RESEARCH ARTICLE

Additional cytogenetic abnormalities in chronic myeloid leukaemia; an experience from Pakistan

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Abstract

Objectives: To evaluate the presence and characteristics of additional karyotype abnormalities in chronic myeloid leukaemia cases.

Method: The cross-sectional study was conducted at the Department of Cytogenetics and Molecular Pathology, National Institute of Blood Diseases and Bone Marrow Transplant, Karachi, from May 2010 to September 2016 and comprised diagnosed chronic myeloid leukaemia patients regardless of age and gender. Baseline cytogenetic evaluation was done on overnight, 24-hrs un-stimulated and 72-hrs stimulated bone marrow cultures, and karyotypes were defined according to the International System for Human Cytogenetic Nomenclature2013. Data was analysed using SPSS 23.

Results: There were 222 cases with a median age of 38 years (range: 12-84 years). The male-to-female ratio was 1.8:1. Chronic myeloid leukaemia was detected in 18(8.1%) patients having additional cytogenetic abnormalities. Among the patients found positive, cytogenetic type was minor in 10(55.55%), major 3(16.66%), complex 3(16.66%), and variant 2(11.11%).

Conclusion: Additional cytogenetic abnormalities were found in 8% of the sample.

Keywords: Additional cytogenetic abnormalities, Chronic myelogenous leukaemia, Bone marrow, Cytogenetics.
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Introduction

Chronic myeloid leukaemia (CML) is a myelo-proliferative neoplasm (MPN) distinguished by the presence of Philadelphia (Ph) chromosome resulting from reciprocal translocation between chromosome 22 and 9 i.e. t(9; 22).[1,2] This translocation leads to the generation of a chimeric gene resulting from the fusion of the Abelson Tyrosine-protien Kinase ABL1 gene on chromosome 9 with the breakpoint cluster region (BCR)gene on chromosome 22 i.e. BCR-ABL1 fusion protein having constitutive ABL kinase activity.[1,2] At diagnosis, majority CML cases harbour the classic t(9;22) (q34;q11) or its variant as the only cytogenetic abnormality.[1] The less common variant translocations that involve a third or even a fourth chromosome in addition to chromosomes 9 and 22, or have cryptic translocations, are not identified by routine cytogenetic techniques.[1] Most common additional cytogenetic abnormalities (ACAs) are double Philadelphia (+Ph), +8, and i(17q) that occur in approximately 70% cases and are termed major-route abnormalities.[2-5] Other ACAs encountered less commonly are t(3;12), t(4;6), t(2;16), and t(1;21), which are called minor-route abnormalities.[2,4] ACAs are reported globally in 5-12% CML patients at diagnosis.[1,4] European Leukaemia Net (ELN) recommendations suggest that the presence of ACAs at diagnosis represent an alarming sign, which warrants careful monitoring of the patient.[1] On the other hand, ACAs that appear subsequently over the course of the treatment are called clonal evolution.[1,2] Most studies have focussed on clonal evolution[5] which has become the routine protocol to assess treatment response. Relatively fewer studies have reported the effect of ACAs prior to therapy.[1,5] The current study was planned to observe the frequency of ACAs in CML cases.

Patients and Methods

The cross-sectional study was conducted at the Department of Cytogenetics and Molecular Pathology, National Institute of Blood Diseases and Bone Marrow Transplant, Karachi, from May 2010 to September 2016 and comprised diagnosed chronic myeloid leukaemia patients regardless of age and gender. After approval from the institutional review board, the sample was raised using non-probability, purposive sampling technique. Those included were patients having being diagnosed with CML on the basis of morphological and cytogenetic analysis. Patients already under treatment for CML were excluded. After taking written informed

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consent from the subjects, baseline cytogenetic evaluation was done on overnight, 24-hrs un-stimulated and 72-hrs stimulated bone marrow cultures by using standard protocol. Cytogenetic was done at metaphase. Interruption of cell cycle was done by using incubation and colcemid to avoid cells enter in anaphase. Then cells were put into hypotonic solution along with Carnoy's fixative. These cells were used for analysis. G-bands via trypsin using Giemsa (GTG) banding technique was used, and karyotypes were defined according to the International System for Human Cytogenetic Nomenclature (ISCN) 2013.[6] Karyograms were built using Meta-system. Data was analysed using SPSS 23. Median and range were calculated for quantitative variables and frequencies and percentages were calculated for qualitative variables.

Results

There were 222 cases with a median age of 38 years IQR-19 (range: 12-84 years). The male-to-female ratio was 1.8:1. Median values included spleen size 18.6cm (range: 12-20cm, IQR: 8cm), haemoglobin (Hb) 8.1g/dl (range: 6.4-13g/dl IQR: 6.6g/dl), platelet count 259x10^9/L (range: 117-1837x10^9/L IQR: 1720x10^9/L), total leukocyte count (TLC) 161.8x10^9/L (range: 13.7-397x10^9/L IQR: 383.3x10^9/L), eosinophil 6% (range: 1-74% IQR: 73%), basophils 6% (range: 2-18% IQR: 16%) and blast cells 2% (range: 3-13% IQR: 10%). ACAs were detected in 18(8.1%) patients. With cytogenetic type being minor in 10(55.55%) cases, major 3(16.66%), complex 3(16.66%), and variant in 2(11.11%) cases (Table).

Discussion

The median age of the sample was 38 years at CML diagnosis which is lower compared to previous data.[4,5] Male-to-female ratio was 1.8:1, and this is in line with literature.[4] Cytogenetics is routinely done to monitor treatment response which is conventional part of the treatment protocol, but limited data is available with regard to baseline analysis which must be done before starting treatment as it adds important prognostic information.[5] The presence of ACAs at baseline categorising the disease in accelerated phase is still a matter of debate and remains controversial.[5] The current study found baseline cytogenetic abnormality in addition to Ph chromosome in 18(8.1%) patients, which is comparable with local data.[7,8] Another study in Pakistan revealed ACAs in 2.2% at diagnosis.[9] On the other hand, studies done in Italy and Romania identified ACAs in 5% at baseline.[1,10] A study done in Taiwan revealed ACAs in 10.7% patients.[11]

The current study found major route abnormality and complex karyotype in 3(16%) of our patients. According to a study, major route abnormalities and complex karyotypes are associated with adverse outcome and progression of disease to advanced phase,[4] whereas, according to a study in South Korea, no ACA at baseline except monosomy 7 is associated with adverse outcome and it does not require any change in treatment protocol.[12] The current study found -Y in one (5%) patient, while it has been earlier reported in 8% patients.[10]

The presence of -Y is seen in older individuals frequently

Table: Additional cytogenetic abnormalities at baseline.

Patient #	Karyotype at time of diagnosis (Additional Cytogenetic Abnormalities)	Cytogenetic Type
1	45,X,-Y,t(9;22)(q34;q11.2)[20]	Minor Route
2	46,XY,t(1;17)(p36-q12), t(9;22)(q34;q11.2)[20]	Minor Route
3	47,XY,+8,t(9;22)(q34;q11.2)[20]	Major Route
4	46,XY,add7(p15),t(9;22)(q34;q11.2),del11(q12)[06]/46,XY,idem,-18,+20[6] /47,XY,idem,+20[02]	Complex
5	45, XY, t(9;22)(q34;q11.2), -19[09]/46, XY, t(9;22)(q34;q11.2)[06]	Minor Route
6	46,XX,t(9;22)(q34;11.2)[07]/ 47,XX,idem,+der22t(9;22)[05]	Major Route
7	46,XY,del7(q22),t(9;22)(q34;q11.2)[20]	Minor Route
8	46,XY,t(4;9;22)(p16;q34;q11.2)[20]	Variant(v;22)
9	46,XX, del(7q), t(9;22)(q34;q11.2)[19]/46,XX,t(9;22)(q34;q11.2)[11]	Minor Route
10	46,XY,t(9;22)(q34;q11.2)[19]/92,idemx2[06]	Minor Route
11	46,XY,t(9;22)(q34;q11.2),inv(9p11)[25]	Minor Route
12	53,XY,+X,+6,+7,+8,t(9;22)(q34;q11.2),+10,+16,+21,+der(22),t(9;22)[15]	Complex
13	48,XY,+8,t(9:22)(q34;q11.2),+der(22) t(9;22),+19[15]	Complex
14	46,XX, t(9;22)(q34;q11.2)[11]/63,idemx2[09]	Minor Route
15	46,XY, t(7;9;22)(q11.2;q34;q11.2)[15]	Variant (v;22)
16	46,XY,t(9;22)(q34,q11.2)[12]/47,XY,idem,+21[5]/46,XY[28]	Minor Route
17	46, XY, +8, t(9;22)(q34;q11.2), -19[15]	Major Route
18	46~74[11]/46,XX[04]	Minor Route

and in some instances can be present as an age consequence solely, but its significance in ACA is debatable.[1]

The current study also found 3-way variant translocations in 2(12%) patients exhibiting ACAs, which is line with a study.[9] One of our patients revealed t(7; 9; 22) (q11.2; q34; q11.2), which is reported to be rare in data.[13] Variant translocations are associated with similar prognostic outcome as those with conventional t(9; 22) (q34; q11.2) when treated with standard treatment.[4] Baseline haematological characteristics of patients presenting with ACAs were also studied. Lower median Hb and platelet count along with higher median eosinophil, basophil count and spleen size was observed in patients having ACAs compared to an Italian study.[1]

The limitations of the current study include failure to calculate the sample size using any calculator, and that BCR-ABL analysis was not conducted.

Conclusion

Additional cytogenetic abnormalities were found in 8% of the sample. On account of scarce data for ACAs in Pakistan, the current study may prove to be a valuable addition to relevant literature.

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References

1. Luatti S, Castagnetti F, Marzocchi G, Baldazzi C, 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; 120:761-7.
2. Marktel S, Marin D, Foot N, Szydlo R, Bua M, Karadimitris A, et al. Chronic myeloid leukemia in chronic phase responding to imatinib: the occurrence of additional cytogenetic abnormalities predicts disease progression. Haematologica.2003; 88:260-7.
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. Fabarius A, Leitner A, Hochhaus A, Müller MC, Hanfstein B, Haferlach C, et al. Impact of ACA at diagnosis on prognosis of CML: long-term observation from 1151 patients of the randomized CML Study IV. Blood 2011; 118:6760-8.
5. Otero L, Ornellas MH, Azevedo AM, Dobbin J, Abdelhay E, Bouzas LF, et al. The Impact of Additional Chromosomal Abnormalities in Response to Imatinib Mesylate Therapy for Chronic Myeloid Leukemia. RevistaBrasileira de Cancerologia. 2007; 53:405-10.
6. Simons A, Shaffer LG, Hastings RJ. Cytogenetic nomenclature: changes in the ISCN 2013 compared to the 2009 edition. Cytogenet Genome Res. 2013; 141:1-6.
7. Ujjan I, Akhund AA, Baig AM, Nizamani GS. The cytogenetic and molecular analysis of chronic myeloid leukemia in a tertiary care hospital of Sindh, Pakistan. Integr Cancer Sci Therap. 2015; 2:164-7.
8. Ujjan, Ikram Din, Akhund AA, Saboor M, Qureshi MA, Khan S. Cytogenetic and Molecular Analyses of Philadelphia Chromosome Variants in CML (chronic myeloid leukemia) Patients from Sindh using Karyotyping and RT-PCR. Pak J Med Sci. 2015; 31:936-40.
9. Syed NN, Usman M, Adil S, Khurshid M. Additional chromosomal abnormalities in philadelphia-positive chronic myeloid leukemia. Pak J Med Sci.2020; 36:208-12.
10. Crisan AM, CoriuD, Arion C, Colita A, Jardan C. The impact of additional cytogenetic abnormalities at diagnosis and during therapy with tyrosine kinase inhibitors in Chronic Myeloid Leukaemia. J Med Life. 2015; 8:502-8.
11. Hsiao HH, Liu YC, Tsai HJ, Hsu JF, Yang WC, Chang CS, et al. Additional chromosome abnormalities in chronic myeloid leukemia. Kaohsiung J Med Sci.2011; 27:49-54.
12. Lee SE, Choi SY, Bang JH, Kim SH, Jang EJ, Byeun JY, et al. The long-term clinical implications of clonal chromosomal abnormalities in newly diagnosed chronic phase chronic myeloid leukemia patients treated with imatinib mesylate. Cancer Genet.2012; 205:563-71.
13. Shan S, Nadeem M, Ara T, Shamsi T. A 3-way Rare Variant Ph Translocation t (7; 9; 22) in a patient with Chronic Myeloid Leukemia.Acta Medica Int. 2015; 2:186-8.