Immuno-hematological analysis of Congenital AML associated with Downs Syndrome

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Abstract
Congenital leukemia is very rare, diagnosed in first month of life and often associated with Down’s syndrome. Congenital Acute myeloid leukemia (AML) is more common over acute lymphoblastic leukemia (ALL). The hematological parameters such as anemia, low hematocrit, reduced RBC count; reduced platelets are distinct characteristics against normal parameters in very closely associated Transient myeloproliferative disorder (TMD). The immunophenotypic markers such as expression pattern of CD13, CD11b and HLADR in combination can be used for distinguishing two diseases. The morphological discrimination between congenital AML and TMD is little difficult and hence combined results of hematological, cytogenetic and immunophenotyping should be considered while concluding the cases. We here report two cases observed in year 2014 in our hospital, at Pune. Both the cases were diagnosed in first week of their life, day 2 (case 1) and day 8 (case 2) with distinct features of increased white blood cell count with myeloid blast, reduced Hb, low hematocrit, reduced RBC count and low platelets count with trisomy 21. Both the cases were CD13-ve and CD11b +ve, hence we conclude these cases as Congenital Acute myeloid leukemia associated with Down’s syndrome. Both the cases survived for less than 15 days after the birth.

Keywords: Surface Markers, FISH and TMD

1. Introduction
Amongst all leukemia, Congenital and neonatal leukemia are very rare malignancy, yet carry high mortality rate [1]. Congenital leukemia is manifested within first few days of life 0-4 weeks, while neonatal leukemia is diagnosed in first 4-6 weeks of life [2-5]. Across congenital leukemia, the acute myeloid leukemia (AML) is more common as compared to Acute lymphoblastic leukemia (ALL) [6-8]. The prognosis is generally poor in congenital leukemia [6, 9]. Children with Down’s syndrome (DS) are at a high risk of developing Leukemia compared to normal genotype [10]. Down’s children are also at high risk of developing transient myeloproliferative disorder (TMD) [11]. TMD is morphologically similar and not easily distinguishable disorder to that of congenital AML. Thus the congenital AML must be carefully distinguished from TMD [10].

The Diagnostic criteria proposed [7, 4] for congenital leukaemia are: (i) presentation in the first 4 weeks of life; (ii) proliferation of immature myeloid, lymphoid or erythroid cells; (iii) Infiltration of these cells into non haematopoietic tissues (iv) Absence of other congenital diseases and (v) Absence of fetomaternal blood incompatibilities such as erythroblastosis fetalis. TMD and AML in DS do not have fine distinguishing characters at hematological and cytogenetic levels, but have immunophenotypic differences from each other and from general AML [10]. Thus both the characters must be taken into consideration while reporting the case of congenital AML. Here we present Immuno-hematological and cytological analyses of two newborn babies with congenital AML associated with DS, reported in our hospital, to illustrate the clinical features and course of this disease.

2. Materials and Methods
2.1) Patients
Two cases of congenital AML with DS were studied in Sahyadri Hospital, Karve road, Pune. The diagnoses were confirmed by the clinical, morphologic and laboratory tests.

2.2) Hematological analysis
The peripheral blood (PB) samples from both babies were collected in separate EDTA vacutainers and used for haemogram, morphological observations and blood grouping. The PB for blood culture testing in blood culture bottles and for C-reactive protein (CRP) plain vacutainers was used. The morphology was observed on PB stained slides of leishman...
under compound microscope using standard laboratory protocol.

2.3) Flow Cytometry analysis:

Flow cytometry was performed on PB (EDTA) using 5 antibody panels against a variety of lymphoid, myelomonocytic, and megakaryocytic antigens. The antibodies (and clones) used (all antibodies from Beckman Coulter) with fluorochromes such as Fluorescein isothiocyanate (FITC), Phycoerythrin (PE), Electron coupled Dye (ECD), Phycoerythrin cyanine 5 (PC5) Phycoerythrin cyanine 7 (PC7) are used in the experiment including, anti CD13-FITC, CD117-PE, CD33-PE, CD14-FITC, CD15-FITC, CD16-ECD, CD11b-PC5, CD56-PE, CD64-PC5, CD11c-PC7, HLA-DR-EC, CD19-EC, CD20-FLTC, CD34-PE, CD3-EC, CD2-PC5, CD8-FITC, CD4-PE, CD5-PE,CD5-PC5, CD7-FITC, CD10-PC5, CD45-PC7, MPO-PC5, CD79a-PE. The sample is processed by a routine laboratory protocol for flow cytometric staining and intracellular staining. All analysis procedures were performed following the manufacturer's instructions. In short, 100 µL of whole blood was mixed with 5 types of antibodies and incubated for 20 min at room temperature in dark. Red blood cells were broken down with lysing solution (VersaLyse solution; Beckman Coulter) for 15 min. Followed by washing, approximately 1 lakh events were analyzed by using a flow cytometer (FC500) and a 32-tube carousel. The flow cytometer was set according to the manufacturer’s instructions, using FlowSet (Beckman Coulter). The intraprep kit (Beckman coulter) was used to stain the intra cytoplasmic antigens. The data was analysed using CytoDiff CXP 2.0; Beckman Coulter [12].

2.4) Fluorescent In Situ Hybridization:

Fluorescent In Situ Hybridization (FISH) test was performed on Peripheral blood (PB). The 200 interphases cells were observed for presence of any abnormality of chromosome 21. The PB samples were allowed to hybridized to chromosome 21 specific probe (Ls121 DNA probe). The Ls121 DNA probe is a spectrum orange directly labeled fluorochrome DNA probe that contain unique DNA sequences corresponding to the D21S259, D21S341 and D21S342 loci located in the 21q22.13 to 21q22.2 region on chromosome 21 [13, 14].The probe was first denatured in hybridization buffer for effective binding. The assay is designed for the detection and quantification of chromosome 21 by (FISH). Results were confirmed by doing routine karyotyping.

3. Results

3.1) Case 1:

The female infant weighing 1080 grams was born to 36 year female after normal vaginal delivery. The baby was born prematurely (28 weeks after gestation), which had head to chest circumference ratio as 1.16 cm and height was 33 cm. Baby cried after physical stimulation (Table 1). The blood pressure was found to be very low 39/21. Gross examination show the respiratory problems with breathing rate 52 /min, heart rate were 132 /min and were slightly feverish 37.2°C. The neurosonography was normal. Abdomen shows mild to moderate ascites. Both kidneys show fullness of Pelvicalyceal system. Skin lesions and hepatomegaly was observed.

The examination of peripheral blood on day 2 of birth revealed that there were 60% circulating blasts with macrocytosis, anisocytosis leukocytosis and thrombocytopenia. She was having blood picture as Hb 10.1g/dL, RBC 2.06x10^6 haematocrit 27.8%, MCV 134.9 fl, MCH 49, M.C.H.C. 36.3 gm/dL, TLC 55.8 x 10^3 cells/µl with neutrophils 24% lymphocytes 12% Monocytes 4%, with giant but reduce platelet count of only 30x10^3 / µl. 29-30 nRBCs was found to be circulating per 100 WBCs. The baby was B Rh⁺ve while mother was O Rh +ve so exclude chances of erythroblastosis fetalis (Table 1). Two days later the WBC count was increased to 64.4x10^3 cells/µl and platelets were further reduce to 14 x 10^3 / µl. The C-reactive protein was negative. Serum TSH was found to be normal, 8.03mIU/L.

Blood cultures were negative and serology testing was not indicative of congenital infection with rubella, cytomegalovirus, herpes virus or syphilis. Cytoplasm was scanty, MPO was negative morphologically.

The neonate was on supportive treatment of vitamin K, IV fluid. The Amputum and Amikacin drugs were given after birth. The morphology of blast shows large nuclei with fine chromatin and prominent nucleoli.

For flow cytometry analysis 49.7% cells were gated with low to moderate side scatter and moderate CD45 positivity (Blast gate).These cells were positive for CD34, CD33, CD11b, CD117, Cyto MPO, CD4, CD7 and CD56. They are negative for CD2, CD3, CD5, Cyto CD3, CD10, CD19, CD20, Cyto CD79a, CD13, CD14, CD15, CD16, CD64 CD11c and HLA DR. FISH test out of 200 interphases observed 190 interphase showed trisomy of 21 (Table 2). Karyotyping of patient was 46 XX +21. The patient died at age of 4 days due to cardiorespiratory arrest and respiratory distress.

3.2) Case 2:

The female infant weighing 1380 grams was born to 20 year female after normal vaginal delivery. The baby was born prematurely, 33 weeks after gestation, with head circumference 30 cm and height was 42 cm. Baby cried after physical stimulation. Patient had one episode of bloody vomiting with one episode of apnea on 2nd day after birth. Patient showed respiratory distress, dysmorphic facies, lethargy and pale appearance. Minor skin lesions were noticed, with no organomegaly.

The examination of peripheral blood on 8th day after birth reveals that there were 59% circulating blasts with macrocytosis, moderate anisopoikilocytosis, mild polychromatia and thrombocytopenia. She was having blood picture as Hb 10.2 g/dL, RBC 2.34x10^6, haematocrit 30.4%, MCV 129.9 fl, MCH 43.6 pg, M.C.H.C. 33.5 gm/dL, TLC
52.6 x 10^3 cells/µl with neutrophils 12% lymphocytes 37% Monocytes 1%, with platelet count of only 36x10^3 / µl (Table 1). Smear shows leukocytosis with shift to left up to blast. Large cells with round to oval nucleus with 1-2 nucleoli seen. P.T. and P.T.T values found to be increased as compare to control and they were 40.3 Sec and 48.4 Sec respectively.

Anisocytosis, macrocytosis, polychromasia, thrombocytopenia and platelet clumps were observed. Occasional nRBCs were found in circulation. The baby was O Rh positive same as mother thus ruled out chances of erythroblastosis fetalis. The C-reactive protein was negative.

Blood cultures were negative and serology testing was not indicative of congenital infection with rubella, cytomegalovirus, herpes virus or syphilis. Cytoplasm was scanty, MPO was negative morphologically.

On admission, baby was received with ET in situ. Initially patient was on Gentamycin than grade up to injection Amikacin and Piptaj (as septic screening was positive) the morphology of blast shows large nuclei with prominent 1-2 nucleoli.

For surface marker analysis, 51% cells gated with low to moderate side scatter and moderate CD45 positivity (Blast gate). These cells are positive for CD34, CD33, CD11b, CD117, CD19 (dim), CD7 CD56 and HLA DR. They are negative for CD2, CD3, CD4, Cyto CD3, CD10, CD20, Cyto CD79a, CD11c, CD13, CD14, CD15, CD16, CD64 and Cyto MPO.

In FISH test out of 200 interphases observed, 185 interphase showed trisomy of 21 (Table 2). Karyotyping of patient was 46 XX +21. The patient died at age of 15 days due to respiratory distress and prematurity with acute myeloid leukemia.

### Comparison of Clinical History, morphology and immunophenotyping of two cases:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Parameter</th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation period</td>
<td>28 week</td>
<td>33 week</td>
<td>MCH</td>
<td>49</td>
<td>43.6</td>
</tr>
<tr>
<td>Weight of baby</td>
<td>1080 gm</td>
<td>1380 gm</td>
<td>MCHC</td>
<td>36.3</td>
<td>33.5</td>
</tr>
<tr>
<td>Age of mother</td>
<td>36</td>
<td>20</td>
<td>Neutrophils</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>Hb</td>
<td>10.1 g/dL.</td>
<td>10.2 g/dL.</td>
<td>Lymphocytes</td>
<td>12</td>
<td>37</td>
</tr>
<tr>
<td>RBC Count</td>
<td>2.05x10^6 cells/µl</td>
<td>2.34 x 10^6 cells/µl</td>
<td>Monocytes</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>WBC count</td>
<td>55846 cells/µl</td>
<td>52600 cells/µl</td>
<td>Thrombocytopenia</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Platelets</td>
<td>30000 /µl</td>
<td>36000 /µl</td>
<td>nRBC/100 WBC</td>
<td>29-30 cells</td>
<td>3-4 cells.</td>
</tr>
<tr>
<td>Blasts</td>
<td>60 %</td>
<td>59 %</td>
<td>CRP</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>27.8</td>
<td>30.4</td>
<td>Aerobic culture and sensitivity till 6th day of incubation</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>MCV</td>
<td>134.9</td>
<td>129.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Comparative results of two cases w.r.t. immunophenotypic studies

<table>
<thead>
<tr>
<th>Surface antigens</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Surface antigens</th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD33</td>
<td>79 % (Moderate)</td>
<td>85 % (moderate)</td>
<td>CD15</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>HLA DR</td>
<td>Negative</td>
<td>43% (moderate)</td>
<td>CD16</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>CytoMPO</td>
<td>55% (dim)</td>
<td>Negative</td>
<td>CD64</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>CD 117</td>
<td>92% (moderate)</td>
<td>97% (moderate)</td>
<td>CD11c</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>CD 34</td>
<td>85% (bright)</td>
<td>83% (bright)</td>
<td>CD20</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>CD 11b</td>
<td>30% (dim)</td>
<td>31% (dim)</td>
<td>CD 2</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>CD7</td>
<td>69% (dim)</td>
<td>67 % (dim)</td>
<td>CD3</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>CD 56</td>
<td>76 % (moderate)</td>
<td>84% (moderate)</td>
<td>CD4</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>CD 13</td>
<td>Negative</td>
<td>Negative</td>
<td>eCD3</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>CD 19</td>
<td>Negative</td>
<td>19% (dim)</td>
<td>CD10</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>CD 14</td>
<td>Negative</td>
<td>Negative</td>
<td>CD5</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

### 4. Discussion

Both the cases presented here have been diagnosed within first week of life. They show increased leukocyte count, presence of myeloid blast and trisomy 21 and hence can be categorized under congenital AML associated with Down’s syndrome rather than neonatal leukemia [2, 4, 6, 15]. As per known reports, this is very rare malignancy, yet carry high mortality rate, our findings are consistent with it [2, 6]. In both the cases studied, we observed minor skin lesions. These lesions are result of infiltration of blast cells and are
called as blueberry muffin rash which is characteristic of congenital AML [4, 6]. The erythroblastosis fetalis and other congenital viral diseases were ruled out in order to assure congenital leukemia rather than TMD [4]. Anemia (low hemoglobin, low hematocrit, reduce RBC count), Cytopenia (reduce neutrophils), low platelets (table1) also helpful in concluding the cases with congenital AML over TMD [10, 16].

Blasts in both cases were CD45+, CD33+, while in case 1 is cyto MPO+ suggestive of myeloid origin (table 2) [2, 10]. Both cases were CD34+ and lacked the more mature myeloid antigens such as CD15 and CD16, similar to normal myeloblasts [10]. The CD14- and CD64- results as well as aberrant expression of CD7+ and CD56+ may appear like TMD as per the previous reports [2], however results are not concrete to label it as TMD [10]. CD13 is expressed on normal myeloblasts at an early stage of development and can be observed in most neoplastic myeloid proliferations is found to be absent in both the cases. CD11b, a more mature myeloid associated antigen not found on normal myeloblasts, TMD or in most cases of AML, was expressed in both cases which may also distinguished the case from TMD [17]. The positive expression of HLA DR in case 2 might be insignificant in conclusion and hence not taken into consideration. The expression of CD19 in case 2 may be aberrant [10].

In summary, based on clinical history, morphology, hematological analysis, cytogenetic study and immunophenotyping we conclude both the cases discussed are very good representative of congenital AML.

References