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GSTT1 null genotype in sickle cell anemia and blood transfusion recurrence – a case report

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ABSTRACT. Sickle cell anemia is one of the most common genetic diseases in Brazil. This disease has an autosomal recessive inheritance pattern with a point mutation on chromosome 11, which is the substitution of an adenine by thymine. This mutation leads to the exchange of a glutamic acid for a valine at residue 6 of the beta globin chain, resulting in an abnormal form of hemoglobin, the so-called hemoglobin S (Hb S). The polymerization of Hb S produces reactive oxygen species, oxidizing agents that promote the oxidation of macromolecules, such as lipids, proteins and DNA. GSTs are enzymes that participate in the conjugation reactions of glutathione to a variety of electrolytic compounds that are potentially toxic and carcinogenic. The We analyzed the *GSTT1* and *GSTM1* gene polymorphisms in a patient with sickle cell anemia in order establish a more efficient clinical approach to treat the patient. The patient under study was 11 years old and sickle cell anemia was confirmed by the Guthrie test. Polymorphism identification was performed by PCR. The genotype identified in the patient was null for *GSTT1* and present for *GSTM1*. In addition, the patient has a recurrent need for blood transfusion.

Key words: Sickle cell anemia; Polymorphism; Prognosis; *GSTT1; GSTM1*

INTRODUCTION

Sickle cell anemia (SCA) is one of the most common genetic diseases in Brazil. Due to the miscegenation feature of our country, SCA affects a great number of black and Caucasian people within the Brazilian population (Albuquerque et al., 2014). It is a hereditary hemolytic anemia, with an autosomal recessive inheritance pattern. The disease is caused by a point mutation on chromosome 11, characterized by a replacement of an adenine residue by a thymine residue (GAG > GTG). At the protein level, a change of valine instead of glutamic acid at position 6 of the β-globin chain leads to the production of hemoglobin S.

The mutation and consequently the amino acid residues substitution contribute to the intramolecular binding of Hb S. The latter has a high probability of polymerizing when exposed to situations of low oxygen tension. Polymerization leads to the production of reactive oxygen species (ROS), potent oxidizing agents that promote the oxidation of lipids, proteins and DNA, which results in cellular and metabolism alterations (Albuquerque et al., 2014; Ansari and Mahmood, 2015). When Hb S is in its deoxygenated state, it polymerizes and alters red blood cell structure, leading to oxidative damage, cellular dehydration, abnormal phospholipid asymmetry and increased vascular endothelial adhesion (Sundd et al., 2018).

Patients with SCA suffer from acute and chronic vaso-occlusive seizures. These physiopathological events of SCA lead to a variety of morbidities such as pain, acute thoracic syndrome, stroke, aseptic necrosis of the bones, leg ulcers and proliferative retinopathy (Liu et al., 1994; Chevalier et al., 2016). Individuals with SCA are predisposed to chronic hemolytic anemia, inflammation, cell adhesion, tissue hypoxia, organ ischemia and tissue infarction (Pierrot-Gallo et al., 2015; Maioli et al., 2016). In addition, SCA patients are predisposed to oxidative stress with continuous production of ROS that can lead to hemoglobin self-oxidation (Ellithy et al., 2015). Oxidative stress is the main feature of SCA (Manfredini et al., 2007; El-Ghamrawy et al., 2014).

The production of ROS is observed during normal metabolism, and when an imbalance occurs between oxidative systems there is structural and functional damage. Free radicals are highly harmful to the body; they injure erythrocytes, proteins and DNA. ROS can react with lipids, producing lipid peroxidation and degradation of membrane lipids, especially in SCA patients, which could lead to physiopathological events (Oztas et al., 2011; El-Ghamrawy et al., 2014). Degradation of membrane lipids and the end products of lipid peroxidation are highly harmful. They reduce the viability of erythrocytes in individuals with SCA. Moreover, SCA patients are daily exposed to oxidative stress due to the imbalance between ROS production and antioxidant enzymes (El-Ghamrawy et al., 2014).

Glutathione S-transferase (GSTs) consist of multifactorial enzymes encoded by at least eight different loci. These enzymes take part in the conjugation reactions of glutathione to a variety of electrolytic compounds, potentially toxic and carcinogenic (Rocha et al., 2007). GSTs are proteins that are abundant in animal cells, found mainly within the cytoplasm but can also be present in organelles. Their extracellular concentration is relatively low, except in bile acid. Several factors such as protein-energy malnutrition, oxidative stress and some pathological conditions reduce the cellular concentration of glutathione (Baba and Bhatnagar, 2018). The deprivation of the activity of GSTs is related

to polymorphisms in their coding genes. The most frequently described GST polymorphisms are related to the *GSTM1* and *GSTT1* genes (Silva et al., 2011).

Polymorphisms on the coding sequences of *GSTM1*, *GSTT1* and *GSTP1* are associated with several diseases such as cancer and inflammatory diseases, which can be related to oxidative stress (Waś et al., 2018). GSTs also have peroxidase activity and can protect tissues from damage caused by oxidative stress (Silva et al., 2011).

Hydroxyurea has been described as a stimulant for the synthesis of fetal hemoglobin (Hb F). Hb F avoids the polymerization of Hb S in individuals with SCA and reduces vessel-occlusive crises and mortality in SCA patients. Studies have shown that hydroxyurea is able to play additional role as a of nitric oxide donor (Gladwin et al., 2002). Moreover, activation of Hb F expression by hydroxyurea can occur by the nitric oxide pathway (Cokic et al., 2003). Clinical studies have demonstrated an antioxidant effect of hydroxyurea on SCA through dosages of glutathione levels (Manfredini et al., 2007; El-Ghamrawy et al., 2014). Due to the mutagenic and carcinogenic action of this drug, cancers and leukemias have been described in SCA patients (McGann and Ware, 2011). In addition, hydroxyurea acts on the S phase of the cell cycle, arresting cell division by inhibiting the activity of the ribonucleotide reductase enzyme, thereby reducing DNA synthesis.

The objective of our study was to analyze the *GSTT1* and *GSTM1* gene polymorphisms in a patient with SCA in order establish a more efficient clinical approach to treat the patient.

MATERIAL AND METHODS

Peripheral blood (15 mL) was collected from the female patient, born in Goiânia-Goiás, Brazil, age 11 years old. A Guthrie test was performed at birth and showed abnormal hemoglobins, with 25.2% of hemoglobin S. Sickling test was performed and the patient was diagnosed with SCA.

The extraction of the genomic DNA from peripheral blood was made using the Kaswi kit® (*Genomic DNA Purification Kit*). The samples were subjected to polymerase chain reaction (PCR) amplification to detect the *GSTT1* and *GSTM1* gene polymorphism according to the protocol established by Frare et al. (2013). DNA segments were identified by a 2% agarose gel electrophoresis and stained with ethidium bromide. The images were captured by the BIORAD photodocumentation system (Bio-Rad, Hercules, California, USA).

This work is in accordance with the guidelines of the National Health Council and the Ethics Committee of the Catholic University of Goiás, under protocol number 76165. The patient under study signed the consent form to participate in the research. Moreover, there was no conflict of interest.

RESULTS

The genotype identified in the patient was null for *GSTT1* and present for *GSTM1* (Figure 1).

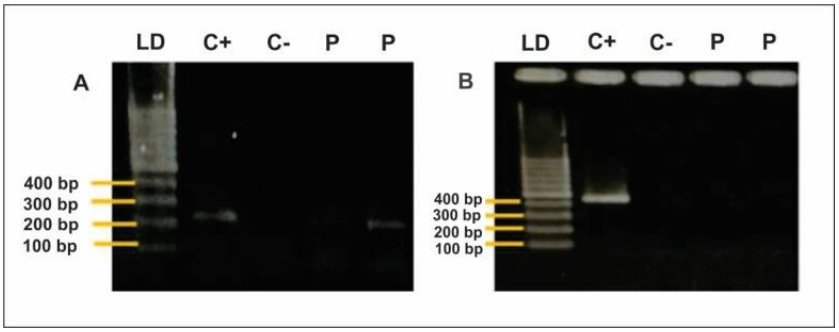

Figure 1. *GSTM1* and *GSTT1* genotyping of a sickle cell anemixa patient. The figure shows a 2% agarose gel stained with ethidium bromide. A – The patient has a present genotype for the *GSTM1* (215pb). B – The patient has a null genotype for the *GSTT1* (480pb). LD: ladder; C+: positive control; C-: negative control; P: patient.

The genotype presented here by the patient (*GSTM1*+/*GSTT1*-) has been identified elsewhere in patients with SCA as well (de Oliveira Filho et al., 2013; Ellithy et al., 2015; Abu-Duhier and Mir, 2017). Ellithy et al. (2015) reported the association of the null *GSTT1* genotype with an increased need for blood transfusions. However, Shiba et al. (2014) did not find that association. Ellithy et al. (2015) also reported the association between the GSTT1 polymorphism and acute thoracic syndrome and vaso-occlusion crises. Shiba et al. (2014) reported that the complications related to SCA are associated with the intense process of oxidative stress, and the GST enzymes protect the body from oxidative stress. They found no significant association between the null genotypes and the frequencies of pain related to SCA, blood transfusion, disease severity or treatment with hydroxyurea.

The patient under study did not develop cases of cute thoracic syndrome and vaso-occlusion crisis. The clinical picture of the patient shows constant infections, including a recent pneumonia with onset of pulmonary embolism, seizures of severe joint pain, usually preceded by blood transfusions. Patient has received packed red blood cells in the last five years for 35 times. Blood transfusions have been performed every 15 days since the second semester of 2017. Analgesia with morphine were used to control the pain during crises. Learning difficulties were reported for this patient.

The study of the GST gene polymorphisms as a molecular marker for the clinical manifestations of SCA and other diseases can offer a better quality of life and prognosis for patients. The detection of GSTT1 polymorphism may provide knowledge to indicate the type of prophylactic measures in order to reduce oxidative stress, antioxidants and the risks of developing severe clinical manifestations of SCA (de Oliveira Filho et al., 2013; Ellithy et al., 2015; Abu-Duhier and Mir, 2017). *GSTM1* and *GSTT1* are efficient molecular markers for diagnosis and clinical prognosis of SCA.

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