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CASE REPORT

CHRONIC GRANULOMATOUS DISEASE: AN UNREPORTED MUTATION

ABSTRACT

Chronic Granulomatous Disease (CGD) is caused by defects in the phagocyte NADPH oxidase and occurs in approximately 1:200,000 births worldwide. It presents with early onset of severe recurrent bacterial and fungal infections. This is a case of a 9-year old male with severe, recurrent bacterial infections since 3 weeks of age. Initial Nitroblue tetrazolium (NBT) reduction tests were normal but a DNA analysis revealed a previously unreported homozygous mutation in *CYBB*, p.S418Y. Dihydrorhodamine (DHR) test showed poor neutrophil oxidation consistent with X-linked CGD. Definitive microbiologic diagnosis is essential for directing therapy for recurrent bacterial and fungal infections. Treatment of infections should be aggressive. Lifelong bacterial and fungal prophylaxis is necessary for prolonged survival. We report a case of confirmed CGD with the previously unreported mutation.

KEYWORDS:
chronic granulomatous disease, recurrent bacterial infections

INTRODUCTION

Chronic Granulomatous Disease is a rare, inherited disorder of the immune system characterized by the inability of the body's phagocytic cells to make reactive oxygen species (ROS) needed to kill certain microorganisms. As a result, patients have increased susceptibility to infections caused by certain bacteria particularly catalase-producing organisms, mycobacteria, and fungi. Rare infections caused by parasites have been also reported in CGD patients[18]. The usual sites of infection are the skin, lungs, lymph nodes, liver, and bones. The most common genetic type affects only boys, and the disease can vary in its severity. Mutations in all five structural genes that comprise the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase have been found to cause CGD. Hemizygous mutations in *CYBB*, encoding gp91^{phox} are inherited in an X-linked pattern and account for 70% of the cases[2]. The other homozygous or compound heterozygous mutations are autosomal recessive (AR) occurring in both sexes and are associated with a milder disease that may considerably be more frequent in populations where consanguineous marriage practices occur. These mutations affect *CYBA*, *Nthe CF1*, *NCF2* or *NCF4*, encoding p22^{phox}, p47^{phox}, p67^{phox} or p40^{phox} respectively[19,20]. The exact incidence is unknown and the prevalence varies among the populations investigated, from 1 case per 1 million to 1 case per 160,000 individuals internationally[21,22]. A mainstay of therapy is the early diagnosis of infection and prompt, aggressive use of appropriate antibiotics and anti-fungal drugs. The currently only curative treatment of this group is allogeneic hematopoietic stem cell transplantation (HSCT)[23].

CASE REPORT

The patient is a nine-year-old male, Filipino, with a recurrent history of fever. He was born to a 31-year old G_2P_1 (1-0-0-1), who had regular prenatal consultations at a private hospital from the second month of gestation. The mother had cough on the 8th month of gestation but no medication was taken. She also had urinary tract infection on the 9th month for which she took cephalexin for 7 days. The patient was born full-term via caesarian section at the same hospital with Apgar score of 8,9, with clear amniotic fluid. Routine hepatitis B and BCG vaccines were given. Newborn screening was normal. He was noted to have jaundice within 24 hours after birth. There were no other pertinent findings. Complete blood count showed white blood cells of $42x10^9$/L with segmenter predominance (83%). He was given ampicillin and amikacin for 7 days for clinical sepsis as blood culture was negative. A repeat of the white blood cell count showed $18.7x10^9$/L segmenter 51%, lymphocyte 41% and platelet of $271x10^3$/L. He was discharged in improved condition without jaundice. On the 23rd day of life, he started to have intermittent low-grade fever for which paracetamol was given and diarrhea. No other signs or symptoms were noted. He was admitted as a case of late-onset sepsis. CBC showed leukocytosis $35.5x10^9$/L with a predominance of neutrophils (51%) and platelet of $358x10^3$/L. Urinalysis, fecalysis, and blood culture were negative, CSF analysis was normal. He was started on ampicillin and gentamicin then later shifted to cefotaxime. He developed healthcare-associated pneumonia and repeated complete blood counts showed persistent leukocytosis. Chest radiograph showed pneumonia with reactive mediastinal lymphadenopathies. Medications were shifted to meropenem, vancomycin, gentamicin, and metronidazole. On the 3rd week of hospital stay, fever persisted. Repeated chest radiograph revealed the progression of pneumonia with reactive mediastinal lymphadenopathies. Meropenem was shifted to ciprofloxacin. He was given intravenous immunoglobulin (IVIG). Mantoux

test showed no induration. Repeat blood culture was negative, while urine culture grew *Candida albicans*. Hence, amphotericin was started. On the 4th week of admission, the patient remained febrile. He was referred to a hematologist and his bone marrow aspirate showed normal results. He was then transferred to a tertiary center for further work-up.

On physical examination upon transfer, the patient was febrile with oral thrush, crackles, and hepatosplenomegaly. Repeat complete blood count showed leukocytes of $22x10^9$/L, segmenters 64%, lymphocytes 31% and platelets of $457x10^3$/L. Gastric acid-fast bacilli (AFB) stain was negative, 2D-echocardiography was normal, CRP 24 mg/L and ESR 72mm/hr, both elevated. Urine cytomegalovirus culture was negative, as well as CMV and EBV IgM. Urine metabolic screening was also unremarkable. Chest CT scan revealed multiple pulmonary nodules, enlarged mediastinal and hilar lymph nodes. He was started on triple anti-TB regimen. Primary immunodeficiency panel work-up using flow cytometry, immunoglobulin panel, and complement assay were all within normal limits. Nitroblue Tetrazolium Reduction Test was 89% (normal range >80%). He was discharged after 10 weeks. Triple anti-TB treatment was continued at home. A repeat chest CT scan after 1 month of anti-TB treatment showed a decrease in the size of pulmonary nodules, paratracheal, prevascular, subcarinal, subaortic and hilar lymph nodes. Whole abdominal CT scan only showed a prominent left hepatic lobe. The patient continued to have several admissions every month due to recurrent pneumonia. He underwent open lung biopsy, and the findings were consistent with tuberculosis and with noted Periodic Acid Schiff (PAS) test positive for hyphal elements within the cells. He was also a treated as a case of fungal pneumonia. Since his discharge up to his 6th year of age, he had multiple monthly admissions and consultations due to recurrent pneumonia, *Salmonella* sepsis, Herpetic gingivostomatitis, Amoebiasis, Ascariasis, Cervical Lymphadenitis, Infectious diarrhea and oral thrush.

He was also noted to have poor weight gain with a Z-score of <-3. Repeat NBT Reduction Test was still normal at 84%. A DNA analysis was sent to the St. Giles Laboratory of Human Genetics of Infectious Diseases at the Rockefeller University in New York USA, which revealed a previously unreported homozygous mutation in *CYBB,* p.S418Y, possibly related to CGD. Dihydrorhodamine (DHR) test showed poor neutrophil oxidation consistent with X-linked CGD diagnosis. At 7 years of age, there was still intermittent recurrence of fever and diarrhea. He had medical consultations in Singapore where further work-ups were done. T-spot TB was non-reactive and Galactomannan Antigen test was negative. Calprotectin showed elevated results (>300ug/g), which is indicative of an active organic disease with inflammation in the gastrointestinal tract. He was maintained on cotrimoxazole and itraconazole prophylaxis. Since then, he had less sick consults and admissions. Although for the past year, due to the irregular intake of prophylaxis, he was admitted for brain abscess. Cranial CT scan showed multi-loculated brain abscesses with the largest lesion measuring 4.8 x 3.8cm associated with vasogenic edema and subfalcine and uncal herniation. Empiric broad-spectrum antibiotics were started and he underwent emergency right frontal craniectomy and evacuation of the abscess. TB culture, aerobic and anaerobic culture of the abscess were negative. The fungal culture noted filamentous fungus although the final result was negative. Serum galactomannan was 0.59. Voriconazole was added on a suspicion of *Aspergillosis* Chest radiograph showed pulmonary tuberculosis on the right upper lobe. The quadruple anti-TB regimen was also started. Tracheal aspirate culture grew methicillin-resistant *Staphylococcus aureus*. Blood and urine cultures were negative. The patient also developed a catheter-related

infection which grew *Pseudomonas aeruginosa*. The patient eventually improved without deficits. Anti-TB treatment and voriconazole were completed for 6 months. He is currently doing well, on regular follow-ups with few sick consults and with regular intake of cotrimoxazole and itraconazole.

DISCUSSION

CGD is primarily a defect in innate immunity. It was first described in 1957 as a fatal granulomatous disorder. Over the last six decades, it has evolved from an immunodeficiency associated with severe infections with poor prognosis, to a disease with effective management potential and high survival rate[2].

Majority of patients with CGD manifest before the age of 5 and primarily affects males, as most are X-linked. Some may be undiagnosed until adolescence due in part to low index of suspicion. Fungal infections are the leading cause of mortality and are often indolent in their presentation[12]. Response to viral infection is normal. Bacterial infections tend to be symptomatic and are associated with fever and leukocytosis. Pneumonia is the most common presentation followed by abscesses (skin, soft tissue, and organs), suppurative lymphadenitis, osteomyelitis and bacteremia/fungemia. Most of these bacterial infections were seen in this patient. The pathogens responsible for the majority of infections are catalase producing bacteria and various fungi especially *Aspergillus species.* In view of response to voriconazole, the brain abscesses seen were most likely caused by this agent. Non-infectious manifestations also affect patients with CGD. They are prone to granulomata of various organs especially gastrointestinal and genitourinary. Other tissues and organs such as the retina, liver, lungs, and bone may also be affected but reasons are unknown. Oral and non-infectious skin manifestations are usually seen in CGD patients and female carriers of *CYBB* mutation. Our patient frequently has aphthous ulcers, diarrhea, and pulmonary infections Growth delay is common as seen in this patient, and failure to thrive can be a presenting symptom as well as compounded by colitis. Growth may improve in late adolescence and may attain normal predicted weight and height[13].

Diagnosis relies on the direct measurement of superoxide production (NBT)[2] and the use of flow cytometry to measure the production of hydrogen peroxide in the presence of peroxidase. The latter is preferable because of its ability to distinguish the X-linked from the autosomal recessive form of CGD, in general. This patient had NBT test done twice with normal results. Confirmatory tests were done in New York and Singapore led us to the diagnosis of an X-linked CGD with a previously unreported hemizygous mutation in *CYBB,* p.S418Y. Most patients with CGD have mutations in the *CYBB* gene that encodes $gp91^{phox}$, located at Xp21.1[14]. We can only surmise the reasons for the normal NBT test since NBT is limited by its subjectivity, need for an experienced technician, and false-negative results that cause the diagnosis of chronic granulomatous disease to be missed. False-negative findings occur when formazan accumulates in cells with low levels of active NADPH oxidase. These patients clinically have the disease, but their NBT test results are negative[17]. Hence, it is important to pursue further diagnostic tests if the initial test results do not correlate with our patient's condition.

The only routine immunization that CGD patients should not have is BCG as it has been associated with disseminated BCG infection[24]. This patient has received BCG vaccine at birth since it is a WHO-recommended vaccination to all newborns. In retrospect, this patient most likely developed disseminated BCG infection after being vaccinated. As early as 3 weeks old, he already presented with fever, hepatosplenomegaly with multiple

pulmonary nodules, mediastinal and hilar lymph nodes in chest CT scan and lung biopsy results consistent with mycobacterial infection.

Disseminated BCG is extremely difficult to treat and the chance of complete eradication is low unless the functional immune response is restored by hematopoietic stem cell transplant[15]. Multiple BCG reactivations can occur in patients with CGD and BCG can remain latent until reactivations take place in adulthood and manifest as disease[24]. Hence it is important for practitioners to be aware of BCG-related complications, which may be the first sign of an underlying immunodeficiency.

Effective management relies on early diagnosis and aggressive management of infectious complications and lifelong antibacterial and antifungal prophylaxis. Prophylactic trimethoprim/sulfamethoxazole at a dose of 5mg/kg/day divided twice daily reduces the frequency of major infections from one episode every year to one every 3.5 years[9]. Prophylactic itraconazole at a dose of 100mg daily for< 13 years or <50kg and 200mg daily for >13 years or >50kg is effective for reducing the frequency of fungal infections[10]. In a large international multicenter randomized placebo-controlled trial, IFN-y was effective at reducing the number and severity of infections by 70% regardless of inheritance pattern, age or use of prophylactic antibiotics at a dose of 50mcg/m^2 subcutaneously three times per week[11]. Allogeneic hematopoietic stem cell transplant (HSCT) is the only known cure for CGD, but majority survive without it, albeit with co-morbidities[2]. Survival has improved greatly over the last decade and is eminently survivable into adulthood. The survival rate is better for the AR form compared with X-linked CGD[3,4,8,25]. However, $p67^{phox}$ and $p22^{phox}$ AR CGD have similar clinical severity than X-linked CGD[26,27]. Hence, the importance of taking daily preventative medication and monitoring should be emphasized to patients and families.

In summary, this is a case of CGD with a nine-year-old male, who presented with recurrent bacterial and fungal infections that started as early as 3 weeks old. Prolonged, recurrent fever is the most common manifestation noted. Complete laboratory work-ups were done which later confirmed a previously unreported hemizygous mutation in *CYBB,* p.S418Y of an X-linked CGD. He is maintained on antibacterial and antifungal prophylaxis with close follow-up.

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