CASE REPORT

Glucose-6-phosphate dehydrogenase deficiency with recurrent infections: case report

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Abstract

Objective: to report a case of rare neutrophil functional disorder with clinical and laboratory findings similar to those of chronic granulomatous disease.

Methods: patient with extremely reduced level of glucose-6-phosphate dehydrogenase and recurrent infections that improved after continuous use of cotrimoxazole. The patient presented leukocytes with defective respiratory burst, similar to what occurs in chronic granulomatous disease.

Comments: the diagnosis of glucose-6-phosphate dehydrogenase deficiency in neutrophils should be considered in any patient with hemolytic anemia whose level of G6PD is extremely low or in any patient that presents recurrent infections as differential diagnosis of chronic granulomatous disease.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency affects over 200 million people around the world. Its frequency is relatively high among African Americans in the United States (13%) and populations in the Mediterranean (5 to 40%). G6PD deficiency is an X-linked enzyme defect, and one of its main signs is the presence of hemolytic anemia. Hemolysis may be triggered by infection and by drugs with oxidative properties, such as acetylsalicylic acid, vitamin K, chloramphenicol and antimalarial drugs. G6PD present in neutrophils and erythrocytes is coded by the same gene, located in the Xq28 chromosome. Over 200 mutations have been reported.

Extremely low levels of G6PD (more than 5% below normal) in neutrophils may be observed in association with rare mutations, causing failure of oxidative metabolism and a consequent reduction in oxygen-dependent phagocytosis.
In these rare cases, patients with G6PD become susceptible to repetition infections. Fewer than 10 cases have been described in the literature. The diagnosis is established assessing the level of G6PD in the neutrophils.3

In the present article, we describe the case of a patient with increased G6PD deficiency in erythrocytes and recurrent infections, with a diagnostic hypothesis of immune system impairment following immunologic investigation.

Case report

We describe the case of a black five-year-old boy presenting recurrent infections starting at 6 months of age: 12 pneumonias episodes, two with pleural effusion, septicemia (1 episode), sinusitis (4 episodes), otitis media (3 episodes), stomatitis (1 episode), skin supplicative lesions (1 episode), and diarrhea (2 episodes). He was hospitalized eight times.

There were no remarkable events during neonatal history. The boy received exclusive breastfeeding until 4 months and mixed breastfeeding until 4 years. At 6 months, he was diagnosed with G6PD deficiency in erythrocytes. The vaccination scheme followed the recommendations of the Brazilian Health Department, without any reactions. The boy had not been to nursery or preschool.

The parents did not present a history of consanguinity. A brother died at age 3 due to sepsis, pulmonary pneumonia, and lung and liver abscess. The father and a sister presented signs of sickle cell anemia.

On physical examination, the child was pale, with hypoplastic tonsils. Lymph nodes were present and without alterations. The boy presented with finger tapering. Liver was 5 cm away from right costal margin and spleen at 8 cm from left costal margin.

Diagnostic assessment revealed sweat Na and Cl without alterations, negative investigation for gastroesophageal reflux, lung scintigraphy without perfusion in the left lung, and granuloma on bronchoscopy. Previous hemograms revealed presence of anemia, leukocytosis, polymorphonuclear leukocytes and eosinophilia. Current hemogram revealed an improvement in hematologic parameters (Table 1). Immunoglobulin levels (IgG, IgA, IgM) were increased (Table 2). Positive antibodies for (IgG) measles and rubella, and negative for HIV. Normal values for CD3, CD4 and CD8 lymphocyte subpopulations. Mantoux reaction = 11mm. Complement system activity was normal (CH50).

Phagocyte evaluation with the nitroblue tetrazolium (NBT) test had the following results: patient 6% versus control 78%; dihydrorhodamine 123 (DHR) determination: phagocytic index = 5 times in the patient and 87 times in controls.

Evaluation of NADPH oxidase activity in leukocytes: superoxide liberation was quantified at 0, 5, 15, 25, 45 and 60 minutes, according to cytochrome reduction and specific inhibition by superoxide dismutase, under spontaneous conditions or stimulation with phorbol miristate acetate (PMA, 30 nM), as described by Condino-Neto et al. (1996).

The results described in Table 3 clearly show that the leukocytes in this patient presenting G6PD deficiency did not trigger an oxidative burst, as is the case of patients with chronic granulomatous disease (CGD). Erythrocyte G6PD dosing was 1.8 to 37 ul/gHb/min (12.1+/− 2.09), revealing a marked G6PD enzyme deficiency.

The diagnostic hypothesis was G6PD deficiency. The child was then started on continuous co-trimoxazole (trimethoprim-sulfamethoxazole combination) (therapeutic dose) with control of infections, improvement in anemia and leukocytosis and decrease in the size of the spleen.

<table>
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<tr>
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<th>Neu</th>
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* after the administration of cotrimoxazole
Leu = leukocytes, Neu = neutrophils, Eos = eosinophils, Bas = basophils, Lym = lymphocytes, Mon = monocytes, HB = hemoglobin, Htc = hematocrit

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Discusson

The bactericidal activity of neutrophils for catalase-positive microorganisms depends primarily on reactive oxygen intermediates (ROI) (superoxide anion, oxygen, and hydrogen peroxide), liberated by the activation of the enzyme NADPH oxidase, in a reaction known as neutrophil oxidative metabolism. Oxidative metabolism (oxidative burst) is characterized by an abrupt increase in the
consumption of oxygen (O$_2$) and by its partial reduction to superoxide anion (O$_2^{-}$). NADPH is a specific donor of electrons for oxygen according to the reaction

\[
\text{NADPH} + 2\text{O}_2 \rightarrow \text{NADP}^+ + \text{H}^+ + 2\text{O}_2^{-}.
\]

When acting as an oxidant, O$_2^{-}$ is reduced to oxygen peroxide (H$_2$O$_2$), which is directly toxic for microorganisms.

The continuous source of NADPH for phagocytes is the hexose monophosphate pathway. G6PD is the first enzyme in this pathway (hexose monophosphate shunt), in which glucose-6-phosphate (Glucose-6-P) is converted into 6-phospho-gluconate (6-PG) at the same time as NADP$^+$ is reduced to NADPH, which in turn is a substrate for oxidative metabolism.

In most types of G6PD deficiency, the levels of neutrophils are between 20 and 75% the normal levels. G6PD levels higher than 5% are apparently sufficient to recycle NADP$^+$ into NADPH at a speed that allows activation of oxidative metabolism and adequate protection against infection caused by microorganisms.

The enzyme NADPH oxidase is made of several components located in the plasmatic membrane of phagocytes. The NADPH oxidase enzyme unit is composed of a protein named cytochrome B 558 [a 91kDa chain (gp91phox) and a 22kDa chain (p22phox)] and of two cytosol proteins (47 and 67 kDa, p47phox and p67phox, respectively). (Figure 1).

A deficiency in any NADPH oxidase subunit leads to impaired phagocytic activity, especially against catalase-positive pathogens, commonly found in CGD. Therefore, both in CGD and in G6PD deficiency there is a defect in the bactericidal capacity of neutrophils.

Microorganisms that produce H$_2$O$_2$ and do not contain catalase (ex. *Streptococcus* and *Haemophilus influenzae*) are easily destroyed by neutrophils in patients with CGD and G6PD deficiency, because their endogenous H$_2$O$_2$ is not destroyed by catalase, and it is capable of supplying deficient cells with toxic oxygen derivatives.

The oxidative metabolism of phagocytes may be evaluated through biochemical and cytochemical methods that detect the production of ROI. The most commonly employed method is NBT reduction, a simple and low-cost test. In the NBT test, phagocytic cells are activated by

### Table 3 - Kinetics of superoxide release by mononuclear (MON) and polymorphonuclear (PMN) leukocytes of patients (PAT) with G6PD deficiency, and healthy controls (CONTR) at 0, 5, 15, 25, 45 and 60 minutes, obtained spontaneously (SPO) or through phorbol miristate acetate (PMA, 30 nM) stimulation

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The results consist of the mean between two experiments, expressed as nmol of released superoxide x 10$^6$ leukocytes.
appropriate stimuli in the presence of yellow stain, which functions as electron receptor and is reduced when in contact with ROI, changing to blue. This stain can be quantified in the cytoplasm of neutrophils, and it is easily visualized in standard microscopes. Another method to assess the activity of oxidative metabolism is direct measurement of O$_2$ consumption, production of O$_2^-$ or production of H$_2$O$_2$. Specific O$_2^-$ dosing is important, since it reveals whether the defect in oxidative metabolism occurred during early stages.

Flow cytometry is also used to diagnose oxidative metabolism defects due to its fast and precise results. The production of H$_2$O$_2$ in PMA-activated neutrophils is measured after staining with DHR. The uptake of DHR by neutrophils causes an increase in fluorescence in the presence of H$_2$O$_2$.

Although detection of O$_2^-$ and H$_2$O$_2$ is more sensitive and specific to evaluate oxidative metabolism, NBT is still the reference method for the screening of CGD. Patients with CGD are unable to reduce NBT to the blue color and, in rare cases, a weak reduction can be detected. Similarly to CGD, a marked G6PD deficiency is also associated with altered NBT test and decreased production of H$_2$O$_2$ and O$_2$.

CGD is a rare primary functional deficiency of the phagocytic system. This genetically heterogeneous entity is characterized by severe and recurrent infections associated with a dysfunction in the phagocytic activity resulting from a failure in the NADPH oxidase system. Mutations in the gene (located on the short arm of chromosome X) that encodes gp91phox (a subunit of the NADPH oxidase system) are responsible for 60 to 65% of the cases of CGD. The remaining 35 to 40% present an autosomic recessive pattern of inheritance. CGD is the best understood phagocytic dysfunction.

The clinical status of patients with G6PD deficiency, in most cases, is that of hemolytic anemia. When the deficiency is marked, the clinical presentation is similar to that of CGD. Recurrent infections by catalase-positive microorganisms (Staphylococcus aureus, enterobacteria such as Salmonella, Serratia marcescens, Escherichia coli, Nocardia, fungi such as Candida albicans and especially Aspergillus) are frequent. There are reports of sepsis due to Chromobacterium violaceum in patients with CGD and G6PD deficiency, suggesting an association between these dysfunctions and susceptibility to infection by this pathogen.

The most commonly affected sites are the lungs, the liver, the skin, the gastrointestinal tract and the lymph nodes. Osteomyelitis, when present, is of difficult resolution. There are reports of lesions in the eyes, including the cornea and retina. Death usually results from fungal infections, especially caused by Aspergillus.
Bacteria undergo phagocytosis but are not destroyed; thus, these patients tend to develop chronic abscesses and granulomas, which may lead to non-infectious complications such as hepatosplenomegaly, lymphadenopathy, hypergammaglobulinemia, chronic diarrhea and granulomatous obstruction in certain organs, sometimes with a fatal outcome. 9

Clinical signs and symptoms appear during the first two years of life in most patients. Some children present the first symptoms in the neonatal period. Lymphadenopathy is present in almost all cases, and is usually one of the first manifestations of the disease. Hepatic and perihepatic abscesses are sometimes the first findings. 9

The treatment of G6PD deficiency implies preventing hemolysis. In severe cases, it is similar to CGD, with an indication for prophylaxis with co-trimoxazol, since this drug reduces the incidence of infection and life-threatening risk. 3,9 Despite the fact that this drug triggers hemolysis in patients with G6PD deficiency, because it presents oxidative properties (1), studies and reports in the literature indicate that co-trimoxazol rarely causes hemolysis in the population with G6PD deficiency. 14

The prophylaxis with antifungal drugs is not indicated, since its efficacy is questionable and it may have important collateral effects. 2 An early and aggressive use of parenteral antibiotics is indicated to resolve infections. 3,9

In life-threatening infections, leukocyte transfusion may be useful, although its application is rare. The obstruction by granulomas in vital organs may recede with the use of hydrocortisone. This treatment, however, is controversial, and should be used with care. 9

Interferon-gamma (INF-γ) activates macrophages in vivo and in vitro since it stimulates NADPH oxidase and enhances the synthesis of nitric oxide by neutrophils (NEU) and mononuclear cells (MON), increasing their microbiocide activity in healthy individuals. 6 Therapy with human recombinant interferon-gamma (rHuIFN-γ) was recently used in patients with CGD with reduction of the relative risk for severe infection in 70% of the cases. 6 Although the oxidative metabolic activity was not restored in most patients, some variants of CGD - such as the defect in gp91phox and DHR, for diagnosis of functional defects of phagocytes, both tests were clearly altered. G6PD levels were then measured due to the diagnosis of G6PD deficiency when the patients was 6 months old, after investigation of anemia. The enzyme dosing carried out in erythrocytes revealed marked deficiency, which led to the conclusion that the phagocytic defects of the patient are caused by G6PD deficiency in the neutrophils. Continuous co-trimoxazol was introduced with important improvement; since the start of this treatment, the patient has not presented any bacterial infections or clinical and laboratory signs of hemolysis.

The CGD was initially diagnosed, it was considered a fatal granulomatous disease. Currently, patients with this diagnosis have a better prognosis, but mortality and morbidity are still significant. 7 Despite the use of aggressive treatment, many patients with CGD die before adolescence. 2 The prognosis of severe G6PD deficiency is not well established due to the small number of cases described. 3

References


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