Occurrence of Acute Myeloid Leukemia in Young Pregnant Women

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Abstract: Although acute leukaemia is rare in pregnancy its importance lies in its life-threatening potential, both to the child and the mother. The possibility of vertical transmission of leukemic cells increases the attention devoted to these patients and their offspring. Three cases of pregnant young women (15–17 years of age) with AML are presented. This series of cases is the first report where gene abnormalities such as ITD mutations of the *FLT3* gene and *AML1/ETO* fusion genes were screened in pregnant AML patients and their babies, so far. Unfortunately, very poor outcomes have been associated to similar cases described in literature, and the same was true to the patients described herein. Although very speculative, we think that the timing and possible similar exposures would be involved in all cases.

Keywords: acute myeloid leukemia, pregnancy, *AML1*/*ETO*, *FLT3* mutation

Introduction

The incidence of acute myeloid leukemia (AML) increases with age and is more frequent above late sixties. As AML's incidence begins to rise after 20 years-old, it occurs in young women of childbearing age (Bhatia and Neglia, 1995; Lichtman and Liesveld, 2001); however, the association with a coexistent pregnancy is rare (1 in 75,000 pregnancies). Even being rare, acute leukemia (AL) is the most frequent malignant disease observed in pregnancy (D'Emilio et al. 1989; Greenlund et al. 2001). These cases are instigating due to the possibility of vertical transmission as well as the toxic effects on the child resulted from treatment (Dilek I et al. 2006).

Evidences argue for a multistep pathogenesis of AML (Bhatia and Neglia, 1995). For instance, it has been shown that the *AML1-ETO* or *CBFB-MYH11* fusion genes, resulting from t(8; 21)(q22; q22) translocation and inv(16)(p13; q22), respectively, can block myeloid differentiation but do not cause frank leukemia (Okuda et al. 1998; Castilla et al. 2004). A second genetic pathway in addition to the fusion protein was suggested to be required to lead to an overt leukemia phenotype. Constitutively activate signaling molecules, such as *FLT3* or *RAS* family members are strong candidates (Nakao et al. 1996; Abu-Duhier et al. 2001).

Intimate contacts between the conceptus and the mother are bi-directional: the feto-placental tissues need nutrition and a suitable environment in homeostatic condition whereas the mother influenced by the placental factors adapts her metabolism and immune system (Thellin and Heinen, 2003). Whether and how the coexistent pregnancy might modulate the AML or vice-versa is still to be answered and environmental exposure is also a factor that should be considered in such cases.

We present herein the clinical and biological characteristics of three pregnant teenagers that developed AML in the third trimester of pregnancies and had poor outcomes. Wondering to understand the coincident cases diagnosed in our laboratory, we screened molecular alterations in the mothers' and their babies' samples, seeking for the most commonly found genetic alterations in AMLs.

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Material and Methods

Subjects

During the period of October 2003 to May 2006, specimens [bone marrow (BM) aspirates and/or peripheral blood (PB)] from cases described were referred to our laboratory to perform immunophenotyping and molecular analysis for diagnostic purpose, and prior to any leukemia treatment.

Morphology and immunophenotyping

The initial diagnosis of AL was established based on morphology analysis on BM and/or PB smears and classified according to criteria described by the *French-American-British* (FAB) group. Fresh cells from BM aspirates were separated using Ficoll-Hypaque® (Pharmacia LKB, Uppsala, Sweden) and subjected to immunophenotypic analyses. Cell surface antigens were detected by flow cytometry after monoclonal antibody labeling using the following panel: TdT, aMPO, CD34/HLA-DR, CD15/CD15, CD10/CD19/CD45, CD41/CD61, CD34/CD56 and CD33+CD13/HLA-DR. The final immunological classification was performed based on the criteria defined by the *European Group for the Immunological Characterization of Leukemias* (EGIL) (Bene et al. 1995).

Molecular analysis

Genomic DNA was isolated from BM and/or PB samples using a standard salting out method previously described (Miller et al. 1988). Total RNA was purified using TRIzol® reagent kit (Gibco/BRL, Life Technologies) according to the manufacturer's instructions. cDNA was generated using Ready-To-Go™ T-Primed First—Strand Kit (Amersham Pharmacia Biotech Inc. 2001), and stored at –20 °C. The integrity of cDNA was examined by amplifying a fragment of the *GAPDH* gene.

The presence of the fusion genes *AML1/ETO, PML/RARA*, *BCR/ABL* and *CBFB/MYH11* was assessed by RT-PCR using primers and conditions slightly modified from the *BIOMED-1 Concerted Action* description (van Dongen et al. 1999). The presence of the fusion genes *MLL/AF4, MLL/AF9* and *MLL/ENL* was assessed by RT-PCR, using conditions (Emerenciano et al. 2006) and primers (Andersson et al. 2001) previously described. Cell lines and known positive patients' samples were used as positive controls. All tests to detect gene fusions were applied for both the patient and their offspring's.

FLT3 internal tandem duplications (FLT3-ITD) were tracked using primers and conditions previously described (Nakao et al. 1996). Detection of *FLT3*-D835/I836 mutations was performed using PCR-RFLP technique previously described (Yamamoto et al. 2001). After amplification of exon 17, PCR products were digested using *EcoRV* enzyme at 37 °C. For observation, both RT-PCR and PCR products were separated by electrophoresis in agarose gels stained with ethidium bromide.

Case Reports

Case 1

A 17 year-old non-white female was admitted at the Hospital Universitário Lauro Wanderley in João Pessoa – Paraíba, Brazil for marked weakness and sore throat for 3 weeks. She was born and always lived in a farm. At the time of admission, October 2003, she was 28 weeks pregnant, febrile, anemic and drowsy. Physical examination revealed adenopathies and painful mass in the internal left leg. No hepatosplenomegaly was observed. PB and BM aspirates were obtained to perform diagnostic tests. The patient gave birth in a Caesarian delivery of a health child without clinical complications. She was treated soon after surgery recovery with an adapted Berlin-Frankfurt-Munich protocol for AML (BFM-AML). Despite of all clinical support during the induction of remission the patient died one month after the beginning of treatment due to respiratory infection and severe digestive hemorrhage.

Case 2

A 15 year-old non-white female was admitted at the Hospital SES in Brasilia- Federal District, Brazil, in October 2004 for marked weakness and pallor. She had been unwell with recurrent fever and infectious episodes. At the time of admission she was 34 weeks pregnant in her first pregnancy. Physical examination revealed adenopathies and hepatosplenomegaly. Laboratorial test were performed and the diagnosis of AML was confirmed. Termination of the pregnancy was performed two weeks later by Caesarian section. Chemotherapy was then initiated, and the patient has also received

induction regime following locally adapted BFM protocol for AML. Due to partial remission, a new regime was performed with Mitoxantrone, high dose Citarabine, Fludarabine and Idarrubicine, but she died short after BM recovery. The offspring is a healthy boy without clinical complications.

Case 3

A 17 year-old non-white female was admitted at the Hospital de Universitário Lauro Wanderley in João Pessoa-Paraíba, Brazil, in July 2005 for marked pallor and anemia unresponsive to treatments. At the time of admission she was at 32 weeks of pregnancy. Physical examination revealed no adenopathies or hepatosplenomegaly. Diagnose of AML was done in PB and BM aspirates. Termination of the pregnancy was performed by Caesarian section at 34 weeks gestation without clinical complications. Chemotherapy was then initiated, and the patient received an induction of three courses of Cytarabine, and doxorubicin followed by consolidation courses with 6-thioguanine and Cytarabine high dose, as the previous cases described. Unfortunately, she died soon after the beginning of the treatment before complete remission of AML, due to a central nervous system hemorrhage.

Table 1. Main laboratorial features of AML cases.

	#1	#2	#3
WBC	202.0×10^6/mL	5.0×10^6/mL	10.9×10^6/mL
Platelets	28.0×10^3/mL	47.0×10^3/mL	52.1×10^3/mL
Blast cells (%)	92	42	32
FAB subtype	M4	M0	M2
Immunophenotype (%)			
aMPO/cCD13	76/95	–/67	22/27
cCD3	–	2	1
cCD22/TdT	–/–	4/12	0/1
cIgM	–	7	1
CD34/CD33+13/CD45	0/1/94	15/96/90	–/80
CD33/CD56	1/1	96/0	58/–
CD41	–	3	–
CD33+13/CD19	1/1	96/5	80/7
CD4/CD8/CD3	1/0/38	12/3/0	27/–/0
CD34/CD7	0/–	15/84	–/–
CD15/CD14	0/–	–/3	–/–
HLA-DR/CD11b	1/0	98/0	35/60
CD61	0	3	2
Fusion genes			
AML1/ETO	(–)	(–)	(+)
PML/RARA	(–)	(–)	(–)
CBFβ/MYH11	(–)	(–)	(–)
BCR/ABL	(–)	(–)	(–)
MLL rearrangements	(–)	(–)	(–)
FLT3 analysis			
Duplication	ITD (340 bp)	WT	WT
TKD Mutation	WT	WT	WT

Abbreviations: WBC: white blood cells count; ITD: internal tandem duplication; TKD: tyrosine kinase domain; WT: wild-type.

Table 1 summarizes the main laboratorial results of immunophenotiping and molecular-genetics analysis of the three AML cases. The morphology aspects of PB and BM smears were compatible with AML in different maturation stages (M0, M2, and M4). Conventional cytogenetics was successful in one patient (2) that showed a 46, XX profile in all mitotic cells. Molecular-cytogenetics tests searching for abnormal fusion genes such as *MLL* rearrangements, *AML1/ETO*, *PML/RARA*, *CBFB/MYH11*, and *BCR/ABL* were performed in all cases and *AML1/ETO* fusion gene was found in case (3). The molecular analysis of *FLT3* gene showed an ITD on in one case (1). Other laboratorial tests such as biochemical and hemotasis profilings were also performed at diagnosis and just before the beginning of chemotherapy protocols. All women were diagnosed with AML in the third trimester of gestation, delivering healthy babies and, all the three babies were phenotypically normal. None of them had been exposed to chemotherapeutics drugs. In all three babies, a PB sample was collected for laboratorial analysis related to maternal leukemia status. They presented normal morphological and molecular features. Their evolution has been normal according to growth and development scale, so far.

Discussion

Review of the literature demonstrated that leukemia during pregnancy occurred in a range of 19 to 45 years old, mean 30, and no case under 19 years had been described (Caligiuri and Mayer, 1989; Chelghoum et al. 2005). To the best of our knowledge this is the first report of pregnant teenagers (15 and 17 years old) with leukemia, diagnosed in a very short period of time. Interestingly, two of them lived in the same region (Paraíba-Brazil) where the main source of economy is tannery, known as very polluted with carcinogenic agents. Although no conclusion may be driven from few cases, the timing and possible similar kind of exposures is very likely to have been the same among them. Therefore, environmental exposures might have been involved in the occurrence of these cases (Bhatia and Neglia, 1995). An epidemiological and exploratory analysis is urgently required to elucidate the occurrence of such AML cases.

Unresponsiveness to treatment, hard to deal with the follow-up and fatal in almost all cases, leukemia in pregnancy is a disease that challenges biological sciences. Up to now, there is no standard approach for this clinical complication and there is no available evidence suggesting how the pregnancy could alter the natural history of AL, and vice-versa (Catanzarite and Ferguson, 1984). The vertical transmission of the mother's leukemic cells to her offspring is possible but very rare (Osada et al. 1990). There are no hints that vertical transmission has happened among our cases, since no clinical, morphological or molecular evidences were detected in the babies.

The clonality patterns of molecular-genetics changes and the natural history that takes account the short latency, makes pediatric leukemia a diverse disease. This distinction has a biological explanation and impacts on etiology. Childhood leukemia in its natural history is not associated to inherited mutant genes, otherwise the concordance rates for leukemia in identical twins with the same acquired genetic abnormality demonstrated by molecular markers associated with acute leukemia, indicate that leukemogenesis starts during the fetal life [reviewed in Greaves and Wiemels, 2003]. The t(8; 21)(q22; q22) is found in approximately 7% of AML and in 20% of *de novo* AML of FAB-M2 subtype (Sandler and Ross, 1997). The translocation involves the *AML1* gene on chromosome 21q22 and *ETO* gene on chromosome 8q22 and is considered to confer good prognosis to the patient. The prenatal origin of this fusion gene has been proved to occur in some cases (Gale et al. 1997). One of the patients with AML-M2 (3) carried out the fusion gene *AML1/ETO* within her blasts. Unfortunately, even carrying this molecular marker, after termination of her pregnancy and under chemotherapy, the patient relapsed and died. As mentioned before, mutations of the receptor tyrosine kinase and/or an ITD of the juxtamembrane domain-coding sequence of the *FLT3* gene is found in 20% of patients with AML, being strongly associated with the tumorigenesis and poor prognosis. In patient (1) ITD mutation of the *FLT3* gene was found as a solely genetic abnormality. Of note is that the coexistence of the genetic alterations has not been found, instead each case presented only one alteration. Alterations in the FLT3 signaling through gain-of-function mutations in the *FLT3* gene itself could potentially contribute to leukemogenesis.

The poor outcome observed among our patients has also been described in a previous report of 10 cases of AL during pregnancy (Ali et al. 2003). All

the babies described here are alive and healthy, while in that report only one healthy neonate was obtained.

We described the clinical and laboratorial findings of three AML cases during pregnancy. It is worthy to emphasize that the ages presented here represent an intermediate range that splits groups wherein ALL or AML prevail. Even considering that the three cases could represent a phenomenon taking by chance, we believe that increasing casuistic in this particular clinical setting is important. Furthermore, genetic studies in pregnant patients with AML are scarce in the literature, and our paper provides such data.

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