Frequency of Three Different Gene Mutations (TEL-AML1, E2A-PBX1 and MLL-AF4) In Acute Lymphoblastic Leukaemia

Abstract

Background and Objectives: Acute lymphoblastic leukemia (ALL) is very common in Pakistan. It is a complex genetic disease involving many fusion oncogene (FO) having prognostic significance. Its incidence may not be uniform in different parts of world because of racial, ethnic and environmental variations. These mutations have important implications for prognosis, drug selection and treatment outcome. It shows that studies on fusion oncogenes become very important for risk stratification and planning of treatment at the start of diagnosis.

Method: We studied fusion oncogenes in 120 pediatric ALL patients from less than one to twenty years of age using RT-PCR and their association with age and gender. It was a cross sectional study carried out in Haematology department, Armed Forces Institute of Pathology, Rawalpindi, Pakistan over a period of one year from 1 Jan 2015 to Dec 2015. Three most common fusion genes i.eE2A-PBX1 t(1;19), TEL-AML1 t(12;21), MLL-AF4 t(4;11) were studied with relevance to their age and gender.

Results: Frequency of MLL-AF4 mutation was found in 5(4.2%) patients. TEL-AML1 was found in 13(10.8%) patients. E2A-PBX1 mutation was found in 12(10%) patients.

Conclusions: These mutations are quite frequent in our ALL patients with different age specificities. Frequencies of some of the oncogenes were different from those reported from other areas of Pakistan. These mutations are helpful in risk stratification and have therapeutic and prognostic significance.

Keywords: Acute Lymphoblastic Leukemia; Mutations; MLL-AF4; TEL-AML1; E2A-PBX1

Abbreviations: ALL: Acute Lymphoblastic Leukemia; FO: Fusion Oncogene

Introduction

Acute lymphoblastic leukemia (ALL) is a neoplasm of lymphoid progenitors that may be of B or T cell lineage (B-ALL, T-ALL) and is most common malignancy of childhood [1]. Its incidence varies throughout the world ranging from 0.9-4.7 per 100,000 children per year [2]. ALL comprises of eighty percent of childhood acute leukemias and there is a striking incidence peak between the ages two to seven years where the incidence is as high as 10 per 100,000 children [3].

The outcome of ALL has improved dramatically in recent decades with cure rates exceeding 80% [4]. However 20% of patients relapse, which carries poor prognosis [4]. Treatment results remain unsatisfactory in adults, with a poor overall prognosis and a long term probability of survival less than 40%. Biological differences in the leukemogenesis between adult and childhood ALL may be the explanation [4].

Genetic abnormalities play a major role in the prognosis and treatment outcome of ALL. It is characterized by various recurring genetic alterations including aneuploidy, structural rearrangements that commonly results in expression of chimeric fusion genes (TEL-AML1), (E2A-PBX1), (BCR-ABL) and rearrangements of MLL are important categories [5,2]. Hypodiploidy and MLL are associated with poor prognosis and high risk of relapse [4, 5]. Different ethnic groups have different genetic mutations.

ALL with TEL-AML1 also called (ETV6-RUNX1) t(12; 21). It carries good prognosis responding well to the treatment. Its frequency in Pakistani population is about 17.8 % [3]. Some author’s claim that this translocation occur prenatally, as 1% of the cord blood cells contained this mutation which later lead to leukemia.

ALL with E2A-PBX1 rearrangements t(1;19) contains transcriptional activation domains of E2A linked to DNA binding domain of PBX1 and the encoded protein inappropriately activates the transcription of genes normally regulated by PBX1 [6]. Patients with this gene fusion frequently present with hypocalcaemia and
coagulopathy and have poor prognosis. Its incidence also varies worldwide and in a Mexican study its incidence is reported to be 11.5% [2].

Structural alterations involving band 11q23 of chromosome 11 are the frequent cytogenetic abnormality in infant ALL reaching up to 80% [5]. In most cases target is MLL gene (mixed lineage leukaemia gene). ALL with MLL gene rearrangements carries bad prognosis with worst outcome in infants. Its incidence is reported to be 16.8% [3] from Pakistan. Patients present with high WBC count and CNS involvement.

Treatment strategy can be planned at the beginning of diagnosis if these genetic abnormalities are known. Patients are divided into low, standard and high risk. Low risk patients are given induction therapy with regimen A, standard risk patients are treated with regimen C, of UKALL 11 protocol. Intensity of the chemotherapy drugs along with their side effects increases in same order. Poor risks are than offered bone marrow transplant after induction, if they are in remission because of high risk of relapse. Treatment of other groups lasts for 2-3 years [4].

**Objective**

We conducted the study to find out frequencies of these mutations i.e. E2A-PBX1 t(1; 19), TEL-AML1 t(12; 21) and MLL-AF4 t(4; 11) in our setup.

**Materials and Methods**

Peripheral blood and bone marrow samples were obtained from pediatric ALL patients admitted to different hospitals of Rawalpindi and Islamabad. They belonged to different ethnic groups. Samples were collected from January 2015-2016. Patients between the ages of less than one to twenty years with confirmed diagnosis of ALL were included. These patients were newly diagnosed with no previous history of any treatment and did not have a prior severe physical illness. 120 samples were processed for molecular Cytogenetics. We studied 3 fusion oncogenes in 120 ALL patients using RT-PCR at the time of diagnosis. The clinical data were recorded at the time of diagnosis.

**RNA Extraction**

Total RNA was extracted from leukemic cells by TriZol LS 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. RT reaction protocol carried out briefly, 3-5μl of RNA was added to 8μl of RT mix containing RiboLock™ RNase inhibitor, M-MuLV reverse transcriptase (Fermentas, USA), random hexamer and reaction was carried out by incubating mixture at 42°C for 60 min, 70°C for 10 min and held at 4°C in the last step.

The cDNA product was amplified with Taq polymerase. Amplification was carried out by Real time PCR in two steps, in first step there is initial denaturation, in second step there are 40 repeated cycles of denaturation, annealing & extension. Fluorescence reading was taken at 60°C step. Amplification plots were generated on ABI 7500 Real Time PCR system (Figure 1 and 2).

**Statistical Analysis**

All the collected data was entered into SPSS version 20.

1. Numerical variables; age, Hb, TLC and percentage of blasts have been presented by mean ±SD.
2. Categorical variable i.e. gender and positive gene mutations on PCR (TEL-AML1, E2A-PBX1 and MLL-AF4) have been presented by frequency and percentage.

**Results**

A total of 120 patients were included in the study. The mean age of the patients was 8.68±6.51 years and there were 68(56.7%) male and 52(43.3%) female patients in the study group. The mean
Frequency of Three Different Gene Mutations (TEL-AML1, E2A-PBX1 and MLL-AF4) In Acute Lymphoblastic Leukaemia

E2A-PBX1 mutation was found in 12(10%) patients. The frequency of E2A-PBX1 was significantly higher in patients aged between 15-20 years. However, there was no statistically significant difference in the frequency of E2A-PBX1 among male patients (14.7% vs. 5.8%; p=.119) as compared to female patients but the difference was statistically insignificant.

MLL-AF4 mutation was found in 13(10.8%) patients. Our results match with those of another local study by Siddiqui R, et al. [7] who observed MLL-AF4 in 5% of pediatric patients with ALL in local population [7]. However much higher frequency has also been reported in local population by Awant, et al. [4] (16.8%) and Faiz M, et al. [8] (14.0%) [3,8]. In a study by Trka J, et al. [17] from Czech republic its incidence is 3.9%. [17]. In another international study its frequency was 39.9% by Janeen JW, et al. [13]. Pandita A, et al. [14] reported its frequency to be 10% [14].

When stratified, all of the MLL-AF4 positive patients were under 2 years of age. Awant, et al. [3] in 2012 however observed two peaks of MLL-AF4 first under 2 years of age (29.4%) and second peak in 8-15 years of age (58.8%) [16]. The frequency of MLL-AF4 mutation was insignificantly higher among female patients (4:1; 7.7% vs. 1.5%; p=.091) as compared to males.

MLL-AF4 mutation was found in 5(4.2%) patients. Results match with those of other local studies by Siddiqui R, et al. [7] who observed MLL-AF4 in 5% of pediatric patients with ALL in local population [7]. However much higher frequency has also been reported in local population by Awant, et al. [4] (16.8%) and Faiz M, et al. [8] (14.0%) [3,8]. In a study by Trka J, et al. [17] from Czech republic its incidence is 3.9%. [17]. In another international study its frequency was 39.9% by Janeen JW, et al. [13]. Pandita A, et al. [14] reported its frequency to be 10% [14].

Discussion

ALL is a heterogenous disease and comprises of many different gene mutations. It is likely to be due to racial and geographic variations and their interactions with environment can lead to difference in their frequencies. These fusion oncogenes usually act by producing different transcriptional factors and act by different cellular pathways [16]. According to genetic abnormalities, patients are divided into low, standard and high risk groups. Armed Forces Institute of Pathology is a big institute in north of the country and it receives patients from very different ethnic background, including patients from tribal areas and Peshawar which are Pathan in origin and patients from Gilgit, Baltistan and Abbottabad and from north of Punjab.

Most of the studies which are carried out on genetic mutations are on BCR-ABL fusion oncogene whose poor prognosis is already well established. Not much work has been done on other fusion oncogenes in this part of the country on large scale.

The mean age of the patients was 8.68±6.51 years and there were 68(56.7%) male and 52(43.3%) female patients in the study group. Iqbal Z [16] (64.7%), Awant, et al. [3] (69.3%) and Faiz M, et al. [8] (73.57%) observed similar male predominance among ALL patients in local population [3, 16, 8]. The hemoglobin of the patients ranged from 5.2g/dl to 10.1g/dl with a mean of 8.00±1.36g/dl. Faiz M, et al. [8] observed hemoglobin in the range of 2.3g/dl to 13.4g/dl with a median of 7.7g/dl [8]. The mean total leukocyte count was 180889.58±195714.63/mm³ and the mean percentage of blasts was 68.44±18.82% in this study.

Table 1: Age related frequency of ALL Mutations in Pakistan (n=120)

| Mutations | Frequency (n) | Percentage | Mean Age (Years) |
|-----------|--------------|------------|------------------|
| TEL-AML1  | 13           | 10.80%     | 14               |
| MLL-AF4   | 5            | 4.20%      | < 2              |
| E2A-PBX1  | 12           | 10.05%     | 15 - 20          |

Table 2: Comparison of Observed Data with Previous Studies of Pediatric ALL Conducted in Pakistan (Percentages)

| Fusion Oncogene | Present Study | Awant T, et al. [3] | Siddiqui R, et al. [7] | Faiz M, et al. [8] |
|-----------------|---------------|---------------------|------------------------|-------------------|
| TEL-AML1        | 10.8          | 17.8                | 3.5                    | 9.7               |
| MLL-AF4         | 4.2           | 16.8                | 5                      | 14                |
| E2A-PBX1        | 10            | 1.9                 | 0                      | 2                 |

Table 3: Comparison of data with international studies

| Gene          | Present Study | Awant T, et al. [3] | Siddiqui R, et al. [7] | Faiz M, et al. [8] |
|---------------|---------------|---------------------|------------------------|-------------------|
| TEL-AML1      | Present Study | 10.20%              |                        |                   |
|               | Mazdaumi SH, et al. [9] | 5%               |                        |                   |
|               | Rahman SA, et al. [10] | 7%               |                        |                   |
|               | Chung HY, et al. [11] | 17.90%           |                        |                   |
|               | Mesquita DR, et al. [12] | 19%             |                        |                   |
| MLL-AF4       | Present Study | 4.20%              |                        |                   |
|               | Pandita A, et al. [14] | 10%              |                        |                   |
| E2A-PBX1      | Present Study | 10%                |                        |                   |
|               | Pandita A, et al. [14] | 5%               |                        |                   |
|               | Jiménez-Moraes S, et al. [15] | 11.50%         |                        |                   |

Citation: Khan A, Ayub M, Ahmed S, Altaf CH, Malik HS (2016) Frequency of Three Different Gene Mutations (TEL-AML1, E2A-PBX1 and MLL-AF4) In Acute Lymphoblastic Leukaemia. Hematol Transfus Int J 2(4): 00045. DOI: 10.15406/hti.2016.02.00045
Frequency of Three Different Gene Mutations (TEL-AML1, E2A-PBX1 and MLL-AF4) In Acute Lymphoblastic Leukaemia

Khan A, Ayyub M, Ahmed S, Altaf CH, Malik HS (2016) Frequency of Three Different Gene Mutations (TEL-AML1, E2A-PBX1 and MLL-AF4) In Acute Lymphoblastic Leukaemia. Hematol Transfus Int J 2(4): 00045. DOI: 10.15406/htij.2016.02.00045

References
1. De Oliveira BM, Valadares MT, Silva MR, Viana MB (2011) Compliance with a protocol for acute lymphoblastic leukemia in childhood. Rev Bras Hematol Hemoter 33(3):185-189.
2. Zhang J, Mullighan CG, Harvey RC, Wu G, Chen X, et al. (2011) Key pathways are frequently mutated in high-risk childhood acute lymphoblastic leukemia: a report from the Children's Oncology Group. Blood 118(11): 3080-3087.
3. Awan T, Iqlbal Z, Aleem A, Sabir N, Absar M, et al. (2012) Five most common prognostically important fusion oncogenes are detected in the majority of Pakistani pediatric acute lymphoblastic leukemia patients and are strongly associated with disease biology and treatment outcome. Asian Pac J Cancer Prev 13(11): 5469-5475.
4. Pui CH, Pei D, Sandlund JT, Ribeiro RC, Rubnitz JE, et al. (2010) Long-term results of St Jude Total Therapy Studies 11, 12, 13A, 13B and 14 for childhood acute lymphoblastic leukemia. Leukemia 24(2): 371-382.
5. Bernt KM, Armstrong SA (2011) Targeting epigenetic programs in MLL-rearranged leukemias. Hematology Am Soc Hematol Educ Program 2011: 354-360.
6. Sabir N, Iqlbal Z, Aleem A, Awan T, Naem T, et al. (2012) Prognostically Significant Fusion Oncogenes in Pakistani Patients with Adult Acute Lymphoblastic Leukemia and their Association with Disease Biology and Outcome. Asian Pac J Cancer Prev 13(7): 3349-3355.
7. Siddiqui R, Nancy N, Naing WP, Ali S, Dar L, et al. (2010) Distribution of common genetic subgroups in childhood acute lymphoblastic leukemia in four developing countries. Cancer Genet Cytogenet 200(2): 149-153.
8. Faiz M, Qureshi AM, Qazi JI (2011) Molecular characterization of different fusion oncogenes associated with childhood acute lymphoblastic leukemia from Pakistan. JAVMS 5(5): 497-507.
9. Mazloumi SH, Madhumathi DS, Appaji L, Prasannakumari (2012) Combined study of cytogenetics and fluorescence in situ hybridization (FISH) analysis in childhood acute lymphoblastic leukemia (ALL) in a tertiary cancer centre in South India. Asian Pac J Cancer Prev 13(8): 3825-3827.
10. Rahmani SA, Ardabili SM, Aghaazadeh A, Jahani M, Izadyar M, et al. (2006) Investigation of TEL-AML1 and BCR-ABL fusion oncogenes in patients affected by acute lymphoblastic leukemia using interphase in situ Hybridization. J. Sci. I. R. Iran 17(1): 17-25.
11. Chung HY, Kim KH, Jun KR, Jang S, Park CJ, et al. (2010) Prognostic significance of TEL/AML1 rearrangement and its additional genetic changes in Korean childhood precursor B-acute lymphoblastic leukemia. Korean J Lab Med 30(1): 1-8.
12. Mesquita DR, Córdoba JC, Magalhães IQ, Córdoba MS, Oliveira JR, et al. (2009) Molecular and chromosomal mutations among children with B-lineage lymphoblastic leukemia in Brazil's Federal District. Genet Mol Res 8(1): 345-353.
13. Janssen JW, Ludwig WD, Borkhardt A, Spadinger U, Rieder H, et al. (1994) Pre-pne-B acute lymphoblastic leukemia: high frequency of alternatively spliced ALL1-AF4 transcripts and absence of minimal residual disease during complete remission. Blood 84(11): 3835-3842.
14. Pandita A, Harish R, Digra SK, Raina A, Sharma A (2015) Molecular cytogenetics in childhood acute lymphoblastic leukemia: a hospital-based observational study. Clin Med Insights Oncol 9: 39-42.
15. Jiménez-Morales S, Peralta EM, Saldaña Alvarez Y, Vera PP, Aguilera RP.

21, 22]. Our finding supports the observation of Mesquita DR, et al. [12] that TEL-AML1 has low incidence in developing countries which may be associated with poor living standards in these countries [12].

When stratified, the frequency of TEL-AML1 was significantly higher in patients aged between 9 to 14 years as shown in table 1. Our observation match with that of Faiz M, et al. [8] who observed 90% of the patients with TEL-AML1 mutation to be aged around 10 years [8]. The frequency of TEL-AML1 was also higher among male patients (3.33:1; 14.7% vs. 5.8%; p=.119) as compared to female patients but the difference was statistically insignificant. Iqlbal Z [16] (2:1) and Faiz M, et al. [8] (4:1) also observed similar male predominance among patients of TEL-AML1 mutation [16, 8].

E2A-PBX1 mutation was found in 12(10%) patients. Jiménez-Morales S, et al. [15] observed a similar frequency of 11.5% in Mexican children with ALL [15]. However, Faiz M, et al. [8] observed E2A-PBX1 mutation in only 2% of patients with ALL in local population [8]. Pandita A, et al. [14] reported its frequency to be 5% [14].

The frequency of E2A-PBX1 was significantly higher in patients aged between 15-20 years. A similar age distribution was observed by Jiménez-Morales S, et al. [15]. However, there was no statistically significant difference in the frequency of E2A-PBX1 among male (1:1; 8.8% vs. 11.5%; p=.623) and female genders. Jiménez-Morales S, et al. [15] however observed much higher frequency of E2A-PBX1 among male patients (5:1) in Mexican patients of ALL [15].

Thus mutations MLL-AF4, TEL-AML1 and E2A-PBX1 were detected in 5(4.2%), 13(10.8%) and 12(10%) patients respectively. MLL-AF4 was seen in children ≤2 years of age. Frequency of TEL-AML1 was highest in 9-14 years and that of E2A-PBX1 was highest in 15-20 years old patients.

Though the results of our study are in line with other local studies in some aspects, yet there are considerable differences. Similar reports about ethnic differences in the disease biology, genetics and treatment outcome have been reported in adult ALL by Sabir N, et al. [23]. These differences can be attributable to population and geographical differences as hypothesized by Mesquita DR, et al. [12]. Considering these differences with other populations and with the local studies with in same population, there is needed to perform a multicenter study with much larger sample size for better estimate of these mutations.

Conclusion
Mutations MLL-AF4, TEL-AML1 and E2A-PBX1 are quite frequent in our ALL patients with different age specificities however they have variable frequencies in different geographical areas. These mutations are helpful in risk stratification and have therapeutic and prognostic significance. In order to benefit from targeted therapy a comprehensive knowledge of these mutations should be known in every population.

Acknowledgement
“We are grateful for the tech support provided by Kazim Shah, Muhammad Ibrahim and Nayar Khan.”

Citation: Khan A, Ayyub M, Ahmed S, Ataf CH, Malik HS (2016) Frequency of Three Different Gene Mutations (TEL-AML1, E2A-PBX1 and MLL-AF4) In Acute Lymphoblastic Leukaemia. Hematol Transfus Int J 2(4): 00045. DOI: 10.15406/htij.2016.02.00045
et al. (2008) BCR-ABL, ETV6-RUNX1 and E2A-PBX1: prevalence of the most common acute lymphoblastic leukemia fusion genes in Mexican patients. Leuk Res 32(10): 1518-1522.

16. Iqbal Z (2014) Molecular genetic studies on 167 pediatric ALL patients from different areas of Pakistan confirm a low frequency of the favorable prognosis fusion oncogene TEL-AML1 (t 12; 21) in underdeveloped countries of the region. Asian Pac J Cancer Prev 15(8): 3541-3546.

17. Trka J, Zuna J, Haskovec C, Brabencová A, Kalinová M, et al. (1999) Detection of BCR/ABL, MLL/AF4 and TEL/AML1 hybrid genes and monitoring of minimal residual disease in pediatric patients with acute lymphoblastic leukemia. Cas Lek Cesk 138(1): 12-17.

18. Iqbal Z, Iqbal M, Akhter T (2007) Frequency of BCR-ABL fusion oncogene in Pakistani childhood acute lymphoid leukemia (ALL) patients reflects ethnic differences in molecular genetics of ALL. J Pediatr Hematol Oncol 29(8): 585.

19. Faiz M, Qazi JI (2010) t (12:21) is underrepresented in childhood B-lineage acute lymphoblastic leukemia in Punjab, Pakistan. J Pediatr Hematol Oncol 12(3): 249-251.

20. Tsang KS, Li CK, Chik KW, Shing MM, Tsui WC, et al. (2001) TEL/AML1 rearrangement and the prognostic significance in childhood acute lymphoblastic leukemia in Hong Kong. Am J Hematol 68(2): 91-98.

21. Lazic J, Tosic N, Dokmanovic L, Krstovski N, Rodic P, et al. (2010) Clinical features of the most common fusion genes in childhood acute lymphoblastic leukemia. Med Oncol 27(2): 449-453.

22. Papadhimitriou S, Patenktis GS, Parcharidou A, Tsakiridou A, Papadakis V, et al. (2008) TEL-AML1 acute lymphoblastic leukemia in greek pediatric population. Pediatrics 121(supple 2): S111-S112.

23. Sabir N, Iqbal Z, Aleem A, Awan T, Naeem T, et al. (2012) Prognostically significant fusion oncogenes in Pakistani patients with adult acute lymphoblastic leukemia and their association with disease biology and outcome. Asian Pac J Cancer Prev 13(7): 3349-3355.

Citation: Khan A, Ayyub M, Ahmed S, Altaf CH, Malik HS (2016) Frequency of Three Different Gene Mutations (TEL-AML1, E2A-PBX1 and MLL-AF4) In Acute Lymphoblastic Leukaemia. Hematol Transfus Int J 2(4): 00045. DOI: 10.15406/htij.2016.02.00045