

Frequency and clinical impact of *ETV6/RUNX1*, *AF4-MLL*, and *BCR/ABL* fusion genes on features of acute lymphoblastic leukemia at presentation

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Abstract

Background: Variations in disease presentation and outcome of leukemia treatment has been associated with the presence of certain mutant genes. Three major translocations (*ETV6-RUNX1*, *BCR-ABL*, and *AF4-MLL*) in acute lymphoblastic leukemia (ALL) have been shown to affect treatment outcome. This study is aimed at assessing the relationship between these translocations and the presence of other indicators of disease severity (white cell count, hemoglobin concentration, platelet count, and hematocrit) in ALL.

Patients and Methods: Forty chemotherapy naïve patients aged between 9 months and 54 years had their marrow samples analyzed for the prevalent mutations. Their clinical and laboratory details on presentation were also obtained.

Results: Abnormal genes detected were *BCR/ABL 1* major transcript in 5 (12.5%), *ETV6/RUNX1* in 2 (5.0%), *MLL/AF4* none and none of the patients had more than one fusion gene. There was no relationship between the presence of these fusion genes and the clinical and laboratory features of ALL. An association exists between the fusion genes and ethnic origin of the patients ($P = 0.005$). There is no significant association between the abnormal fusion genes detected and some laboratory features of prognostic importance, which include total white blood cell count ($P = 0.416$) and FAB subtype ($P = 0.576$).

Conclusion: Presence of fusion the genes *BCR/ABL 1*, *ETV6/RUNX1*, and *MLL/AF4* does not have any impact on the clinical and laboratory features of ALL at presentation.

Key words: Acute lymphoblastic leukemia, *BCR/ABL 1*, *ETV6/RUNX1*, fusion genes, *MLL/AF4*

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Introduction

Acute lymphoblastic leukemia (ALL) is a neoplastic proliferation of lymphoid cells arrested at a premature stage

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of differentiation. It can occur in both children and adults and accounts for 80% of all cases of childhood leukemia^[1,2] and 20% of adult cases.^[3,4] In a study done at University of Benin Teaching Hospital, Benin, to determine the pattern of leukemia incidence in Benin city, the incidence of ALL was found to be 10.3% of 253 cases of leukemia seen between 1990 and 2004 and showing increasing trend at 5 years intervals.^[5] Similarly, in a preliminary report of a prospective study on the neoplastic diseases of the hematopoietic system carried out at University College Hospital Ibadan, 19.5% of the cancers were found to be ALL.^[6]

Accurate characterization of the lymphoid malignant cell requires not only morphology but also, immunologic, cytogenetic, and molecular diagnostic methods. These methods can identify the different subtypes requiring different treatment approaches in terms of either conventional or more intensive therapy according to risk-based stratification and prognosis. This has led to improved treatment outcome over the years with event-free survival rates over 80% being achieved.^[7] The situation is not the same in developing countries, where achieving remission is a major challenge, and the relapse rate is very high; because the different subtypes are not accurately identified to inform the choice of chemotherapeutic treatment.

The clinical and laboratory findings in ALL are varied and have been associated with specific underlying chromosomal abnormalities.^[8,9] The prognostic clinical and laboratory factors include age at diagnosis, initial white blood cell (WBC) count, central nervous system (CNS) involvement at presentation, testicular involvement, gender, and race. Molecular studies in recent times have availed science the knowledge of genetic abnormalities and correlation with the clinical presentation of the disease.^[9-11]

Four major chromosomal translocations found in ALL define distinct clinic-pathological subgroups and have been used in risk stratification for treatment purposes. They include *t(12;21)/ETV6-RUNX1* also known as *TEL-AML1*, *t(1;19)/E2A-PBX1*, *t(9;22)/BCR-ABL*, and *t(4;11)/AF4-MLL*.^[12] Patients with *t(1;19)*, *t(9;22)* or *t(4;11)* respond poorly to chemotherapy and have a poor prognosis.^[13] The cryptic *t(12;21)(p13;q22)* results in the *ETV6-RUNX1* fusion gene formerly called *TEL/AML1* and is known to confer excellent prognosis.^[14]

In this study, the fusion gene transcripts *ETV6-RUNX1*, *BCR-ABL*, and *AF4-MLL* was analyzed. These transcripts are known to occur more frequently in ALL and influence behavior of the leukemic blasts hence are valuable for risk stratification of treatment as they are predictors of prognosis.^[3] They are also useful in the evaluation of minimal residual disease. This study was aimed at determining the frequency of the fusion gene transcripts *ETV6-RUNX1*,

BCR-ABL, and *AF4-MLL* and their effect on the pattern of presentation of ALL.

Patients and Methods

This was a cross-sectional study carried out on newly diagnosed ALL patients in centers from South East Nigeria. Forty chemotherapy naïve patients were recruited, over 6 months (from November 2012 to July 2013) for the study. These were patient whose bone marrow aspiration cytology had more than 20% lymphoblasts. Only patients who gave informed oral and written consent were recruited. Ethical approval was obtained from the University of Nigeria Teaching Hospital Health Research and Ethics review Board, as well as from other centers, from where patients were recruited. This was done in accordance with the Helsinki declaration of 1964. Sociodemographic details of patients were obtained using a questionnaire, which was completed by the investigator.

From each patient, 10 ml of venous blood was obtained and used for full blood count using Sysmex 2000 auto-analyzer (Sysmex Corporation, UK). The RNA extraction was done using guanidine isothiocyanate solution containing ethylenediaminetetraacetic acid 5 mM, guanidine thiocyanate (GTC) 4.0 M, tris citrate 25 mM, sarcosyl 0.5%, and β -mercaptoethanol 25 ml to activate the GTC. RNA was extracted with Thermo scientific GeneJet RNA purification kit (Lot NO 00118481) according to manufacturer's guideline. The purity of the RNA was assessed with BioRad Spectrophotometer (Bio-Rad Laboratories Inc.) read off at A260/A280. The RNA was immediately converted to cDNA for stability by adding 21 μ l of cDNA cocktail to the RNA extracted. The cDNA was tested for quality by running standard polymerase chain reaction (PCR) and agarose gel electrophoresis. TaqMan reverse transcription-PCR was then used for amplification of the target fusion genes using Applied Biosystems thermal cycler Step One software version 2.0 (Life Technologies, Thermo Fisher Scientific Corporation, UK). Each patient sample was run in duplicate and tests were repeated on separate occasions to ensure reliability and reproducibility of the results. Positive standard controls and nuclease-free water as negative controls were also included in the run.

Statistics

A sample size of 40 has been chosen for this study and has 80% clinical power to detect a difference of 16.67% with a significance level (alpha error) of 0.05 (two-tailed analysis). The sample size was calculated using GraphPad StatMate version 2.0 (GraphPad Software Inc., UK). The calculation is based on study design for two proportions. Factors considered in sample size calculation include:

- Mean frequency of the fusion genes from previous studies
- Clinical power of 80% (conventional)

- Alpha level of 0.05 (conventional)
- Effect size (the level of difference to be detected = 16.67%, informed by the average frequency of 15%, and an additional margin of 1.67%).

The expected pattern of distribution of the fusion genes is unknown. The Kendaul Tau-b correlation coefficient was calculated using SPSS 17.0 (IBM Corporation, Armonk NY, USA) and expressed in tables.

Results

A total of 40 patients were included in the study, age ranged from 9 months to 54 years with a median of 16 years and mean 17.8 ± 14.4 years. Most of the patients 42.5% (17/40) were <10 years old. There were 27 (67.5%) males and 13 (32.5%) females with male-to-female ratio of 2.1:1. The patients presented with a constellation of clinical signs and symptoms with fever as the most common presenting symptom and lymphadenopathy as the most common presenting sign [Figure 1]. None of the patients had any feature suggestive of involvement of the CNS; however, one patient (2.5%) presented in an unconscious state and died within 72 h of admission.

The mean hemoglobin concentration at diagnosis was found to be 6.1 ± 2.1 g/dl; mean platelet count $39 \pm 25 \times 10^9/L$ while the mean total WBC count was $55 \pm 71.7 \times 10^9/L$. The mean platelet count was $39 \pm 25 \times 10^9/L$ and blast percentage of $85.3\% \pm 9.8\%$. The FAB L2 subtype was the most common seen in the study. Of the 40 samples analyzed,

L2 subtype constituted 67.5% of ALL while L1 subtype was observed in 32.5% of patients. No L3 subtype was seen in the series.

The abnormal genes detected were BCR/ABL1 major transcript in 5 (12.5%) patients and ETV6/RUNX1 in 2 (5.0%) patients. Of the patients who had the BCR/ABL1 transcript, 4 were <18 years of age while only one patient was more than 18 (54 years). Both patients who had the ETV6/RUNX1 were children, <10 years of age. MLL/AF4 fusion gene abnormality was not found among the patients; neither were there any combinations of abnormal fusion observed. There was no relationship between the fusion genes detected and clinical manifestations of the patients [Table 1], however, there appear to be an association between the fusion genes and ethnic origin of the patients ($P = 0.005$). There is no significant association between the abnormal fusion genes detected and some laboratory features of prognostic importance which include total WBC count ($P = 0.416$) and FAB subtype ($P = 0.576$).

Discussion

Acute lymphoblastic leukemia is invariably fatal and affects predominantly the younger patient population as seen in this study. The mean age of the study group was found to be similar to what was obtained by Olaniyi *et al.* in an audit of acute leukemia in UCH, Ibadan where a mean age of 28 ALL patients they studied was 19 years,^[15] but higher than the study by Nwannadi *et al.* in South-South Nigeria that reported 4 years.^[16] The male-to-female ratio in this study was similar to findings by Okpala *et al.* in Ibadan in their study of 30 patients with ALL to determine prognostic factors.^[17]

Males had a worse outcome than females, and this has been attributed to the involvement of sanctuary sites like testis, which can be a source of relapse. In addition, patients seen in this study appear to have a high tumor burden and invariably poor outcome as shown by the majority of the patients presenting with high leukocyte count ($WBC = 55.9 \pm 71.6 \times 10^9/L$), tissue infiltration, and L2 subtype similar to outcome of the study reported by Williams in Ibadan, South-West Nigeria.^[18]

The clinical importance of determining the underlying aberrant genetic mutation is to characterize adequately the leukemic cells for risk stratification and prognostication, targeted therapy, and modification to achieve improved survival. The majority of the patients in this study did not have any of the mutant genes being screened for, the BCR-ABL1 translocation was the most common type of mutation observed. Furthermore, all the BCR-ABL1 positive

Table 1: Relationship between the fusion genes and clinical/laboratory features at presentation

Parameter (at presentation)	BCR-ABL1 correlation (P)	ETV6-RUNX1 correlation (P)	None correlation (P)
Hb concentration	-0.167 (0.303)	-0.099 (0.542)	0.202 (0.210)
Hematocrit	-0.213 (0.186)	-0.050 (0.760)	0.214 (0.184)
White cell count	0.213 (0.187)	-0.139 (0.392)	-0.105 (0.157)
Platelet count	-0.072 (0.658)	-0.010 (0.951)	0.068 (0.675)
Age	-0.096 (0.563)	0.140 (0.397)	0.003 (0.986)

Hb=Hemoglobin

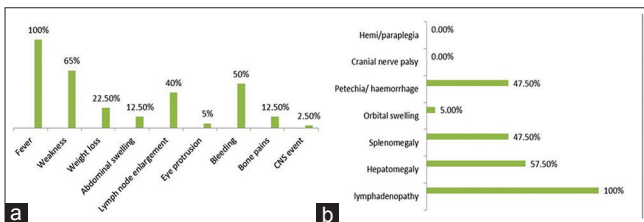


Figure 1: (a) Frequency of symptoms of acute lymphoblastic leukemia (ALL) at presentation. (b) Frequency of symptoms of ALL at presentation

patients were of the major transcript type e13a2 and e14a2, as seen in chronic myeloid leukemia (CML). However, the possibility of CML in blastic transformation in these patients needs to be ruled out. Disease outcome for these patients is poor but would benefit from treatment with tyrosine kinase inhibitors and allogeneic hematopoietic stem cell transplant as noted in some studies.^[19,20]

Half of the childhood ALL patients who screened positively for these fusion genes had the *ETV6-RUNX1*, while the other half had the *BCR-ABL1*. This is similar to findings reported by Romana *et al.* where a high frequency of the fusion gene was detected in childhood B-lineage ALL.^[21] The relatively lower frequency in children may be partly due to the fact that some adult ALLs may be the terminal event of a CML with a rather short chronic phase. The *BCR-ABL1* was found in all the adults who screened positively for the fusion genes being studied.

There were some differences in the frequency of the gene rearrangements detected in this study when compared to reports from other parts of the world. This might be due to genetic diversity that exists among diverse races. In the study done by Noreen *et al.* in Pakistan, (studying 5 fusion genes in adult patients),^[22] *MLL-AF4* was found in 10 (9.7%) patients as compared to none in this study. *BCR-ABL1* was seen in 21 (20.3%) patients as compared to 5 (12.5%) in this study, which included both adults and children. However, some similarity exists in the frequency of *ETV6-RUNX1*; 4.8% in their study versus 5% in this index study though in their study majority of the patients who had this fusion gene were adults. In 33 patients, no fusion gene was detected, and *MLL-AF4* fusion gene was undetectable in this study. This may be explained by the presence of other partner gene rearrangements involving *MLL*, which could have been present and include *AF9*, *AF10*, and the over 60 partner genes recorded in literature.^[19]

Limitations of the study

The low sample size though representative of the study population may not be completely adequate to assess some minor clinical outcomes.

Conclusion

The fusion genes detected had no impact on the clinical and laboratory features at presentations of the disease. There was no difference in the pattern of presentation of ALL in this study when compared to other previous studies. However, there seems to be some variability in the frequency of fusion genes observed in most ALL patient groups across various geographical and racial populations. This observation deserves a multi-racial analysis as this may indicate varied etiological factors, which may be peculiar to different populations.

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Conflicts of interest

There are no conflicts of interest.

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