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Research Article

# Frequency of MLL Gene Rearrangement AF4 t(4;11) in Adult Acute Lymphoblastic Leukemia - A Single Institute Experience

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**Keywords**
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**Abstract**

**Background:** Mixed lineage leukemia (MLL) is one of the most frequently involved genes in hematologic malignancies, in particular in some forms of acute leukemia, both lymphoblastic and myeloid. In adult ALL, MLL gene re-arrangement is found in 7% cases as reported in previous studies. MLL gene rearrangement is associated with poor prognosis. MLL gene rearrangements arise from fusions of this gene at 11q23 with a large number of partner genes. In ALL, the most common gene partner is the AF4 gene on chromosome 4q21 resulting in a t(4; 11) (q21; q23) rearrangement.

**Objective/Rationale:** This study was planned to estimate the frequency of MLL gene rearrangement AF4 t(4;11) in patients of acute lymphoblastic leukemia because this rearrangement is associated with unfavorable prognosis.

**Material and methods:** It was a cross-sectional observational study. All newly diagnosed cases of adult ALL were included in the study. Total 101 patients were included, 88 had B-cell lineage ALL and 13 had T-Cell lineage ALL. Institutional review board approved the study. Informed consent was obtained and details were noted in a Performa including age, gender, clinical features, complete blood count (CBC), and bone marrow aspirate and biopsy report. Diagnosis was done on the basis of WHO classification of neoplasm 2008. RT- PCR method was applied to detect MLL gene rearrangement AF4 t(4;11).

**Results:** Out of 101 patients, 34 were females and 67 males, with mean age of 34.5 ± 18.9 (range 18-50 years in both females & males). MLL gene rearrangement AF4 t(4;11) was detected in 08(7.9%) patients of ALL. The mutation was observed in 03(37.5%) males out of 67 and 05(14.7%) females out of 34. Significantly more MLL gene rearrangement was found in patients with pre-B-Cell ALL (75%), then in pre-T-Cell ALL (25%).

**Conclusion:** MLL gene rearrangement AF4 t(4;11) has been detected in 7.9% cases of ALL in our study. Further larger studies are needed to validate our data and follow up required for elucidation of clinical significance of mutation in this group of patients.

## ABBREVIATIONS

CBC: Complete Blood Count; MLL: Mixed Lineage Leukemia; ALL: Acute Lymphoblastic Leukemia; RT-PCR: Reverse Transcriptase Polymerase Chain Reaction; WBC: White Blood Cell; MDS: Myelodysplastic Syndrome; RBC: Red Blood Cell; TLC: Total Leucocyte Count; Hb: Hemoglobin; LDH: Lactate Dehydrogenase; CNS: Central Nervous System; HSCT: Hematopoietic Stem Cell Transplantation

## INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a heterogeneous disease, both in terms of its pathogenesis and the groups of peoples that it affects. Pathogenesis of this disease involves a number of deregulated pathways controlling cell proliferation and differentiation. Acute lymphoblastic leukemia (ALL) comprise for less than 20% of adult acute lymphoblastic leukemia [1-5]. The t(4;11)(q21;q23) is a frequent cytogenetic

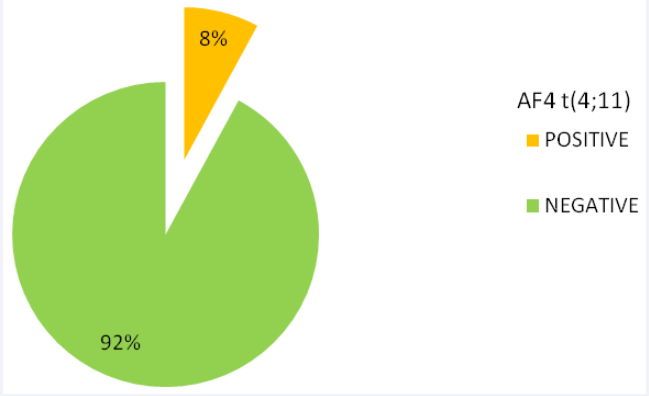


**Figure 1** Frequency of AF4 t(4;11) in All Patients N=101.

abnormality seen in this disease [6]. This translocation fuses the ALL1 (MLL, HRX, Hrtx1) gene to the AF4 gene. This fusion gene identifies a subset of ALL with aggressive clinical course and poor outcome [7,8]. In addition, the ALL1 (MLL)/AF4 fusion is associated with a pro-B immunophenotype [9]. Rearrangements involving 11q23/MLL locus are frequently encountered in acute leukemia. About 70%-80% of infant acute leukemia has 11q23/MLL abnormalities, associated with a distinct genetic profile and poor prognosis [10]. The most common translocations involving 11q23/MLL include t(9;11) (p22;q23), t(4;11) (q21;q23), and t(11;19) (q23;p13) [11].

The t(4;11) translocation is associated with unfavorable presenting features such as very high white blood cell (WBC) count [12,13], and high blast cell count. Due to this association with age, in infancy the pro-B immunophenotype and t(4;11) have often been associated with poor prognostic factors.

Chromosomal rearrangements involving the mixed-lineage leukemia (MLL) gene (also known as ALL-1, HRX, and HTRX1), located at the chromosomal band 11q23, have been associated with many different types of hematological malignancies [14].

They include reciprocal translocations, inversions and segmental insertions. These rearrangements result in the generation of in frame fusion transcripts with various partner genes from more than 60 distinct gene loci [14].

The MLL gene forms many fusions genes with a variety of partner genes by chromosome translocations, and form abnormal fusion genes in leukemogenesis [15-18]. Currently, up to 70 different partners of MLL fusion genes have been confirmed, all correlated with high risk acute leukemia [19,20].

## MATERIALS AND METHODS

This study was conducted at the National Institute of Blood Diseases and Bone Marrow Transplantation (NIBD). The study was approved from the institutional ethics committee. The non-probability sampling technique was used to include the samples in the study. Informed consent was obtained from patients prior to enrolment into the study and a Performa was subsequently filled which consisted of the following details: age, gender, clinical features, CBC, bone marrow aspirate and biopsy report details if available.

### Inclusion criteria

Newly diagnosed cases of ALL patients between 18-50years age including both male and female were included in this study.

### Exclusions criteria

Patients diagnosed as ALL receiving treatment, de novo cases of acute myeloid leukemia, and those who developed acute leukemia from MDS were excluded. Patients who received conventional chemotherapy or on any tyrosine kinase inhibitor were also excluded.

### Data collection procedure

**RNA extraction:** RNA was extracted from whole blood using triazole method; following procedure was followed:

Two ml blood was taken in a tube. RBC lysis buffer was added in a ratio of 1:3 and kept on ice for 15-20 minutes. Centrifugation was done at 1400 rpm for 10 minutes at 4°C. Supernatant was carefully decanted. It was transferred in 1.5 ml micro centrifuge. Further centrifugation was performed for 3 minutes at 3000 rpm at 4°C. After carefully decanting the supernatant 800-1000ul trizole solution was added and re-suspended. After addition of 200ul chloroform ($CHCl_3$), it was vortexes for 15 second. Then the sample was allowed to stand on ice for 5-10 minutes. Centrifugation at 1400rpm for 15 minutes at 4°C was done again. Equivalent volume of ice-cold isopropanol was added and vortexed for 15 seconds. Centrifugation process repeated again at 1400rpm for 15 minutes at 4°C and supernatant was carefully removed. The pellet was rinsed with 500ul ice-cold 75% ethanol. Centrifugation was done again at 7500 rpm for 8 minutes at 4°C and supernatant was carefully removed. RNA pellet was formed using vacuum dryer for about 10 minutes. RNA pellet was re-suspended in 20-50ul RNA free water. It was incubated on heat block at 55-60°C for 10 minutes and stored at -80°C. cDNA (complementary DNA) was isolated from RNA using AFFIMETRIX First-strand cDNA synthesis kit.

**PCR mix preparation:** We took patients' cDNA 2ul + qPCR mix (2x) 10ul + MLL/AF4 probe/primer mix 5ul + water (nuclease free). Pipetting gently up and down several times and the PCR strips placed in real time instrument mixed it.

**Real time PCR Procedure:** Using ANA GENE BIOTECH and by labeling the slot as per strip label, plate setup was programmed. The sequence of labeling of slots should be the same way as strips kept in the machine. We placed the strips in the thermal cycler to run 40 cycles. We selected the channel for acquisition (Green/Yellow). FAB green channel for translocation detection and HEX yellow channel for internal control.

## RESULTS

Out of a total 101 ALL patients 34(33.6%) were females and 67(66.3%) were males. The mean age was 34.95 ± 18.92years (range: 18-50 years); in male 34.44 ± 18.45 years, while in female the mean age was 34.63 ± 18.35years. Table 1 shows the mean age, Hb, TLC, platelet count and percentage of blast count with 95% confidence interval, range and median inter-quartile range. Of 101 patients, 86 patients were of B-Cell lineage and 15 were T-Cell lineage according to the WHO classification of Acute Lymphoid Neoplasm 2008.

**Table 1**: Descriptive Statistics of Variables.

| Variables | Mean ± SD | 95%CI | Median (IQR) | Max-Min | AF4 t(4;11) Positive (n=08) Mean ± SD | AF4 t(4;11) Negative (N=93) Mean ± SD |
|---|---|---|---|---|---|---|
| Age (years) | 27.41 ± 10.6 | 25.3 to 29.5 | 23(16) | 50-18 | 25.1 ± 12.3 | 27.6 ± 10.56 |
| Hb (gm/dl) | 9.6 ± 2.54 | 9.1 to 10.1 | 9.8(3.9) | 15-3.7 | 8.7 ± 2.31 | 7.89 ± 2.26 |
| TLC (x $10^9$/l) | 43.5 ± 94.3 | 24.9to 62.1 | 7.4(20.3) | 431-0.4 | 114.7 ± 105.1 | 38.12 ± 54.5 |
| Plt (x $10^9$/l) | 89.4 ± 105.6 | 68.5-110.2 | 42(107) | 463-0 | 65.5 ± 95.9 | 53.61 ± 83.2 |

Figure 1 shows the result of frequency of MLL gene rearrangement AF4 t(4;11). Out of total 08(8%) positive for the mutation 03(37.5%) males and 05(62.5%) were females. The mutation was seen in 06(75%) out of 86 pre-B-Cell ALL and 02 (25%) out of 15 pre-T-Cell ALL. Analysis of the MLL gene mutation AF4 t(4;11) status was also done in different age groups shown in Table 2.

## DISCUSSION

The presence of t(4;11)(q21;q23) with expression of the fusion gene MLL AF4 characterizes a subset of ALL with aggressive clinical features. These patients, at the time of diagnosis, frequently have an elevated WBC, massive hepatosplenomegaly or lymphadenopathy, higher lactate dehydrogenase (LDH) values, and frequent Central Nervous System (CNS) involvement, with a poor clinical outcome both in infants and in adults [21,23].

Cytogenetic and molecular analysis of leukemic cells at diagnosis is necessary for the prognostic stratification of ALL patients at diagnosis of disease because these factors are independent of predicting clinical outcome of patients. Stratification of ALL patients according to cytogenetic and molecular characterization helps in establishing the best post remission therapy for individual patients, including the possibility of consolidation treatment, intensification and allogeneic hematopoietic stem cell transplantation (HSCT).

Our study has several limitations. The first and major problem is that analyses were based on observational studies rather than prospective controlled studies. The results reported here should therefore be interpreted carefully by physicians, WBC counts and ratios of favorable cytogenetics were not identified as sources of heterogeneity, we still cannot rule out the potential effect of other factors, such as differences in treatment, distribution of the intermediate and adverse cytogenetic aberrations, which were not examined in our analysis.

There are, nevertheless, a number of findings that are reproducible across the studies. For example, in terms of the clinical features at presentation, the most striking finding is the association between the presence of a t(4;11), a high WBC and massive hepatosplenomegaly. Consistent with this, a number of studies have also shown that t(4;11) are associated with a high serum level of (LDH) at diagnosis, although this data is not available from the majority of studies. About 5% of adult B-Cell acute lymphoblastic leukemia (B-ALL) are characterized by t(4;11)(q21;q23), which confers peculiar features to this B-ALL subtype, including a very immature immunophenotype and poor prognosis [20].

**Table 2:** Frequency of AF4 t(4;11) in All Patients with Respect to Age (N=101)

| Age Groups | N | AF4 t(4;11) | Percentage |
|---|---|---|---|
| **≤ 34** | 75 | 6 | 8% |
| **> 34** | 26 | 2 | 7.60% |

In this study, MLL gene rearrangement AF4 t(4;11) positive rate was 7.9 % (08), and this is near to previous report[1]. In this study large population of patients were pre-B cell, ALL which are more prone to have AF4 t (4;11) as describe in previous studies. In other paper published in Italy by F. Marchesi, K. Girardi, and G. Avvisati also indicates 2-7% of patients of acute lymphoblastic leukemia(ALL) showing t(4;11)(q21;q23)/MLL-AF4 positivity [21-23].

Our study is comprised of small pool of patients to compare different characteristics likes age, gender and types of ALL with others studies. In this study positive cases included 03(37.5%) males and 05(62.5%) females; all are adults, which is also not similar with previous studies. One study of Tamai H in 2010 have suggested that higher incidence of MLL gene rearrangement AF4 t(4;11) in younger compared to older ALL patients.

Referring from the aggressiveness of AF4 t(4;11) positive ALL in this study, different treatment strategy is required for treatment of this selective population. Considering the fact that molecularly targeted agents is blocking specific pathway, specifically targeted molecular agents can be a potential option in this patient population.

## CONCLUSION

MLL gene rearrangement AF4 t(4;11) has been detected in 7.9% cases of ALL in our study. Following the discovery of MLL gene rearrangement AF4 t(4;11) in patients was associated with aggressive clinical behavior. Patients with MLL gene rearrangement t(4;11) at diagnosis frequently associated with elevated white blood count, massive hepatosplenomegaly or lymphadenomegaly, frequent Central Nervous System (CNS) involvement and higher LDH values. Furthermore, in view of the limitations of current prognostic risk stratification in ALL, there is also considerable interest in whether MLL gene rearrangement AF4 t(4;11) were a useful molecular marker for predicting response to therapy and long-term outcome in patients with ALL.

Further larger studies are needed to establish MLL gene rearrangement a mandatory test for all patients and follow up required for elucidation of clinical significance of mutation in this group of patients.

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