Case study

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY CO-INCIDENT WITH AUTOIMUNE HAEMOLYTIC ANEMIA IN AN ADOLESCENT FEMALE: A CASE REPORT.

ABSTRACT

Background: Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency is a genetic disorder with a deficiency or absence of G6PD, an enzyme required for the proper function of red blood cells. The pattern of inheritance for *G6PD* gene mutation is sex-linked recessive. It thus affects males and either homozygous or heterozygous females whose unaffected X chromosome is inactivated (lyonized females). Autoimmune hemolytic anemia (AIHA) is a disorder characterized by premature destruction of red blood cells (RBC) by autologous RBC autoantibodies whose presence is demonstrated by a positive direct antiglobulin (Coombs) test (DAT). AIHA is classified into primary or idiopathic and secondary types based on the absence or presence of an underlying disease. In Nigeria AIHA is rare in children but secondary forms are common in teenagers**.** Chronic granulomatous disease (CGD) is a rare, genetic, immune disorder characterized by neutrophil dysfunction and deficiency or absence of G6PD in both neutrophils and RBC. CGD has both sex-linked recessive and autosomal recessive pattern of inheritance. Therefore CGD with G6PD could occur in a female with X chromosome anomaly in whom co-existence of an immune disorder and G6PD deficiency could worsen haemolysis.

Aim: To highlight the possible existence of the rare chronic granulomatous disease.

Case: To describe a case of acute hemolytic anaemia on a background of G6PD deficiency, positive direct Coombs test and a seeming neutrophil dysfunction in an adolescent female.

Case report: We report a case of a G6PD deficient 14 year-old female with systemic inflammatory response syndrome, marked neutrophillia yet mild toxic granulation, steroid-responsive hemolytic anemia and positive direct Coombs test.

Conclusion: C6PD deficiency and AIHA may co-exist in an adolescent female possibly as part of CGD but a definitive diagnosis of CGD is required.

Keywords: G6PD deficiency, AIHA, Chronic granulomatous disease, Adolescent female, steroid-responsive.

INTRODUCTION

G6PD deficiency is a genetic metabolic abnormality caused by deficiency of G6PD an enzyme critical for the proper function of red blood cells (RBC). The *G6PD* gene is located on the long arm (q) of the X chromosome (Xq28) of RBC and neutrophils [1] The precise mutation is known for 186 of the 400 reported genetic variants[2]. The enzyme has been classified into I to V in order of increasing enzyme activity and decreasing disease severity [1]. In cases with a family history, the *G6PD* gene mutation is inherited in an X-linked recessive manner affecting males and heterozygous females with unfavorable X-inactivation (lyonized females), in many cases mutation occurs as a new (sporadic or de novo) mutation [3,4].

The *G6PD* catalyzes the formation of coenzyme NADPH, which protects cells from oxidative damage. A mutation in the *G6PD* gene results in low levels of functional G6PD, which in turn leads to low levels of NADPH and a depletion of an antioxidant known as glutathione, which is necessary to protect the cell's hemoglobin and its cell wall from highly reactive oxygen radicals[3]. This reduction in NADPH makes red blood cells more susceptible to destruction from oxidative stress than other cells. When individuals with very low enzyme activity are exposed to trigger factors red blood cells are broken down prematurely. Triggers of hemolysis in G6PD-deficient persons include certain drugs such as quinine-based antimalarials and sulphur

containing antibiotics, ‘camphor’ (naphthalene balls), certain infectious diseases and eating fava beans (favism). In the absence of trigger factors most people with G6PD deficiency are normal. Acute hemolytic anemia (AHA) develops about 2-3 days following exposure to the trigger. When the body cannot compensate for accelerated loss features of severe anemia develop, these include fatigue, pale color, jaundice even in the neonate, shortness of breath, tacchycardia, dark urine, splenomegaly and heart failure. Most episodes are self-limited. Individuals of all races and ethnic backgrounds are affected with varying severity, Africa being among those with the highest prevalence rates [5].

Case presentation

A fourteen year-old female presented with complaints of paleness of the body, yellowness of eyes, generalized body weakness, high grade fever, abdominal pain and passage of cola-coloured urine. She was having a normal menstruation. The patient is the third child in a non-consanguinous monogamous family with 6 children; 2 boys and 4 girls. Her mother is 40 years old while father is 50. Both parents attained primary education and are in socioeconomic class IV. Patient had no such personal or family history previously. No causative medications were identified. Respiratory rate was 36 cycles per minute and heart rate was 147 beats per minute. Both were significantly deranged >2SD from normal for age and sex. She had high grade pyrexia of 39 degree Celsius, was icteric, dyspneic, markedly pale, her spleen was not enlarged, she had lower abdominal tenderness. Hematocrit was 9% and mean oxygen saturation was 90% in room air. Laboratory evidence was of intravascular haemolysis (peripheral blood film evidence of bone marrow erythroid hyperplasia but with no spherocytes). Blood film also noted many band

neutrophils but only mild toxic granulations. Complete blood count from the specimen in the EDTA bottle was done by automation as described by Araoye and Luzattoo and showed hemoglobin (Hb) of 3 g/dl, normal platelet count and neutrophillic-leucocytosis. G6PD was deficient as determined by the fluorescent spot method [6]. Direct Coomb's test was positive for 'warm' antibodies. Genotype was AA and blood group was 'A' Rhesus (Rh) positive. Plasmodium falciparium malaria parasite was seen on blood film. Blood culture was not done. Other investigations carried out include a retroviral screen which was negative. Urine analysis showed haemoglobinuria and nitrite positivity. Few white blood cells and normal red blood cells without cast were identified on urine microscopy probably due to the menstruation. Abdomino-pelvic ultrasonography revealed mild enlargement of the kidney and spleen.

She was immediately transfused with 'O' Rh positive packed red blood cells, intra-nasal oxygen, intravenous anti-malarial and antibiotic medications were given. Fever gradually resolved but hemolysis persisted with worsening hypoxemia and progressively dropping hematocrit despite transfusion of additional 4 pints of 'A' Rh positive blood in daily aliquots. All blood unit were negative for hepatitis B and C antigen, HIV and VDRL but were not screened for G6PD deficiency. She responded to oral prednisolone as she became independent of blood transfusion and her hemoglobin increased by 2g/dl with no further drop. She was counseled concerning her G6PD status. Hematocrit on discharge was 16% and patient's condition was stable. A week after discharge patient was still in stable clinical condition, had same discharge Hb value although with urinalysis evidence of low grade hemolysis. She was commenced on tapering doses of prednisolone. A repeat G6PD status is planned as patient is still being followed-up at the time of this report. Informed consent from parents and her primary caregivers as well as the Ethical approval from the Health Research Ethical Committee were obtained for this report.

The diagnosis of G6PD deficiency is based on the identification of characteristic features via a thorough clinical evaluation and demonstrating decreased activity of the G6PD enzyme through either a quantitative assay or a screening test such as Beutler fluorescent spot test [6]. Beutler fluorescent spot test [6] is a rapid and inexpensive test that visually identifies NADPH produced by G6PD which causes the blood spot to fluoresce under ultraviolet light. When the blood spot does not fluoresce, the test is positive. False negative results occur during active hemolysis the test is therefore best done 2–3 weeks after a hemolytic episode. Elevated serum lactate dehydrogenase (a marker of hemolytic severity), reticulocytosis, Heinz bodies and bite cells on blood film are supportive.

Management of G6PD deficiency is by avoiding triggers, discontinuation of causative drug, treatment of the infectious trigger, administration of intravenous fluids and blood in severe cases.

G6PD deficiency has been described in association with a rare immune disorder called chronic granulomatous disorder (CGD) [7,8,9]. CGD is a Sex- linked recessive and Autosomal recessive disorder with defective neutrophil function caused by mutation of a gene that encodes one of the proteins in the NADPH oxidase complex [9]. In CGD patients neutrophils are incapable of superoxide production, essential for the killing of fungi and catalase positive bacteria [8,9]. Activation of the NADPH-dependent oxidase requires stimulation of the neutrophils conversely effective neutrophil phagocytosis requires activation of NADPH-dependent oxidase[9]. Few CGD patients with G6PD deficient neutrophils also have erythrocytes lacking the enzyme; these patients have chronic hemolysis [9]. Complete absence of the enzyme has also been described [10]. CGD is a rare disease with an incidence of four to five per million individuals [9]. Approximately 65% of patients with CGD are as a result of mutations in the X-chromosome gene, most are males. The clinical presentation, attack rate and severity of infections are

exceedingly variable [9]. This is highlighted by Gray et al [10] among 3 male siblings of a Canadian family. The onset of clinical signs and symptoms vary from early infancy to young adulthood [9]. Granuloma formation and inflammatory processes especially of the skin are a hallmark of CGD. [9]. Female X-linked carriers of CGD have been reported with an increased incidence of autoimmune phenomen [7,11].

The hallmark of Autoimmune hemolytic anemia (AIHA) is the presence of autologous RBC autoantibodies usually demonstrated by a positive direct antiglobulin (Coombs) test (DAT) followed by destruction of the antibody coated RBC in the reticulo-endothelial system [12,13]. AIHA is classified into 'warm' and 'cold' or primary and secondary [14,15,16]. Antibodies (IgG) bind to RBCs at 37 °C (98.6 °F) in warm antibody AIHA [14] while the cold-reactive autoantibodies (IgM) bind optimally to RBCs at temperatures below body temperature in cold antibody AIHA [14,15]. Few patients have mixed autoantibodies AIHA [15]. Primary/idiopathic AIHA occur in the absence of an underlying disease while secondary forms occur together and remit with correction of a suspect disease or when the suspect disease causes an immunologic aberration [16]. Causes of secondary warm AIHA include chronic lymphocytic leukemia (CLL), lymphomas, Systemic lupus erythematosus (SLE) [16]. B-cell lymphoproliferative disorder, *Mycoplasma pneumoniae* infections in adolescents or young adults, infectious mononucleosis and chickenpox in children have been implicated in secondary cold antibody AIHA. Drugs such as second- and third-generation cephalosporins, cefotetan, and ceftriaxone[17] are also implicated in immune mediated injury to RBCs where autoantibodies may bind to RBC-drug complex or to RBC without the presence of drug [17]. A careful history of drug exposure should be elicited.

Eighty to ninety percent of adult cases of AIHA are caused by warm antibodies, cold agglutinin disease accounts for the rest being more common in women than in men [16]. Primary warm antibody AIHA accounts for about half of all cases, while paroxysmal cold hemoglobinuria (PCH) make up 2-5% and Donath-Landsteiner one-third of cold AIHA, both being most common in children [18]. Insidious onset of variable symptoms of anemia based on rate of hemolysis is typical of warm antibody AIHA. Secondary forms present with symptoms and signs of the underlying disease. Patients with idiopathic cold agglutinin disease have either a chronic hemolytic anemia or episodic, acute, self-limited hemolysis.

Hematocrit levels range from less than 10% to compensated near-normal in warm antibody AIHA, mild to moderate anemia with hematocrit levels as low as 15-20% in chronic cold agglutinin disease, a rapid fall in hematocrit during a paroxysm in PCH and similar to those of warm antibody AIHA or PCH in drug-induced immune hemolytic anemia depending on the mechanism of the drug-induced immune process [14,19]. Leukopenia, neutropenia, occasionally immune thrombocytopenia (Evans syndrome) have been reported in warm antibody AIHA [19]. Leucopenia noted early during a PCH paroxysm may be followed by leukocytosis[18]. Hemolysis may lead to consumptive depletion of complement levels. Blood film evidence of hemolysis with polychromasia (reticulocytosis), spherocytes, RBC fragments, nucleated RBCs, and erythrophagocytosis by monocytes is common to all types of AIHA. RBC autoagglutination may be seen in the blood film and in chilled anticoagulated blood from patients with cold-antibody AIHA. The DAT may be positive for anti-IgG alone in idiopathic and drug-associated (alpha-methyldopa) warm AIHA, positive for anti-IgG and anti-C3 in patients with SLE-associated and idiopathic warm AIHA and positive for anti-C3 alone in cold agglutinin disease, warm AIHA with low affinity IgG antibody, in some drug-associated cases and in PCH [14].

The laboratory findings of drug-induced AIHA may resemble those of warm antibody AIHA or a cryopathic hemolytic syndrome, depending on the mechanism of the autoantibody induction and the target antigen [17]

Children with warm antibody AIHA have a rapid response to glucocorticoids. Infants and adolescents have an insidious onset with tendency to become chronic [20] in the older [21]. The mortality rate ranges from 10 to 30%, higher in those with chronic AIHA and in Evans syndrome [19,21]. Idiopathic cold agglutinin disease is benign, self-limited in post infectious type and PCH [18]. However, death may result from infection or severe anemia or sometimes from an underlying lymphoma. The Donath-Landsteiner antibody and PCH patients may survive for years. Prognosis is good for drug-induced immune HA with cessation of hemolysis and a negative DAT obtained shortly after discontinuation of the drug but for cases of drug-induced autoantibodies which may remain positive for months. Treatment of AIHA include the use of steroids, IV immunoglobulin, splenectomy, avoidance of triggers, withdrawal of drugs as well as avoidance of blood transfusion except for life threatening anemia.

DISCUSSION

G6PD deficiency is fairly common in Nigerian children while AIHA is rare; 15.3% overall prevalence of G6PD deficiency and 13 cases of AIHA (7 females and 6 males) over a 10 year period in Western Nigeria [12,22]. CGD is also a rare disease with an incidence of four to five per million individuals [9]. G6PD in Nigerian male and female children has been reported as 24.1% and 6.6% respectively, the Igbo children having the 2nd highest [22]. AIHA is also more common in males but has an adolescent female preponderance [23]. The index patient is an

adolescent female. While the precise incidence of AIHA is not known, the number of affected children (<20 years old) are estimated to be less than 0.2/100,000 with the highest rates seen in pre-school age children [23,24,25]. Thus AIHA can be seen throughout childhood, but of note should prompt more attention when seen in **teenagers** as they are more likely to have an underlying systemic illness[23] this seems to mirror our patient. G6PD has been reported in lyonized heterozygous or homozygous females [3], raising the suspicion in our patient. G6PD deficiency and AIHA are disorders associated with acute and or chronic hemolysis. Therefore, coincidence of both disorders could exacerbate hemolysis as evident in the index patient. The hemolytic episode in AIHA is usually preceded by an acute infection [26] suggested by the systemic inflammatory response syndrome with leucocytosis in our patient although a blood culture was not done to identify the causative organism.

The absence of previous hemolytic episodes may imply an acute event with the fact that the patient was stable with a hematocrit of 16% at discharge and follow-up suggesting a compensated chronic low grade hemolysis. G6PD status was deficient despite been assayed at time of hemolysis when new RBCs would normally show normal activity. Intravascular hemolysis noted in this patient rather than extravascular support G6PD deficiency over AIHA. However the positive direct Coombs test and the slightly enlarged spleen on sonogram support extravascular hemolysis of AIHA. Moreover, absence of splenomegaly in 78% AIHA patients has previously been reported with median spleen size of 0.5 cm in secondary cases [28]. Mild toxic granulation and marked neutrophillia with the suggested degree of infectivity imply a defective neutrophil function as may be found in CGD.

This study agrees with the view that AIHA is more common in females and an adolescent female with AIHA is likely to have an underlying illness [23]. In this instance AIHA being coincident

with G6PD deficiency raises the question of an existence of the rare, sporadic, chronic granulomatous disease (CGD) [9,10] Similar to our observation, females with homozygous inheritance of the defective G6PD gene, complete absence of G6PD and co-incident non-spherocytic hemolytic anemia in chronic granulomatous disease (CGD) have been described [9,10]. Fewer individuals with apparent CGD having neutrophils and erythrocytes deficient in G6PD activity have also been described [3]. Both group of patients have chronic hemolysis [3,4]. Additionally, CGD patients have been reported with an increased incidence of autoimmune phenomena also in female X-linked carriers [11,28]. This study, having identified clinical and laboratory features of both AIHA and G6PD deficiency in an adolescent female creates the need for studies regarding the possible relationships between these two disorders in this scenario. For screening of CGD, the nitroblue tetrazolium (NBT) dye test or the more accurate **flow cytometry** test using dihydrorhodamine 123 (DHR) which detects oxidant production because it increases fluorescence when oxidized by H_2O_2 may be used [29]. Molecular studies would also be useful in delineating the index patient as either a lyonized heterozygote or homozygous G6PD deficient female and a co-incident carrier of the CGD gene with autoimmune phenomena. These specialized tests could not be done in our patient due to financial constraint as well as unavailability of materials and personnel. Asymptomatic CGD patient has been reported [10], likewise our patient had no hallmark of CGD. Our patient did not have the four criteria required for a diagnosis of SLE although AIHA has been reported to precede SLE as the only manifestation by months or years before serological confirmation [30]. Our patient had normal platelet count thereby ruling out the diagnosis of Evan's syndrome [31].

The study also shows that steroid therapy (prednisolone) is quite effective in acute AIHA suggesting an AIHA induced by IgG rather than IgM [32,33]. Our patient's Coombs test also

supported a warm reacting autoantibody but a monospecific antibody test was not done. It is noteworthy that red cell transfusion could be useful in life-threatening anemia, otherwise is rarely effective [26].

Although the majority of cases of warm ALHA are acute and carry a better prognosis with the possibility of spontaneous resolution within 6 months, infants and adolescents are said to have an insidious onset with tendency to become chronic [19] the chronic being more difficult to treat.

Similar to Salawu and Durosinmi's report [12], our adolescent female patient had no history of chronic haemolysis but an insidious onset of symptoms with prompt response to glucocorticoids. She therefore needs monitoring for chronicity and a repeat G6PD assay because of unscreened blood transfused. Prevalence of G6PD deficiency in blood donor has been reported as high in a part of the country.

CONCLUSION: C6PD deficiency and AIHA may co-exist in an adolescent female possibly as part of CGD but a definitive diagnosis of CGD is required.

LIMITATION: Failure to establish a definitive diagnosis of CGD, genetic anomaly or SLE.

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