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# Clinical and laboratory repercussions in patient with hemoglobin SD-Punjab disease: a case report

## *Repercussões clínicas e laboratoriais em paciente com hemoglobinopatia SD-Punjab: relato de caso*

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## ABSTRACT

We report the first case of hemoglobin SD-Punjab disease, a rare form of sickle-cell disease, in the state of Bahia. Detection was possible by a test for the identification of hemoglobin (Hb) variants with the high-resolution liquid chromatography technique. By means of the molecular study of chromosomal polymorphism with beta-globin S gene, the Bantu haplotype was observed. According to studies, there is strong association between the prevalence of Bantu haplotype and reduced levels of fetal Hb and Hb D-Punjab as a stimulating factor for S polymerization, what contributes to the hematological disorders of the disease and organ damage, as gallstones and aseptic necrosis of the femoral head.

**Key words:** sickle-cell anemia; hemoglobinopathies; haplotypes; blood protein electrophoresis.

## INTRODUCTION

Sickle-cell anemia (SCA) is the most common blood disorder in the world, with significant prevalence in the Brazilian population, especially in the Southeast and Northeast regions[1-3]. It is characterized by alterations in the globin genes, causing defective hemoglobin (Hb) synthesis, and the pathological alterations of the sickle-cell condition[4]. It is frequent in the states with a large portion of Black ancestry, above all that of Bahia. Due to extensive miscegenation, there was wide distribution of abnormal hemoglobins, what originated heterozygous presentations of sickle-cell disease (SCD), and the rare associations of hemoglobin variants, among which the compound heterozygote SD-Punjab or SD-Los Angeles stands out[1, 3]. According to studies, such as that conducted by Rezende *et al*. (2016)[3], patients with Hb SD-Punjab develop symptoms similar to those displayed by SS homozygotes[2-7].

Hb D-Punjab, in its turn, is a subtype of hemoglobin D (HbD), first described by Itano in 1951[5], characterized by a glutamine mutation for glutamic acid at codon 121 of beta-globin gene[2, 4, 5, 7]. HbD-Punjab in homozygous state is rare, and its occurrence is documented in few cases in the literature: seemingly asymptomatic individuals with blood count parameters within the reference values[8]. However, this D-Punjab variant, when associated with HbS, apparently causes anemia and a clinical course similar to severe cases of SS[2-4, 6, 7]. About this SD-Punjab association, pioneering studies such as those by Nagel (1984)[9] and Adachi *et al*. (1988)[5] already demonstrated that the HbS polymerization rate is higher when HbD-Punjab is present, what produces a picture of hemolytic anemia and vaso-occlusive events with painful crises, similar to what happens in homozygotes for HbS[2-5].

Besides HbD-Punjab as a modulating factor of HbS polymerization[3, 5], other factors related to polymorphism of HbS gene help explain diversity in relation to clinical manifestations of SCD[4, 5]. At the genetic level, five different patterns were discovered of combination of polymorphic sites in chromosomes with beta-globin S gene. Those patterns are called haplotypes[6] and they are: Bantu, Benin, Senegal, Cameroon and Arab-Indian[4, 9]. The discovery of haplotypes permitted better understanding of the disease clinical variability. Some Brazilian studies found correlation between severe clinical manifestations of SCD and fetal Hb (HbF) and the haplotype of beta-globin S gene[4, 5, 7, 9, 10]. An example of that is the high prevalence of individuals with the Bantu haplotype and reduced levels of HbF (< 5%) with severe symptoms[7, 9].

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Few pieces of information exist on the rare associations of hemoglobin variants in the Brazilian population. About that subject, Bonini-Domingos (1993)[1] screened a significant number of individuals with hemoglobinopathies from all regions of the country; just one case of the SD-Punjab association was identified, what corresponds to 0.92% of the 109 cases of rare associations of hemoglobin variants[1]. Researches on the prevalence of HbD-Punjab in Brazil found similarities with data obtained in researches conducted in Afro-Americans, with estimates ranging from 0.1% to 0.4%[2]. Studies such as those permitted demonstrate the need of information about prevalence, phenotypic manifestations, and diagnostic aspects of the compound SD-Punjab[1, 2]. Therefore, the current work is intended to report the first study of a clinical case of HbSD-Punjab in the state of Bahia, correlating its clinical and laboratory findings with the data and discussions of scientific literature.

## CASE REPORT

A 36-year-old female patient sought for medical assistance in November, 2015, at a municipality in the inner state of Bahia, complaining of hip joint pains. Over the last three years she had evolved with frequent episodes of hip arthralgia, needing treatment in urgency services to alleviate pain. She had an 18-year-old diagnosis of SS hemoglobinopathy, and a 6-year-old diagnosis of gallstones with cholecystitis, surgically treated with a total cholecystectomy early after diagnosis. She also informed that over the 18 years' clinical follow-up of SCD, she presented innumerable painful crises and received transfusion of red cell concentrate in two occasions, with relief of the painful picture. The physical examination showed regular general state. Cardiorespiratory evaluation without abnormalities. Abdomen was painless on palpation. Intense pain was caused by mobilization of right and left hip joints.

In September 2015, the patient was diagnosed with aseptic necrosis of femoral head (ANFH), after conduction of several exams, among which nuclear magnetic resonance (NMR) of right and left hip. The clinical findings suggestive of ANFH showed changes of bone marrow signal in all bone structures, which, according to the exam, related to a possible hematological disorder. Right hip: osteonecrosis affecting femoral head and neck, with accentuated chondral thinning of hip joint cartilage in T1. Left hip: osteonecrosis of femoral head with hyperintensity (at T1) in crescent format associated with signs of collapse/fracture and loss of spherical shape of femoral head. In the occasion, the cause of osteonecrosis was investigated: rheumatoid factor (RF), anti-SSA/RO and anti-SSB/LA antibodies, and antinuclear factor (ANF) were all negative. Osteocalcin, calcium, and phosphorus were within the reference ranges.

Given the clinical evaluation and the results of the cited exams, new tests were ordered: blood count {red blood cells = 3.23 million/mm$^3$ [reference interval (RI) = 4-5.2 million/mm$^3$}; hemoglobin = 7.9 g/dl (RI = 12-16 g/dl); hematocrit = 23.6% (RI = 35%-46%); reticulocyte count = 4.5% (0.6%-2.55% for adults); poikilocytosis with the presence of drepanocytes and codocytes. A new test for identification of hemoglobin variants using high-performance liquid chromatography (HPLC) presented: HbS = 43.8%; HbA2 = 4.2%; HbF = 1.7%; and the presence of probable HbD-Punjab = 50.3%. HbS gene polymorphism was also investigated, by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), which identified the presence of haplotype Bantu or Central African Republic (CAR) [- + - - - -].

Through exams, the diagnosis of hemoglobinopathy with Hb variant D-Punjab, anemia, and ANFH was achieved, as a possible complication of the SCD. These data allowed diagnosis after exclusion of other causes of symptoms.

## DISCUSSION

In the reported case, the patient's diagnosis 18 years before was SCD (HbSS), as the electrophoresis method then did not enable differentiation between HbS and HbD. However, in the second electrophoresis described in the current case presentation, by the HPLC method, it was possible to verify the presence of abnormal Hb, probably D-Punjab. Many individuals with HbSD-Punjab are no doubt underdiagnosed, for not all the electrophoresis methods permit identification of HbD-Punjab[1-4, 8]. The difficulty in finding individuals who are homozygous for HbD-Punjab seems to be even greater, because the few reports published in the literature show that in the homozygous state for HbD-Punjab, individuals are apparently asymptomatic[2, 3, 8]. However, in the heterozygous compound HbSD-Punjab form, there are reports of serious symptoms[3, 6].

The patient of the current case presented hemoglobin and hematocrit below RI at the blood count, and reticulocyte count above the parameter considered adequate, what indicates an episode of severe anemia and a possible hemolytic state. The presence of codocytes, poikilocytosis and depranocytosis was also detected, what is commonly found in SCAs. Other relevant pieces of information are stored in the pathological backgrounds of the report: several painful crises, and the conduction of a transfusion

of red blood cell concentrate, as well as the history of gallstones and cholecystectomy. Studies carried out by Oberoi *et al*. (2014)[6] and Rezende *et al*. (2016)[3] assessed cohorts of HbSD-Punjab patients and also observed moderate or severe anemia[3, 6], as well as complications such as painful crises, gallstones, and ANFH, and the need for blood transfusion by some patients[6].

Recent studies have associated the HbSD-Punjab variant with more severe clinical manifestations than other rare combinations of hemoglobin variants[2, 3, 6]. Therefore, individuals with SD-Punjab disease apparently evolve with a clinical course similar to that of homozygotes HbSS[2], with physiopathological events contributing to the severity of the picture, with hemolysis of erythrocytes and vaso-occlusive events, some of the most common complications[4-7]. About this subject, Nagel (1984)[9] published a study on HbSD-Punjab, in which patients with clinical symptoms similar to the HbSS genotype had episodes of hemolysis and shorter lifespan of erythrocytes, besides vaso-occlusion[7, 9] phenomena. Adachi *et al*. (1988)[5] also demonstrated that the low polymerization rate of HbS is higher when HbD-Punjab is present[5], speculating, however, on the role of HbD-Punjab and getting in perspective, in this case in special, what most literature points: that in the S heterozygous inheritance, the polymerization tendency and the vaso-occlusive events[2, 4, 11] are reduced.

Two genetically determined characteristics that are among the largest SCD modulators are described in the literature: the haplotype associated to beta-globin S gene and the HbF[4, 9, 11]. About those characteristics, the molecular study of the current case revealed the haplotype Bantu in the Sβ gene; and hemoglobin electrophoresis, a percentage of 1.7% HbF, a value significantly lower than the percentages of HbF found in studies of adults published in the scientific community even for individuals with SCD SS. The averages of HbF, for example, in works conducted by Figueiredo *et al*. (1996)[12] and Galiza Neto *et al*. (2005)[10] were 6.35% and 7.61%, respectively. In this context, the literature shows strong correlation between the prevalence of individuals with Bantu haplotype and HbF levels lower than 5%[4, 9-11].

HbF at an intraerythrocytic environment exerts a protective function against deoxygenation of hemoglobin, which competes with the consequent HbS polymerization and change in the conformation of normal erythrocyte into sickle-shaped cells, an event that contributes to the severity of SCD[4, 9, 10].

NMR of the hips performed in the current case revealed the radiological alterations common to a hip joint necrotic degenerative process. The lesion mechanism leading to osteonecrosis of femoral head occurs initially by the accumulation of sickled erythrocytes in the microcirculation that irrigates the femoral head[4, 6, 11]. The small blood vessels that perfuse the region provide slow blood flow and limited arterial supply[4, 11, 13]. With low oxygen delivery, HbS molecules form polymer aggregates that result in the formation of "sickle-shaped erythrocytes". These erythrocytes suffer a process called gelation[4, 11], stiffening their walls and losing their deformability when moving through the venous sinuses[4], and have a property of adhesion to the endothelium of these vessels, determining the vaso-occlusive events, hypoxia, tissue lesion, evolving to bone necrosis[4, 11, 13]. The reduced femoral head blood flow provokes degeneration in trabecular architecture, collapse of the subchondral bone, and secondary arthrosis in up to 70% of the cases[4, 13].

Studies suggest that the clinical progression of femoral necrosis in sickle-cell patients occurs at a higher frequency that has been described in other non-traumatic diseases[13]. ANFH leads to disability proportionate to pain, progressively worsening with load and even at rest[4, 11, 13]. Pains mainly affect in the inguinal region, gluteal muscles, and anterior region of the thigh[11, 13]. ANFH prevalence in the SCD is high, reaching 50%, and the bilateral involvement occurs in 40%-90% of patients[13]. This SCD complication requires, most of times, definitive intervention with arthroplasty, considered a procedure with high indices of morbidity and mortality[4, 11].


## RESUMO

*Reportamos o primeiro caso de hemoglobinopatia SD-Punjab, uma forma rara da doença falciforme, no estado da Bahia. A detecção ocorreu pelo exame para identificação de hemoglobinas (Hb) variantes com técnica de cromatografia líquida de alta resolução. Através do estudo molecular do polimorfismo no cromossomo com gene da betaglobina S, verificou-se a presença do haplótipo Bantu. Segundo estudos, existe forte associação de prevalência do haplótipo Bantu e níveis reduzidos da Hb fetal e da Hb D-Punjab como fator de estímulo à polimerização da S, o que contribui para os distúrbios hematológicos da doença e a lesão de órgãos, como cálculos biliares e necrose asséptica de cabeça de fêmur.*



***Unitermos:*** *anemia falciforme; hemoglobinopatias; haplótipos; eletroforese das proteínas sanguíneas.*

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