Shwachman-Diamond syndrome: first molecular diagnosis in a Brazilian child

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Herein the first molecular diagnosis of a Brazilian child with Shwachman-Diamond Syndrome is reported. A 6-year-old boy was diagnosed with cystic fibrosis at the age of 15 months due to recurrent respiratory infections, diarrhea and therapeutic response to pancreatic enzymes. Three sweat tests were negative. At the age of 5 years, he began to experience pain in the lower limbs, laxity of joints, lameness and frequent falls. A radiological study revealed metaphyseal chondrodysplasia. A complete blood cell count showed leukopenia (leukocytes: 3.1-3.5 x $10^3/\mu L$), neutropenia (segmented neutrophils: 15-22%), but normal hemoglobin, hematocrit and platelet count. A molecular study revealed biallelic mutations in the Shwachman-Bodian-Diamond Syndrome gene (183-184TA-CT K62X in exon 2 and a 258+2T-C transition) confirming the diagnosis of Shwachman-Diamond Syndrome. A non-pathologic, silent nucleotide A to G transition at position 201 was also found in heterozygosis in the Shwachman-Bodian-Diamond Syndrome gene. This is the first report to describe a Brazilian child with molecular diagnosis of Shwachman-Diamond Syndrome, a rare autosomal recessive disorder characterized by exocrine pancreatic insufficiency, intermittent or persistent neutropenia and skeletal changes. Other characteristics include immune system, hepatic and cardiac changes and predisposition to leukemia. Recurrent bacterial, viral and fungal infections are common. The possibility of Shwachman-Diamond Syndrome should be kept in mind when investigating children with a diagnosis of cystic fibrosis and normal sweat tests.

Keywords: *Leukopenia/genetics; Exocrine pancreatic insufficiency/genetics; Cystic fibrosis; Bacterial infections; Humans; Male; Child; Case reports*

Introduction

The Shwachman-Diamond Syndrome (SDS) is a rare autosomal recessive disorder characterized by exocrine pancreatic insufficiency, intermittent or persistent neutropenia and skeletal changes[1-4]. Other clinical characteristics include immune system, hepatic and cardiac abnormalities and predisposition to leukemia[1].

The syndrome was first described in 1964 by Shwachman et al.[5]. However, its molecular basis was only identified in 2002; this demonstrated that approximately 90% of the patients had biallelic mutations in the Shwachman-Bodian-Diamond syndrome gene (SBDS) located on chromosome 7[1,3].

The relative incidence of 1:76.538 live births suggests that SDS is relatively more common than previously thought[6]. There is a higher prevalence in males with a ratio of 1.7:1[7-9]. The frequency of SDS in Brazil is not known, nor is the presence of possible molecular changes associated with this pathology.

The aim of this report is to describe for the first time the molecular diagnosis of a Brazilian child with SDS.

Case report

A 6-year-old boy was diagnosed with cystic fibrosis at the age of 15 months due to recurrent respiratory infections, diarrhea and therapeutic response to pancreatic enzymes. Three sweat tests were negative.

At the age of 5 years, the patient began to experience pain in the lower limbs, laxity of joints, lameness and frequent falls. There was no history of fractures, liver or kidney disease, use of medications associated with osteoporosis or rickets, lack of sunlight exposure, low calcium or vitamin D diet, or reports of metabolic bone disease or bone dysplasia in the family. There is no evidence for consanguinity. On physical examination his weight was 16 kg (10th centile), height 103 cm (5-10th centile), with P1/G1 Tanner pubertal stage. The liver was palpable 3 cm below the right costal margin. He also presented discrete genu varum, hyperextensible elbow joints and increased anteroposterior diameter of thorax. Review of medical records showed chronic leukopenia (leukocytes: 3.1-3.5 x $10^3/\mu L$), neutropenia (segmented neutrophils: 15-22%), but normal hemoglobin, hematocrit, and platelet count. A radiological study showed metaphyseal chondrodysplasia, sclerosis and pseudocysts in radial, humeral, ulnar, tibial and femoral metaphysis (Figure 1). Bone densitometry showed a z-score of -1.6.

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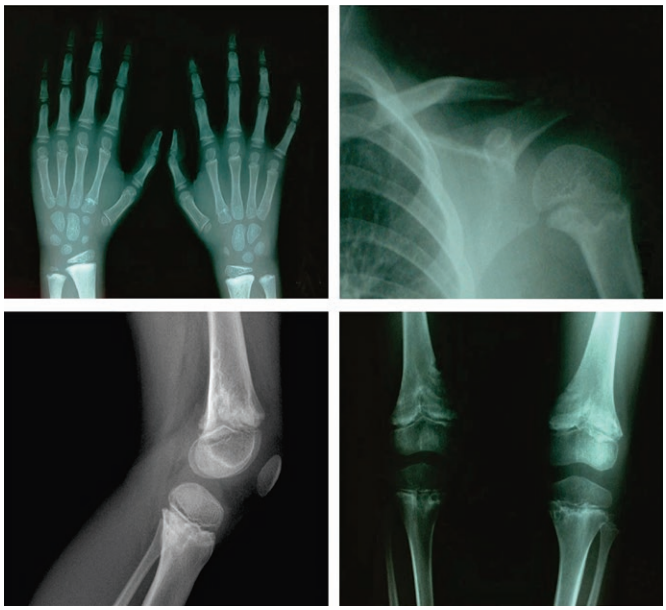

Figure 1 – Metaphyseal chondrodysplasia, sclerosis and pseudocysts in radial, humeral, ulnar tibial and femoral metaphysis

Evaluation of serum calcium, phosphorus, alkaline phosphatase, 25(OH)Vitamin D, 1,25(OH)Vitamin D, parathyroid hormone, liver and kidney function, thyroid function, electrolytes, lipase, amylase, blood sugar, immunoglobulin (IgG, IgA and IgM) and CD4 and CD8 lymphocyte counts were all normal. Hemoglobin electrophoresis showed: Hemoglobin (Hb) A1: 91.7%; Hb A2: 2.6%; Hb F: 5.7%. Blood sugar, insulin and an oral glucose tolerance test were normal, in spite of a raised glycated hemoglobin level of 6.5% (normal: < 5.6%). Fecal fat excretion tested positive and an abdominal ultrasound revealed increased pancreatic echogenicity. Sanger sequencing[8,9] demonstrated known pathogenic mutations in exon 2 of the SBDS gene: 183-184TA - CT K62X (Stop codon no exon 2) and 258+2T-C transition. In addition, a non-pathologic silent nucleotide A to G transition at position 201 in the SBDS gene was also detected in heterozygosis.

Discussion

The most common hematologic abnormality in SDS is persistent or intermittent neutropenia caused by bone marrow hypoplasia[1,10]. Normochromic-normocytic anemia or macrocytic anemia with thrombocytopenia may occur. All these findings were present in this current case. Approximately 80% of patients have high levels of hemoglobin F[8]. Bone marrow disease can progress to aplastic anemia, myelodysplastic syndrome or acute myeloid leukemia[1,7].

Recurrent bacterial, viral, and fungal infections, particularly, otitis, sinusitis, pneumonia, septicemia, osteomyelitis, and cutaneous infections are common. The main mechanisms responsible for these infections are neutropenia, defects in neutrophil chemotaxis, defects in lymphocyte-mediated immunity, reduced numbers of B cells, and low immunoglobulin (IgG) serum levels[1].

The pancreatic endocrine function is generally intact but exocrine pancreatic dysfunction caused by acinar cell hypoplasia is usually diagnosed in the first year of life with intestinal malabsorption, steatorrhea, failure to thrive, and low levels of fat soluble vitamins[8]. The diagnosis is confirmed by increased fecal fat excretion in the absence of cholestatic liver disease or intestinal failure, and by measurement of fecal elastase[8]. Trypsinogen is usually low in under 3-year-old children but normal in older patients. Moreover, the isoamylase serum is not useful in under 3-year-old children because serum levels are usually low in this age group[1]. Imaging studies usually show a small pancreas replaced predominantly by fat[4]. Unlike cystic fibrosis, the sweat test that measures the amount of chloride in the sweat is normal.

Hepatomegaly is found in approximately 15% of patients, and 50-75% have elevated liver enzymes[1]. Hepatomegaly and elevation of transaminases may eventually disappear, but cholestasis may persist and liver microcysts may develop in older patients[1]. The patient described here had hepatomegaly, a common feature in SDS.

The main skeletal abnormality is metaphyseal dysostosis involving the head of the femur, knees, humeral head, wrists, ankles and vertebrae[1]. The radiological study of this patient showed metaphyseal chondrodysplasia of the radius, ulna, tibia and femur. Abnormalities of the rib cage, sliding femoral epiphysis, digit abnormalities, spinal deformities, osteopenia and osteoporosis have also been described. The patient described in this case report had increased anteroposterior diameter of the chest, a bone densitometry with a z-score of -1.6, and normal calcium, phosphorus, alkaline phosphatase and vitamin D.

Insulin-dependent diabetes, growth hormone deficiency, hypogonadotropic hypogonadism, hypothyroidism and short stature are endocrine changes described in SDS[11]. Impaired growth involves multiple factors including pancreatic insufficiency, feeding difficulties, recurrent infections and metaphyseal dysostoses. In this patient, glycated hemoglobin of 6.5% (upper normal limit) reinforces the need for vigilance regarding the development of diabetes.

The diagnosis of SDS is based on the clinical phenotype and is particularly challenging in older individuals in whom symptoms such as steatorrhea may have disappeared and the neutropenia may have changed to a cyclic pattern. Therefore, SDS should be suspected in children with poor weight gain, abnormal stools and neutropenia, as well as in any child suspected of having cystic fibrosis but with a negative sweat test[5]. In spite of repeated negative sweat tests, this patient was being treated for cystic fibrosis.

Approximately 90% of patients have mutations of the SDS gene located in the 7q11 centromeric region of chromosome 7[1]. This gene apparently acts during the mitotic process by promoting microtubule stabilization and actin polymerization, avoiding genomic instability and predisposition to neoplasias[1,7]. Among several mutations described, the Ca. 258 + 2T and Ca. 183_184 TA>CT mutations correspond to 74% of the cases[12]. Approximately 10% of patients do not show identifiable mutations in the SDS gene[3] meaning that a negative test does not rule out the syndrome. The molecular study in this patient's report showed previously described mutations in exon 2 of the

SBDS gene: 183-184TA-CT K62X (Stop codon in exon 2) and a 258+2T-C transition.

This is the first report to describe the molecular diagnosis of SDS in a Brazilian child and highlights the need to investigate SDS in all children treated for cystic fibrosis with normal sweat tests.

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