General Aspects of Pathophysiology, Diagnosis and Treatment of Sickle Cell Disease

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Abstract - Since its first report, sickle cell disease (SCD) has attracted the attention of many health professionals and researchers, because its affects about 24.6% of the world population and can cause the death of up to 90% of patients in Africa Sub-Saharan Africa with SCD before 5 years of age. Hemolysis, vaso occlusive crises, pain crises, chronic inflammation, risk of infarction, thrombosis and stroke are realities constantly faced by the patients affected by this hematologic disorder. The stages of the pathophysiological process, the pathways of activation and molecular aspects are still not fully understood, hence, the patients affected dependent on medical follow-up, periodic examinations, use of medications and even periodic blood transfusions. The objective of the present study was to carry out a bibliographic review aiming at a direct and clear approach to the pathophysiological aspects of the disease discovered and to link them with laboratory tests, drugs used and the recent discoveries made through the research with MicroRNAs.

Keywords - Sickle cell diasease, diagnosis, hydroxyurea, MicroRNAs.

I. INTRODUCTION

In 1910, sickle cell disease (SCD) was described by Dr. James Herrick in a case report of a 20-year-old patient with severe anemia accompanied by lower limb ulcers, pulmonary complications, and jaundice [12]. Due to the elongated erythrocyte morphology, similar to the sickle shape, in 1945, the disease was named sickle cell disease [21]. Years later, SCD was described as result of a mutation in the sixth codon, in the polypeptide chain of β - globin on chromosome 11, thus being classified as a genetic disease of autosomal recessive inheritance. This mutation is responsible for the substitution of a single nitrogenous base: adenine (A) by a thymine (T), leading to the erroneous production of glutamic acid instead of valine. Hence, there is the formation of an anomalous hemoglobin, called HbS [7, 35].

In healthy individuals, there are three distinct types of hemoglobin: hemoglobin A (HbA), hemoglobin A2 (HbA2) and fetal hemoglobin (HbF) [21, 38]. Thus, the HbS gene can be combined with HbA, resulting in the manifestation of the heterozygous genotype (HbAS) [11]. There are also cases in where there is the combination of HbS with other hemoglobinopathies, such as HbD, beta-thalassemias, HbC, among others [21, 28]. This association is in a way beneficial process, because it causes a dilution effect, which reduces the contact between the SCD erythrocytes, difficulting the formation of a polymer [21].

Some authors consider that SCD be a protection against malaria. Research has shown that in heterozygous phenotypes, the morphological alteration of erythrocytes results from a defense process against *Plasmodium sp*. SCD erythrocytes act facilitating the phagocytosis process of the parasite and through the low availability of oxygen, reducing the development process of the etiologic agent of malaria. However, certain stages of this mechanism remain obscure, being so-called "Malaria Hypothesis" [8].

The phenotype of sickle cell anemia presents its first manifestations between 6 months and 2 years of age, due to the decrease in the availability of HbF, which slows the polymerization processes of HbS, difficulting the early visualization of the signs and symptoms of hematological disorder [7, 34]. During first infancy, when HbF decreases, the first characteristic symptom is pain crisis. In addition, due to functional asplenia, children with SCD when compared to healthy children have a higher risk of developing infections, such as those caused by Streptococcus pneumoniae and parvovirus B19 [7].

The pathophysiology of SCD is initiated in situations with low oxygen tension, where HbS undergoes polymerization. This especially occurs in organs where blood flow is slower or in areas where the terminal blood supply is limited. However, this fact does not avoid that lungs and brain can be affected [16]. The polymerization and deoxygenation of HbS are responsible for the morphological alteration, rigidity and decrease in the erythrocyte deformation capacity [15, 35]. This process includes the morphological changes of the red blood cells, hemolysis, chronic inflammation, cells with high adhesion, increased oxidative stress and dysfunction/activation of the endothelial cells, which cause an acute vaso - occlusion process [31, 32].

The vessel occlusion is a multicellular and multi-stage process, being initiated through the interaction between erythrocytes, activated leukocytes, endothelial cells, platelets and plasma proteins [15, 39]. The adhesion between leukocytes and erythrocytes activates the endothelial pathway leading to events of hypoxia, oxidative stress, reduced availability of nitric oxide (NO) and inflammation [15, 31]. The occlusive vessel process associated with ischemia and reperfusion with consequent injuries and endothelial activations results in a chronic inflammatory process, which is maintained through the reduction of NO availability, elevated levels of inflammatory cytokines and the presence of oxidative stress [30].

In addition, the morphological alteration of erythrocytes alters the functionality of the sodium and potassium pump, leading to loss of water and potassium, generating erythrocytes

denser and more susceptible to polymer formation. Membrane alterations are triggered by the rearrangement of the spectrin-actin protein, phosphatidylserine externalization, with subsequent generation of free radicals and acceleration of apoptosis process. Due to hypoxia, infarction of several types of tissue can occur, especially those where there is acid pH [20]. This morphological alteration of the erythrocytes leads to obstruction of the blood flow in the capillary vessels and hemolysis, which can lead to bone marrow necrosis, splenic alterations, vasoconstriction, acute thoracic syndrome, among others [3, 15].

The activation of endothelial cells is responsible for increased expression of surface adhesion proteins, such as selectin, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), fibrinogen, fibronectin and CD36 [13, 36]. In this way, endothelial activation favors the capture of erythrocytes and leukocytes, which in turn also have increased adhesive properties [21].

The SCD individuals have activated leukocytes in the blood vessel, facilitating adhesion to the vascular endothelium, especially when there is inflammation. SCD leukocytes have twice as many adhesive properties as leukocytes from healthy subjects [21, 40]. Once adhered the leukocytes have the ability to perform secondary adhesions with erythrocytes leading to physical obstruction of the microcirculation [40]. Research has shown that SCD patients also present a numerical increase in the amount of neutrophils and eosinophils, but the participation of eosinophils in the process has not yet been clearly established [39].

Thus, the inflammatory process of the airways in SCD patients, is characterized by the intense participation of eosinophils, being the lungs more vulnerable to vessel occlusion due to its anatomy. Research suggests that asthma is associated with acute chest syndrome (ACS) and pain crises in SCD patients, especially children. SCD children diagnosed with asthma have the first episode of acute chest syndrome earlier. They require more transfusions and are affected by episodes of more frequent pain, consequently they need a longer hospitalization time [4]. The pathophysiological relationship, as well as the triggering factors of asthma attacks in SCD patients are still not fully understood. At the moment only treatments during asthma crisis have shown results [33].

Increased mechanical fragility and loss of erythrocyte elasticity are directly proportional to HbS concentration. Thus, another remarkable mechanism is hemolysis. Extravascular hemolysis is caused by SCD erythrocyte phagocytosis, whereas intravascular hemolysis is a consequence of lysis of SCD erythrocytes [1]. Thus, patients with this pathology usually present splenomegaly, due to the repetitions of vaso - occlusive episodes, hence may have fibrosis and spleen atrophy [15, 21].

II. PROPOSAL

This paper has the objective of carrying out a review of the literature on the pathophysiology of SCD, as well as the presentation of the new findings and treatments performed until the moment. This work was carried out through researches in books and articles about the theme, aiming to present to students, researchers and health professionals a concise and clear view of the main aspects of the disease.

III. RESULTS AND DISCUSS

It is estimated that 222.785 of SCD births around the world occur every year. About 24,6% of the world 's population is affected by SCD, being populations in sub - Saharan Africa, parts of the Mediterranean and midwestern India most affected [19, 21]. However, due to population migrations, this pathology also reached Europe and the Americas, being classified as the most common hereditary hematological disorder of the United States [18, 25]. In developed countries, in 1967, the mean mortality from SCD was around 20 years of age, being resulted from infections [29]. In the 1990s, the life expectancy of SCD men was 42 years and 48 years for women [26]. Between the 1980s and 1990s, there was a decrease of 68% in the mortality of SCD children between 0 and 3 years old. [41].

Currently, cohort studies conducted between 2000 and 2007 demonstrate the absence of SCD deaths before 18 years of age in developed countries [27]. Developing countries have limitations on mortality data for these patients. Retrospective studies show that due to the presence of infections and differences in health access in urban and rural areas, 50-90% of SCD children are afflicted by premature death in sub-Saharan Africa [28]. Thus, neonatal screening tests are extremely important because its help in the early detection of SCD. The child death affects about 25-30% of SCD children worldwide. One of these factors is the great possibility of congestion in the red pulp by sequestration of SCD erythrocytes, resulting in auto-splenectomy [25].

The diagnosis of SCD is performed by blood count total, solubility test, fetal hemoglobin and hemoglobin A2 measurement, and neonatal screening. However, the solubility test is not indicated for children less than 6 months of age, because during this period there are low HbS levels and high concentration of HbF [7].

Confirmation of SCD is only obtained with the detection of HbS by electrophoresis [21]. Hemolytic anemia present in SCD is associated with moderate reductions in hematocrit, hemoglobin, and count erythrocytes. The hemogram of these patients demonstrates normocytic and normochromic anemia. Biochemical tests demonstrate unconjugated hyperbilirubinemia; high lactate dehydrogenase concentrations; and low concentrations of haptoglobin [2].

During the last decade, interest in microRNAs (miRNAs) has increased exponentially after the discovery of its importance in biological processes such as development, cell proliferation and apoptosis [24]. The miRNAs were first mentioned in the year 1993, when Rosalind C. Lee noted that the Lin 4 gene was responsible to regulated of *Caenorhabditis elegans* developmental events, through the down-regulation of the Lin-14 protein level, which is responsible for the first stage of larval development of *Caenorhabditis elegans* [17].

Currently, 2578 human mature miRNAs have been identified, but the majority biological function remains

unknown. It is estimated that about one-third of the human genome is post-transcriptionally controlled through miRNAs and changes in its expression levels may contribute to the pathophysiology of various diseases, including SCD and associated diseases such as asthma, acute chest syndrome, thrombosis, stroke, among others. [10]. The miRNAs are classified as small non-coding RNAs, located mainly in the introns, responsible for silencing through mRNA cleavage or protein translation blockade [10, 24].

Research done in recent years has shown that comparing healthy individuals with SCD patients, it is possible to note changes in the expression profile of miRNAs between them. Among these alterations 40 miRNAs are differentially express in SCD platelets, suggesting the existence of a direct connection of the expression of the miRNAs with the platelet functions, being able to influence other blood cells and vascular endothelial cells [14]. In this context, studies with SCD have related the 146a, 155 and 21 miRNAs with the immune system. The miRNA-146a has an anti-inflammatory function by suppressing NF-kB activation, while miRNA-21 is responsible for the expression of NF-kb and STAT3. However, miRNA-155 has a pro-inflammatory action [37].

During a hemolytic crisis, SCD patients may need of red blood cell transfusion, when the hematocrit has a decrease of greater than or equal to 20%. Blood transfusion helps in the recovery process; however, it exposes the patient to the risk of contracting blood-borne diseases. In addition, with the blood transfusion, there creation of antibodies against less immunogenic blood groups occurs, hence there is the difficulty of blood compatibility for future transfusions [39].

Another recurrent problem blood transfusion are iron overload, which can be detected by laboratory tests such as iron levels, ferritin level, iron binding capacity and transferrin. [21]. Iron toxicity is directly linked to iron not bound to transferrin, leading to its deposition in organs, triggering the onset of hepatic cirrhosis and hepatic insufficiency and hyperpigmentation of the skin [5, 21]. In such cases, the indicated treatment is the use of iron chelating drugs such as deferoxamine; deferiprone and deferasirox [5].

In order to avoid vaso-occlusive crises and consequent hemolytic crisis and prolonged hospitalizations, many drugs have been researched and developed. Currently the most commonly used drug is hydroxyurea, a hydrocarbon that is responsible for impeding the exit of G1/S phase cells from the cell cycle, being widely used in the treatment of neoplasias of the hematopoietic system [34]. In SCD, hydroxyurea acts to increase HbF synthesis and increase NO bioavailability. As a consequence, there is inhibition of platelet aggregation and decreased expression of vascular endothelial adhesion molecules, reducing patient mortality by 40%. However, some patients present unsatisfactory responses to hydroxyurea treatment, especially when the use of this drug is continuous, leading to a decrease in efficacy [8].

Another therapeutic approach used is increased synthesis and availability of HbF. The 5-azycytidine, has a preventive effect on the first complications of SCD by increasing HbF, leading to a reduction of up to 20% of symptoms [6]. In turn, 5-Aza-2'-deoxycytidine is a drug with lower toxicity and lower tumor induction potential when compared to 5-azacytidine [8]. The sodium phenylbutyrate showed satisfactory results in the short term, however, in the long term, it generates tolerance to patients [9]. Thalidomide withdrawn from the market in the 1960s, due to its poor fetal formation, was once again the target of research due to its inhibitory properties of TNF-α. Derivatives of thalidomide such as lenalidomide and pomalidomide also have the ability to induce HbF synthesis [38].

During the occlusive vessel process, the bioavailability of arginine is low. Thus, intervention through supplementation with arginine helps in the rapid reversal of vaso-occlusive processes [15, 22, 23]. Arginine, when metabolized by arginase, is converted to nitric oxide, which acts directly on the vascular endothelium. Nitric oxide has vasodilatory action, decreases platelet aggregation and endothelial activation and neutrophil adhesion, causing increased blood flow and consequent reoxygenation, favoring the dispersion of the polymers [8].

Inhalation of nitric oxide gas has been shown to be effective during vaso occlusive crises and is of great utility in the reduction of acute chest syndrome. However, the high cost and difficulty of manipulation hinders the access of this therapy by a large part of the population of developing or underdeveloped countries [8, 22, 23]. In addition, phytomedicines over the years have proven quite effective. The ethanolic extract of *Cajamuscajam sp.* plant has been shown to reduce the degree of erythrocyte deformation during hypoxia [8, 21].

IV. CONCLUSIONS

Even with more than a century of its discovery, certain pathophysiological factors of SCD remain obscure, as a new guide for pathophysiological understanding, the MiRNAs present great potential for new discoveries. The development of future treatments the miRNAs has proved very promising, since a treatment at the molecular level improved the quality of life since childhood, dispensing the use of drugs and the needs of blood transfusions. However, this reality of miRNA manipulation is still a distant future, because the studies are still recent and there is still much to be clarified.

More current alternatives are expected to launch more effective drugs in the market, as well as better attention to these patients through periodic exams, appropriate treatment with hydroxyurea, genetic counseling, dissemination of the disease in developing and underdeveloped countries, among others. These measures are fundamental, since the earlier the detection of SCD, the better the living conditions of these patients.

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