Molecular Diagnosis of Shwachman-Diamond Syndrome Presenting with Pancytopenia at an Early Age: The First Report from Turkey

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Abstract A three-month-old boy presented with growth failure, skeletal abnormalities, otitis media and pancytopenia. Exocrine pancreatic insufficiency was confirmed by low levels of fecal elastase. He was diagnosed as Shwachman-Diamond syndrome by clinical and laboratory findings. The diagnosis was confirmed by sequence analysis for SBDS gene on chromosome seven revealing compound heterozygous mutation, which are c.258+2T-C and c.183-184TA-CT. Matched unrelated donor screening for hematopoietic stem cell transplantation was initiated. Unfortunately, he died of respiratory difficulty at 5 months of age. Our case is the youngest patient whose presumptive Shwachman-Diamond syndrome diagnosis was confirmed by molecular analysis.

Keywords Immune deficiency · Pancytopenia · Schwachman-Diamond Syndrome

Introduction

Shwachmann-Diamond syndrome (SDS) is an autosomal recessively inherited cancer predisposition syndrome characterized by pancreatic insufficiency and bone marrow failure which presents with mainly neutropenia, anemia and thrombocytopenia. It was firstly described in 1960s and its estimated incidence is one per 75.000 live births [1–3]. Skeletal changes, growth failure, dental anomalies,

immunological defects and recurrent infections are the other features of SDS.

To the best of our knowledge, our patient is the youngest case with SDS diagnosed by molecular analysis and reported from Turkey. Meanwhile, we aimed to remind both clinical and laboratory features of SDS.

Case Report

A three-month old boy presented to our emergency department with fever and purulent discharge from left ear for 1 week. He was born after a full term gestation with a birth weight of 2,860 g (between 10th and 25th percentiles). Due to his short upper limbs and narrow thoracic cage, he had been diagnosed as hypochondroplasia previously in an other centre. But, when his pancytopenia was noticed, he was referred to our hospital. His parents were not consanguineous but the first pregnancy of his mother resulted in abortus.

His weight and height were 3.3 kg (below 3rd percentile) and 54 cm (below 3rd percentile), respectively. His extremities were short and thoracic cage was narrow apparently. Petechiae were seen on the neck and upper thorax region. Purulent discharge in the left external auditory canal was noticed. Otoscopic examination yielded perforated otitis media. Anterior fontanelle was 2 × 3 cm and open. He was tachypenic but no auscultation finding was noted. Cardiovascular system evaluation was normal. The liver was palpable 2 cm below the costal margin on the midclavicular line.

His initial blood count revealed hemoglobin; 6.1 g/dl, leukocyte; $3.9 \times 10^9/l$ and platelet; $9 \times 10^9/l$. Mean corpuscular volume was 99 fl. Reticulocyte count was 0.1 %. Peripheral blood smear yielded 12 % polymorphonucleated cell, 6 % monocyte and 80 % lymphocytes. Serum liver

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and renal function tests were all normal. C-reactive protein was 3.45 mg/dl.

Serum vitamin B12 and folic acid levels were 1,029 pg/ml (200–600 pg/ml) and 18 ng/ml (5–20 ng/ml), respectively. Viral serology—including parvovirus and human immune deficiency virus—was negative. Due to his persistent pancytopenia, bone marrow aspiration was performed. Light microscopic evaluation of smears revealed moderately cellular bone marrow with 10 % normoblast. Despite the peripheral blood neutropenia, no stage arrest was noticed at myeloid differentiation. Increased histiocytes and prominent hemaphagocytosis—macrophages engulfing both thrombocytes and erythrocytes—were also noted. Megakaryocyte dysplasia was apparent. Unfortunately, no metaphases was obtained for cytogenetic evaluation.

Radiographic evaluation of the bones showed short extremities and ribs, pelvic hypoplasia but no metaphyseal dysostosis or costochondral thickening. His echocardiography and cranial ultrasonography were normal. Abdominal ultrasonography yielded bilateral increased echogenicity in renal medulla and dilatation of left renal collecting system also mild hepatomegaly. Blood and urine amino-acid analysis were normal. Urinary calcium, oxalate and citrate excretion were also normal.

Serum IgG and IgA were 1630 mg/dl (normal range : 273–1,660 mg/dl) and 173 mg/dl (normal range: 0–100 mg/dl), respectively. Serum IgM was also normal. Lymphocyte subset analysis showed low CD19 with the level of 3 % (normal range: 11–45 %). Sweat chloride test was 26 meq/l. Fanconi aplastic anemia was excluded by a negative DEB test. Although his mother didn't describe prominent diarrhea and pancreatic image by ultrasonography was normal, pancreatic amylase was found as 1.23 IU/l (17–123 IU/l). Fecal elastase level was 50 µg/g (normal value >200 µg/g). Due to exocrine insufficiency, oral pancreatic enzyme replacement therapy was started.

He was supported by thrombocyte and erythrocyte transfusions. Parenteral ampicillin-sulbactam therapy was started for otitis media. Granulocyte stimulating factor was also begun for his neutropenia. Sequence analysis for SBDS gene on chromosome seven revealed compound heterozygous mutation, which are c.258+2T-C and c.183-184TA-CT. Because he had no HLA matched related donor, matched unrelated donor screening was started as soon as possible. He was discharged from the hospital after the cessation of the treatment. Unfortunately, it was learned that he died in a local health care centre several weeks later due to respiratory difficulty.

Discussion

SDS is a rare bone marrow failure syndrome with an estimated incidence of 1 of 75,000 live births but it should

be diagnosed as earlier as possible due to increased risk of myelodysplastic syndrome and acute myeloid leukemia [3]. The responsible genetic defect—SBDS gene on chromosome 7q11—was first described by Boocock et al. [4] in 2003 (MIM ID *607444). The exact role of SBDS gene has not been understood fully yet. It is thought that it may involve in ribosomal biogenesis and RNA processing [5]. Most patients with SDS—like our patient—have compound heterozygous mutations of SBDS whereas no mutations were detected in 10–30 % of patients with SDS [6]. The most common described mutations are splice site mutation; 258+2T-C in one allele, followed by 183-184TA-CT mutation in the other one. SBDS-negative SDS patients may have more severe hematological involvement but milder exocrine pancreatic disease [7].

A consensus on diagnostic criteria of SDS was accepted in 2002 (Table 1) [8]. Persistent diarrhea, malnutrition and growth failure due to fatty replacement of pancreatic acinar tissue are most prominent during infancy and improve spontaneously in up to 50 % of patients by age [9]. Mean birth weight is at the 25th percentile, however; by 6 months of age, mean weights and heights decrease below the 3rd percentile of age like our patient.

Hematological findings appear usually beyond the infancy. Neutropenia (88 %)—usually intermittent—is the most common hematological finding at presentation. Anemia, thrombocytopenia and trilineage aplasia/dysplasia can be seen. Bone marrow cellularity reveals a wide spectrum changing from aplastic to hypercellular. Dysplasia—like in our patient—can be seen [3]. The bone marrow evaluation of our patient showed normal cellularity. Normoblasts vacuolization was noticed. Despite the peripheral neutropenia, no myeloid stage arrest was observed. Increased histiocytes and prominent hemaphagocytosis—macrophages engulfing both thrombocytes and erythrocytes—

Table 1 Diagnostic criteria of SDS

Fulfilling at least two of the following criteria

- (1) At least two of the following:
 - (a) Chronic cytopenia(s) detected on at least two occasions over at least 3 months
 - (b) Reduced marrow progenitors
 - (c) Persistent elevation of hemoglobin F
 - (d) Persistent red blood cell macrocytosis (not caused by nutritional deficiency)
- (2) At least one of the following:
 - (a) Evidence of pancreatic lipomatosis
 - (b) Reduced levels of at least two pancreatic enzymes adjusted to age
- (3) Positive genetic testing
- (4) First degree-family member with Shwachman-Diamond syndrome

were also noted. It was thought that hemaphagocytosis may be associated with his infection and play a role in apparent pancytopenia. But, because five of the HLH criteria were not obtained, HLH 2004 protocol was not initiated [10].

Neutropenia, neutrophil dysfunction and thoracic deformity may increase the risk of recurrent viral, bacterial even fungal infections—particularly upper and lower airway infections—which are the major reasons of mortality in SDS patients. Neutrophil defects in mobility, migration and chemotaxis due to cytoskeletal/microtubular defects have also been reported [3]. Our patient presented with perforated acute otitis media that was treated successfully but unfortunately died of respiratory failure at five months of age despite the prophylaxis with granulocyte-colony stimulating factor and cotrimaxazole.

The presentation with pancytopenia in early infancy—like in our patient—is obviously rare. Usually, the disease becomes apparent with isolated neutropenia complicated by recurrent infections after the first year of life. If skeletal changes are not present, delay in the diagnosis is almost inevitable. Besides; cardiac, renal and endocrinological involvement were also reported [11].

In SDS, the estimated risk for the development of myelodysplastic syndrome and acute myeloid leukemia is 19 % at 20 years and 36 % at 30 years [11, 12]. Leukemia was reported in 26 SDS patients at a median age of 14 years (range:1.5–43 years) [13]. So, certain diagnosis should be confirmed as early as possible. The only definitive therapy for bone marrow failure is hematopoietic stem cell transplantation but its timing is still debating. The reduced intensity conditioning regimen might decrease the organ failure especially pulmonary and cardiac complications. SBDS gene analysis should be performed for matched related donors before the transplantation.

Herein, we report an infant with SDS who presented with pancytopenia and was diagnosed by molecular analysis at early months of age. Up to our knowledge, this is the youngest patient whose presumptive SDS diagnosis confirmed by molecular analysis and reported from Turkey.

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Conflict of interest The authors state that they have no conflict of interest.

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