

RESEARCH ARTICLE

Prognostically Significant Fusion Oncogenes in Pakistani Patients with Adult Acute Lymphoblastic Leukemia and their Association with Disease Biology and Outcome

Noreen Sabir^{1#}, Zafar Iqbal^{1,2,8,10#*}, Aamer Aleem^{1,3#}, Tashfeen Awan^{1#}, Tahir Naeem^{1,4}, Sultan Asad⁵, Ammara H Tahir^{1,6}, Muhammad Absar^{1,4}, Rana MW Hasanato⁴, Sulman Basit⁷, Muhammad Azhar Chishti⁷, Muhammad Faiyaz Ul-Haque², Ahmad Muktar Khalid⁸, Muhammad Farooq Sabar⁵, Mahmood Rasool⁹, Sajjad Karim⁹, Mahwish Khan^{1,10}, Baila Samreen^{1,11}, Afia M Akram¹, Muhammad Hassan Siddiqi¹, Saba Shahzadi¹, Sana Shahbaz¹, Agha Shabbir Ali¹², Amer Mahmood¹³, Muhammad Akram¹⁴, Tariq Saeed¹⁴, Arsalan Saleem¹⁵, Danish Mohsin¹⁵, Ijaz Hussain Shah¹⁶, Muhammad Khalid¹⁶, Muhammad Asif¹⁷, Mudassar Iqbal^{1,18,19}, Tanveer Akhtar¹

Abstract

Background and objectives: Chromosomal abnormalities play an important role in genesis of acute lymphoblastic leukemia (ALL) and have prognostic implications. Five major risk stratifying fusion genes in ALL are BCR-ABL, *MLL-AF4*, *ETV6-RUNX1*, *E2A-PBX1* and *SIL-TAL1*. This work aimed to detect common chromosomal translocations and associated fusion oncogenes in adult ALL patients and study their relationship with clinical features and treatment outcome. **Methods:** We studied fusion oncogenes in 104 adult ALL patients using RT-PCR and interphase-FISH at diagnosis and their association with clinical characteristics and treatment outcome. **Results:** Five most common fusion genes i.e. BCR-ABL (t 9; 22), *TCF3-PBX1* (t 1; 19), *ETV6-RUNX1* (t 12; 21), *MLL-AF4* (t 4; 11) and *SIL-TAL1* (Del 1p32) were found in 82/104 (79%) patients. *TCF3-PBX1* fusion gene was associated with lymphadenopathy, *SIL-TAL1* positive patients had frequent organomegaly and usually presented with a platelets count of less than $50 \times 10^9/l$. Survival of patients with fusion gene *ETV6-RUNX1* was better when compared to patients harboring other genes. *MLL-AF4* and BCR-ABL positivity characterized a subset of adult ALL patients with aggressive clinical behaviour and a poor outcome. **Conclusions:** This is the first study from Pakistan which investigated the frequency of 5 fusion oncogenes in adult ALL patients, and their association with clinical features, treatment response and outcome. Frequencies of some of the oncogenes were different from those reported elsewhere and they appear to be associated with distinct clinical characteristics and treatment outcome. This information will help in the prognostic stratification and risk adapted management of adult ALL patients.

Keywords: Acute lymphoblastic leukemia - ALL - adult - fusion oncogenes - Pakistan

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Introduction

Acute lymphoblastic leukemia (ALL) is the commonest

malignancy in children but is less common in adults. Although remarkable progress has been made in the treatment of ALL in children with cure rates of around

¹Hematology, Oncology and Pharmacogenetic Engineering Sciences Group, Health Sciences Laboratories, Faculty of Biological Sciences, Department of Zoology, University of the Punjab, ⁵Centre for Advanced Molecular Biology, ⁶National Centre of Excellence in Molecular Biology, ¹⁰Institute of Molecular Biology and Biotechnology & Centre for Research in Molecular Medicine, University of Lahore, ¹²Post Graduate Medical Institute & Institute of Child Health, ¹⁴Department of Oncology, Allama Iqbal Medical College and Jinnah Hospital, ¹⁵University of Health Sciences, Lahore, ⁸School of Biological Sciences, University of Sargodha, Sargodha, ¹¹Sind Institute of Urology and Transplantation, Karachi, ¹⁶Department of Oncology, Punjab Medical College and Allied Hospital, Faisalabad, ¹⁷Department of Biotechnology and Informatics, (BUIEMS), Quetta, Pakistan, ²Department of Pathology, ³Department of Medicine, ²Molecular Genetic Pathology, ³Hematology/Oncology Division, Department of Medicine, ⁴Microbiology Section, ⁷Biochemistry Research Section, ¹³Embryonic Stem Cell Unit, Department of Anatomy, College of Medicine and King Khalid University Hospital, King Saud University, Riyadh, ⁹Centre of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia, ⁶Montefiore Medical Centre, NY, USA, ¹⁸Asian Medical Institute, Kant, ¹⁹National Surgical Centre, Bishkek, Kyrgyzstan #Equal contributors *For correspondence: mianzafaram@yahoo.com

80%, treatment results remain unsatisfactory in adults, with a poor overall prognosis and a long term probability of survival less than 40 % (Pui et al., 2004; Bassan., 2005). Biologic differences in the leukemogenesis between adult and childhood ALL is the most likely explanation for this discrepancy. There are significant differences between the childhood and adult ALL as indicated by the prevalence of various genetic abnormalities and other factors. Genetic abnormalities play a major role in the prognosis and treatment outcome of ALL; however, primary genetic abnormalities alone may not be sufficient to induce leukemogenesis (Andreasson et al., 2001; Moorman et al., 2007). Although the impact of genetic factors including specific translocations and DNA ploidy is well characterized in childhood ALL, the prognostic significance of these abnormalities in adult ALL is less well defined (Moorman et al., 2007).

It is likely that the distribution of molecular genetic subtypes of ALL may not be uniform in different parts of the world because of the racial and genetic variations that exist among different populations. Previous studies indicate that ALL in adults is a heterogeneous disease with differences in the clinical characteristics and molecular abnormalities in various ethnic groups (Iacobucci et al., 2012). Therefore, it is important to study the disease biology and underlying molecular pathways in different populations in order to develop effective treatment strategies. Acquired recurrent chromosomal abnormalities in the malignant cells are the hallmarks of acute leukemia and define different subsets of the disease in ALL patients (Harrison & Feroni, 2002). The discovery and characterization of these genetic lesions has increased our understanding of the biology of the disease, has shaped our current classification system of ALL, and is used to direct therapy. The development of treatment strategies resides in defining the molecular pathways underlying the pathogenesis of the disease. In order to improve the outcome in adult ALL, it is essential that targeted therapies are developed aiming at specific genetic lesions. Knowledge about the genetic abnormalities obtained by molecular cytogenetics is essential to develop targeted therapies, and to incorporate them in treatment protocols. This work aimed to detect the chromosomal translocations and underlying fusion oncogenes in adult ALL patients and study their association with clinical features and treatment outcome.

Materials and Methods

Patients

From March 2009 to February 2012 a total of 128 patients from 4 different centers in Lahore, Pakistan, participated in the study. According to the inclusion criteria, untreated patients between the age of 16 and 70 years with the morphological and cytochemically confirmed diagnosis of any of the three French-American-British (FAB) subtypes of ALL were eligible. Diagnosis was confirmed with immunophenotyping when it was available. Patients were excluded if they had a prior malignancy, severe systemic illness, or a psychiatric disorder. All patients had adequate renal and hepatic

function. Informed consent was obtained from all the patients and the study was approved by the ethical committees of the participating centers.

Leukemia diagnosis

Immunophenotypic data from the local institutions were used after central review, cytometric analysis and panels of monoclonal antibodies were used for indirect immunofluorescence staining. The criterion for surface marker positivity was at least 20% expression of the leukemia blast cell population. B-lineage antigen expression was defined as CD19 or CD20 positivity and T-lineage antigen expression as CD5 or CD2 reactivity. Expression of the common ALL antigens was assessed by CD10 reactivity. It was recommended that pretreatment blood specimens be submitted for molecular cytogenetic analysis for the presence of the fusion genes to the HOPES Group. Fusion genes were studied in 104 adult ALL patients using RT-PCR and interphase fluorescent in situ hybridization (FISH), and their association with clinical features and treatment outcome was analyzed.

RNA extraction

Total RNA was extracted from leukemic cells by Trizol reagent according to the manufacturer's instruction.

Synthesis of complementary DNA (cDNA)

RNA was reverse-transcribed to cDNA for using as template in PCR reaction. The reverse transcriptase (RT) reaction is catalyzed by enzyme 'Reverse Transcriptase' in the presence of random hexamer primers which are the short pieces of synthetic DNA complementary to 3' tail of target mRNAs of fusion genes. RT-reaction protocol and other reaction conditions were adopted from van Dongen (Van Dongen et al., 1999). Briefly, 10 μ l of RNA was added to 10 μ l of RT-reaction mixture containing 5X RT buffer, 25 mM dNTPS, 10mM random hexamer primers, RiboLockTM RNase inhibitor, M-MuLV Reverse Transcriptase (Fermentas, USA) and DEPC- treated water. Reaction was carried out by incubating mixture of the template, random hexamers and DEPC treated water at 70°C for 10 min. Then rest of the reagents were added and incubated at 42°C for 60 min, 70°C for 10 min and held at 4°C in the last step. The integrity of cDNA was assessed by amplification of housekeeping genes GAPDH.

RT-PCR amplification

PCR primers and nested-PCR protocols for the detection of five fusion gene were adopted from Van Dongen (Van Dongen et al., 1999). Primers used in this study were synthesized by the National Centre of Excellence in Molecular Biology, Lahore, Pakistan. For the 1st round of nested PCR, a 50 μ l PCR reaction was performed containing 5x PCR buffer with KCl, 25mM MgCl₂, 25mM dNTP Mix, DEPC water, Taq DNA Polymerase (Fermentas, USA), primer (forward and reverse) and cDNA as a template. For second round PCR, product of first round PCR was used as template along with the forward and reverse primers. Thermal cycling conditions for PCR were initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation

Table 1. Frequency of 5 Common Fusion Oncogenes in Adult ALL Patients, Male to Female Ratio and Their Distribution According to 3 Age Groups

Fusion oncogene	Chromosomal abnormality	Male-Female Ratio	Age 16y-29 y No (%)	Age 30-59 y No (%)	Age ≥60y No (%)	Total
<i>TCF3-PBX1</i>	t (1;19) (q23;p13)	7.5:1	10 (-58.8)	6 (35.3)	1 (5.8)	17 (16.3)
<i>MLL-AF4</i>	t (4;11) (q21;q23)	2.3:1	6 (60)	4 (40)	0	10 (9.7)
BCR-ABL	t (9;22) (q34;q11)	3.2:1	4 (19)	16 (76.19)	1 (4.8)	21 (20.3)
ETV6- RUNX1	t (12;21) (p13;q22)	7:0:1	3 (60)	2 (40)	0	5 (4.8)
SIL-TAL1	Micro deletion 1p32	4.8:1	19 (65.5)	9 (31)	1 (3.45)	29 (27.9)

Table 2. Analysis of Patients' Treatment Outcome

Parameter	Number (%)
Patients eligible	104
Fusion genes detected	82 (79)
Complete remission (CR)	79 (76)
Induction failures	25 (24)
Induction deaths	3 (2.9)
Died in remission	16 (15.4)
Relapsed	60 (57.7)
Continued CR	3 (2.9)

of double stranded DNA at 95°C for 30 sec, annealing of primers to DNA template at 65°C for 60 sec and extension to form multiple copies of DNA strands at 72°C for 60 sec, followed by a final extension at 72°C for 7 minutes. Agarose gel (1.5%) was used for electrophoresis of PCR products followed by visualization by UV transilluminator. All recommended precautions were taken to avoid contaminations. Appropriate negative and positive controls were included in each amplification experiment.

Interphase fluorescent in situ hybridization (interphase FISH)

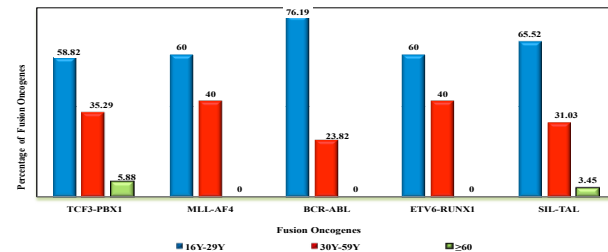
Interphase FISH was used to confirm our findings of RT-PCR analysis of fusion oncogenes. All probes and kits were purchased from Abbot Laboratories (Illinois USA) and FISH procedures were carried out according to the manufacturer's instructions. Stained slides were analyzed using a FISH analyzer system (Leica microscope; CytoVision 4.0, Applied Imaging, Biosciences Centre, Newcastle, UK).

Treatment protocol

Induction therapy was given over a four-week period in two phases. Phase-I included dexamethasone, vincristine and danuorubicin. In phase-II cyclophosphamide and cytarabine were given. Consolidation therapy was administered every four to six weeks with six alternating cycles of methotrexate, L-asparaginase, high dose cytarabine (recommended dose reduction for patients >70 years of age). Patients also received prophylactic central nervous system (CNS) intrathecal chemotherapy. Maintenance protocol included pulses of dexamethasone, daily mercaptopurine, weekly methotrexate, and monthly vincristine.

Response criteria

Patients were considered to be in CR when the results of BM examination were normal (including <5% blasts and >25% cellularity), the neutrophil counts were greater than $1.5 \times 10^9/l$, the platelet count was greater than

**Figure 1. Frequency Distribution of 5 Common Fusion Oncogenes in Adult ALL Patients in Different Age Groups**

$100 \times 10^9/l$, and all extramedullary disease had resolved. Statistical Analysis

We used convenient sampling technique in this study to collect the data; therefore, we used non-parametric tests to analyze the data. Chi Square test was used to study the association between different oncogenotypes and clinical and laboratory parameters of leukemia patients. Kaplan and Meier method was used to calculate the median survival times, while Breslow's test was used to study the survival differences between various patient groups.

Results

Molecular Cytogenetic analysis

One hundred and four blood samples of adult ALL patients were subjected to molecular cytogenetic analysis. Of these, 82 (79%) ALL patients were found to have five common fusion oncogenes with the distribution of *TCF3-PBX1* in 17 (16.3%) patients, *MLL-AF4* in 10 (9.7%), BCR-ABL in 21 (20.3%), *ETV6-RUNX1* in 5 (4.8%) and SIL-TAL1 in 29 (27.9%) patients (Table 1, Figure 1). There was 100% concordance between RT-PCR and FISH results.

Patients' characteristics

Eighty-two out of 104 (79%) patients with a median age of 26 years (range 16-70) were found to have fusion genes. There were 62 (76%) males and 20 (24%) females. Initial WBC counts ranged from $0.5-455 \times 10^9/l$ (median $17 \times 10^9/l$), platelet counts ranged from $8-557 \times 10^9/l$ (median, $55 \times 10^9/l$), and hemoglobin levels ranged from 4.0-16.5 g/dl (median 9.6 g/dl). Eighty-four percent of patients had WBC counts $<30 \times 10^9/l$, and rest had WBC counts greater $>30 \times 10^9/l$ while 3% of the patients had hyperleukocytosis. Fifty-six percent of patients had a fever or infection before chemotherapy. Sixty-nine patients (66.3%) had a mediastinal mass, 26/104 (25%) had splenomegaly, and 21/104 (20.2%) had hepatomegaly. Palpable lymphadenopathy was present in 43/104 (41.3%)

Table 3. Comparison of Clinical Characteristics of Adult ALL Patients with Different Fusion Genes (n=82)

Clinical parameters	BCR-ABL	ETV6-RUNX1	MLL-AF4	SIL-TAL1	TCF3-PBX1
	No. (%) N=21	No. (%) N=5	No. (%) N=10	No. (%) N=29	No. (%) N=17
Sex					
Male	15 (71)	4 (80)	7 (70)	24 (83)	15 (88)
Female	6 (29)	1 (20)	3 (30)	5 (17)	2 (12)
Age					
16y-29 y	4 (19)	3 (60)	6 (60)	19 (65)	10 (59)
30y-59y	13 (62)	2 (40)	3 (30)	9 (31)	6 (35)
≥60y	4 (19)	0	1 (10)	1 (3)	1 (6)
WBC count					
<30,000	11 (52)	4 (80)	3 (30)	16 (55)	14 (82)
>30,000	10 (48)	1 (20)	7 (70)	13 (45)	3 (18)
Hepatomegaly					
No	11 (52)	2 (40)	5 (50)	24 (83)	15 (88)
yes	10 (48)	3 (60)	5 (50)	5 (17)	2 (12)
Splenomegaly					
No	5 (24)	5 (100)	5 (50)	21 (72)	14 (82)
Yes	16 (76)	0	5 (50)	8 (28)	3 (18)
Lymphadenopathy					
No	3 (14)	4 (80)	5 (50)	21 (72)	2 (12)
yes	18 (86)	1 (20)	5 (50)	8 (28)	15 (88)
Platelet count					
<50,000	20 (95)	1 (20)	9 (90)	6 (21)	5 (29)
>50,000	1 (5)	4 (80)	1 (10)	23 (79)	12 (71)
CR					
<4weeks	4 (19)	4 (80)	6 (60)	20 (69)	11 (65)
>4weeks	14 (66)	1 (20)	1 (10)	1 (3)	3 (18)
No remission	3 (14)	0	2 (20)	8 (28)	3 (18)

patients. CNS disease as confirmed by spinal cytology, was found in 6/104 (5.7%) patients. According to the FAB criteria, 19% cases were L1, 76% were L2, and 5% were L3. Immunophenotyping was available in 41 cases to study the surface markers.

Response to therapy

Twenty five (24%) patients had to be excluded on the basis of statistical criteria when response was analyzed. Seventy-nine of 104 (76%) eligible patients achieved a CR (Table 2). Three patients died before hematopoietic recovery after induction chemotherapy. Thirty-six (46%) of the responders were in CR within 30 days from the first treatment. Forty-three (54%) patients required more than 30 days to achieve CR either because of slow recovery of blood counts or marrow cellularity. Seventy-six percent of remissions were achieved within 42 days. Sixteen patients died in remission. Treatment response was strongly associated with age (Table 2). Fifty-five (93.2%) of the 59 patients less than 30 years old achieved a CR, as compared to 23 (62.2%) of 37 patients between the ages of 30-59 years, while only one (12.5%) out of 8 patients 60 years or older achieved CR (P<.001) (Figure 2).

Fusion genes

MLL-AF4: *MLL-AF4+* fusion gene was expressed in 10 (9.7 %) patients and the clinical characteristics of the *MLL-AF4+* patients are shown in Table 3. At diagnosis these patients frequently had an elevated WBC count, hepatosplenomegaly, lymphadenopathy and CNS involvement. The presence of t(4;11)(q21;q23) with the expression of fusion gene *MLL-AF4* characterizes a subset

Table 4. Frequency Of Relapses According To The Fusion Oncogenes: FO (N=82)

Fusion Oncogenes	Frequency of FO (%)	Relapse frequency (%)
<i>TCF3-PBX1</i>	17 (20.7)	13 (76.5)
<i>MLL-AF4</i>	10 (12.2)	6 (60)
BCR-ABL	21 (25.6)	17 (81)
<i>ETV6-RUNX1</i>	5 (6.1)	2 (40)
SIL-TAL1	29 (35.4)	22 (76)
Total	82 (79)	60 (73)

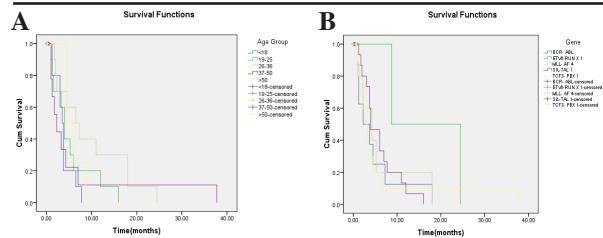


Figure 2. Survival of Patients. A) Survival of patients according to the age groups. Out of 79 patients, 20 patients in the age group 26-36 years had a better survival than other age groups. There was no significant difference between the survivals of other age groups (p=0.153). B) Survival of patients according to the fusion oncogenes. Survival of patients with gene *ETV6-RUNX1* was better than the patients with other fusion genes. However there was no significant difference among survival of patients with other fusion oncogenes (p=0.153)

of adult ALL patients with aggressive clinical features and a poor outcome.

SIL-TAL1: The *SIL-TAL1+* fusion gene was expressed in 29 (27.9 %) patients (Table 1). Clinical characteristics of the *SIL-TAL1+* patients are shown in Table 3. *SIL-TAL1+* patients frequently presented with lymphadenopathy, organomegaly and low platelet counts (<50×10⁹/l). The immunophenotyping data available in 24 of *SIL-TAL1+* patients indicated this fusion gene was associated with T-ALL. *SIL-TAL1* positivity was significantly associated with low platelet's count and lymphadenopathy (p<0.001) and patients in this subgroup had poor survival (Figure 3).

BCR-ABL: Clinical characteristics of the *BCR-ABL+* patients are shown in Table 3. *BCR-ABL* fusion gene was expressed in 21 (20.3%) patients (Table 1). The prevalence of *BCR-ABL* positivity increased with age and was more common between the 30-59 year age group. *BCR-ABL+* patients frequently had high leukocyte count (p<0.001) and splenomegaly (p<0.001). *BCR-ABL+* ALL was associated with a 10% lower chance of CR as compared to *BCR-ABL* negative disease and a poor overall prognosis, with a median survival of 8 months (Figure 3).

ETV6-RUNX1: Clinical characteristics of *ETV6-RUNX1* positive patients are given in Table 3. The *ETV6-RUNX1* fusion gene was expressed in 5 (4.8%) cases (Table 1). There were 4 males and one female and all our *ETV6-RUNX1* positive patients were young below the age of 46 years. Immunophenotyping data was available in two patients and they had B-ALL lineage. One of the patients presented with massive organomegaly and most of the patients had total leukocytic counts below 30×10⁹/l. In most of the cases remission was achieved in less than four weeks and *ETV6-RUNX1*-positive patients had a favorable prognosis during the first few years (Figure 3). These clinical features suggest that *ETV6-RUNX1* positive patients have a relatively good prognosis (Figure 3)

although their long-term survival was negatively affected by late relapses.

TCF3-PBX1: Clinical characteristics of *TCF3-PBX1* positive patients are shown in Table 3. The *TCF3-PBX1* fusion gene was expressed in 17 (16.3 %) cases (Table 1). Majority of the patients with *TCF3-PBX1* were males (15) and only 2 females with ages between 16 to 29 years. Hepato-splenomegaly was uncommon but *TCF3-PBX1* positivity was associated with lymphadenopathy ($p=0.005$). Most of the positive cases had a total leukocyte counts below $30 \times 10^9/l$ along with anemia and thrombocytopenic at presentation. Immunophenotyping data were available in nine patients and all cases belonged to common ALL (CD10⁺, CD19⁺) lineage. In 11 cases remission was achieved in less than four weeks and 3 patients achieved late remission.

Discussion

Although some of the cytogenetic abnormalities have shown to be independent prognostic factors in pediatric and adult ALL, prognostic significance of cytogenetic abnormalities other than BCR-ABL (t 9; 22) and *MLL-AF4* (t 4; 11) is less well defined in adult ALL with the same abnormalities being reported as both good or poor risk in different studies (Faderl et al., 1998; Thomas et al., 2004; Mancini et al., 2005). The most likely reason for this is the genetic heterogeneity of the disease. The most common fusion oncogenes in adult ALL other than the BCR-ABL (t 9; 22) include *TCF3-PBX1* (t 1; 19), *ETV6-RUNX1* (t 12; 21), *MLL-AF4* (t 4; 11) and *SIL-TAL1* (Del 1p32).

We found that 82 (79%) of the adult ALL patients harbored one of the five fusion oncogenes with the distribution of *TCF3-PBX1* in 17 (16.3%) patients, *MLL-AF4* in 10 (9.7%), BCR-ABL in 21 (20.3%), *ETV6-RUNX1* in 5 (4.8%) and *SIL-TAL1* in 29 (27.9%) patients. There were significant differences in the frequency of some of these rearrangements when compared to reports from other parts of the world. For example Mancini et al found only one patient with *ETV6-RUNX1* fusion gene in a much bigger series, and hardly any patients with this abnormality in one of the largest studies in ALL, MRC UKALLXII/ECOG 2993 trial, while it was present in five of our patients indicating a much higher prevalence of this rearrangement in our population (Mancini et al., 2005; Moorman et al; 2007). Similarly *TCF3-PBX1* rearrangement was found in 17 (16.3 %) of our patients, again much more common than previously reported (Thomas et al., 2004; Mancini et al., 2005; Burmeister et al., 2010). The frequency of *MLL-AF4* rearrangement also appears to be higher in our patients (Mancini et al., 2005; Moorman et al; 2007). As expected, the CR rates of adult ALL varied among our patients harboring different fusion genes.

Among the various gene rearrangements, the t(4;11) (q21; q23)/*MLL-AF4* positive ALL is rarely observed in adult patients. In our adult ALL patients, *MLL-AF4* frequency was 9.7%. It was associated with FAB L1 or L2 morphology, immature immunophenotype, B-cell lineage, high leukocyte counts and poor outcome. The t(4;11) [*MLL-AF4*] associated disease is generally considered

to be a high risk ALL subtype, characterized by a poor clinical outcome (The Group Français De Cytogénétique Hématologique., 1996; Pui et al., 2008) which is in accordance with our results.

The frequency of BCR-ABL rearrangement in our patients is similar to other studies i.e., around 20-25%. Faiz et al. (2010) reported a higher frequency of BCR-ABL rearrangement in adult ALL patients from Pakistan. However, they only used RT-PCR for their fusion gene studies without confirmation of their PCR results using any validated techniques like cytogenetic analysis or FISH (Faiz et al., 2011). Iqbal and Akhtar used RT-PCR and cytogenetics interphase FISH techniques and reported the frequency of BCR-ABL to be around 50% in adult ALL patients (Iqbal and Akhtar., 2006). However, they studied the patients from North Western Frontier Province (now Khyber Pakhtunkhwa) and Federal Area of Pakistan (Iqbal, 2006), reflecting a totally different ethnic group and geographic area of Pakistan, which further strengthens our hypothesis of ethnic and geographic differences in the genetics of leukemia. These findings of Iqbal (2006) and Iqbal and Tanveer (2006) were again confirmed in another study by the same group using interphase FISH techniques (Iqbal et al., 2009).

BCR-ABL fusion gene was also associated with a poor prognosis in our study which was in accordance with previous findings (The Group Français de Cytogénétique Hématologique, 1996; Pui et al., 2008). Treatment with tyrosine kinase inhibitors (TKIs) in combination with chemotherapy has produced promising results in this subgroup of patients, although problems have emerged with drug resistance, and relapses are common without a stem cell transplant (Moorman et al., 2007; Soverini et al., 2007; Xing et al., 2012). Unfortunately TKIs were not available for our BCR/ABL+ patients which resulted in lower CR rates and relapse of most of the patients. Identification of this genetic entity at diagnosis and incorporation of TKIs in the treatment of BCR/ABL+ ALL is crucial for an optimal outcome (Fielding., 2010).

The t(12;21)[*ETV6-RUNX1*] is uncommon and detectable by FISH or PCR analysis in about 3 % of adults with B-ALL. Patients generally have a favorable prognosis (Burmeister et al., 2010). Survival of our patients with *ETV6-RUNX1* was better than the patients with other fusion genes.

TCF3-PBX1 (t 1;19) fusion gene may be found in up to 5% of adult ALL patients. We found a much higher frequency of this rearrangement (17/104, 16.3 %). It was more common in younger adults, organ infiltration was less common in this group of patients, and the bone marrow mostly showed L2 morphology. The immunophenotypic profile appears to be distinctive for this ALL subgroup and they usually have common ALL (CD10⁺, CD19⁺) profile. Because patients carrying this abnormality have a high relapse rate, they need to be identified at presentation and considered for intensified treatment protocols (Devaraj et al., 1995; Foa et al., 2003). However, recent studies indicate this adverse prognosis can be overcome by more intensive chemotherapy such that this subgroup of ALL is considered to have a favorable prognosis now ((Burmeister et al., 2010).

Various important prognostic factors in ALL other than genetic abnormalities include age of the patient, WBC count and response to initial therapy. We also found that age was an important prognostic factor in this cohort. The CR rate decreased from 90% for patients below the age of 30 years, to 14% for patients older than 50. There was a corresponding increasing risk of resistant disease with increasing age from 3% to 19% ($p=0.004$). The CR rate did not decrease significantly with increasing WBC count, although the 7 patients with WBC count more than $100 \times 10^9/l$ had a CR rate of only 52% but it was not statistically significant ($p=0.983$). Our findings are in accordance with the previously reported literature (The Group Français de Cytogénétique Hématologique., 1996; Pui et al., 2008; Burmeister et al., 2010).

This study shows the prevalence of different fusion genes and their association with clinical parameters as well as treatment outcome. It also indicates that based upon molecular cytogenetic evaluation at diagnosis and its integration with clinical characteristics, patients can be stratified into different prognostic groups. Previous studies have shown that treatment can be improved by more intensified treatment protocols at least in some of the ALL patient subgroups with poor outcome although high relapses may remain a problem (The Group Français de Cytogénétique Hématologique., 1996; Pui et al., 2008; Burmeister et al., 2010). Our data and its comparison with previous reports reflect the ethnic and geographic differences in biology and treatment of adult ALL, and indicates a strong interplay of genetic and environmental factors affecting the outcome of the disease. More comprehensive and large scale studies using advanced genetic techniques like microarray and whole genome sequencing are required to further explore the genetic heterogeneity of adult ALL and its correlation with disease biology and treatment outcome. This will further help in better prognostic stratification and improvement of treatment outcome.

In conclusion, this is the first study from Pakistan which investigated the frequency of 5 fusion oncogenes in adult ALL patients, and their association with clinical features, treatment response and outcome. Frequency of some of the oncogenes was different from reported elsewhere and they appear to be associated with distinct clinical characteristics and treatment outcome. This information will help in the prognostic stratification and risk adapted management of adult ALL patients.

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References

- Andreasson P, Schwaller J, Anastasiadou E, et al (2001). The expression of ETV6/CBFA2 (TEL/AML1) is not sufficient for the transformation of hematopoietic cell lines in vitro or the induction of hematologic disease *in vivo*. *Cancer Genet Cytogenet*, **130**, 93-104.
- Bassan R (2005). Evolving strategies for the management of high-risk adult acute lymphoblastic leukemia. *Haematologica*, **90**, 1299.
- Burmeister T, Gökbuğet N, Schwartz S, et al (2010). Clinical features and prognostic implications of *TCF3-PBX1* and *ETV6-RUNX1* in adult acute lymphoblastic leukemia. *Haematologica*, **95**, 241-6.
- Devaraj PE, Foroni L, Janossy G, et al (1995). Expression of the E2A-PBX1 fusion transcripts in t (1; 19) (q23; p13) and der (19) t (1; 19) at diagnosis and in remission of acute lymphoblastic leukemia with different B lineage immunophenotypes. *Leukemia*, **9**, 821-5.
- Faderl S, Kantarjian HM, Talpaz M, Estrov Z (1998). Clinical significance of cytogenetic abnormalities in adult acute lymphoblastic leukemia. *Blood*, **91**, 3995-4019.
- Faiz M, Iqbal QJ, Qureshi A (2011). High prevalence of BCR-ABL fusion transcripts with poor prognostic impact among adult ALL patients: report from Pakistan. *Asia Pac J Clin Oncol*, **7**, 47-55.
- Fielding AK (2010). How I treat Philadelphia chromosome-positive acute lymphoblastic leukemia. *Blood*, **116**, 3409-17.
- Foa R, Vitale A, Mancini M, et al (2003). E2A-PBX1 fusion in adult acute lymphoblastic leukaemia: biological and clinical features. *Br J Haematol*, **120**, 484-7.
- Harrison CJ, Foroni L (2002). Cytogenetics and molecular genetics of acute lymphoblastic leukemia. *Rev Clin Exp Hematol*, **6**, 91-113.
- Iacobucci I, Papayannidis C, Lonetti A, et al (2012). Cytogenetic and molecular predictors of outcome in acute lymphocytic leukemia: recent developments. *Curr Hematol Malig Rep*, **7**, 133-43.
- Iqbal Z (2006). Frequency of chromosomal abnormalities and corresponding fusion oncogenes in acute lymphoblastic leukemia (ALL) patients of Pakistan and its implication in differential diagnosis and prognosis of leukemia. *Haematologica*, **91**, 65.
- Iqbal Z, Tanveer A (2006) Incidence of different fusion oncogenes in acute lymphoblastic leukemia (ALL) patients from Pakistan: possible implication in differential diagnosis, prognosis, treatment and management of ALL. *Haematologica*, **91**, 64.
- Iqbal Z, Akhtar T, Iqbal M, et al (2009). First comprehensive report of strong interplay of genetic and environmental factors as well as high degree of ethnic and geographical variations in biology of leukemia as manifested by frequencies of common fusion oncogenes of prognostic significance associated with different Leukemic subtypes in Pakistani population. In: Online proceedings American Association for Cancer Research 100TH Annual Meeting, April 18-22, Colorado, Denver.
- Mancini M, Scappaticci D, Cimino G, et al (2005). A comprehensive genetic classification of adult acute lymphoblastic leukemia (ALL): analysis of the GIMEMA 0496 protocol. *Blood*, **105**, 3434-41.
- Moorman AV, Harrison CJ, Buck GA, et al (2007). Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): analysis of cytogenetic data from patients treated on the medical research council (MRC) UKALLXII/eastern cooperative oncology group (ECOG) 2993 trial. *Blood*, **109**, 3189-97.
- Pui CH, Evans WE (2006). Treatment of acute lymphoblastic leukemia. *N Engl J Med*, **354**, 166-78.
- Pui CH, Robison LL, Look AT (2008). Acute lymphoblastic leukaemia. *Lancet*, **371**, 1030-43.

- Pui CH, Sandlund JT, Pei D, et al (2004). Improved outcome for children with acute lymphoblastic leukemia: results of total therapy study XIII B at St Jude children's research hospital. *Blood*, **104**, 2690-6.
- Soverini S, Colarossi S, Gnani A, et al (2007). Resistance to dasatinib in Philadelphia-positive leukemia patients and the presence or the selection of mutations at residues 315 and 317 in the BCR-ABL kinase domain. *Haematologica*, **92**, 401-4.
- The groupe franGais de cytoghetique hematologique (1996). Cytogenetic abnormalities in adult acute lymphoblastic leukemia: correlations with hematologic findings and outcome. *Blood*, **87**, 3135-42.
- Thomas X, Boiron JM, Huguët F, et al (2004). Outcome of treatment in adults with acute lymphoblastic leukemia: analysis of the LALA-94 trial. *J Clin Oncol*, **22**, 4075-86.
- Van Dongen JJ, Macintyre EA, Gabert JA, et al (1999). Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. *Leukemia*, **13**, 1901-28.
- Xing H, Yang X, Liu T, et al (2012). The study of resistant mechanisms and reversal in an imatinib resistant Ph+ acute lymphoblastic leukemia cell line. *Leuk Res*, **36**, 509-13.