Leukemia Research Reports 10 (2018) 20–25

Contents lists available at ScienceDirect

Leukemia Research Reports

journal homepage: www.elsevier.com/locate/lrr

Detection of KIT mutations in core binding factor acute myeloid leukemia

Passant Badr[a], Ghada M Elsayed[b,*], Dalia Negm Eldin[c], Bahia Y Riad[d], Nayera Hamdy[e]

[a] BSc Biotechnology, Faculty of Science, Cairo University, Cairo, Egypt
[b] Professor of Clinical Pathology and Oncologic Laboratory Medicine, National Cancer institute, Cairo University, Cairo, Egypt
[c] Lecturer of biostatistics, Department of Biostatistics and Cancer Epidemiology, National Cancer Institute, Cairo University, Cairo, Egypt
[d] Professor of Organic Chemistry, Faculty of Science, Cairo University, Cairo, Egypt
[e] Professor of Clinical Pathology and Oncologic Laboratory Medicine, National Cancer institute, Cairo University, Cairo, Egypt

ARTICLE INFO

Keywords:
CBF-AML
KIT mutations
FLT-3 mutations
HRM analysis

ABSTRACT

We have investigated the frequency and the effect of *KIT* mutations on the outcome of patients with CBF-AML. 69 patients (34 pediatrics and 35 adults) with CBF-AML were enrolled in the study. The frequency of *KIT* mutations was higher in adults compared to pediatrics (22.9% and 14.7%, $p = 0.38$) respectively. Leukocytosis $\geq 20 \times 10^9$ /L was significantly associated with pediatrics compared to adults. t(8;21)(q22;22) was significantly associated with thrombocytopenia in adults. We conclude that no significant difference is found between *KIT* mutated and unmutated CBF-AML in adults and pediatrics. Children with CBF-AML present with leukocytosis. t(8;21) is associated with thrombocytopenia.

1. Introduction

Core binding factor acute myeloid leukemia (CBF-AML) represents 4–12% of all AML, 15% of adults and 25–30% of pediatrics. Patients with CBF-AML are characterized with high complete remission (CR) rates (86–88%), however, 30–50% of patients relapse, and the 5-year survival is only 50% [1].

Mutations in the *KIT* gene are the most common (15–45%) in CBF-AML. The *KIT* gene is located on, chromosome band 4q11-12 and encodes a 145-kDa transmembrane glycoprotein that is a member of the type III tyrosine kinase family. Binding of stem cell factor (KIT ligand) to the KIT receptor activates downstream signaling pathways important for cell proliferation, differentiation, and survival [2]. *KIT* mutations result in ligand- independent activation and most commonly affects the extracellular portion of the receptor (exon 8), and the tyrosine kinase domain (exon 17). Mutations affecting the juxta-membrane domain (exon 10 and 11) are less common KIT mutations have been associated with poor outcome in CBF-AML [3].

The national comprehensive cancer network (NCCN) guidelines have included *KIT* mutations as a prognostic marker that can change CBF-AML from favorable to intermediate risk group [4]. In contrast, the European Leukemia Net did not add KIT mutations in the routine workup for patients with CBF-AML Unlike the cytogenetic classification, the outcome of CBF-AML is heterogeneous [5].

The aims of this work were to analyze the different clinical and prognostic characteristics of CBF-AML and to investigate the prevalence and prognostic effect of *KIT* mutations (exon 8 and exon 17) on the outcome of this group of AML patients.

2. Materials and methods

2.1. Patients

Patients were recruited in a period of two years, retrospectively and prospectively from June 2014 to June 2016. 765 patients were diagnosed with AML, 234 pediatrics and 531 adults. A total of 69 patients (34 pediatrics and 35 adults) were diagnosed with CBF AML and included in this study. Sub classification of AML according to French-American-British (FAB) subtypes was based on morphology, cytochemistry (chloroacetate esterase and myeloperoxidase) and immunophenotyping. The diagnosis CBF-AML was confirmed by the detection of inv (16)(p13q22) or t(8,21) (q22;q22)/RUNX1-RUNX1T1 fusion genes using reverse transcriptase–polymerase chain reaction (RT-PCR). All patients gave informed consent and the study was approved by the Institutional Review Board (IRB), (201516028.4) of the National Cancer Institute (NCI), Egypt.

All patients received the 3 and 7 induction chemotherapy at the NCI, induction chemotherapy consisted of Adriamycin 30 mg/m2 for 3

Abbreviations: CBF-AML, core binding factor AML; PCR-RFLP, Polymerase chain reaction restriction fragment length polymorphism; HRM, High resolution melting curve analysis; NCCN, National comprehensive cancer network; ELN, European Leukemia Network; FLT-3-ITD, FLT3 internal tandem duplication

* Corresponding author.
E-mail addresses: passant.badr@nci.cu.edu.eg (P. Badr), ghada.elsayed@nci.cu.edu.eg (G.M. Elsayed), Nayera.e-shakankiry@nci.cu.edu.eg (N. Hamdy).

https://doi.org/10.1016/j.lrr.2018.06.004
Received 1 May 2018; Received in revised form 5 June 2018; Accepted 29 June 2018
Available online 21 July 2018

days and ARAC 100 mg/m2 by continuous infusion for 7 days, after complete remission high-dose cytarabine 3 g/m2 IV over 3 h every 12 h on days 1, 3, and 5 for four cycles was given for consolidation (**5**). Achievement of CR was defined by the detection of less than 5% blasts in normocellular BM. Overall survival (OS) was measured for all living patients from the date of entry to the date of death or last time follow up. Disease free survival (DFS) was calculated from the date of CR to the date of relapse in the first CR.

2.2. Detection of C-KIT mutations

DNA extraction: Genomic DNA was extracted from BM samples using GeneJET Whole Blood Genomic DNA Purification Mini Kit (#K0781- Thermo Scientific). DNA quantity and quality were checked using Thermo Scientific NanoDrop™ 1000 Spectrophotometer.

2.3. HRM analysis for KIT exon 8 mutations

HRM analysis was used for the analysis of *KIT* exon 8 mutations. All samples were tested in triplicates on 7500 fast real-time pcr-Applied Biosystems. Positive and negative controls were included in each run. Twenty nanograms of DNA were amplified in 20 µl reaction volume containing 0.5 µM forward and reverse primers designed by [6] and 10 µl of MeltDoctor™ HRM (Applied Biosystems) master Mix with its thermal profile for 45 cycles with a ramp of 0.02 ˚C/ S. The expected PCR product was 219 bp.

Upon completion of the run, data were analyzed as fluorescence versus temperature graphs (temperature shifting, difference plots, and derivative melting curves) using High Resolution Melting (HRM) Software version 2.0 (#4,397,808).

2.4. PCR amplification and cycle sequencing for KIT exon 8 mutations

Samples positive for exon 8 mutations with same primers were confirmed by sequencing. PCR products were purified using QIAquick PCR Purification Kit (#28,104). Cycle sequencing was performed using BigDye™ Terminator v3.1 Cycle Sequencing Kit and the sequencing product was purified using Centri-Sep™ Spin Columns (#401,762) according to manufacturer instructions. Sequencing products were then resuspended with 10 µl of Hi-Di™ Formamide (#4,311,320), incubated at 95C for 5 minutes and then chilled on ice for 5 min. Bidirectional sequencing was performed on the Applied Biosystems™ 3500 Genetic Analyzer. Sequencing traces were analyzed by Applied Biosystems SeqScape Software v2.5. The analysis of data was done according to Gene Bank accession number (U63834.1).

2.5. Fragment analysis for KIT exon 8 mutations

Fragment analysis was used to help in the analysis of indel mutations of *KIT* exon 8. PCR reaction was performed as previously described by [7] using a Fluorescently labeled forward primer (FAM). Next, these PCR products were added to 7 µL of Hi-Di™ Formamide (#4,311,320) and 2 µL of GeneScan™ 500 LIZ™ dye Size Standard (#4,322,682).The mixture was then injected to Applied Biosystems™ 3500 Genetic Analyzer, analysis and verification of fragments size done using GeneMapper® Software Version 4.1 Microsatellite Analysis.

2.6. PCR-RFLP for KIT exon 17 mutations (D816)

KIT exon 17 was amplified as previously described by [8]. PCR products were digested by AatII (10 U/µL) (#ER0991) at 37 ˚C overnight. Heterozygous samples create 106, 85 and 21 bp fragments, while wild samples create 85 and 21-bp fragments.

2.7. Statistical analysis

Descriptive statistics were calculated for all variables. Patient follow-up was updated on April 1, 2017. Disease-free survival (DFS) and relapse-free survival (RFS) were estimated from the date of complete remission. Differences in proportions were assessed using the v2 or Fisher exact statistic. Survival was plotted with Kaplan–Meier curves and the data for the various groups were compared with independent T-test, [9]. All survival estimates were reported 1 standard error (SE). All P values were 2-sided, *P* value less than 0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 24.0 software statistical package (SPSS, Chicago, IL, USA).

3. Results

3.1. Comparison of initial clinical and laboratory characteristics of CBF-AML between pediatrics and adults patient's groups

Mean age was (7.1 ± 4.7 vs 32.1 ± 10.2) years for pediatrics and adults respectively, and median was (6.0 (1.0–16) vs 30.0 (19–59)) years for pediatrics and adults respectively. The prevalence of CBF-AML was 69/765 (9%), (14.9%, 6.36%) for pediatrics and adults respectively. t(8;21)(q22;22) was detected in (64.7% vs 62.9%, $p = 0.87$) for pediatrics and adults respectively. Inv(16) (p13q22) was detected in (35.3% vs 37.1%, $p = 0.87$) for pediatrics and adults respectively. Leukocytosis $\geq 20 \times 10^9$ /L was significantly associated with pediatrics compared to adults, (64.7% vs 40%, $p = 0.04$). The frequency of FLT3-ITD and FLT-3 TKD mutations was (2.9% vs 5.7%, $p = 0.57$, 2.9% vs 8.6%, $p = 0.3$) for pediatrics and adults respectively. 26 pediatrics and 26 adults were followed till the end of induction chemotherapy, 61.5% of pediatrics and 69.2% of adults achieved complete remission with no significant difference, $p = 0.771$. The total death rate was 31(44.9%). Four (12.9%) patients died before starting chemotherapy from disease progression and 12 (38.7%) patients died during induction chemotherapy. Death was contributed mainly to infections and febrile neutropenia. OS at 6 months was significantly higher in adults compared to pediatrics (63.3% vs 35.7%, $p = 0.043$).

3.2. Comparison of initial clinical and laboratory characteristics between inv (16) and t(8;21) in CBF AML

Inv (16) (p13q22) was significantly associated with FAB subtypes M4 + M5 and t(8;21))(q22;22) was significantly associated with M1 + M2 in pediatrics and adults ($p < 0.001$, $p = 0.004$) respectively.

In pediatrics, inv (16) (p13q22) CBF-AML was associated with hepatomegaly, splenomegaly and lymphadenopathy compared to t (8;21) (q22;22) (41.7% vs 22.7%, $p = 0.27$; 25% vs 13.6%, $p = 0.64$; 8.3%vs 4.5%, $p = 1$) respectively. inv (16) (p13q22) positive CBF-AML was also associated with anemia (HGB ≤ 8 g/dl) and leukocytosis $\geq 20 \times 10^9$/L compared to t (8;21)(q22;22) (91.7% vs 59.1%, $p = 0.06$; 83.8% vs 54.5%, $p = 0.14$) respectively.

In adults, t(8;21)(q22;22) was significantly associated with thrombocytopenia $\leq 20 \times 10^9$/L compared to inv (16) (50% vs 7.7%, $p = 0.01$). However, t(8;21)(q22;22) was significantly associated anemia (HGB ≤ 8 g/dl) with compared to inv (16) (p13q22) (72.7% vs 30.8%, $p = 0.03$) respectively. No significant difference was found in response to induction chemotherapy was found between in inv 16 compared to t(8;21) in pediatrics and adults ($p = 0.18$, $p = 0.19$ respectively, Table 1.

3.3. Effect of the initial clinical and laboratory characteristics on OS of CBF-AML

When we investigated the effect of different pretreatment clinical and laboratory parameters on the OS at 12 month period for adults and 6 months for pediatric CBF-AML, because the number of pediatric cases