



Case Report

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Libyan Boy with Autosomal Recessive Trait (P22-phox Defect) of Chronic Granulomatous Disease

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ABSTRACT

Chronic granulomatous disease (CGD) is a primary immune deficiency disorder of the phagocytes. In this disorder, phagocytic cells (polymorphonuclear leukocytes and monocytes) cannot produce active oxygen metabolites and, therefore, cannot destroy the ingested intracellular bacteria. Clinically, patients with CGD usually have recurrent bacterial and fungal infections caus-

ing abscess and granuloma formation in the skin, lymph nodes and visceral organs.

In this report, we present a boy from Libya with a rare autosomal recessive trait of CGD (defect of p22-phox) who has chronic lung disease following multiple severe pneumonia attacks. The case we present suffered from bloody diarrhea since the third month of

his life. He also had recurrent episodes of fever, and later, developed persistent cervical lymphadenitis and failure to gain weight. CGD is a very rare condition worldwide. It is also not recognized here in Libya, and usually not in the list of differential diagnosis for chronic pulmonary infections. We advise that pediatricians and general practitioners who treat chronic cases of lung diseases (with or without chronic diarrhea) should consider primary immunodeficiency disorders in the hope that early diagnosis and treatment may prevent chronic complications especially of the respiratory tract. Furthermore, we state that, to the best of our knowledge, this is the first documented case of CGD from Libya.

INTRODUCTION

Chronic granulomatous disease is a hereditarily determined illness, characterized by inability of the body's phagocytic cells to destroy certain microorganisms. As a consequence, patients with CGD have an increased susceptibility to infections caused by certain bacteria and fungi (frequently *Staphylococcus aureus*, and *Aspergillus* species, respectively) [1]. This insufficiency in microbial

killing is usually caused by a defect in the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme complex. NADPH is the enzyme that generates the microbicidal respiratory burst [2, 3]. The deficiencies of oxidative metabolism are usually detected with either the nitroblue tetrazolium (NBT) dye test or the dihydrorhodamine (DHR) oxidation test. These tests are based on the ability of products of oxidative metabolism to alter the oxidation states of reporter molecules so that they can be detected microscopically (in the case of NBT) or by flow cytometry (in the case of DHR) [4]. However, NBT test, although more conventionally used, is sometimes not sufficiently sensitive. Therefore, many investigators have preferred the use of the DHR test [5].

In CGD, phagocytes manage to ingest bacteria normally; however they can not destroy them. Patients usually have an elevated incidence of mucosal inflammatory disorders such as colitis, enteritis, and gastric outlet obstruction. A cutaneous disease occurs in 60-70% of patients. CGD is an extremely rare disease that occurs in about 1 in every quarter of a million live births [6].

CASE REPORT

A Libyan boy first presented to the pediatrics department of Tripoli Medical Centre (Libya) when he was 3 months old suffering from loose stool with streaks of blood. He also had frequent episodes of fever at first presentation to the hospital; later, he developed persistent cervical lymphadenitis and failed to gain weight. The boy is the 3rd child (of 4 children) from related Libyan parents. He was born in the 35th week of gestation with a birth weight of 2400 grams. He was vaccinated with BCG on the second day of his life. The first child of the family (a girl) had a similar medical history, with persisting pneumonia, suspected tuberculosis, and anemia. She died in Libya in 2004. The patient's older brother is normal. However, his younger sister was found to be a carrier of the disease; but otherwise healthy and has no significant susceptibility to infections up to the present date.

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The patient presented to the University Children's Hospital of Bonn (Germany) at the age of 10 months; and because of a positive tuberculin skin test and previous history of BCG vaccination, an infection with BCG was suspected. He was put on antimyco-

bacterial regimen (rifampicin and isoniazid). The patient was later transferred to Charite Children's Hospital of Berlin to continue his treatment, and further investigations were carried out. A lymph node of the left axilla was surgically removed, and a culture from the biopsy grew bacteria. Additionally, cytomegalovirus (CMV) was detected in the urine without apparent disease. These findings have led to a suspicion of a primary immune deficiency disorder.

At the age of 12 months, he again showed recurrent episodes of pneumonia, diarrhea and enlargement of lymph nodes. He was treated with antibiotics several times, with good results. A few months later, following an immunological profile for his case, a negative NBT and DHR assay was detected. The final diagnosis of chronic granulomatous disease was confirmed by molecular analysis, which revealed a defect in the p22-phox component of the CYBA gene.

The results of his investigations were as follows: Stool analysis revealed the presence of *Clostridium difficile*; however, no parasites were found. CMV DNA was present in urine. But the aspergil-

lus antigen was not detected in his blood. The excised cervical lymph nodes (LN) yielded the growth of *Enterococcus faecalis* and *Klebsiella pneumoniae* on culture. There was no growth of mycobacteria on culture of fluids from the bronchial lavage performed. Gastroduodenoscopy and colonoscopy showed redness and swelling of the mucous membrane of the stomach and the colon, respectively. Furthermore, signs of colitis and tendency for superficial bleeding were evident.

Immunoglobulin levels against tetanus, diphtheria and pertussis (DPT) were normal. Serum antibody levels were as follows: IgG 1697 mg/dl, IgA 161 mg/dl, IgM 100 mg/dl. Indicating that humoral immunity of the patient was functioning normally. His haemoglobin level was moderately low (9.9 g/dl). Normal values were detected for sodium, potassium, calcium, chloride, creatinine, transaminases, LDH and alkaline phosphatase.

Ultrasound imaging (USI) showed enlarged cervical LN (maximum diameter 22 mm), and slightly enlarged mesenteric LN (11 mm).

Furthermore, the liver was moderately enlarged (Figure 1).

X-rays of the chest showed that the left diaphragm was lower than the right, and the left lung was smaller than the right, with central infiltrates on both sides. The heart was shown to be displaced to the left side.

A high-resolution computed tomography (CT) scan of the chest confirmed the X-ray readings and showed hypoplastic left lung with diffuse dystelectasis; however, no other specific infiltrates were found (Figure 2).



Figure 1: Ultrasound image showing a mild hepatomegaly.

Magnetic resonance imaging (MRI) of the chest confirmed the

USI findings and revealed multiple enlarged cervical LN, and one large mediastinal LN on the right side in addition to a nonspecific mass situated on the right paravertebral area at the level of chest vertebral body number 11 and 12.



Figure 2: High-resolution CT-scan showing a left hypoplastic lung.

Positron emission tomography (PET) showed focally intense signals of the upper lobe of the right lung (paravertebrally) and in the glandula submandibularis (cervical LN), and a less intense signal in the inferior lobe of the left lung (Figure 3).

PET-CT Fusion technique [7] which allows accurate correlation

of great anatomic details (from the CT scan) and informative metabolic information (from the PET scan) was used, and it confirmed the CT scan findings (Figure 4). Genetic analysis of the patient's NADPH oxidase revealed a deletion of 7 base pairs (bp) in exon 5 on both alleles of the p22-phox gene. The bp deleted were from 323 to 329 (the investigation was done in Dresden, Germany).

DISCUSSION

The exact incidence of CGD is unknown. In the USA, it is estimated that CGD affects approximately one infant per quarter of a million live births [6, 8]. Worldwide, however, the incidence of the disease varies among the populations studied, with variations from 1 case per 160 thousands individuals to 1 case per 1 million people [9]. Surprisingly, for Libya, and after a thorough literature search, we confirm that this is the first reported case of CGD.

Four genes have been connected to CGD: the CYBB gene, encoding the gp91-phox subunit; the CYBA gene, encoding for p22-

phox; the NCF1 gene, encoding p47-phox; and the NCF2 gene which encodes for p67-phox.

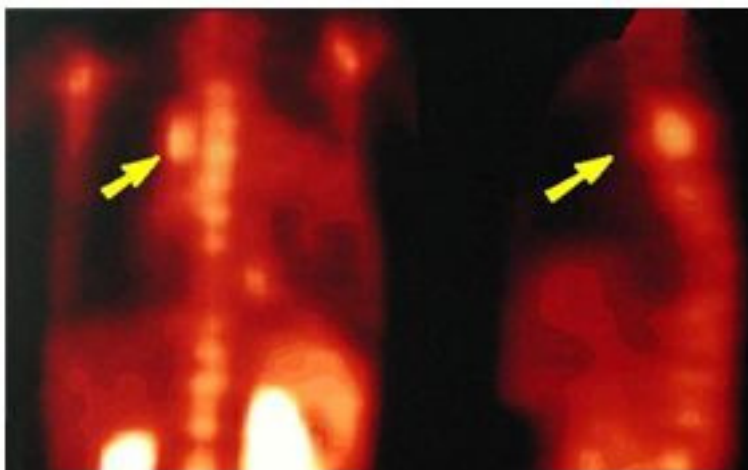


Figure 3: PET image showing intense signals on the upper lobe of the right lung (yellow arrow).

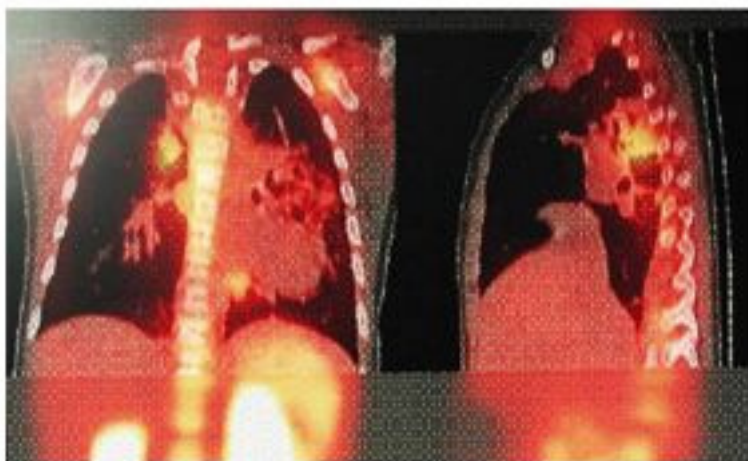


Figure 4: PET-CT Fusion scan showing the location of the focally intense signals from the upper lobe of the right lung paravertebrally, and the less intense signals from the left lung, indicating a hypoplastic left lung.

CGD is frequently inherited (60% of cases) in an X-linked recessive manner where most patients are boys, who have hemizygous mutations on the X-linked gene coding for gp91-phox. However, among the other subtypes of CGD, the autosomal recessive forms (40% of cases) may be associated with milder disease. The extent to which environmental factors and secondary genetic defects may influence the course of the disease is still not understood [10, 11].

A wide variety of molecular defects have been described in the genes for the gp91-phox component, the p22-phox component, and the p67-phox component. These defects include frame shifts; deletions; and non-sense, missense, splice-region, and regulatory region mutations [11]. The protein, p40-phox, has been involved in the regulation of the NADPH oxidase, but no individual with a mutation in this protein has been found so far. A new variant of CGD has been described; this form is caused by an inhibitory mutation in Rac2,

which regulates activity of the neutrophil respiratory burst and actin assembly. Mutations in the genes for p67-phox (NCF2) and p22-phox (CYBA) are usually the rarest, accounting for fewer than 10% of cases of CGD [12].

Morbidity due to infections remains significant, particularly in those with the X-linked type. Currently, the yearly mortality rate is a little more than 1.5% per year for persons with autosomal recessive CGD and over 5% for those with X-linked CGD [10, 11]. The long-term survival rates of patients who present with symptoms after the end of their first year of age is significantly better than that of patients whose illness starts in infancy [13]. Fortunately, since the introduction of prophylactic antibacterials and anti-fungals the prognosis for patients with CGD has greatly improved, with patients frequently living to see their 30th and 40th birthdays [12, 13].

In our case and because of the detection of *Clostridium difficile* in stool and *Enterococcus faecalis* and *Klebsiella pneumoniae* in the excised cervical lymph node the patient was put on Metronidazole and Imipenem for 3 weeks.

Following this treatment the patient's condition has improved greatly and the degree of inflammation (as monitored by the level of CRP and leukocyte count) has decreased. Although generally, the patient became better, nevertheless, fresh blood spots were still noticeable in stools. Because nonspecific colitis was assumed to be associated with his condition, he was treated with oral prednisone at an initial dose of 1 mg/kg body weight (BW). However, the two anal fissures which were found on physical examination, might explain the fresh blood streaks of the stool in some of the instances. While in Germany, the patient was vaccinated with Pneumococcus vaccine and with the Measles, Mumps and Rubella (MMR) a few months later. Therefore, with the previous vaccines he took (in Libya) his vaccination status is now considered complete.

In view of the fact that patients with CGD usually have recurrent bacterial and fungal infections, which may lead to abscess formation in the skin, lymph nodes and viscera, our patient was put on a lifelong antibiotic (Cotrimoxazole, 5mg TMP/kg BW) and antimycotic (Itraconazole 10mg/kg BW) prophylaxis plan. Nevertheless,

the patient was advised to have a regular liver function tests. The general condition of our patient is now satisfactory and is gaining weight gradually. He is now 2 years and 9 months of age. The last respiratory system examination revealed a clear chest with normally breathing lungs devoid of infections. His parents are pleased with the outcome of the treatment so far, and are constantly monitoring his condition with regular follow-up visits to the hospital.

Alternative methods of treating CGD include interferon-gamma injections [14], white blood cells (WBC) infusions, and bone marrow transfusion (BMT) [15]. Gamma-interferon, although widely used in the USA, in Europe it is usually preserved for the difficult and persistent infections. The route of administration (an injection given twice weekly) is also a drawback. Transfusion of WBC from healthy donors is sometimes used but not preferred for its obvious side effects in the form of transfusion reactions that can occur especially after repeated transfusions. Gene therapy is also an option for CGD cases, where patients' own stem cells are removed and the defective genes replaced with normal genes before reinjecting them

back to the patient [16].

By presenting this typical case of CGD, the authors advise that primary immune deficiency diseases, particularly CGD, should be considered in cases of chronic unresponsive infections, especially in children. After confirming the diagnosis, all cases of CGD should be put on a life-long aggressive antibiotic and antifungal prophylaxis treatment; and for infections, special antibiotic and antifungal therapy regimes should be started for periods up to 3 times longer than that for patients with other similar conditions. Early detection and treatment of such congenital disorders will certainly decrease the many complications that may arise in the course of the disease. Furthermore, CGD patients should have frequent and regular consultations with their health care providers, and have access to CGD resources for information in the form of booklets and websites dedicated to the disease [17].

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