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MYELOID LEUKEMIA ASSOCIATED WITH DOWN SYNDROME WITH COMPLEX KARYOTYPES: REPORT OF TWO CASES - EXPERIENCE AT A TERTIARY CARE HOSPITAL

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ABSTRACT
Down syndrome is the most common chromosomal abnormality in the live newborn. It is associated with increased incidence of malignancies. Approximately, 1 in 100-200 DS children develop acute leukemia with approximately equal numbers of acute lymphoid and myeloid leukemias. Down associated myeloid leukemia is usually megakaryoblastic type and exhibit characteristic flow cytometry pattern. They express CD45, CD34, CD33, CD38, CD36, CD56, HLA-DR, CD7, and at least one of the megakaryocytic markers CD41, CD42a, or CD61. Less frequently, the blasts express CD4, CD13, CD11b, CD265 (glycophorin A), orCD15. These blasts do not express the lymphoid markers such as CD3, CD5, CD19, and CD20.Here we wish to present two cases of *myeloid leukemia associated Down syndrome* with *complex karyotypes*. Of these two cases, one case was suspected to have Down syndrome due to characteristic flow cytometry pattern and was later confirmed on karyotyping.

INTRODUCTION

Down syndrome (DS) is the most common cytogenetic abnormality at birth (1/700). These individuals display various developmental anomalies like delayed milestones, craniofacial dysmorphy, cardiovascular defects and learning disabilities [1]. Individuals with DS have a 50 fold increase in incidence of acute leukemia (AL) during the first five years of life compared to non DS individuals. Paradoxically, individuals with DS have decreased frequency of solid tumours. 50 % of acute myeloid leukemia (AML) in DS is Acute Megakaryoblastic Leukemia (AMKL) beyond neonatal period [1, 2]. Since this type of disease in unique to children with DS the term myeloid leukemia of DS encompasses both myelodysplastic syndrome and AML as there are no biological differences in DS individuals between myelodysplastic syndrome and AML as per WHO 2008 [2].

Flow cytometry plays a crucial role in diagnosis of AML in DS as the blasts exhibit a characteristic immunophenotype. They are positive for CD117, CD 13, CD33, CD7, CD4, CD42, TPO-R, IL 3R, CD 36, CD41, CD61, and CD 71 and are generally negative for Myeloperoxidase (MPO), CD14, CD15 and glycophorin A. CD 34 is negative in 50% of cases and CD41 is negative in 30% cases [2, 3].

AML in DS is a unique biological subtype, which is quite different from AML in non DS children. The paradox of increased risk of leukemia and heightened sensitivity to chemotherapy has been described [4]. Therefore, a standardized dose reduced AML therapy should be considered in children with DS [5]. As the blasts in DS are MPO negative they can easily be mistaken as lymphoid blasts. Acute lymphoblastic leukemia (ALL) occurs in 1 in 300 children with DS [7]. Many groups have

reported that ALL in DS have inferior event free survival and overall survival when compared with children with ALL but without DS. Factors contributing to inferior outcome in children with ALL with DS include increased risk of relapse and increased morbidity and mortality resulting from toxic side effects of therapy [6, 8].

Here, we wish to present two cases of myeloid leukemia associated with DS with complex karyotypes. One of these cases was diagnosed as ALL and was on treatment and DS was overlooked. In our centre only on Flow cytometry analysis, DS was suspected and confirmed on karyotyping.

MATERIALS AND METHODS

Flow immunocytometry was performed by direct multicolour immunofluorescent technique on bone marrow aspirates. Four color panels of monoclonal antibodies included evaluation of membrane (CD3,CD 4, CD 5, CD 8, CD10, CD 11c, CD 13, CD 14, CD15, CD19 , CD 33, CD 34, HLA-DR, CD 41a, CD 25, CD 117, CD 235a and CD45) and cyotoplasmic antigens (TdT, CD3, MPO, CD79a). Samples were analyzed on a Beckmen Coulter FC 500 flow cytometer. Blast cell populations were sequentially gated and analyzed according to a pattern of CD 45 antigen expression and side light scatter cell characteristics (CD 45/SSC). At least 10000 blast cells per gate per tube were collected. Results were considered positive by a criterion of ≥20% labeled cells for membrane as well as ≥10% for cytoplasmic antigens as determined by mean fluorescence intensity. Final characterization and classification of leukemic samples were performed according to the guidelines given by WHO[2]. All samples were stained as per manufacturer's protocol and cocktails were used wherever possible. All reagents were CE/IVD approved.

Cytogenetic analyses were performed by direct preparation of unstimulated bone marrow cells and following 24 h of culture in RPMI 1540 culture medium with 25% fetal calf serum at 37 C. The G-banding method was used for the preparation of the metaphase cell preparation. The Karyotype was arrayed according to the depictions and descriptions given in the International system for Human Cytogenetic Nomenclature 2008 (ISCN, 2009). The abnormality was found in 20/20 metaphases examined.

Bone marrow aspirates were methanol fixed and stained with Leishman-Giemsa stain. Bone marrow biopsies were fixed with acetic acid-zinc-formalin fixative, paraffin embedded and sections cut on a microtome with 2-3 micron thickness.

CASE REPORTS

Case 1

This 4 yrs old boy initially presented in civil hospital at Gwalior, India with history of recurrent fever, weakness and malaise. General examination revealed mild hepatomegaly. There was no lymphadenopathy, splenomegaly, sternal tenderness or bleeding tendency. Hemogram showed Hb 9.2 gm/dl, TLC 34000/cmm with 60% blasts and reduced platelets (90,000/cmm). The blasts were MPO negative and diagnosis of ALL was made. Patient was treated with steroids along with antibiotic cover. Cytogenetics was not asked for nor clinical examination revealed any stigmata of DS. However, patient after initial response did not progress satisfactorily. Then, Patient was referred to our centre. At the time of presentation his Hb was 9.3 gm/dl, TLC 9490/cmm with 10% blasts and reduced platelets (70,000/cmm). His bone marrow done at this juncture was markedly hemodiluted with few blasts which were MPO negative. Bone marrow biopsy and flow cytometry were also not contributory. Patient was re-evaluated after few days. TLC had risen to 29720/cmm with 39% blasts which were MPO negative (Fig 1). Bone marrow aspirate and bone marrow biopsy also showed acute leukemia (MPO negative). Flow Cytometry was performed on bone marrow aspirate sample. The gating strategy was FSC Vs SSC, SSC Vs CD45. 24.1 % cells were gated in area of interest and sequential gating for ontogeny of cells revealed gated(Dim), CD34, CD33, HLA-DR, CD7, CD4 and CD41 and negative for cMPO, CD13, CD11c, TdT, and other cells were positive for CD45 lymphoid markers (Fig 2a-f). Based on the findings of Flow cytometry which revealed a pattern typical of Myeloid Leukemia associated with DS as per WHO 2008, same diagnosis was given and cytogenetics was asked for. Cytogenetics revealed a complex karyotype 49, XY with the presence of translocation involving the p arm of chromosome #10 and q arm of chromosome #11 at bands 10p13 and 11q21 along with trisomies of chromosomes # 8, # 10 and # 21 (Fig 3).. Leukemic diagnosis comprehensive profile showed PML-RARA, t (8; 21), RUNX 1 and Inv (16) gene rearrangements to be negative. Patient was started on BFM 98-DS protocol. Induction phase was with AIE (Cytarabine, reduced dosage of Idarubicin, Etoposide). Post induction bone marrow examination was suggestive of remission. Consolidation was instituted with AI (Cytarabine, reduced dosage of Idarubicin) + haM (cytarabine and reduced dose of mitoxantrone) and intensification was done with HAE (cytarabine and etoposide). Patient is presently in remission and on follow up.

Case 2

This 2 yrs old female child, a previously known case of DS presented with 10 days history of low grade fever with splenomegaly. Evaluation revealed 70 % blasts on peripheral smear examination. Same was confirmed on bone marrow examination. The blasts were MPO negative (BM/444/14 & B/3025/14). Flow Cytometry was performed on bone marrow aspirate sample. The gating strategy was FSC Vs SSC, SSC Vs CD45. 70.6 % cells were gated in area of interest and sequential gating for ontogeny of cells revealed gated cells were positive for

CD45 (Dim), CD34, CD33, CD 13, CD7, CD4 and CD41 and negative for cMPO, HLA-DR, CD11c, TdT, and other lymphoid markers (Fig 4a-f). Thus diagnosis of Myeloid leukemia associated with DS as per WHO 2008 was made.

Leukemic diagnosis comprehensive profile showed PML-RARA, t (8; 21), RUNX 1 and Inv (16) gene rearrangements to be negative. Karyotyping revealed a complex karyotype 46XX, t(1;5)(q21;q13), der (21;21)(q10;q10), +21, add (22)(q13)[16]/46, idem, del(3)(q12q25)[4] (Fig 5). Patient was started on BFM 98-DS protocol. Induction phase was with AIE. Post induction bone marrow examination was suggestive of remission. Consolidation was instituted with AI + haM and intensification was done with HAE. Post consolidation bone marrow however revealed partial remission with residual disease of 8% blasts. Bone marrow transplant is being contemplated with possibility of salvage chemotherapy as back up.

Fig 1. Bone marrow biopsy

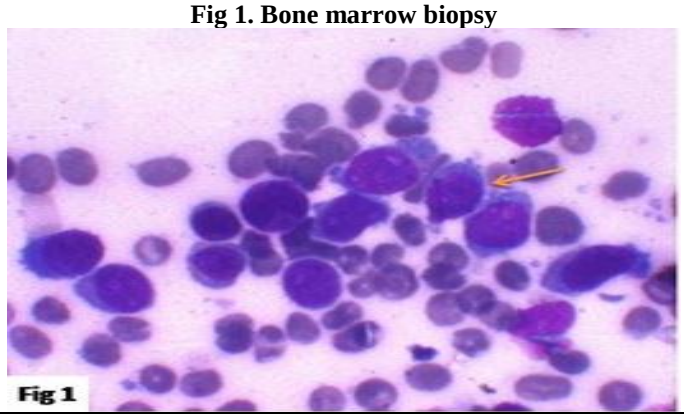


Fig 2.Gating stategy was SSC Vs CD45, Gated cells were positive for CD34, Gated cells were positive for CD33 & HLA DR, Gated cells were positive for CD7, Gated cells were positive cells for CD4, Gated cells were positive for CD41

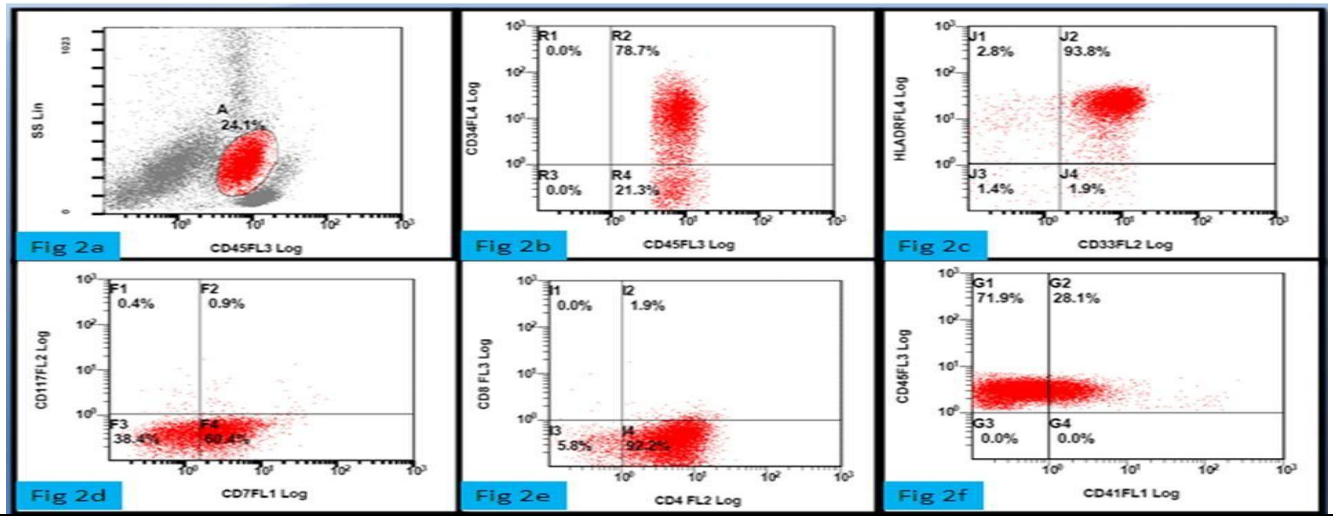


Fig 3. Interpretation : 49, XY with the presence of translocation involving the p arm of chromosome #10 and q arm of chromosome #11 at bands 10p13 and 11q21 along with trisomies of chromosomes # 8, # 19 and # 21

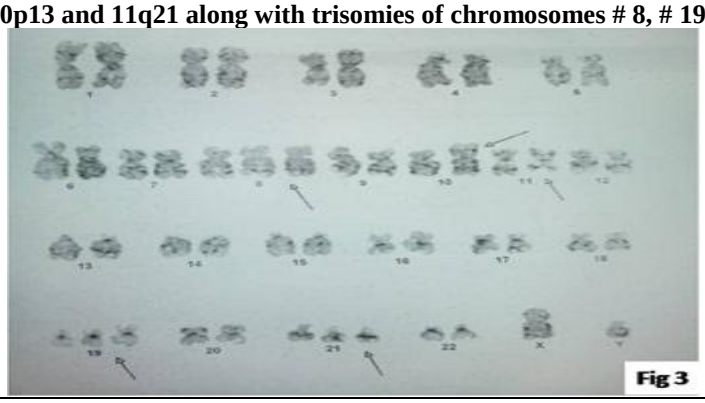


Fig 4. Gated cells were positive for CD34, gated cells were positive for CD33, gated cells were positive for CD13, Gated cells were positive for CD7, Gated cells were positive for CD4, 21% of gated cells were positive for CD41

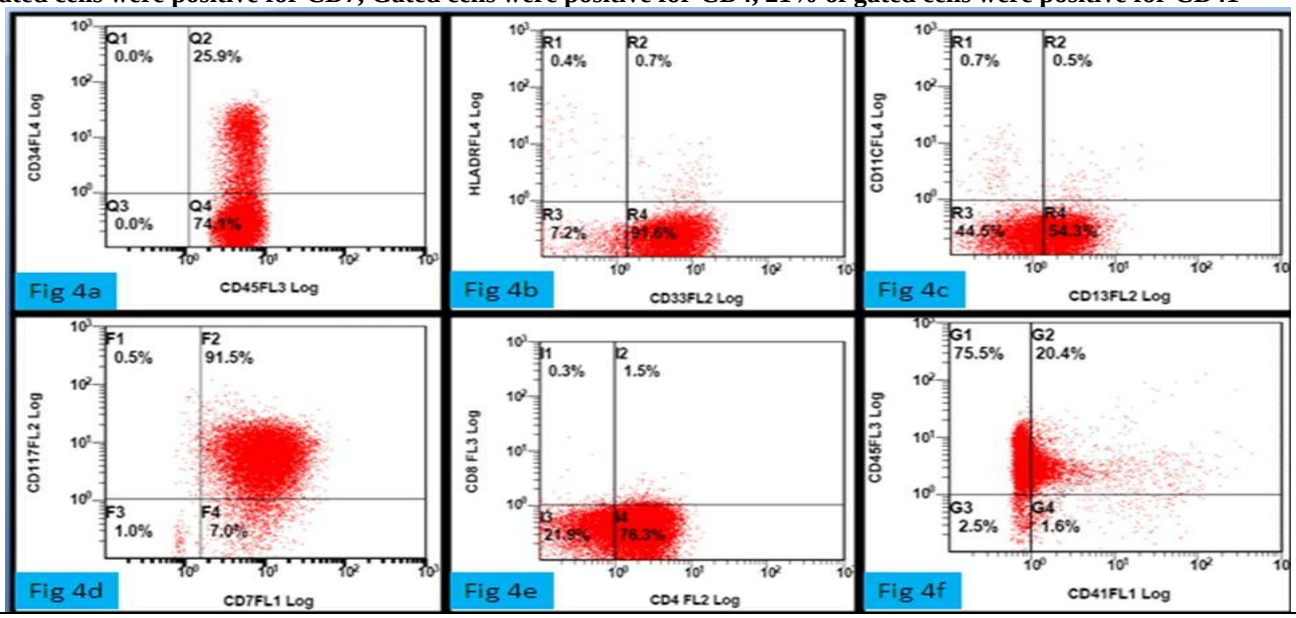
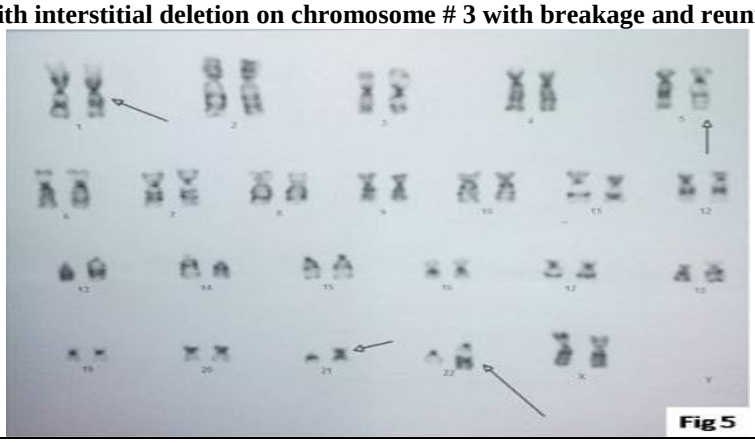


Fig 5. Interpretation : Analysis of 20 metaphases reveal 16 metaphases with translocation involving chromosomes # 1 and # 5 at bands 1q21 and 5 q13, another homologous translocation involving both chromosomes # 21 at bands 21q10 and 21q10, along with extra chromosome # 21 and added material on the long arm of chromosome # 22 at band 22q13. Another 4 metaphases reveal 46 chromosomes with the presence of same chromosomal abnormalities as mentioned above along with interstitial deletion on chromosome # 3 with breakage and reunion bands 3q12 and 3q25.



DISCUSSION

The natural history of leukemia in children with DS suggests that trisomy 21 directly contributes to the malignant transformation of hematopoietic cells. In addition, somatic mutations of the *GATA1* gene have been detected in nearly all DS AMKL cases and are notably absent in non-DS AMKL [1]. There is a well-recognized preceding transient abnormal myelopoiesis (TAM), occurring in the neonatal period in 10% of infants with DS[2]. TMD is a clonal pre-leukemia phase characterized by an accumulation of immature megakaryoblasts in the fetal liver and peripheral blood [9]. The clinical presentation of neonates with TAM varies widely from a healthy appearance to bruising, respiratory distress, fulminant hepatic failure, even *hydrops fetalis* or death in 15–20% of cases that have been diagnosed. Overall, though, the majority of cases resolve spontaneously with normal blood counts at a mean of 84 days. The incidence of TAM may be underestimated as not all cases come to medical attention. The median age of presentation of TAM, based on pooled data from > 200 neonates, is 3–7 days [10]. After a period of 1–3 years, a subset of these children (20–30%), develop AML. In a series of 112 patients with AMKL, the median age of DS patients was 1.8 years vs. approximately 8 years in non-DS cases [5]. Manifestations in patients with AMKL include anemia, thrombocytopenia, myelofibrosis, organomegaly, extensive skeletal lesions, and leucocytosis although white blood counts are lower than in non-DS. CNS involvement is unusual [1].

DS is a unique model for studying the association of leukemia biology and therapeutic response because on one hand trisomy 21 increases the risk for leukemia several

fold and on other hand leukemia is highly sensitive for chemotherapy [4, 11, 12]. Although all DS children have trisomy 21, only 10% develop TAM and less than 1% develop AL [13]. These observations support the hypothesis that DS leukemogenesis is a multistep process in which the progenitor cells acquire multiple genetic lesions during progression to AL. As trisomy 21 alone is not sufficient for development of AL, discovery of GATA-1 mutations was found in nearly all the individuals with TAM/AL [14]. GATA 1 mutations contribute to leukemia by providing a block in the maturation of megakaryocytic differentiation. Even though presence of trisomy 21 and GATA1 mutations appears adequate for TAM, it is insufficient for leukemogenesis because majority of TAM resolves spontaneously. This spontaneous resolution points out the fact that specific environment is required for proliferation of these cells. Possible hypothesis for this is that TAM arises in the fetal liver and spontaneous regression of TAM after birth occurs due to loss of permissive liver environment [15]. Thus although TAM cells are not frankly leukemic, they acquire additional lesions which in turn results in overt leukemia. These additional lesions could be mutations in p53, altered telomerase activity or additional karyotypic abnormalities, trisomy 8 being the most common [13, 16]. One of our patients had additional trisomy 8.

The two patients were treated as per AML-BFM 98 for DS patient protocol which became standardized treatment regimen for treatment of AML in DS[5]. This study proved that a low dose regimen was superior to AML-BFM 93 protocol I terms of 3 year survival, relapse and early deaths [5]. Highly chemosensitive nature of DS blasts was brought out by various studies [2, 4, 11, 12]. This is because overexpression of gene present on chromosome 21 may alter expression of genes on other chromosomes that subsequently affect hematopoiesi. For example, gene encoding cystathionine β synthase (CBS) located on 21q22.3 is expressed at 12.5 times greater in DS-AMKL as compared to non DS-AMKL. Increased activity of CBS causes lower levels of homocysteine, methionine, S-adenosylhomocysteine and S-adenosylmethionine [17]. Altered levels of these results in altered methylation and functional folate deficiency. This may result in increased rates of DNA mutation. Interestingly, it is possible that the greater sensitivity to chemotherapy shown by patients with DS-AMKL results from overexpression of CBS [13, 17].

The complex karyotype seen in these two cases have not been reported so far in India. The largest studies in India were carried out by N Chandra et al and Frenny Sheth et al on the cytogenetic profile of DS in India [18, 19]. Recently a report of multiplex reverse transcriptase PCR for diagnosis of acute myelosis in an infant with DS revealed a cryptic t (15; 17) which was missed on conventional karyotyping highlighting the need for further studies to assess other possible mutations in multi-step carcinogenesis of AL in DS was emphasized [20].

To conclude, myeloid leukemia of DS, a new entity described in WHO 2008 (ICD – O 9898/3) presents with typical findings where blasts are MPO negative and have a typical flow pattern as described above. MPO negativity can cause erroneous diagnosis of ALL and methotextrate may be administered which is harmful in these patients because of heightened chemosensitivity. Hence it is important to be aware of this entity and its flow pattern for accurate diagnosis and thus optimal treatment.

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