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Case Report

Role of multiplex reverse-transcriptase PCR for diagnosis of Acute myelosis in an infant with Down's syndrome

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year.² However, there may be diagnostic difficulty in some cases as there have been reports of TMD at later ages (second or third month of life), as well as cases of “congenital leukemia”.⁴ Hematologic and cytogenetic differences between these disorders also have been described. TMD tends to manifest with normal hematocrit and platelet counts, whereas AML generally exhibits cytopenias.¹ Blasts in TMD usually have only the constitutional Trisomy 21, whereas blasts in AML may show additional complex cytogenetic abnormalities.⁵ One of the few modalities available to establish the diagnosis would be by identifying one of the 28 possible mutations associated with AL. We present a case report on the use of Multiplex RT-PCR in the diagnosis of Acute myelosis in Down's syndrome.⁶

Introduction

Children with Down syndrome (DS) are at a higher risk of developing Acute leukemias compared with the general pediatric population.^{1,2} Neonates with DS also may develop a transient myeloproliferative disorder (TMD), an abnormal proliferation of myeloid blasts in the blood that resolves without therapeutic intervention.³ TMD and acute myeloid leukemia (AML) in DS show strikingly similar morphologic features.¹ The main difference in the clinical presentation of these disorders is the age of onset, with TMD occurring during the first few days of life and AML usually manifesting after 1

Case report

A 34-year-old lady at 37 weeks period of gestation with uncomplicated pregnancy gave birth to a male baby after an emergency LSCS due to spontaneous rupture of membranes with meconium stained liquor. Antenatally, a triple test was done in civil laboratory which was negative and ultrasonography showed no congenital anomalies. The baby was asymptomatic for the first two days after which he developed hypoglycemia. Routine investigations done in civil hospital showed evidence of sepsis with leukemoid picture on

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peripheral smear. The baby was transferred to our hospital, where physical examination revealed a lethargic baby with weak cry and stigmata of Down syndrome, which included upward slanting of palpebral fissures, depressed nasal bridge, bilateral simian crease and micrognathia (Fig. 1A). Systemic examination revealed hepatosplenomegaly and a Grade 2 precordial ejection systolic murmur. Investigations revealed Hemoglobin 10.6 gm/dL, Total Leucocytic Count 1,32,000/cumm, Differential Leucocytic Count: Blasts 12%, Meta-myelocytes 07%, Myelocytes 03%, Band forms 10%, Basophils 13%, Eosinophils with precursors 12%, Monocytes 05%, Neutrophils 33%, Lymphocytes 05% with Platelet count of

60,000/cumm. The blasts had basophilic cytoplasm with coarse basophilic granules and cytoplasmic blebbing suggestive of Megakaryoblastic morphology (Fig. 1B).

Flow cytometric immunophenotyping (FCI) was done on the peripheral blood (PB) sample which showed blasts positive for CD34, CD117, CD13, CD33, CD7, CD41 (Fig. 1D), but negative for anti-Myeloperoxidase, CD14, HLA-DR, TdT and other lymphoid markers (CD3, CD10, CD19, CD22) (Fig. 1D). These findings are quite characteristic of blasts of TAM. Bone marrow (BM) aspirate smears showed 20% blasts with similar morphology as PB. X-ray showed cardiomegaly and echocardiography revealed persistent pulmonary hypertension of

Fig. 1 – (A) Syndromic neonate with depressed nasal bridge and macrognathia. (B) Megakaryoblast with basophilic cytoplasm, cytoplasmic blebbing (Leishman Stain, $\times 100$). (C) Karyotypic studies showing Trisomy 21. (D) FCI with blasts showing CD13, CD33, CD34, CD117 but negative for cMPO. (E) MT-PCR showing presence of 325 bp fusion gene PML-RARA.

Fig. 1 – (continued).

newborn. On karyotyping 47, XX + 21 was confirmed (Fig. 1C). Repeated PB counts showed decrease in blasts percentage to 08% with the morphology of the blasts changing to typical type I blasts.

Initial neonatal course was marked by respiratory distress and hypoglycemia followed later by respiratory distress and features of neonatal sepsis. Blood culture grew *Klebsiella pneumoniae*; sensitive to Imipenem. The neonate then underwent single volume exchange transfusion twice for hyperleukocytosis in addition to other supportive measures. In spite of therapy, the neonate developed severe pneumonia and succumbed to his illness on day eighteen of life.

Mutational analysis for 28 possible mutations on archival mRNA extracted from the blasts was performed by Multiplex RT-PCR using the Hemavision Multiplex RT-PCR kit. Archival RNA was converted to cDNA, which was used for the subsequent 2 PCR reaction. First split out reaction showed a positive band in lane 4. The second split out of lane 4 showed a positive band in lane 4 at 325 bp, corresponding to t(15; 17) (q22; q12) encoding for the PML/RAR α fusion gene (Fig. 1E), a cryptic mutation missed on conventional karyotyping. This showed that the blasts were clonal in nature, thus establishing the diagnosis of Acute leukemia though other investigations

favoured a diagnosis of TAM. And as per the latest WHO 2008 guidelines, the mere presence of the recurrent translocations such as PML/RARA is sufficient to make a diagnosis of Acute leukemia irrespective of the number of blasts.¹ Hence a post mortem final diagnosis of Acute promyelocytic leukemia was made.

Discussion

Children with DS are at increased risk of several hematological disorders like Acute leukemia (AL), acute myelofibrosis of childhood and TAM. DS children have 20-fold increased risk of developing both acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) compared to non-DS children¹ with 70% of cases being Acute Megakaryoblastic leukemia (AMkL).

Apart from TAM and AML (Megakaryoblastic) other forms of Acute leukemia, both ALL and AML, affects neonate with Down's syndrome. TAM is the most common myelosis in neonatal period while only a few case reports of AL are available in this period. There is 150 fold increase in AML between 2 and 5 years of age.¹ Prevalent approach in diagnosis

of Acute myelosis in a Down's syndrome neonate would be on the basis of morphology and flow cytometry.¹ But, the clonal nature of the cells can be ascertained accurately by molecular techniques alone. Many mutations are known in a transformed Acute leukemia in neonates with Down's syndrome, most common being the GATA mutation which is seen in Megakaryoblastic AML (70%). Though the GATA mutation was not analyzed, the evidence of PML/RARA fusion gene proves the clonal nature of the myeloid cells.⁶ This highlights the prevalence of other possible mutations in a setting of Acute myelosis in Down's syndrome child, which may be part of the initial phase of multi-step carcinogenesis known to occur in leukemias. It also helps in applying possible theranostic methods of treatment, as in this case, the infant could have benefited from the use of ATRA.

Conflicts of interest

All authors have none to declare.

REFERENCES

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW, eds. *WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues*. Lyon: IARC; 2008:110–144.
2. Webb D, Roberts I, Vyas P. Haematology of Down syndrome. *Arch Dis Child Fetal Neonatal Ed*. 2007;92:F503–F507.
3. Zipursky A, Brown EJ, Christensen H, Doyle J. Transient myeloproliferative disorder (transient leukemia) and hematologic manifestations of Down syndrome. *Clin Lab Med*. 1999;19:156–167. vii.
4. Granzen B, Bernhard B, Reinisch I, Skopnik H, Mertens R. Transient myeloproliferative disorder with 11q23 aberration in two neonates with Down syndrome. *Ann Hematol*. 1998;77:51–54.
5. Vyas P, Crispino JD. Molecular insights into Down syndrome-associated leukemia. *Curr Opin Pediatr*. 2007;19:9–14.
6. Hoffbrand AV, Catovsky D, Tuddenham Edward GD, eds. *Postgraduate Hematology*. 6th ed. 2011:395–414.