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CASE SERIES

Retrospective Study of Three Cases of Congenital Leukemia with Clinical Presentations and Particular Cytogenetic Abnormality

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ABSTRACT

BACKGROUND

The goal is to assess the prognosis of cytogenetic abnormality, because cytogenetic abnormality is rarely encountered in clinical practice.

METHODS

We retrospectively report three cytogenetic abnormality cases with clinical, cytogenetic, and genetic characteristic.

RESULTS

All cases occurred within one month of birth and had prominent hepatosplenomegaly, including acute myeloid leukemia (case 1, case 2) and acute leukemia (case 3). Moreover, case 1 appeared as leukemia cutis at birth, case 2 was born with respiratory distress, and both showed hyperleukocytosis. The R-banded karyotype detected cytogenetic abnormality in three cases, case1 with 46,XY,t(8;12)(q21;p13), case 2 with 47,XX,+21 and case 3 with 46,XY,t(6;X)(q22:p12), respectively. Especially in case 1, reverse transcription–polymerase chain reaction analysis showed MLL-AF10 rearranged.

CONCLUSION

In our studies, all cases had not received chemotherapy and survived about 1 months - 2 months. It suggests that cytogenetic disorders are closely related to disease development and likely result in fatal outcome if untreated. Thus, we proposed that a proper treatment decision is urgently needed in congenital leukemia.

KEYWORDS

Congenital leukemia; Cytogenetic abnormalities; R-banded karyotype; Fluorescence in situ hybridization; Reverse transcription-polymerase chain reaction

INTRODUCTION

Congenital leukemia (CL) is defined as occurring within the first 6 week of life, with an incidence of CL ranging from 1 per million to 5 per million live births and accounting for <1% of all childhood leukemia [1,2]. In two thirds of patients, it manifests as an acute myeloid leukemia (AML), frequently with monocytic /monoblastic characteristics. Most other cases are acute lymphoblastic leukemia (ALL), particularly B lineage, but some are mixed phenotype or blastic plasmacytoid dendritic cell neoplasms [3].

From cases reported, some reviewers hold the view that CL needs treatment to cure. Many chemotherapies have been used and some neonates achieved remission [4-7]. In contrast, other studies reported that the patients with normal karyotype achieved spontaneous remission without receiving treatment [8,9]. Presently, whether CL requires treatment remains controversial. Besides, the toxicity of the chemotherapeutic agents in the infants is unclear.

In this paper, we discussed three cases of CL, and illustrated the clinical, hematologic, pathologic observations.

MATERIALS AND METHODS

Case Enrolled

We searched the database of the children patient's (≤12 years old) data at the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University during the years of 01.01.2006 and 01.01.2020. A total of 3 cases with congenital leukemia were collected. The ethics committee of the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University had approved our data collection and sample analysis. The patient's guardians completed the informed written consent in accordance with Declaration of Helsinki.

Morphology and Flow Cytometric Immunophenotyping

Bone marrow (BM) aspirate smears were reviewed in this case. Cases were classified according to French-American-British (FAB) criteria. Cell surface antigens were detected by flow cytometry (Becton, Dickinson and Company, FACSC antTM-II, Franklin Lakes, NJ, USA) and further analyzed by extended panels designed to characterize acute leukemia (AL) according to the methods described previously [10-12].

Conventional Karyotyping

BM cells were cultivated for 24 hours - 48 hours without mitogen stimulation and harvested for chromosomal examination in a standard way. At least 20 metaphases R-banded by Wright's stain were examined, and the ISCN nomenclature (2009) was used to describe chromosomal abnormalities.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Analysis

Total RNA was extracted from the patient's BM cells at the time of diagnosis. RT-PCR analyses were performed, following the manufacturer's instructions.

Fluorescence in Situ Hybridization (FISH)

We used commercially available probes to detect 3 cases by inter/meta FISH. Interphase signals were evaluated in 200 nuclei of marrow cells. Images were captured by a Nikon 80-A1 fluorescent microscope and analyzed with image analysis software AI.

Whole-Exome Sequencing

Genomic DNA was isolated from BM leukocytes through standard phenol/chloroform extraction protocols. DNA quality and quantity were assessed by agarose gel electrophoresis and a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). A total of 2 μg of genomic DNA from each sample was used for library construction with an Agilent SureSelect Library Prep Kit according to the manufacturer's protocol. Exome libraries were captured using an Agilent SureSelect Human All Exon v5 Kit (Agilent Technologies, Santa Clara, CA, USA) and were sequenced with an Illumina HiSeq 4000 through a 150-bp paired-end run (Illumina, San Diego, CA, USA).

RESULTS

Between 2006 and 2020, a total of 434 children patients (≤12 years old) were diagnosed with AL, but only 3 patients (including 1 female and 2 males) were enrolled in our project (3/434).

Case 1

The patient was the product of a normal full-term pregnancy (about 39 weeks). The antenatal period was uneventful, with ultrasonography findings within normal ranges. The male neonate was 3.6 kg. After birth, blue-violaceous dermal nodules on his skin appeared. Apgar scores were 9 (due to skin) and 10 at 1 and 5 minutes of life, respectively. After 42 minutes, the neonate was referred to the neonatology. Upon examination, six dome-shaped nodules with purple ± blue were observed predominantly on the trunk, with a few lesions on the face and the scalp. In addition, the biggest mixed echo mass can be seen and located under the jaw about 20 × 30 mm, the boundary is clear and shows poor activity. The neonate also had signs of hepatosplenomegaly, which was further examined by ultrasound and showed the liver 19 mm was below the costal margins while the spleen (thick 20 mm) was palpable at the costal margins. The kidney and pancreas were normal. Right testis showed hydrocele cavity effusion; whereas, the left testis was normal.

On admission, blood sample analysis showed hyperleukocytosis 71.1 × 109/L, hemoglobin (Hb) 139 g/L, platelet (PLT) 71 × 109/L and red blood cell 3.74 × 1012/L. A peripheral blood smear demonstrated circulating blasts (72%), while a BM aspiration showed approximately 61% blasts of myeloid lineage. BM aspirates showed active proliferation of leukemic cells. In addition, it was also accompanied with little granules and loose reticulate (Figure 1A). Dysplasia of small megakaryocyte and megaloblasts were found in Figure 1B and Figure 1C. The naive cells morphological features of peripheral blood are similar with BM (Figure 1D). The peroxidase (POX) is partially weakly positive, naphthyl-acetate esterase (NAE) staining of non-specific esterase was strongly positive, and nonspecific esterase staining is significantly inhibited by NaF (Figure 2A - Figure 2C). The results

are consistent with an M5 subtype. In addition, blood, stool, and urine culture were negative. The serologic testing was not indicative of congenital infection with syphilis, HIV, HAV, HBV, and HCV. Immunophenotyping of the BM showed the cells to be positive for surface CD117 +, CD33+, HLA-DR+, CD64+, CD56 +, CD4+, and CD15+ and negative for surface CD13-, CD36-, CD14-, CD7-, CD19-, and CD3-. A pathologic diagnosis of AML with non M3 was given.

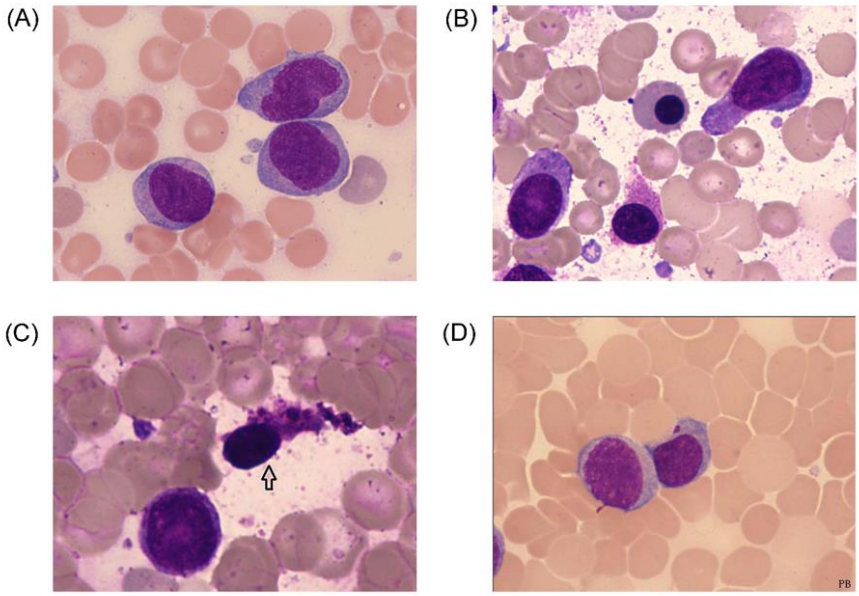

Figure 1: Bone marrow morphologic features (case 1). **(A)** BM blasts had obvious irregular nuclei (including 1 to 3 nucleolus), loose reticulate and granules. Cytoplasm is present as gray blue. **(B)** BM smear shows megaloblasts. **(C)** BM smear shows small megakaryocyte (arrow). **(D)** The morphological features of peripheral blood are similar with bone marrow.

Besides, the platelet gradually decreased over 6 days of hospital stay. The low platelet level can cause important organ bleeding, such as intracranial hemorrhage, and may induce hemorrhagic shock and disseminated intravascular coagulation. So, on this day, 33 ml human O Rh-positive platelets transfusion to the patient and increased platelets to 103 × 109/L. However, the level declined quickly to 75 × 109/L. The leukocytes were sustained at high level with blasts. Babies with hyperleukocytosis often accompanied with respiratory distress with hypoxia and acidosis, cardiac failure and renal failure.

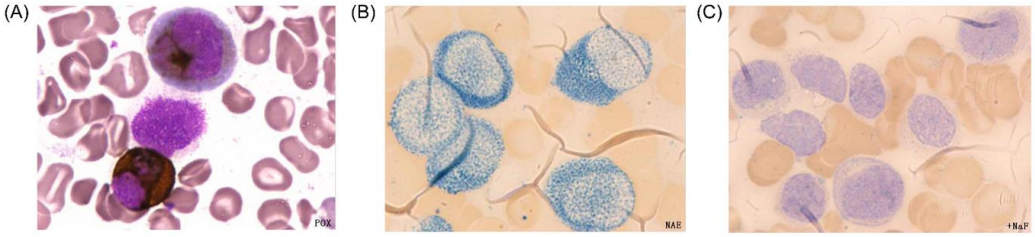

Figure 2: Cytochemical stain of bone marrow (case 1). **(A)** The bone marrow cells showed strongly positive for peroxidase staining. **(B)** NAE (Naphthyl-acetate esterase) staining of non-specific esterase was strongly positive. **(C)** Nonspecific esterase staining is significantly inhibited by NaF in NaF inhibition experiment.

Cytogenetics of the BM revealed an abnormal male karyotype of 46,XY,t(8;12)(q21;p13) by R-banded technique (Figure 3A). However, the karyotype of parents is normal by G-banded banding technique in peripheral blood, as well as his sister. Metaphase FISH analysis with GLP RUNX1-RUNX1T1 dual color fusion probe (located at 21q22/8q22), GLP ETV6-RUNX1 dual color fusion probe (located at 12p13/21q22) and GLP C-MYC dual color break-apart probe (located at 8q24) revealed 8q22 (including ETO gene) translocation to TEL gene (located at

12p13), and this further explains that the breakpoint occurred before 8q22 (Figure 3B - Figure 3D). However, this translocation was also confirmed by whole exome sequencing. In addition, MLL-AF10 fusion gene was detected by RT-PCR, but FISH studies did not detect MLL gene rearrangement.

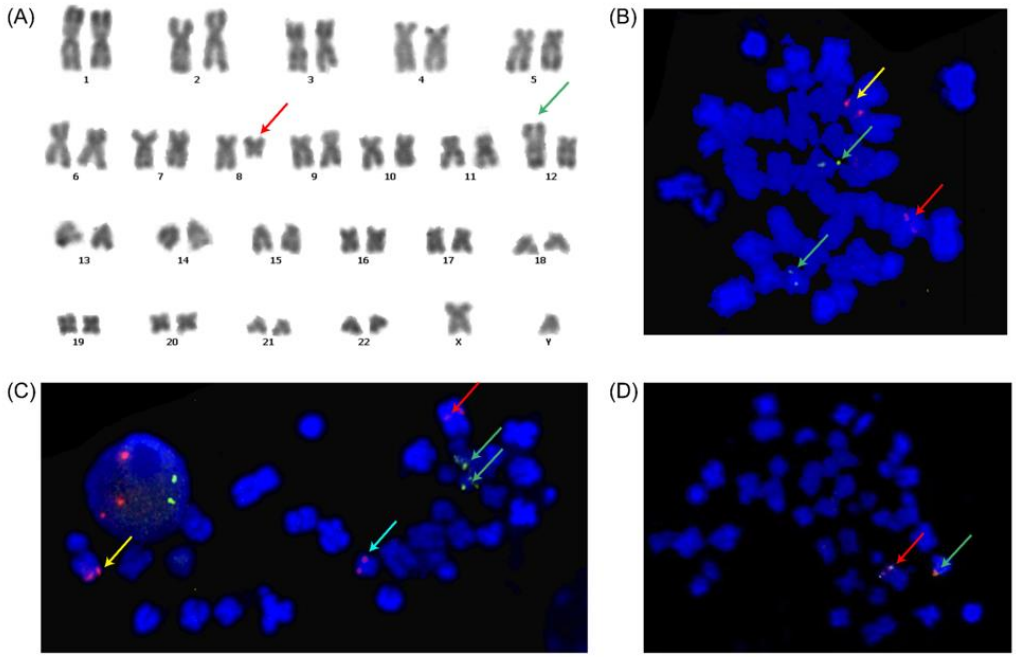

Figure 3: Karyotype and fluorescence in situ hybridization (FISH) analysis (case 1). **(A)** karyotype (R-banding): 46,XY,t(8;12)(q21;p13). **(B)** Metaphase FISH analysis with GLP RUNX1- RUNX1T1 dual color fusion probe (located at 21q22/8q22) revealed 2G2O signals. Two green signals were on the two native chromosome 21 (green arrow). Two red signals were located on the der (12)t(8;12) (q22;p13) (yellow arrow) and native chromosome 8 (red arrow), respectively. **(C)** Metaphase FISH analysis with GLP ETV6-RUNX1 dual color fusion probe (located at 12p13/21q22) revealed 2G3O signals. Two green signals were on the two native chromosome 21 (green arrow). Three red signals were on the der(12)t(8;12) (q22;p13)(red arrow), der(8)t(8;12)(q22;p13) (blue arrow) and native chromosome 12 (yellow arrow), respectively. **(D)** Metaphase FISH analysis with GLP C-MYC dual color break-apart probe (located at 8q24) displays 2 fusion signals, two yellow fusion gene signals on the native chromosome 8 (green arrow) and der(12)t(8;12)(q22;p13) (red arrow), respectively. **Note:** G = Green, O = Red.

Since the disease had low chances of achieving complete remission, a conservative attitude with palliative care was adopted. The patient eventually died at the age of 1 month due to cerebral hemorrhage.

Case 2

A 39 weeks full-term 3.5 kg female neonate was found to have difficulty in breathing after birth, but responded well to oxygen treatment. Apgar scores were both 10 after 1 and 5 minutes, respectively. Physical examination revealed a 4 cm hepatomegaly below the costal margin and a 3cm splenomegaly below the costal margin. Her peripheral blood showed Hb 120 g/L, white blood cell (WBC) 77.74 × 109/L and PLT 452 × 109/L. A peripheral blood film showed trilineage dysplasia and pleomorphic blasts (>50%).

Following transfer to our hospital, her body temperature increased to 38.5°C and she still had difficulty breathing. The blood sample analysis showed hyperleukocytosis (115.7 × 109/L), blast cell count 52%, Hb 135 g/L, and PLT 394 × 109/L. Flow cytometry immunophenotyping of these cells was positive for CD7, CD33, CD34, CD117, and HLADR. BM aspiration revealed BM infiltration by medium-sized immature cells (55% blast cells) with obvious irregular nuclei, the cytoplasm is grey-blue and filled with prominent granules (Figure S1A). Besides,

BM smear also shows small megakaryocytes (Figure S1B). Cytogenetic analysis revealed a 47,XX,+21 karyotype in 20 cells examined, but she had no clinical features of down syndrome, and the karyotype also confirmed by FISH (Figure S2A, S2B). However, RT-PCR and FISH studies showed no MLL rearrangement (Figure S2C).

1 Supplementary Figures

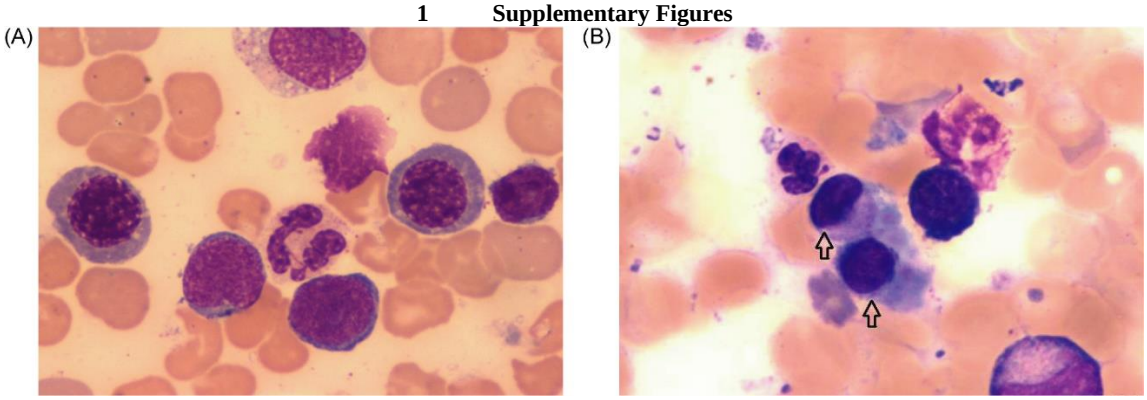

Figure S1: Bone marrow morphologic staining features (case 2). **(A)** BM blasts had obvious irregular nuclei, the cytoplasm is grey-blue, and filled with granules. **(B)** BM smear shows small megakaryocyte (arrow).

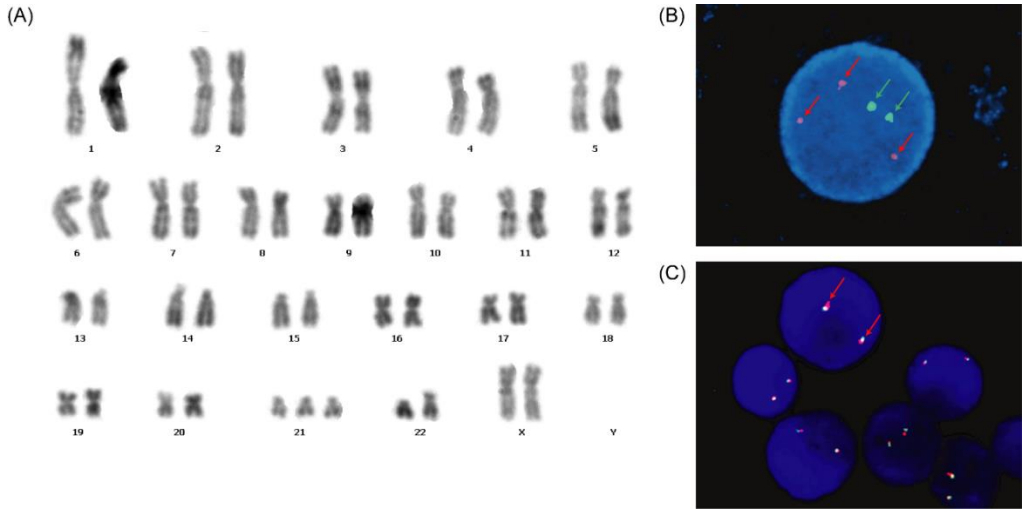

Figure S2: Karyotype and fluorescence *in situ* hybridization (FISH) analysis (case 2). **(A)** karyotype (R-banding): 47,XX, +21. **(B)** FISH analysis with the GLP 13/21 dual color probe (located at 13q14/21q22). There were two green signals (green arrow) on native chromosome 13×2, three red signals on native chromosome 21×2 and extra chromosome 21 (red arrow), respectively. **(C)** FISH analysis with GLP *MLL* probe (located at 11q23). The picture shows two yellow signals (red arrow).

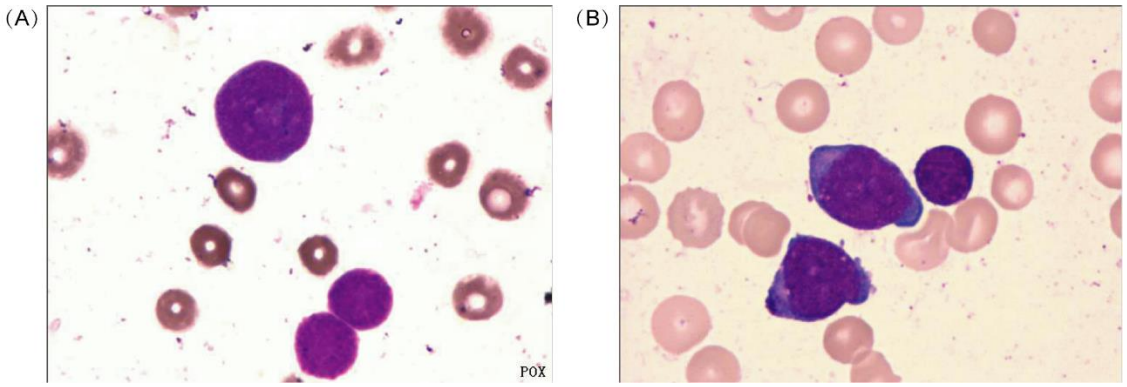

Figure S3: Bone marrow morphologic staining features (case 3). **(A)** The bone marrow cells showed negative for peroxidase staining. **(B)** BM blasts were obvious irregular, the cytoplasm is grey-blue, and had granules.

Furthermore, the neonate underwent distress intrauterine and found amniotic fluid pollution before, so we had to consider it as neonatal pneumonia and leukemia. Then, she underwent the antibiotic therapy treatment starting on the second day after birth. Despite this, the blood count still revealed hyperleukocytosis (85.9 × 109/L) and C-reactive protein increased gradually to 49 mg/L. It implied the treatment did not make an effect. The patient eventually died at the age of 1.5 months due to respiratory failure.

Case 3

The child birth weight was 3.85 kg. Child was clinically pale, which was found at 1 month after birth. The blood count revealed severe anemia (Hb 59g/L), WBC 9.2 × 109/L, and PLT 35 × 109/L. A peripheral blood film showed pleomorphic blasts (10%). So, he was transfered to our hospital.

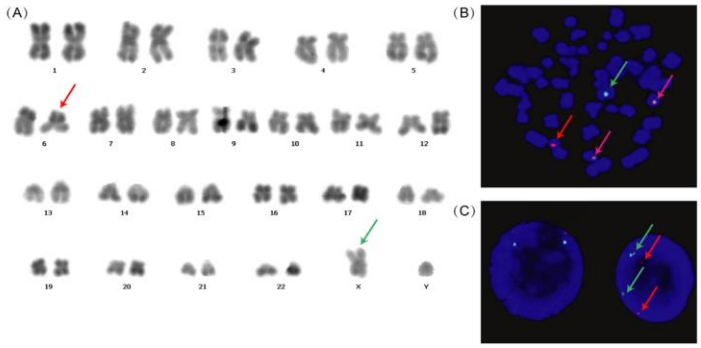

Figure 4: Karyotype and fluorescence in situ hybridization (FISH) analysis (case 3). **(A)** karyotype (R-banding): 46,XY,t(6;X)(q22:p12). **(B)** FISH analysis with the CSP18/CSP X/CSP Y color probe (located at18p11.1-q11.1/Xp11.1-q11.1/Yp11.1-q11.1). In metaphase, one green signal (green arrow) on chromosome X, one red signal (red arrow) on chromosome Y, and two pink signals (pink arrow) on chromosome 18 (2G2O2P) were observed. **(C)** FISH analysis with the CLP D7S522/CSP7 dual color probe (located at7q31/7p11- q11). In interphase, two green signals (green arrow) and two red signals (red arrow) on native chromosome 7 × 2, respectively.

On the day of admission, the baby received a transfusion of 50 mL human O Rh-positive platelets which increased to 82 × 109/L. However, it gradually declined to 63 × 109/L. A detailed physical examination revealed a firm hepatosplenomegaly with liver 3 cm below right costal margin, spleen 2 cm below left costal margin, but the other abnormal findings were not observed. Flow cytometry showed the blasts CD117 with HLA-DR (14.1%) were positive. BM aspirations showed negative for peroxidase staining (Figure S3A). Besides, BM blasts were obviously irregular, the cytoplasm is grey-blue, and granules were observed (Figure S3B). The karyotype was 46,XY,t(6;X)(q21:p12) (Figure 4A). It was further confirmed by CSP18 / CSP X / CSP Y color probe and CLP D7S522 / CSP7 dual color probe. It revealed that the translocation occurred on chromosome X and chromosome 6 (Figure 4B, 4C). RT-PCR and FISH studies showed no MLL rearrangement.

Due to the progression of his condition, the neonate did not receive symptomatic treatment. Unfortunately, he died because of massive gastrointestinal hemorrhage after 1 month.

DISCUSSION

CL is a rare, progressive disease and overall survival is short. Only few case series have been published so far. Available case reports are not enough to establish the clinical and biological characteristics of this disease. In this study, we discussed three cases of CL.

In our case, MLL gene rearrangement was detected by RT-PCR in case 1, which was detected in 70% of both AML and ALL infants [13]. This rearrangement was strongly correlated with myelomonocytic and monoblastic phenotypes (M4 and M5, FAB classification), which was consistent with our case (diagnosis of M5) [14]. Due to the MLL-AF10 rearrangement, the patient was classified as “high risk”, which implied poor prognosis and often become refractory to treatment [15]. According to literature, infants with t(4;11), t(9;11), and t(11;19) chromosome translocations might have a worse outcome than infants with other types of MLL gene rearrangements[16,17]. Thus, the infant died after 1month without chemotherapy.

Meanwhile, our aberrations with t(8;12)(q21;p13) and t(6;X)(q22:p12) were first reported in CL. Especially for case 1, there was no previous family history of leukemia. Furthermore, CNV examination of their (parents and patient) peripheral blood showed the child chromosome 8q22 and 12p13 exists with abnormal copies, which is consistent with FISH findings. The results were further confirmed by meta-FISH showing that breakpoint had taken place in the TEL gene (located at 12p13) and the translocation may have occurred around 8q21.3. Their parents did not show aberrant CNV. We proposed that embryos factor may be the cause of this disease evolution, but a valid hypothesis has not yet been substantiated.

In addition, the abnormal karyotype group may be relevant with clinical characteristics and result in poor prognosis compared with normal karyotype [18]. An estimation of the prevalence of CL patients with abnormal karyotype has been impossible until now. From our review, all cases with abnormal karyotype had short survival. Thus, cytogenetic abnormalities are a strong indicator of poor prognosis and badly need treatment. In addition, BM aspiration showed massive infiltration of blast cells in three cases, especially of case 1 and case 2 where small megakaryocytes were detected. Moreover, case 1 also showed megaloblasts. This phenomenon revealed AL accompanied with dysplasia and implied significantly poor outcome [19].

In conclusion, our patients had unique abnormal chromosomes, genes, and BM infiltration pattern. These factors and the age of these cases were responsible for their poor outcome. If a proper chemotherapy treatment were found, the survival of this case maybe longer. Hence, a proper treatment in which to cure congenital leukemia with genetic abnormality is badly needed.

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Author Contributions

RQY collected and analyzed data, interpreted the results, and wrote the manuscript; QL provided critical ideas and critically reviewed the manuscript; CCW, JC, and JXW contributed to the SPSS software design and analyzed data. ZL, HH and WJG collected and analyzed data and interpreted the results; LHL, RQY and XHZ designed the study, analyzed data, and wrote the manuscript; QL designed the study, performed experiments, and interpreted the results; all authors reviewed and approved the final draft of the manuscript.

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