Frequency of 11q23/MLL gene rearrangement in Egyptian childhood acute myeloblastic leukemia: Biologic and clinical significance

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Abstract

Background: Molecular cytogenetic abnormalities involving 11q23 are among the most common cytogenetic abnormalities in acute myeloid leukemia (AML) patients. **Aim of the work:** we aimed to evaluate the frequency of MLL/AF9 fusion gene in *de novo* AML patients, its impact on clinical features, and its prognostic significance. **Patients and Methods:** Twenty-eight children patients with AML and 20 healthy controls were subjected to complete clinical examination and laboratory investigations including, complete hemogram and bone marrow (BM) examination. Diagnosis was based on FAB morphologic and immunophenotypic criteria. Detection of (MLL/AF9) fusion gene was assessed by dual color fluorescent *in situ* hybridization (FISH). Follow-up were carried out clinically and by blast count in BM, and response to therapy to detect the outcome of the disease. **Results:** The incidence of MLL-fusion gene MLL/AF9 in AML cases was about (6/28) (21%). Four patients with MLL/AF9 fusion gene were newly diagnosed, two cases were at relapse and no patient at remission showed positivity. As regard the clinical outcome, five out of six MLL positive cases died, three of them during induction and two during relapse. The FAB AML subtypes with MLL/AF9 fusion were one M2, three M4, and two M5. **Conclusion:** MLL-fusion gene MLL/AF9 was found in about 21% of studied AML patients when assessed by FISH technique and this is of high clinical relevance as most of these abnormalities have been associated with poor prognosis.

Key words: Acute myelobastic leukemia, 11q23/MLL gene. Dual color FISH.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous clonal disorder characterized by autonomous proliferation and impaired differentiation of hematopoietic progenitor cells and account for approximately 20% of pediatric leukemias.[1] Approximately, two-thirds of children with AML are cured with intensive multi-agent chemotherapy.[2] Multiple chromosomal and gene rearrangements have been identified in AML, such as MLL, PML/RARA, DEK/CAN, and AML1/ETO.[3] MLL-fusion proteins are potent inducers of oncogenic transformation, and their expression is considered to be the main oncogenic driving force in ~10% of AML patients. These oncogenic fusion proteins are responsible for the initiation of a downstream transcriptional program leading to the expression of factors such as MEIS1 and HOXA9, which in turn can replace MLL-fusion proteins in overexpression experiments.[4]

MLL gene is located on chromosome 11q23 and is considered one of the most common genetic abnormalities in AML. The most common MLL gene rearrangements in AML are the t (9;11) (p22;q23), t (6;11) (q27;q23), t (11;19) (q23;p13.1) and t (11;17) (q23;q21). The AF9 gene at chromosome band 9q22 and the ENL gene at chromosome 19q13, which encode transcriptional factors are common partner genes.[5] Translocations involving chromosome 11q23 frequently occur in pediatric AML and are associated with poor prognosis although some studies suggest that patients with t (9;11)(p22;q23) have a more favorable prognosis.[6]

Aim of the work

This study aimed to evaluate the frequency of MLL/AF9 fusion gene t (9;11)(p22;q23) in AML patients, its impact on clinical features, and its prognostic significance.

Patients and Methods

This study was conducted on 28 patients with AML Who were attendants to Pediatric Oncology Unit of Tanta University Hospital in the period from October 2012 to October 2013. Their ages ranged from 12-16 years, with males: Females of 19:9 that is, nearly 2:1. In addition, 20 apparently healthy children matched in age and sex were taken as the control group. Informed consent was obtained from all patients and controls with approval of the Ethical Committee for Human Research in Tanta University Hospital.

All patients and controls were subjected to complete clinical and laboratory investigations including complete hemogram, bone marrow (BM) examination. Diagnosis was based on FAB morphologic and immunophenotypic criteria. Collection of samples from patients and controls were taken at the time of diagnosis as follows: 1 ml BM or 2 ml blood in EDTA tubes for complete blood count and BM morphologic, cytochemistry and immunophenotyping and another 1 ml BM and/or 2 ml blood in sterile preservative free heparin tubes for fluorescent *in situ* hybridization (FISH) technique.

Follow-up of patients was carried out clinically and by blast cell count in BM on day 28 after two courses of induction chemotherapy according to AML-protocol (MRC-10). In addition to all trans-retinoic acid in AML-M3.[7]

Course 1 Ara-C, daunorubicin, etoposide

Daunorubicin 50 mg/m2 intravenous (IV) days 1, 3, and 5, cytosine arabinoside (Ara-C) 100 mg/m2 IV bolus every 12 hours days 1-10 (20 doses), etoposide 100 mg/m2 IV 1 hour infusion days 1-5 and intrathecal cytarabine in age adjusted doses at the time of diagnostic lumber puncture.

Course 2 Ara-C, daunorubicin , etoposide

Daunorubicin 50 mg/m2 IV daily on days 1, 3, and 5, cytosine arabinoside (Ara-C) 100 mg/m2 IV bolus every 12 hours on days 1-8 (16 doses), etoposide 100 mg/m2 IV daily (1 hour infusion) days 1-5 and intrathecal cytarabine in age adjusted dosing on day 1.[7]

Cell culture and fluorescent *in situ* hybridization

Peripheral blood and/or BM specimens were cultured for 48 and 72 h at 37°C in Roswell Park Memorial Institute medium supplemented with 10% fetal bovine serum without the addition of any mitogen (unstimulated). Colcemid (0.02 µg/ml) was added to the cultures 30 min before harvest. After 30 min of hypotonic treatment with 0.075 M KCl, the cells were fixed with methanol and acetic acid (3:1) and cells were made into slide preparations.

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Fluorescent *in situ* hybridization assay: Was performed according to (Vysis, Abbott) manufacturer's instructions, hybridization mixture (10 µl) was then applied to each slide, which was cover slipped and sealed. Hybridization solution contained (hybridization buffer, purified water, and the specific probe). Specific probe (LSI) MLL/AF9 dual color dual fusion translocation probes (Vysis, Downers Grove, IL) for detection of t (9;11)(p22;q23) was used. Hybridization was performed for 10 h at 37°C in a humidified chamber. Posthybridization washes consisted of rinses in 0.4 × SSC at 37°C and 2 × SSC at room temperature. Finally, nuclei were counterstained with 4□,6-diamidino-2-phenylindole dihydrochloride. Cells were analyzed under a fluorescence microscope equipped with quips spectra vision hardware and software.

Counting 200 nuclei per slide and scored for the presence or absence of the fusion gene examined.

To determine the positive cut-off level of MLL/AF9 fusion gene t (9;11)(p22;q23), FISH control experiments were performed on the 20 control samples. The proportion of nuclei with these fusion genes were counted among 200 cells in each of the control samples. Mean of aberrant signals ± 3 standard deviation (SD) was set as the normal range. Range of fusion gene MLL/AF9 is 0-2, mean value ± SD are 1.15 ± 0.9333 and cut-off values ± 3 SD for MLL/AF9 t (9;11)(p22;q23) fusion genes are 1.6499.

Evaluation criteria

In a normal cell without the MLL/AF9 fusion gene, two orange signals representing normal copies of MLL and two green signals representing normal copies of AF9 are observed, whereas an abnormal nucleus showing one orange, one green, and two fusion (101G 2F) signal pattern [Figure 1].

Statistical analysis

Statistical presentation and analysis of this study was conducted, using the mean, SD and Chi-square test using SPSS version 16.

Results

MLL-fusion gene t (9;11) (MLL/AF9) was found in 6 out of 28 patients (21%). Four cases were newly diagnosed, and two cases were at relapse. Most of the positive cases were younger (2 years old and the relapsed cases were below 4 years, and all were males). No patients at remission showed positivity for MLL/AF9 fusion gene.

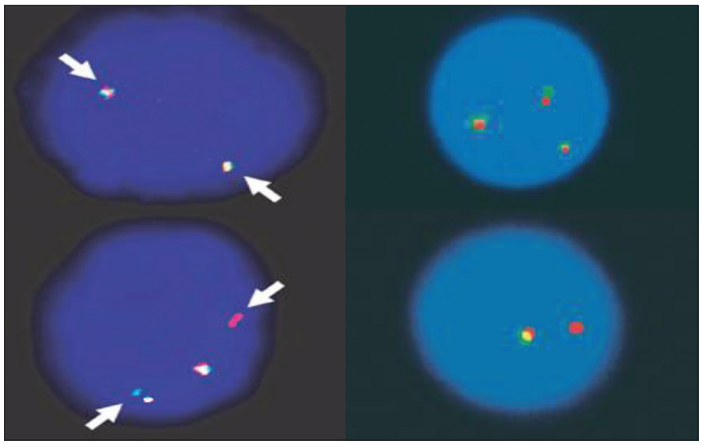

Figure 1: MLL/AF9 fusion gene t(9;11) dual color, dual fusion fluorescent *in situ* hybridization

The initial leukocytic counts in cases with MLL/AF9 fusion gene was more than 50,000/mm3, hemoglobin (Hb) level was <9 g/dl and higher peripheral blasts, which were significantly different from those lacking it. FAB subtypes of MLL/AF9 fusion positive AML cases were one M2, three M4, and two M5.

As regard the clinical outcome, five out of six MLL/AF9 fusion positive cases died, three of them during induction and two during relapse.

The only positive MLL/AF9 fusion case who achieved continuous complete remission was 10 years old, presented with low leukocytic count and classified as FAB-M2.

Discussion

Acute myeloid leukemia is a heterogeneous disease with diverse genetic abnormalities and variable responsiveness to therapy.[8] For instance, the translocations t (8;21), t (15;17) and inv (16) or t (16;16) herald a favorable prognosis, whereas other cytogenetic aberrations, such as, 11q23 abnormalities, indicate intermediate or worse prognosis.[9] Molecular cytogenetic abnormalities in AML are the strongest independent prognostic indicators and these are being used to influence choice of therapy.[10]

This study aimed to evaluate the frequency of MLL/AF9 fusion gene t (9; 11)(p22; q23) in childhood AML cases, the impact of this mutation on the clinical features and disease outcome by using dual color FISH [Table 1].

In this study, MLL/AF9 fusion gene rearrangement t (9;11) was found in 6 out of 28 AML cases (21%). Four cases of them were newly diagnosed and the other two cases were at relapse. The newly diagnosed positive cases were younger (2 years old) and the relapsed cases were below 4 years and all were males. Our results were in agreement with He *et al*. 2012[11] and Launay *et al*. 2014[12] who found that MLL gene rearrangements involving the band 11q23 are the most common genetic alteration encountered in infant AML. These results were also reported in many studies, which stated that AF9 gene at 9p22 is one of the most common fusion partner genes with MLL gene.[13]

In the current study, no significant association between t (9;11) and patient's sex, Hb concentration and platelet count was detected. The patients who had MLL/AF9 fusion showed a trend to have higher white blood cells counts and higher blast percentage, which is concomitant with that reported by Muñoz *et al*. 2003.[14]

As regarded the clinical outcome, five out of six MLL positive cases died, three of them during induction and two during relapse. The only positive MLL case who achieved

Table 1: Sensitivity, specificity, accuracy, PPV and NPV for detection of 11q23/MLL gene rearrangement (MLL/AF9) t(9;11) by FISH technique

Statistical analysis	MLL/AF9 fusion %
Sensitivity	71.42
Specificity	100
PPV	100
NPV	90.9
Accuracy	92.59

For MLL/AF9 fusion no case showed false positive and only two cases were false negative by FISH. PPV=Positive predictive value, NPV=Negative predictive value, FISH=Fluorescent *in situ* hybridization

Table 2: Comparison between different studies regarding 11q23/MLL gene rearrangement

Parameters	The current study	He *et al.* 2012[11]	Wang *et al.* 2013[16]
Method of detection	Dual color FISH	RT-PCR with MLL-dual color FISH	By RT-PCR and sequencing
Frequency	(6/28) 21%	(28/234) 11.97%	Only studied two cases
Frequent AML subtypes	One M2, three M4 and two M5	Most of patients (24/28) (85.7%), were M4/M5	One patients was diagnosed as AML-M2 and one patients AML-M5
Outcome	Five out of six MLL/AF9 fusion positive cases died, three of them during induction and two during relapse	Complete remission after treatment for 28 cases with MLL rearrangements was 53.8%, compared with 90.5% for (M4/M5 AML with other karyotypic abnormalities or normal karyotype)	The two patients got hematologic complete remission after induction but one of them relapsed and died during consolidation therapy with intermediate-dose cytarabine

FISH=Fluorescent *in situ* hybridization, RT-PCR=Reverse transcriptase-polymerase chain reaction, AML=Acute myeloid leukemia

complete continuous remission was 10 years old, presented with low leukocytic count and FAB-M2. These results are in agreement with Tuborgh *et al*. 2013[15] who found that MLL gene rearrangements are associated with poor prognosis, Wang *et al*. 2013[16] who found that patients with t (11;22)(q23;q11.2) were vulnerable to relapse after conventional chemotherapy and had poor prognosis and He *et al*. 2012[11] who found that 11q23/MLL rearrangements is highly correlated with poor prognosis and recommended intensive chemotherapy and stem cell transplantation to ameliorate the clinical outcome of 11q23/MLL rearrangements [Table 2].

FAB morphologic subtypes of MLL/AF9 fusion gene positive cases were one case of M2, three cases of M4, and two cases of M5 which were in agreement with He *et al*. 2012[11] who found 24 cases with M4/M5 out of 28 AML patients with 11q23/MLL-fusion gene rearrangements (85.7%) [Table 2].

The presenting features associated with the expression of MLL-fusion transcripts (age 2 years, high initial leukocytic count, high percentage of BM blasts and high peripheral blood blasts) are known to be associated with a poor prognosis. The unfavorable prognosis conferred by MLL-fusion denotes that the presence of this fusion gene was significantly associated with a poor overall survival status. This was in agreement with several studies,[15-17] which reported that MLL gene translocation has been associated with poor prognosis and short survival.

Conclusion

MLL-fusion gene MLL/AF9 in AML cases was about 21% of studied AML patients when assessed by FISH technique and this is of high clinical relevance as most of patients with these abnormalities have been associated with poor prognosis. Therefore, it should be added to the list of molecular abnormalities used as risk stratifying features in AML patients.

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