Chronic granulomatous disease

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Abstract

Chronic granulomatous disease is a rare inherited disorder characterised by inability of phagocytes to generate reactive oxygen species needed for intracellular killing of phagocytosed microorganisms. We report the case of an 8-month-old male child with recurrent chest infections and perianal abscess that had no response to conventional antibiotic treatment. His two elder brothers died due to similar complaints at the ages of 4 and 5 months. Four elder sisters were healthy and alive. This history indicated that the patient might have X-linked chronic granulomatous disease. A definite absence of superoxide activity in the patient's granulocytes detected by dihydrorhodamine test and nitroblue tetrazolium dye reduction test confirmed this diagnosis.

Keywords: Chronic granulomatous disease, Perirectal abscess, Nitrotetrazolium, Dihydrorhodamine.

Introduction

Chronic granulomatous disease (CGD) is a relatively rare genetic disorder characterised by defect in respiratory burst activity of phagocytes that is associated with intracellular killing of phagocytosed microorganisms.[1] In CGD, the primary defect is associated with the key enzyme, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Without this enzyme superoxide and other microbicidal reactive species cannot be generated. NADPH oxidase is composed of 5 subunits; two are membrane-bound, gp91phox (CYBB gene) and gp22phox (CYBA gene), while three are cytosolic components, p47phox (NCF1 gene), p67phox (NCF2 gene) and p40phox (NCF4 gene).[2] CGD is most commonly inherited as X-linked, constituting 70% of the reported cases. The remaining 30% of cases are autosomal recessive (AR), associated with mutations in the other subunits.[3]

CGD patients are susceptible to infections caused by catalase positive organisms, which consume cytoplasmic hydrogen peroxide and survive intracellularly. Common infection causing pathogens are Staphylococcus aureus, Serratia marcescens, Burkholderia cepacia, and Aspergillus.

The diagnostic tool that measures neutrophil oxidative activity is dihydrorhodamine (DHR) assay by flow cytometry that can identify X-linked and AR forms of CGD and also helps in identification of X-linked carriers.[4]

Here we report the case of an 8-month-old male child with recurrent chest infections and perianal abscess that had no response to conventional antibiotic treatment.

Case Report

An 18-month-old boy was referred to Immunology Department of the Armed Forces Institute of Pathology (AFIP) in March 2013 for detailed workup of suspected immunodeficiency disorders. The case was managed from March 2013 to January 2014. The patient had had several episodes of pneumonia since 5th day of his life. At presentation, his parents reported chest infection, along with perianal abscess and oral ulcers for the preceding two weeks. He had a history of two previous hospital admissions with complaints of pneumonia when he received two courses of antibiotics, including clindamycin and cefipime. Anti-tuberculin treatment (ATT) had been initiated following positive quantiferon gold test at 2 months of age during his first hospital admission.

Two elder male siblings of the patient had died of recurrent chest infections at the ages of 4 and 5 months. Four elder female siblings were alive and healthy.

On examination, his respiratory rate was 62/min and his body temperature was 39.2°C. On physical examination of respiratory system, there was mild chest retraction and rales in both lungs. The patient was responsive and alert with no neurological deficit. Total leukocyte count (TLC) was 17,500/ul with 68% neutrophils and 26% lymphocytes. C-reactive protein (CRP) was 24mg/dl and erythrocyte sedimentation rate (ESR) was 110mm/hr. Chest X-ray revealed consolidation in both lungs. Blood cultures grew methicillin-sensitive staphlococcus aureus (MSSA). Cerebrospinal fluid (CSF) and urine cultures were sterile. The results of primary laboratory tests at the time of first and second admissions were noted (Table-1).

The immunological workup (Table-2) revealed

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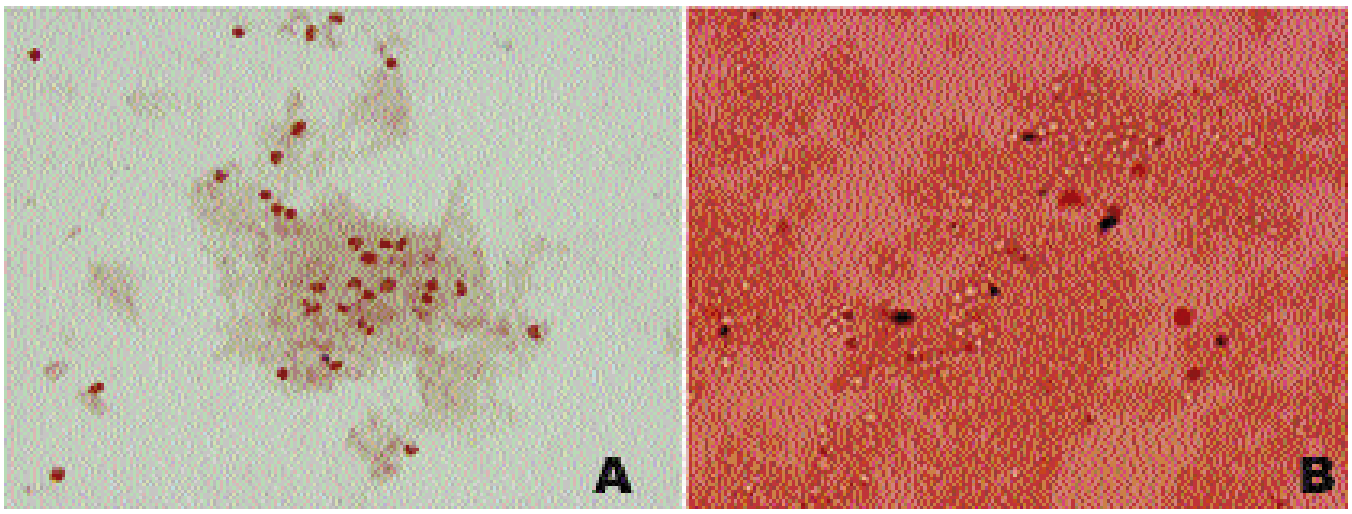

Figure-1: The NBT test in the CGD patient and normal control. A) The negative NBT test in this CGD patient. B) A normal NBT test in a control.

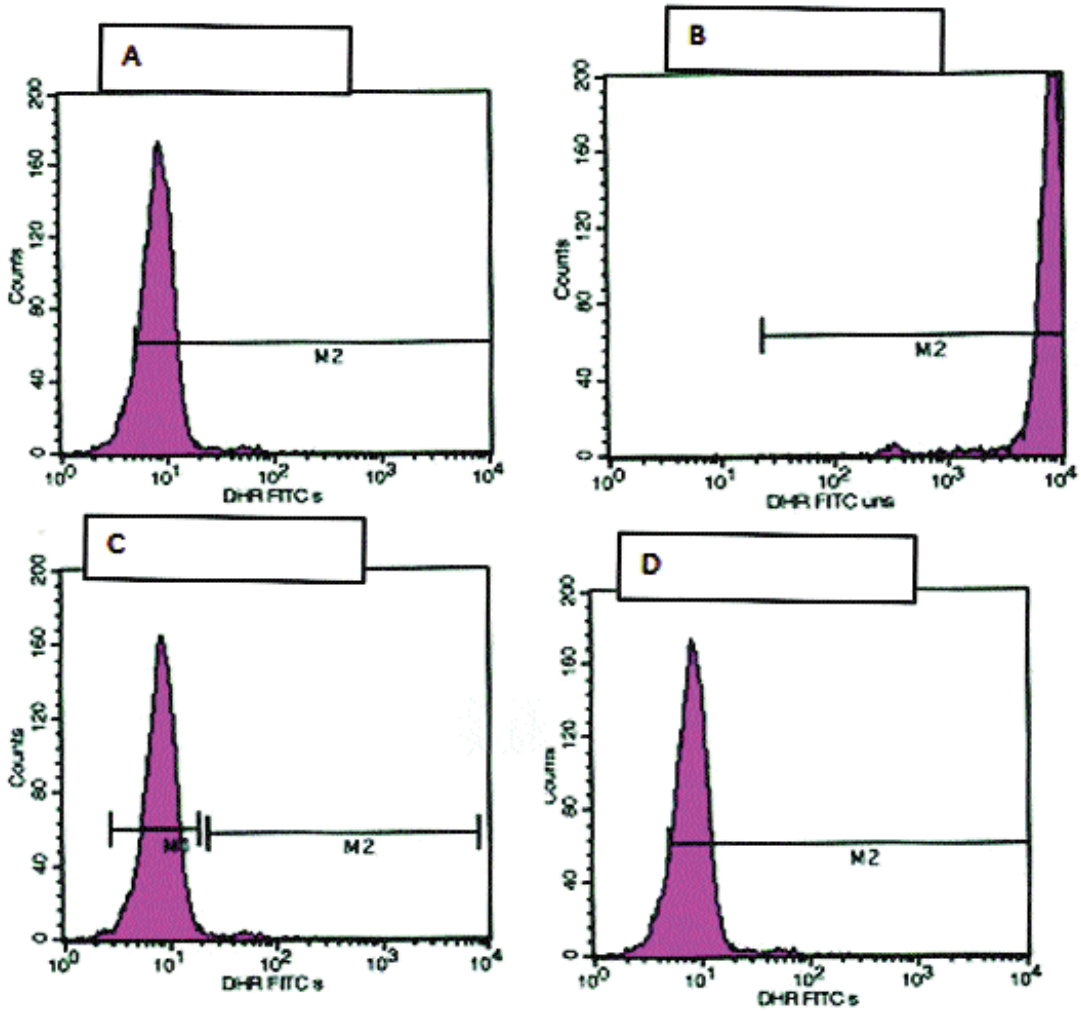

Figure-2: (A, B) The result of the neutrophil burst activity test in the 8-month-old patient with CGD. In contrast to the control. (C,D) There were defects with a right sided shift and a fluorescence peak of low intensity in this patient.

Table-1: Primary laboratory test at the time of 1st and 2nd hospital admission.

Lab tests	Results (1st admission) 24th Dec,2011	Results (2nd admission) 12th Jan,2012	Comments
TLC	21,500/ul	19,800/ul	___
DLC	Neutrophils 81%, Lymphocytes 17%	Neutrophils 78%, Lymphocytes 20%	___
Haemoglobin	11.4 g/dl	12.3 g/dl	___
Platelets	327	340	___
ESR	110 mm fall after one hour	112 mm fall after one hour	___
CRP	48 mg/l	48 mg/l	___
Blood Culture	Negative (after 7 days)	Negative (after 7 days)	___
Urine Culture	Negative	Negative	___

TLC: Total leukocyte count
DLC: Differential Leukocyte count.
ESR: Erythrocyte sedimentation rate
CRP: C-reactive protein.

Table-2: Immunological test.

Lab Tests	Results	Age specific reference range
Immunoglobulin G	15.8	3.1 - 13.8 g/l
Immunoglobulin A	1.7	0.3 -1.2 g/l
Immunoglobulin M	1.9	0.5 - 2.2 g/l
Immunoglobulin E	12 IU/ml	<120 IU/ml
Component 3	0.8 g/l	0.7 - 1.7 g/l
Component 4	0.4 g/l	0.2 - 0.5 g/l
Haemolytic complement CH50	82 CH50 units	78 CH50 units
Cluster of Differentiation (CD)19 + B lymphocytes	19%	19 % -31%
CD3 + T lymphocytes	60%	58 % - 67 %
CD4+ T lymphocytes	28%	38 % - 50 %
CD8+ T lymphocytes	33%	18 % -25%
Nitroblue tetrazolium test (NBT)	Negative	___
Dihydrorhodamince (DHR)123 ()		
Neutrophil's oxidative Index (NOI)	9	205-765

Immunoglobulin G (IgG), IgA, IgM, IgE, Haemolytic complement (CH50), Component 3 (C3), C4 levels within his age-matched reference ranges. Lymphocyte subset was carried out and his T and B cells were normal for his age. Laboratory evaluation for neutrophil's oxidative burst was performed and diagnosis of chronic granulomatous disease (CGD) was confirmed by both negative Nitroblue tetrazolium test (NBT) and lack of dihydrorhodamine (DHR) fluorescence following neutrophil stimulation against phorbol myristate acetate (PMA). After the diagnosis was communicated to the consultant paediatrician, the patient was started on parenteral clarithromycin 40mg twice daily, Ceftazidime 250mg once daily, Imipenim 100cc thrice daily along with ventolin nebulization once daily. The patient responded to the antibiotics after two weeks of proper compliance. As his clinical condition improved and his acute phase inflammatory markers returned to normal, prophylactic antibiotics trimethoprim/sulfamethoxazole suspension 5ml once daily were started. The patient was still in hospital at the time of this Report, and paediatricians were considering Interferon-y (IFN-Y) preparation for the patient.

Discussion

CGD is an immune deficiency disorder characterised by early onset of recurrent fungal and bacterial infections due to defects in superoxide generating system of neutrophils. Early diagnosis requires high index of suspicion along with aggressive and prompt treatment of infections. In this case, perianal abscess and recurrent pneumonia during infanthood points towards a primary immune deficiency. In CGD, the clinical presentation varies from patient to patient. As shown in this case, lungs are infected most commonly, manifesting as tuberculosis or pneumonia. Other common infections are lymphadenitis, osteomyelitis, liver or subcutaneous abscess. Several CGD patients with Bacille-Calmette-Guerin (BCG) disease and tuberculosis (TB) have been described in regions endemic for TB.[5] Assuming that prevalence of CGD in Pakistan is similar to international prevalence, 4-5 new patients of CGD should be identified in the country per year, but only 1-2 new patients are diagnosed at each centre with CGD, suggesting that many children with CGD may not be correctly diagnosed.

NBT is the conventional laboratory tool to screen for CGD but it has been replaced in many laboratories by DHR assay, a flow cytometer equivalent.

Laboratory diagnosis of CGD can be achieved by performing flow cytometric analysis to evaluate NADPH oxidase activity (oxidative burst) using a fluorescent marker of superoxide generation. DHR is highly sensitive, reproducible and rapid assay that quantitates oxidative

capacity of neutrophils using fluorescent probe DHR 123.

The histogram of stimulated, DHR-loaded granulocytes from a patient with X-linked disease forms a unimodal narrow peak, but typically has little fluorescence increase over that of the unstimulated control cells. In contrast, comparative data from AR patients show a moderate fluorescence increase after stimulation, with a characteristically wide peak.[6] In our patient, death of two male siblings with repeated infections, absent nitroblue dye reduction by neutrophils of the patient along with the characteristic narrow unimodal peak of stimulated neutrophils projecting same fluorescent intensity as that of unstimulated peakI was highly suggestive of his X-linked (XL) mode of inheritance.

XL-CGD carriers can be detected using DHR assay, revealing a mosaic pattern. However, it should be kept in mind that one-third of the X-linked cases of CGD are sporadic with mothers showing normal DHR pattern. Therefore, genetic testing remains the most reliable way of carrier identification and to establish genotype.

Prophylactic trimethoprim-sulphamethoxazole reduces the frequency of bacterial infections with CGD.[7] Most abscesses require surgical drainage for therapeutic as well as diagnostic purposes. Effective antibiotic prophylaxis, aggressive surgical and medical intervention and vigilant surveillance for signs of infection has improved prognosis.

Subcutaneous administration of IFN-γ reduces the frequency of infections significantly by stimulating super oxide production but the cost remains prohibitive in resource-limited settings. Stem cell transplantation is curative in CGD. However, in patients with no human leukocyte antigen (HLA)-identical family donor, gene therapy is an effective therapeutic alternative that provides CGD patients with genetically modified autologous haematopoietic stem cells.[8]

Conclusion

Improved recognition of CGD and early diagnosis is the cornerstone in reducing morbidity and mortality so that prophylaxis may be initiated early and infections may be treated promptly. Availability of DHR test makes possible early diagnosis and identification of X-linked and AR forms of the disease. In addition, the test may be utilised as a screening test to identify female carriers of X-linked disease, keeping in mind that definite identification rests on detection of mutations in the target genes.

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