out of 79 CML patients with ACAs. The difference in observation of the most common additional abnormality in these studies (absent Y chromosome) as compared to ours (trisomy 8) may be accounted to following facts–

The selection criteria employed, type of study conducted and size of the study groups in the above mentioned studies were entirely different from ours i.e., Patients (559) with previously untreated Ph and BCR-ABL–positive CML in early CP were enrolled in 3 concurrent studies by Luatti et al[17] and imatinib mesylate treatment was given in different regimens and response was noted; while Clinical and cytogenetic data of 1151 of 1311 patients with Ph+ and BCR-ABL+ CP-CML were investigated prospectively by Fabarius et al[12]. Duration of disease and treatment were not included as selection criteria in our study and study group was only of 52 analyzed patients out of 66 enrolled.

In the present study all the other additional chromosomal abnormalities, except trisomy 8, were equally prevalent seen in one case each (14.3%).

Thus our study has established the trisomy of chromosome 8 as most common additional chromosomal abnormality found in Philadelphia positive CML cases which should be kept in mind while performing routine investigatory karyotyping of these patients.

CONCLUSION

In the present study we have observed the occurrence and relative frequency of additional chromosomal abnormalities and tried to find their association with various important parameters such as the stage of disease, Philadelphia chromosome positive state and patient characteristics like age, sex, etc. All of these findings and discussion of our study have established that most common ACA in CML is trisomy 8. Trisomy 8 and other abnormalities affecting the 8q24 region are very important, because this includes the gene locus for the c-Myc gene. C-Myc is a key player in cell growth and differentiation and a correlation between high c-Myc expression and CML progression has been reported. Our results have re-emphasized the importance of trisomy 8 and its role in the pathogenesis of CML. Early identification of these abnormalities may help in adapting to a more appropriate therapeutic approach.

REFERENCES

1. Phan CL, Xavier Sim YH, Isa RA, Yegappan S, Chang KM. Clonal Expansion of Co-Existing Ph-Negative Unrelated Cells in Ph-Positive CML during Imatinib Mesylate Therapy. Ann Clin Pathol. 2016;4(1):1063.
2. Rowley J. A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. Nature (Lond). 1973;43:290-93.
3. Baccarani M, Deininger MW, Rosti G, Hochhaus A, Soverini S, Apperley JF, et al. European Leukemia Net recommendations for the

management of chronic myeloid leukemia: 2013. Blood. 2013;122:872-84.

4. Cortes J, O'Dwyer ME. Clonal evolution in chronic myelogenous leukemia. Hematol Oncol Clin N Am. 2004;18:671–84.
5. Mitelman F, Levan G, Nilsson P, Brandt L. Non-random karyotypic evolution in chronic myeloid leukemia. Int. J. Cancer. 1976;18:24-30.
6. Heim S, Mitelman F. Secondary chromosome aberrations in the acute leukemias. Cancer Genet. Cytogenet. 1986;22:331-338.
7. Bakshi SR, Brahmbhatt MM, Trivedi PJ, Dalal EN, Patel DM, Purani SS, et al . Trisomy 8 in leukemia: A GCRI experience. Indian J Hum Genet. 2012;18(1):106–108.
8. Heim S, Mitelman F. Multistep cytogenetic scenario in chronic myeloid leukemia. In: Advances in Viral Oncology. Vol. 7 (G. Klein, Ed.). New York: Raven Press; 1987.p.53-76.
9. Shaffer LG, McGowan-Jordan J, Schmid M, editors. ISCN (2013): An In¬ternational System for Human Cytogenetic Nomenclature. Basel: S. Karger, 2013.
10. Wan TSK. Cancer cytogenetics: methodology revisited. Ann Lab Med. 2014;34:413-425.
11. Chavan D, Ahmad F, Iyer P, Dalvi R, Kulkarni A, Mandava S et al. Cytogenetic Investigation in Chronic Myeloid Leukemia: Study from an Indian Population. Asian Pacific J Cancer Prev. 2006;(7):423-426.
12. Fabarius A, Leitner A, Hochhaus A, Muller MC, Hanfstein B, Haferlach C, et al. Impact of additional cytogenetic aberrations at diagnosis on prognosis of CML: long-term observation of 1151 patients from the randomized CML Study IV. Blood. 2011;118(26):6760-68.
13. Boronova I, Bernasovsky I, Bernasovska J, Sotak M, Petrejcikova E, Bozikova A et al. Detection of Philadelphia chromosome in patients with chronic myeloid leukemia from the Presov region in Slovakia(1995-2004). Bratisl Lek Listy. 2007;108(10-11):433-436.
14. Bozkurt S, Uz B, Buyukasik Y, Bektas O, Inanc A, Goker H et al. Prognostic importance of additional cytogenetic anomalies in chronic myeloid leukemia. Med Oncol. 2013;30(1):443.
15. Mohamed AN, Pemberton P, Zonder J, Schiffer CA. The effect of imatinib mesylate on patients with Philadelphia chromosome-positive chronic myeloid leukemia with secondary chromosomal aberrations. Clin Cancer Res. 2003;9(4):1333-337.
16. Syed NN , Usman M, Adil S, Khurshid M. Additional chromosomal abnormalities in Philadelphia-positive chronic myeloid leukemia. Hematol Oncol Stem Cell Ther. 2008;1(3):166-70.
17. Luatti S, Castagnetti F, Marzocchi G, BaldazziC, Gugliotta G, Iacobucci I, et al. Additional chromosomal abnormalities in Philadelphia-positive clone: adverse prognostic influence on frontline imatinib therapy. A GIMEMA Working Party on CML analysis. Blood. 2012;120(4):761-7.