Research Article

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Medical & Clinical Case Reports Journal

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Vol: 1 & Iss: 2

Pediatric Acute Megakaryoblast Leukemia & Down Syndrome -An Experience of A Single Institute

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Citation: : Trivedi PJ, Patel DM, Kazi MM, Varma PK, Patel AB (2023) Pediatric Acute Megakaryoblast Leukemia & Down Syndrome -An Experience of a Single Institute. *Medi Clin Case Rep J.* 1(2), 24-28.

Received: 21 June, 2023; **Accepted:** 06 July, 2023; **Published:** 10 July, 2023

A B S T R A C T

Cytogenetic abnormalities in Down Syndrome (DS) are unique and emphasise upon the association between chromosome 21 and cancer. DS patients are high risk patients to develop leukemia specifically AMKL- a FAB subtype AML-M7 as compared to any other subtype of AML and ALL. Patients with DS shows GATA mutation and have better outcomes as compared to Non-DS AMKL. Their laboratory and clinical features are unique. Because of the development of uniform treatment protocols, there is an improvement in the clinical outcomes for DS-associated leukemia over the years. In our cytogenetic laboratory, we have studied 3 pediatric DS patients with AMKL. Bone marrow and Peripheral blood lymphocytes were used for conventional cytogenetic and Fluorescence in Situ Hybridization technique. Using standard protocol metaphases achieved and GTG banding was carried out for karyotype analysis. In FISH technique Locus specific Identifier RUNX1T1/RUNX1 probe was used. Trisomy 21 was confirmed in stimulated and unstimulated blood culture and by FISH technique. All patients have better survival with standard induction therapy. The present study highlights the screening of all DS patients and their follow up study to understand the progression to AMKL for tailoring therapy regimen.

Keywords: Acute Megakaryoblastic Leukemia; Chromosomal abnormalities; N Down syndrome; Fluorescence in-situ Hybridization

Introduction

Approximately 1/700 to 1/1000 new-borns develop Down Syndrome (DS) and carry trisomy 21 as a constitutional cytogenetic abnormality [1]. DS children are more prone to acquire leukemia in childhood as compared to children without DS and they do not develop solid tumors as often as down syndrome [2].

Approximately 3% of AML and MDS patients along with trisomy 21 showed other chromosomal abnormalities but their prognosis is not yet clear [3]. A small number of myeloid patients show tetrasomy of chromosome 21 and of pentasomy of chromosome 21q, which often presents as isochromosome or isodicentric chromosome 21 [4]. AML-M7 is a rare form of AML with megakaryocytic differentiation in greater than 50% of the neoplastic myeloblasts, evident by morphology and immunophenotype[5]. In the French American- British (FAB) classification, it is also known as Acute megakaryoblastic leukemia (AMKL). In children with DS, the risk of developing AMKL is increased 500-fold as compared to the non-DS population; and the risk of developing Acute Lymphoblastic Leukemia (ALL) is 20- fold greater in children with DS [2].

Before establishment of leukemogenicity a preleukemic condition is raised known as Transient Myeloproliferative Disorder (TMD), & 30% of new borns with DS represents characterised by a GATA binding protein 1 (*GATA1*) mutation. Almost all DS-AMKL cases are associated with a GATA1 mutation [6,7] and 80-90% of DS-AMKL shows a favorable response to chemotherapy [8-10]. Athale et al reported that non-DS children with AMKL are usually, not associated with

GATA1 mutation and often have a poor clinical course with a 5- year event free survival of 22 to 28% [11].

Due to the presence of trisomy in the patient, oxidative stress is developed leading to altered folate metabolism. This predisposes the patient to acquire mutations in *GATA1* gene, which further leads to the development of TMD. It has been shown that mutated GATA-1 could not suppress E2F transcription in Fetal liver cells [12].

The present study aimed to correlate the laboratory and clinical finding s of pediatric AML patients with DS. This research article presents three pediatric DS patients who developed acute megakaryoblastic leukemia (AML-M7/AMKL). The study was approved by the institutional review board and patients' general consent was taken. Here, this research article will provide a base for future investigations on the impact of gain of chromosome 21 in hematological cancer, with a further goal of discovering new vulnerabilities and develop novel targeted therapies to improve long term outcomes for DS and non-DS patients.

Case Details

Patient 1: 1-A 2-year-old male child suffering from low grade fever, weakness was admitted to Gujarat Cancer and Research Institute, Ahmadabad, India. His bone marrow report was AML-M7 and in conventional cytogenetic report showed trisomy 21 prior to the course of therapy. On admission the laboratory investigations revealed White Blood Cell count 4800/cmm with low platelet count 9000/cmm. Atypical cells with high N:C ratio. Basophilic to polychromatic scant cytoplasm and blebbing is seen. On bone marrow aspiration examination report showed, partially Diluted, Megakaryocytes not Seen. Suppressed erythropoiesis, PAS Stain-Negative, Sudan Black B-Negative. Blast Cells 51 %.Myelocytes- 7 %, Polymorphs- 20 %, Lymphocytes- 5 %, Inter Normoblasts-7 %, Late Normoblasts- 10 %. Blasts are medium to large sized with high nucleocytoplasmic ratio, open chromatin, conspicuous nucleoli, and moderate amount of granular cytoplasm. Erythropoiesis & granulopoiesis is suppressed. Megakaryocytes not seen. In peripheral smear-RBC - Normocytic normochromic mixed with few microcytic cells with occasional nucleated RBCs. WBC - Mild leukocytosis with blasts. Blast-35, P-27, L-32, E- 00, M-06. Severe thrombocytopenia. So, diagnosis was Acute Leukemia morphologically myeloblastic (AML). Serum LDH was 663U/L. Serum Creatinine was 0.2 mg/dl. In molecular study, FLT3 ITD / D835 Mutation by PCR-RFLP was negative. In chest X-Ray report Presence of ill-defined soft tissue opacities is noted involving bilateral lung fields. TSH of patient was high as 10.5 uIU/ml.

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Patient was treated with Daunorubicin and cytarabine in combination for first two months of admission and then with cytarabine. Bone marrow aspiration was again carried out after 2 months of treatment. The aspirate of normocellular bone marrow smear showing erythroid series with normoblastic maturation. Myeloid series with normal granulocytic precursors. Megakaryocytes seen, with normal morphology. PS RBC - Normocytic normochromic cells. WBC - Count within normal limits. P-74, L-22, E-01, M-03. Platelets Count adequate. MP not seen. Diagnosis is an AML & marrow is in remission.

Patient 2: A 8-months female patient was admitted in The Gujarat Cancer & Research Institute, Ahmedabad for leukemia work up. peripheral blood showed low platelet count 23000/cmm. Bone Marrow aspiration report showed adequate Material with Hypercellular marrow. Altered M: E Ratio. Megakaryocytes were suppressed. Few mature forms seen. Suppressed erythropoiesis, few normoblasts seen. PAS Stain was negative, Sudan Black B 76 % Myelocytes-3 % PolyMorphs-10 %, Eosinophils- 5 %, Lymphocytes-5 %, Late Normoblasts- 1 %. Bone Marrow report showed hypercellular marrow with adequate cell trails with around blasts-76%. Blasts are large cells with high N:C ratio, round/oval nucleus with open chromatin Eosinophils (5%) Erythroid series markedly suppressed Occasional normoblasts seen. Megakaryocytes suppressed few mature forms seen. In peripheral smear RBC were sparsely distributed. Microcytic hypochromic RBC. Marked leukocytosis. Blast-87, P-09, E-04. Moderate Thrombocytopenia. Acute leukemia morphologically myeloblastic. Serum LDH was 4470U/L. Uric Acid (UA) was 0.49 mg/dl. Flow cytometry, on peripheral blood showed 88% blasts were gated using CD45 V500c vs. side scatter. The blasts mainly expressed myeloid markers CD13, CD117, CD33 and CD41a along with CD34. Aberrant expression of cCD3 and CD19 was seen. Final diagnosis was Acute Megakaryoblastic Leukemia with aberrant cytoplasmic CD3 expression. In molecular study, FLT3 ITD / D835 Mutation by PCR-RFLP was negative. In chest X-Ray report showed normal. Liver is enlarged in size measures 10 cm and shows normal echopattern. Patient was treated with hydroxy urea (500mg) but unfortunately patient was lost to follow up.

Patient 3: A 2-months female patient was admitted in The Gujarat Cancer & Research Institute, Ahmedabad for leukemia work up. Patient had hypothyroidism & Down syndrome. Peripheral blood showed Hemoglobin was 5.3 g/dl, RBC 2,070,000 cells/µL, WBC. 21700 cells/µL platelets 356000 cells/µL, Polymorphs 27.1 % Eosinophils

2.6 % Lymphocytes 30.8 % Monocytes 24 % Basophils 15.5 %. Absolute Neutrophil Count (ANC) 5860 /uL Absolute Lymphocyte Count (ALC) 6650/uL. Serum LDH was 817U/L. Serum Uric acid 8.94 mg/dL. Immunophenotyping report and other investigation was not carried out as patient refused to take treatment and was lost to follow-up.

Materials and Methods

Conventional Cytogenetics

Bone marrow and peripheral blood sample was collected ascetically in Sodium Heparinized vaccuatte. In conventional cytogenetic study, stimulated and unstimulated peripheral blood sample was carried out to rule out the constitutional cytogenetic abnormality by long term and short-term culture respectively as per standard protocol and slides were banded using Giemsa Trypsin G banding technique. Good morphology metaphases were captured in Zeiss automatic karyotyping system and

analysis using IKAROS software and karyotype description was done using ISCN 2020 guidelines [13].

Fluorescence in situ hybridization (FISH)

Image capturing, and processing were carried out using Epi-fluorescence microscope equipped with an ISIS FISH imaging system (Metasystems, Germany) (AXIO Imager.Z2, Zeiss, USA) equipped with appropriate filter sets. FISH was performed using *RUNX1/RUNX1T1* LSI probe. In LSI *RUNX1/RUNX1T1* probe, *RUNX1* gene was tagged with Spectrum green and *RUNX1T1* gene tagged with Spectrum Orange.

Results

Conventional Cytogenetic & FISH results

Conventional chromosome analyses at diagnosis of GTG banded metaphases were carried out. Total 20 metaphases were karyotyped. All metaphases of stimulated showed 47, XY,

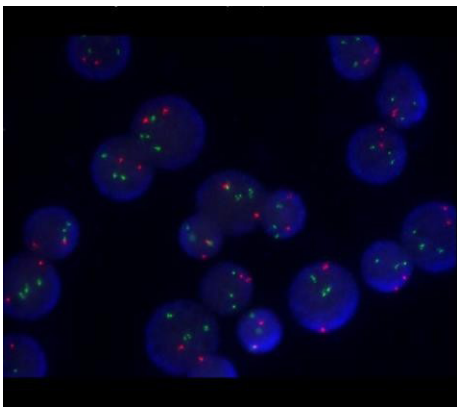
+21c [20] & unstimulated culture metaphases showed 47, XY, +21[20] (Figure 1A & 1B) respectively) in patient 1.

Stimulated culture G banded karyotype result showing 47,XY,+21c	Unstimulated culture G banded karyotype result showing 47,XY,+21 Unstimulated culture G banded karyotype result showing 47,XY,+21
Figure 1A	Figure 1B
Stimulated culture G banded karyotype result showing 47,XX,+21c	Unstimulated culture G banded karyotype result showing 47,XX,+21
Figure 2A	Figure 2B

Identifier probe showing three green signals of indicating three copies of RUNX1 gene Conventional chromosome analyses at diagnosis of GTG banded metaphase were carried out for a female patient (patient 2 & patient 3). Total 20 metaphases were karyotyped in each patient. All metaphases of stimulated showed 47, XX,+21c[20] & unstimulated culture metaphases showed 47,XX,+21[20] (Representative (Figure 2A & 2B) respectively.

FISH was performed in all 3 patients using *RUNX1/RUNX1T1* LSI probe and results showed three green signals for AML1 gene (Representative image (Figure 3).

Representative Fluorescence in Situ Hybridization Results with Runx1t1/Runx1 Locus Specific Identifier Probe Showing Three Given Signals of Indicating Three Copies of Runx1 Gene



Discussion

The constitutive abnormality such as trisomy 21is mostly due to non-disjunction during meiosis & it is the most frequent cytogenetic abnormality primarily in 95% of cases. [14-16]. Hematological malignancies are frequently presented with several structural as well as numerical alterations of chromosome 21. Long arm of chromosome 21 bears approx. 329 genes [17].and several have been suggested as possible mediators of leukemogenesis through increased dosage effects. The chimeric transcripts resulting from fusion of genes present on chromosome 21 such as TEL-AML1, AML1-ETO, FUS-ERG etc. have major functional impact and has widely been studied in various neoplastic conditions. However, it is largely unknown whether there is some role of gain of chromosome 21. On the other hand, the whole chromosome gains of 21 is a continuing abnormality observed in several types of acute leukemia, found in up to 35% of cases.

DS children harbour constitutive trisomy 21 and thereby highlight the association between gain of chromosome 21 and leukemogenesis, bringing them to a higher risk of developing acute leukemia as compared with other children [18]. Almost all newborns with down syndrome are presented with several quantitative and/or qualitative disorders of the myeloid compartment such as macrocytosis, dysplastic platelets, leukocytosis, and on average 4% or more blasts in the peripheral blood. These signify an intrinsic effect of trisomy 21 in such children [19]. Typical features of Transient MD are circulating peripheral megakaryoblasts with the immunophenotype .

CD33/38/117/34/7/56/36/71/42b thrombocytopenia, variable presence of leukocytosis [1]. in accordance with these all three patients have low platelet count and leukocytosis with high blast count. Also, in immunophenotype, results showed positive for CD13/CD33/CD117/CD34/CD41a expression. TMD spontaneously resolves within the first months of life, suggesting that mechanisms regulated by the Fetal microenvironment maintain perturbed hematopoiesis. Up to 30% of children with DS who have classically defined TMD develop ML–DS before 5 years of age. Our all patients developed myeloid leukemia before age of 5 years.

Reports suggest that trisomy 21 whether constitutive or acquired is a key factor in hematological malignancies [18]. There is a stepwise progression of trisomy 21 and TMD, by pathogenesis with a higher procurement of genetic alterations (including GATA1 mutations). Next Generation Sequencing identifies have ‘silent TMD,’ defined by a peripheral blast count of ≤10% and GATA1 gene mutation in ~20% of neonates with DS.

The 5-years overall and event-free survival of patients with ML -DS and with non-DS is 89– 93% and 87–90% respectively resulting in their good prognosis as compared to AML children. [20, 21] In the present work, study of GATA mutation was not possible in all patients.

According to Gamis et al [22]. DS patients may develop signs of myelodysplasia. This condition is characterized by progressive anemia and thrombocytopenia, dysplastic erythroid cells, and megakaryocytes in the bone marrow. Such conditions may result in difficulty to aspirate because of the presence of myelofibrosis. The development of AML is preceded by this myelodysplastic phase, and those disorders are often referred as "myeloid leukemia of DS. Due to these reasons, bone marrow aspiration was not possible in patient 3, Buitenkamp et al & Salazar et al, reported that ML–DS children with DS–ALL have a poor outcome compared with non-DS children with ALL. The poor outcome in children is due to higher relapse rates, increased risk of infection, treatment related mortality and induction failure. [23,24] however, there is an improvement in clinical outcomes for DS-associated leukemia which is due to development of uniform treatment protocols facilitated by international cooperative groups.

The comprehensive understanding of molecular pathogenesis underlying DS-associated leukemia is done owing to the recent characterization of genetic landscape. The remission rate of 80 to 90% is observed in DS-AMKL, presenting a favorable response to chemotherapy [25]. In present study all patients have myeloid lineage leukemia -AML-M7 and all three patients are alive till date and patient 1 is in remission. There are subtle reasons for such an association, however it strongly suggests that somatic +21 is clonally selected during leukemia development and that megakaryocytic and B-cell progenitors are intensely susceptible to the increased dosage of chromosome 21 genes.

AML-M0, M1/M2, and M6 have also been described in ML-DS, but less frequently. AMKL development initiates with the trisomy and this is followed by the addition of GATA1 mutations leading to TDM. The next step is acquisition of mutations in the cohesin components (53%), CCCTC- binding factor (20%), or other epigenetic regulatory factors (EZH2, KANSL1) by a previous TDM clone resulting in further progression of AMKL [26].

Conclusion

Screening for genomic and genetic aberrations in clinical settings is of utmost importance in order to get information for prognostic risk stratifications and thereby proper management of AML patients. This study will present a glimpse of our knowledge on DS-associated leukemia that addresses clinical features, therapy, and molecular mechanisms of leukemogenesis. This will be considered in parallel to other hematological malignancies harboring somatic +21 with a view to discover new vulnerabilities and develop novel targeted therapies to improve long term outcomes for DS.

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