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An Iranian Patient Suffering from Chronic Granulomatous Disease, with Mutation in the NCF1 Gene

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Abstract

CGD is an innate immunodeficiency characterized by an increased susceptibility to recurrent infections and granulomatous inflammation. CGD results from the loss of phagocyte superoxide production caused by a failure of the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme. It is caused by recessive mutations in any of four genes that encode subunits of the NADPH oxidase.

The most common autosomal recessive form of CGD is p47phox encoded by the NCF1 gene which is clinically milder. In this case study, we report a boy with lung abscess and recurrent oral thrush presentations. Whole exome sequencing (WES) test was performed to identify the underlying genetic mutation in this patient. WES of the patient reported a homozygous deletion mutation in the NCF1 gene (NM_608512: exon2: c.75_76delGT). Our data shows that early detection of NCF1 mutation has a wide heterogeneity in clinical manifestations of the patients.

Keywords: chronic granulomatous disease, NCF1 gene, nicotinamide adenine dinucleotide phosphate oxidase enzyme, p47Phox.

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Introduction

Chronic granulomatous disease (CGD) is an uncommon primary immunodeficiency disorder with reported prevalence of approximately 1 in 200000 to 1 in 250000 (1). Patients with CGD have an increased susceptibility to recurrent severe bacterial and/or fungal infections and excessive hyperinflammatory responses (2-6).

The underlying mechanism of CGD is characterized by the imbalance between generation and removal of reactive oxygen species (ROS) in phagocytes. The inability of phagocytes to produce sufficient amount of ROS prevents them from eliminating bacterial and fungal pathogens. Conversion of molecular oxygen into potent ROS in phagocytes depends on an enzyme called nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase). NADPH oxidase consists of spatially separated six structural components, namely, $gp91^{phox,}$ $p22^{phox}$, $p47^{phox}$, $p40^{phox}$ and GTPase Rac that associate in a stimulus-dependent manner to form active enzymes. Five genes encoded each component of this enzyme complex: $gp91^{phox}$ by the *CYBB* gene (cytochrome b-245 beta subunit) located on chromosome X, $p22^{phox}$ by the *CYBA* gene (cytochrome b-245 alpha subunit) located on chromosome 16, $p47^{phox}$ and $p40^{phox}$ by the *NCF1* (neutrophil cytosolic factor 1) and *NCF4* (neutrophil cytosolic factor 4) genes located on chromosomes 1 and 22, respectively. Patients with CGD have a mutation in one of the five genes, leading to inappropriate activation of NADPH oxidase, and defective ROS production in their phagocytes (7, 8).

Catalytic core of the enzyme is composed of two subunits gp91phox and p22phox which are integral membrane proteins. Downstream signaling composed of p47phox phosphorylation, followed by an entire assembly translocation to the membrane in order to form an active enzyme complex with b588 (9).

In western countries, disease-causing mutation in approximately 65% to 70% of CGD patients is seen in the *CYBB* gene encoding gp91phox with an X-linked pattern of inheritance and severe clinical phenotype. Defect in other components of NADPH oxidase causes the autosomal recessive form of CGD in almost 30% of patients with variable clinical manifestations, from very mild to severe clinical phenotype. The most common responsible mutation for autosomal recessive (AR) form of CGD occurs in the *NCF1* gene encoding p47phox. Defects in the gene encoding p22phox, p67phox and p40phox are extremely rare (10-13).

Unlike western world, autosomal recessive inheritance of CGD is the most common form of the disease in eastern countries due to the high rate of consanguinity (13-15). The location of the *NCF1* gene, encoding p47-phox, is on chromosome 7q11.23 with about 15000 base pairs length and is composed of 11 exons with a size of 390 amino acids and a molecular mass of 47 kDa (16). The most common mutation in the *NCF1* gene is a 2-bp GT deletion at the beginning of exon 2, accounting for more than 95% cases of p47phox defects (17).

CGD is characterized by various clinical manifestations depending on the disease-causing mutation. Nonetheless, patients with X-linked form of CGD show more severe phenotype compared to patients with AR inheritance. Type of mutation in AR patients could also have potential effect on the severity of disease (13).

Here we report the clinical and immunological features of a 4-year old boy who presented with recurrent abscess and oral thrush due to a mutation in the NCF1 gene.

Case presentation

The patient is a 4-year old boy born from consanguineous marriage. He was doing well until 3 months of age when he developed liver abscess. He presented with fever at the age 20 months and the following work-up revealed lung abscess. His medical history was also significant for recurrent oral thrush since he was 2 years old. The family history revealed an abortion with unknown etiology. Otherwise, there was no history of recurrent infection, death with unknown cause or recurrent infection in his family. Given the

history of recurrent abscess, primary immunodeficiency was suspected and the patient was referred to our clinic for further clinical and laboratory investigations. Immunological work-up revealed high levels of immunoglobulin (Ig) G and IgE, whereas the level of IgA and IgM were normal. The specific antibody responses to tetanus and diphtheria vaccines were normal. His total leukocyte count was 7250/µL with 38.3% lymphocytes and 43.3% neutrophils. The diagnosis of CGD was confirmed by both abnormal neutrophil nitroblue tetrazolium (NBT) slide test and Dihydrorhodamine (DHR) Flow Cytometry Test **(Table 1).**

Table1. Laboratory workup of the CGD patient

Parameters	Patient's Value	Normal Ranges
WBC	7.2	4-10 ($\times 10^9$/L)
Lymph	2.7	2.3-5.4 ($\times 10^9$/L)
PMN	3.1	1.5-8.5 ($\times 10^9$/L)
PLT	329	150-450 ($\times 10^9$/L)
Hb	11.4↓	12-16 (mg/dL)
IgG	2158↑	650-1410 (mg/dL)
IgG1	3009↓	3060-9450 (mg/dL)
IgM	118	55-210 (mg/dL)
IgA	373	83-255 (mg/dL)
IgE	> 2500↑	up to 15 (IU/ml)
CD3	58	39-73 (% of lymphocytes)
CD4	38	25-50 (% of lymphocytes)
CD8	15	11-32 (% of lymphocytes)
CD19	27	17-41 (% of lymphocytes)
CD20	27	17-40 (% of lymphocytes)
C3	243	890-1870 (mg/dL)
C4	46	160-380 (mg/dL)
Anti T	0.32	> 0.1 immunization protection present (IU/ml)
Anti D	0.6	> 0.1 immunization protection present (IU/ml)
NBT	0*	90-95%
DHR+PMA	8.7*	(NOI= Neutrophil Oxidative Index) >100

*; posetive, ↓ or ↑; denote results below or above normal range for age respectively; WBC, white blood cell; Lymph, lymphocytes; PMN, polymorphonuclear leukocyte; PLT, platelet; Hb, hemoglobin, CD, cluster of differentiation; Ig, immunoglobulin; Anti T,Anti-tetanus; Anti D, Anti Diphtheria; NBT, nitroblue tetrazolium; DHR, Dihydrorhodamine; PMA, phorbol myristate acetate; µl, microliter; mg, milligram; dl, deciliter

A genetic study for the mutation of NCF1 gene encoding the p47phox, the most common gene mutation in autosomal recessive CGD, was done and the mutation was found. This mutation was known as delta GT that deletes two DNA building blocks from the NCF1 gene in an area called exon 2 (c.75_76delGT). It is caused by gene conversion with one of two NCF1 pseudogenes, and leads to complete deletion of the NCF1 gene. This mutation has been confirmed by genescan assay and found in both parents in heterozygous form.

Discussion

In this study, we reported an Iranian patient suffering from CGD, with mutation in the *NCF1* gene. AR CGD is caused by mutations in the genes encoding the subunits of the phagocyte NADPH oxidase complex, for instance, *CYBA, NCF1*, and *NCF2.* The most frequent AR CGD form is caused by mutations in the *NCF1* gene (6). As a result, CGD phagocytes cannot proficiently produce superoxide (O2-) and fail to kill ingested pathogens. The mutation in these genes often leads to life-threatening bacterial and fungal infections, which usually takes place during childhood. Severe infections mainly occur by *Staphylococcus* and *Aspergillus* species (6, 7). Common infectious syndromes include pneumonia and lung abscesses, skin and soft tissue infections, lymphadenopathy, suppurative lymphadenitis, osteomyelitis, and hepatic abscesses as well as granulomatous inflammatory responses (15, 16). The manifestations of CGD are diverse, such as recurrent pyogenic abscess formation in regional lymph nodes, pulmonary parenchyma, and liver (12-14). In our study, liver and lung abscesses were detected in the patient. Other studies have reported various frequencies of abscesses in CGD patients. Movahedi et al. have reported 7% liver abscess in their cases, which was lower than other studies such as Winkelstein JA et al. (2, 18). Liver abscesses are rife complications of CGD (19). Lublin et al. have reported 14% hepatic abscesses in their patients

(20).

Furthermore, Winkelstein et al. had been detected 27% hepatic abscesses in their CGD registry (2). In the largest cohort of Blancas et al., their Mexican CGD patients were reported to present mouth ulcers 26%, pulmonary abscess 23%, and hepatic abscess 16% (21). Moreover, Wolach et al. performed two different studies on CGD patients in Israel, and they reported the frequencies of hepatic and lung abscesses in these patients to be 53%-39% in hepatic abscesses and 36%-41% in lung abscesses (4, 14). In this study, we described a CGD patient from consanguineous parents with mutations in the NCF1 gene and high levels of IgG and IgE, normal levels of IgA and IgM, as well as normal B-cell and T-cell subset counts. Immunoglobulin levels of CGD patients are often normal or elevated (22). Similarly, 21 Indian patients with AR-CGD and Del GT mutation in the NCF1 gene were diagnosed in a study conducted by Kulkarni1 et al., in which 62% of the patients belonged to a consanguineous parents. In these patients, immunoglobulin levels were raised in 50% of the patients with normal numbers of B- cells and T-cells. This study reported the phenotypic heterogeneity and the clinical presentations ranging from asymptomatic to severe presentation leading to an early death or required organ transplantation (15).

Patiroglu et al. present a case of CGD whose features were similar to selective IgA deficiency (SIgAD) and hyperimmunoglobulin E syndrome (HIES). Laboratory findings in this patient showed elevated IgG and normal IgM levels (23). In another study, different immunological phenotypes were reported in 20 CGD patients, who revealed normal Ig levels (24). Keleó et al. showed a five-year-old boy with CGD who demonstrated low levels of IgG, IgM and IgA, and increased IgE levels (25). Moreover, Hanoglu et al. reported a case of CGD with hypogammaglobulinemia features and the need for intravenous immunoglobulin (22). The considerable prevalence of the AR over the XLR form is caused by the high rate of consanguinity, which was also showed among CGD patients. In XLR-CGD, each new mutation has a 50% chance of resulting in a new patient, whereas in AR- CGD, both parents need to be carriers (4). Similarly, in our patient, mutation was confirmed by genescan assay and was found in both parents in the heterozygous form. Altogether, these findings show that mutation in the NCF1 gene could be involved as a genetic etiology for patients with hypergammaglobulinemia and recurrent abscesses.

Conclusions

we reported a boy with a mutation in the NCF1 gene, along with hepatic and pulmonary abscesses, no significant infections and normal lymphocyte counts. Our data showed that early detection of NCF1 mutation is truly important for timely management of CGD patients. Wide heterogeneity is observed in clinical manifestations of patients with Del GT mutation. More studies are required to understand the underlying mechanisms of this phenotypic heterogeneity.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgment

Written informed consents were obtained from the patients`s legal guardians for publication of this case report and its accompanying information.

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