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Frequency of two transcripts of *BCR-ABL 1* fusion gene (p210 and p190) in acute lymphoblastic leukemia and chronic myeloid leukemia by reverse transcriptase polymerase chain reaction

Abstract

Objective: To determine the frequency of two transcripts of BCR-ABL 1 fusion gene (P210 & P190) in acute lymphoblastic leukemia and chronic myelogenous leukemia by reverse transcriptase polymerase chain reaction.

Study design: A cross-sectional study.

Place and duration of study: The Armed Forces Institute of Pathology (AFIP), Rawalpindi, from December 2012 to January 2014.

Methodology: 147 diagnosed patients of CML and ALL were subjected to real time reverse transcriptase polymerase chain reaction. For PCR,2-3ml of venous blood in EDTA was collected .RNA extraction was done by Trizol Reagent LS (MRC, USA) and cDNA was synthesized using reverse transcriptase and gene specific primer. Real time- PCR was done on ABI-7500.The positive samples were identified when fluorescence exceeded threshold limit.

Results: Out of 147 samples, 85 were with diagnosis of CML. All of them (100%) showed P210. 58 patients had diagnosis of ALL. Out of these BCR-ABL1 is detected in 11(18.9%) patients. 9(81.8%) expressed P190 whereas P210 was present in 2(18.1%) patients. 4 patients in lymphoid blast phase of CML were identified. 3(75%) of them had PP210 and 1(25%) shows both (P190 & P210) the transcripts.

Conclusion: All CML patients expressed P210 transcript whereas ALL patients mostly showed P190. These transcripts expressing specific tyrosine kinase protein have diagnostic as well prognostic significance.

Keywords: chronic myelogenous leukemia, acute lymphoblastic leukemia, real time polymerase chain reaction, BCR-ABL1

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Maria Altaf,[1] Saleem Ahmed Khan,[1] Ayesha Khan,[2] Ammara Arsalan[1]
[1]Department of Hematology, AFIP, NUMS, Pakistan
[2]Department of Hematology, CMH Malir, Pakistan

Correspondence: Maria Altaf, Department of Hematology, AFIP, NUMS, Pakistan, Email aysharao@yahoo.com

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Introduction

BCR-ABL1 is the defining molecular lesion of chronic myelogenous leukemia(CML) and also found in a subset of acute lymphoblastic leukemia(ALL).[1] This unique genetic aberration on chromosome 22 arises as a consequence of reciprocal translocation of ABL1 gene from chromosome 9, next to BCR gene on chromosome 22.[2] History of hybrid BCR-ABL gene started in 1960 when an abnormal shortened chromosome 22 termed philadelphia chromosome was described in leukemic cell of patients with CNL,13 years later in 1973 Janet Rowly identified BCR-ABL fusion gene as hybrid of two normal chromosome that have fused together.[3] 90-95% of patients of CML have BCR-ABL fusion gene in which major breakpoint cluster region(M-bcr) is associated with production of e13/e14a2 which translated into P210kDa oncoprotein.[4] Chimeric BCR-ABL1 mRNA is also seen in adult ALL patients.[5] Around 63% of BCR-abl positive all patients genomic breakpoints occurs in the first intron of BCR gen(m-bcr)and BCR –ABL gene resulting from fusion of first exon(e1) of BCR with second (a2) of ABL1 gene. The e1a2 mRNA translates in P190kDa. However 27% of Philadelphia positive ALL patients harbor P210.[6]

The fusion protein has increased tyrosine kinase activity[7] and affects multiple cellular processes including intracellular signaling, apoptosis, transcriptional regulation and cellular adhesion result in deregulated proliferation. The natural history of CML progresses through three phases from chronic to blast crises.[8] The biology of CML is complicated by transformation of CML into lymphoid blast phase (LBP). Secondary lymphoid blast phase of CML shows exclusively P210 but is associated with new kinase domain mutations and/or amplification of Philadelphia chromosome.[9]

Both BCR-ABl transcripts can be detected by reverse transcriptase polymerase chain reaction (RT-PCR).In this new era of molecular study and gene specific diagnosis identification of P190 in CML is important as presence of this molecular abnormality is associated with aggressive course of disease[10] and likely transformation into acute leukemia. Whereas, presence of BCR-ABL in ALL is a poor prognostic marker[11] and finding P210 in Philadelphia positive ALL guides us that patient might have evolved from underlying CML.[12] The aim of this study is to find the frequency of P190 and P210 in CML and ALL patients.

Methodology

This cross-sectional, study was conducted at the department of Heamatology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from December 2012 to January 2014. A total of 147 newly diagonsed

patients of CML and ALL were included in this study. After approval of the ethical committee of AFIP, patient reassurance and consent, 2-3 ml of venous blood was drawn from the antecubital vein by aseptic technique in ethylene diamine tetra-acetic acid (EDTA) for CBC and PCR. Blood counts were performed on Sysmex KW-21 automated hematology analyzer. Total leucocyte count of all individuals was recorded. For RT-PCR, RNA was extracted from peripheral blood leucocytes by using Trizole-LS reagent (USA). Complimentary DNA (cDNA) was synthesized by mixing 8 µl of extracted RNA with deoxy nucleotide triphosphates (dNTPs), RT buffer, M-MLV enzyme and RNAase inhibitor. A gene specific primer was added to tube: ABL-R 5'-GGCCACAAAATCATACGTGCA. The synthesized cDNA was mixed with PCR mix that contains dNTPs, magnesium chloride etc. Along with DNA Taq polymerase and following BCR-ABL primers and probes were used.

Primers/probe for P190

BCRF (5CTGGCCCAACGATGGCGA)

ABLR (5CCCTTCAGCGGCCAGTAGCATCTGA)

ABLP (5CACTCAGACCCTGAGGCTGAA)

Primers/probe for P210

BCRF (5TCCGCTGACCATCAAYAAGA)

ABLR (5CCCTTCAGCGGCCAGTAGCATCTGA)

ABLP (5CACTCAGACCCTGAGGCTCAA)

Samples were analyzed by running on ABI-7500 Sequence Detection Sysrem (Applied Biosystem).thermal cycling parameters for PCR consisted of initial 10 minutes at 95°C,followed by 40 biphasic cycles of 15 seconds at 95°C for denaturation and 1 minute at 60°C for annealing and extension. Positive and negative controls were run along with test samples. All the collected data were entered in Statistical package for Social sciences (SPSS) version 17. The analyzed variables included numerical data like age and qualitative data like gender.

Results

Out of 147 patients enrolled in the study, 85 were of CML and 58 were of ALL. Out of 85 CML patients 52 were males (61%) and 24 were females (29%). Male to female ratio is 2:1:1. The age of patients ranged between 11-80 years. The mean TLC in CML group was 139±83. Minimum TLC was 12.3×10^9/l. Descriptive statistics are shown in Table 1. On PCR all 85 patients of CML (100%) were positive for P210.

Table 1 Descriptive stats of age. Blood in BCR-ABL positive CML

CMLn (85)	Minimum	Maximum	Mean	Standard deviation
Age (yrs)	11	80	40	±16
TLC (10^9/l)	12	298	139	±83
Hb (g/dl)	7	16	10.6	±2.3
Platelet (10^9/l)	15	900	333.1	±157.3

Table 2 Descriptive stats of age, blood count, in BCR-ABL positive ALL

ALL n (11)	Minimum	Maximum	Mean	Standard deviation
Age (yrs)	2	54	27	±16
TLC (10^9/l)	13.0	269.9	111.7	±76.0
Hb (g/dl)	6	11	8.5	±1.50
Platelet (10^9/l)	7	45	17.2	±12.5
Peripheral blast count	89	96	92.3	±2.2

58 patients were with the diagnosis of ALL.BCR-ABL1 was detected in 11 patients (19%). Out of these 11 patients, 8(72.7%) were male and 3(27.2%) female. The age of patients ranged between 2-54 years, mean age was 27±16 years. Minimum TLC was 13.0×10^9/l and maximum was 269.9×10^9/l. Mean TLC was 111.7±76.0 in this group. Descriptive statistics were shown in Table 2. On PCR, 9 patients (81.8%) were found positive for P190 whereas 2(18.1%) expressed P210 in it.

4 patient of CML were identified that presented with lymphoid blast phase. All 4 of them were male with ea TLC of 70.7±43. On PCR, 3 has P210 whereas one patient expressed both p190 and P210.

Discussion

Around 90-95% of cases of CIVIL and 10-25% of ALL harbours reciprocal translocation between chromosome number 9 and 22. This results in the formation of Philadelphia chromosome which is characterized by the presence of hybrid BCR-ABL fusion gene.[12] Depending on breakpoint of BCR gene, three types of BCR-ABL fusion gene are formed, involving exon2 of ABL gene but different exons of BCR gens. The transcript type of 13_a2/e 12_a2 encodes p190 protein[13] whereas fusion gene e1a2 (usually found in Philadelphia positive ALL) encodes p 190 protein. All of these transcripts exhibit dysregulated proteim kinase activity compared to normal ABL product. All of these transcripts exhibit dysregulated protein kinase activity compared to normal ABL product. As a result there is excessive tyrosine phosphorylation of many intracellular proteins.

As there are differences in clinical presentation, evolution of disease, the prognostic implications and various therapeutic options separation of the two transcripts become important. The study showed that out of 85 CIVIL patients, 52 were males (61%) and 24 were females (29%). The male predominance in our study population is comparable with local and international statistics/most of studies show slight male predominance. In our study male to female ratio was 2.1;1.

The age of the patient with CML ranges from 11 to 80 years with mean age of 40±16 years. This is comparable with some local studies

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but not with international studies. In a local study by Saad et al, it was concluded that mean age of male patients with CML was 36.5 years, and for female I was 39 years.[14] In another study done by Sharma et al (India) has shown median age of 35 years in CML patients which also matches with our study.[15] In international studies and Western literature the median age of disease presentation is around 67 years whereas range of age is almost the same.

The principal observations of this study are the results of PCR. In CML there is 100% positivity for P210. Where as in ALL, BCR-ABL fusion gene is found in 11% of patients. Out of these 11 BCR-ABL positive patients,[2](18.2%) expresses P210 while[9](81.8%) has the expression of P190. The results of this study are in concordance with the results of local and international studies.

Few studies are available in our population because of unavailability of advance modern diagnostic facilities and majority of laboratories in Pakistan are doing multiplex –PCR for the detection of BCR-ABL, in which primers of P 190 and P210 are combined. In a local study by Suhaib et al,[16] found 100% positivity of PT-PCR for P210 in 10 CML patients (untreated) in Pakistan population. This is a relatively smaller study group also they use multiplex PCR for the detection of BCR-ABL.

In this study BCR-ABL is defected in 11(19%) ALL cases, which is comparable with international studies and no local study is available on this particular subject to the best of our knowledge. Out of 11 BCR-ABL positive ALL patients, 8 were males (72.7%) and 3 were females (27.2%). The age of the patients with ALL ranged from 2 to 54 years with mean age of 27±16 years. Mean TCL in the ALL group was 111.7 x 10^9/1. Minimum TLC was 13.0 x 10^9/l and maximum was 269.9 x 10^9/l. The patients with ALL who harbor BCR-ABL fusion gene have mean of blast count of 92% in their peripheral blood.

In one study by Prateek Bhatia[17] in year 2013, the incidence of BCR-ABL in ALL is 67% (10 out of 15) in adults and 33%(5/15) in children. Another study done in Germany[18] in year 2002 selected 342 BCR-ABL positive patients of ALL. 26277% showed the presence of P190 while P210 is present in (22%) patients.[19] The rate of co-expression was 3% Another study conducted in Malaysia[19] selected 7 BCR-ABL positive ALL patients, 5(71.4%) expresses P129 whereas 2 of them (28.5%) have P210. The results of our study are similar to these international studies. A study doe USA in year 2008[9] selects 26 CML patients that have under gone transformation into lymphoid blast phase. All of then shows the presence of P210 in them.

The result of the study suggest that identification of BCR-ABL fusion transcript is useful in defining prognosis, as finding studies sharing P190 I CML is associated with aggressive course of disease and short live response to TKI.[20] Accurate identification of BCR-ABL in ALL is also important in defining prognosis[21] and this finding is absolute requirement for imatinib therapy.[22] Additionally presence of P210 gives us a clue that patient might have evolved from underlying CML.[9] The ultimate goal of all the advance diagnostic facilities is to contribute to improvement in health o patients.

Conclusion

P210 is the most frequent transcript type in CML, whereas P190 is the usual transcript in BCR-ABL positive ALL patients. Identification of BCR-ABL is important in ALL patients as it helps in risk stratification of the disease.

Acknowledgments

None.

Conflicts of interest

The authors declare no conflicts of interest.

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