An Atypical Case with Chronic Granulomatous Disease and Kabuki Syndrome

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ABSTRACT

Chronic granulomatous disease (CGD) is a rare inherited immunodeficiency that arises from defects in the NADPH oxidase complex, primarily affecting the respiratory burst in neutrophils. Kabuki syndrome (KS) is a rare genetic syndrome and often present with facial, skeletal, visceral and cardiac anomalies, immunological defects and varying degrees of mental retardation. A 20-year-old male was admitted with the complaints of the recurrent abscess. He had a history of recurrent abscesses and long-term antituberculosis treatment. Cytochemical and morphological analysis, Sanger sequencing and whole-exome sequencing were used for the diagnosis of CGD. Both AR-CGD (p67phox defect) with homozygous c.229C>T nonsense mutation in NCF2 gene and heterozygous nucleotide change c.3983G>A in the KMT2D gene causing a novel missense mutation p.Arg1328Gln resulted in Kabuki syndrome. To our knowledge, this is the first report of both CGD and Kabuki syndrome combined in a single patient. CGD is always considered for the differential diagnosis during BCGitis history and recurrent skin abscess.

INTRODUCTION

Chronic granulomatous disease (CGD) is a rare inherited immunodeficiency that arises from defects in the NADPH oxidase complex, primarily affecting the respiratory burst in neutrophils. A mutation in any one of the genes encoding the components p22phox, p47phox, p67phox and p40-phox of the leukocyte NADPH oxidase leads to autosomal recessive (AR)-CGD, whereas a hemizygous mutation in the CYBB gene encoding gp91phox may cause X-CGD (1). Kabuki Niikawa–Kuroki syndrome (KS) was initially described in Japan, as a rare disease approximately seen 1:32,000 newborns. KS has a wide range of symptoms, including characteristic dysmorphic (facial) features, mental retardation, short stature and skeletal anomalies and immunological defects (2). Type 1 Kabuki syndrome (KS1; OMIM #147920) is inherited in an autosomal dominantly accounted for 55–80% of the patients. KS1 arises from mutations in (histone-lysine N-methyl transferase 2D) KMT2D gene on chromosome 12q13 and may occur in many patients sporadically (2). Arnold Chiari type I malformation is characterized by caudal herniation of the cerebellar tonsils and this finding has been described only infrequently in association with Kabuki syndrome (3).

In this report, we describe a very rare case who was operated for the Chiari malformation type I. The case was identified both an AR-CGD with a nonsense mutation in the NCF2 gene and KS with a novel missense mutation in KMTD2 gene. All the mutations were explaining the infectious, radiological, neurological and morphological manifestations of the patient.

CASE REPORT

A 20-year old male patient was admitted to the infectious diseases department with complaints of fever and multiple abscesses on the sternal and axillary region. He was born into a consanguineous Turkish family, living in central Anatolia. He had a history of progressive axillary lymphadenopathy after receipt of BCG vaccination, recurrent skin abscess and long term antituberculosis treatment in his childhood. The patient had a history of surgery during the adolescence period when he was 12-years old to treat the progressive foot drop manifestations due to the Arnold Chiari type I malformation. It was shown to be related to herniation of the tonsils below the foramen magnum by 5 mm in MRI scan, causing significant syringomyelia and occipital bone deformity. After the insertion of a ventricle-peritoneal shunt, his neurological symptoms were completely resolved, but the neuromuscular paresis was only partially resolved. Multiple axillary and sternal abscesses, which was 5x3 cm in diameter was drained percutaneously, can be seen on physical examination. The aerop, fungal, Nocardia and mycobacterial culture of the abscess did not
yield any microorganism. Vegetation was not observed in echocardiography for further investigations. Fiber optic bronchoscopy was performed because of nodular consolidation in the upper lung lobe and lower lung lobe posterobasally in thorax tomography. However, there was no bacterial fungal and mycobacterial growth was seen in the Broncho alveolar lavage specimen. Antituberculosis treatment was initiated because of granulomatous inflammation in the axillary lymph node sampling and PPD measurement of 30 mm. He was referred to the Immunology Department for recurrent pulmonary infections and skin abscess. Although the patient had normal lymphocyte counts and subsets, the percentage of CD8 T cells was elevated and the ratio of CD4/CD8 T cells was inverted, 3/7. Alpha-beta T cell 76% and gamma-delta T cell 24%. IgG and IgA concentrations were elevated to 1850 mg/dL and 546 mg/dL, respectively. Functional study of neutrophils by the dihydrorhodamine 1,2,3 (DHR) assay showed a stimulation index (SI) 1.4 fold (normal range 60–100 fold), which was very low and diagnostic for CGD (Fig. 2). The mother did not have a specific mosaic pattern in DHR assay. Expression of flavocytochrome b558, the membrane unit of NADPH oxidase consisting of a heterodimer of gp91phox and p22phox, was checked in patient sample only by p22phox antibody clone 44.1 (Santa Cruz Biotechnology, Santa Cruz, Calif), which was negative if either p22phox or gp91phox was lost, which is normal in our patient (1). MPO expression was also in the normal range at cytometry (Fig. 2). Thus, our investigation focused on AR-CGD genes (NCF1 and NCF2). This family was living in a small town where we have previously diagnosed five CGD patients with a mutation in the NCF2 gene (1, 4). We assumed that there might be an ancestral relationship between these families.

First, a homozygous c.229C>T mutation was found by Sanger sequencing the exons and exon/intron boundaries of the NCF2 gene (OMIM, 608515), resulting in p.[Arg77X], which causes loss of p67phox protein from NADPH complex, and the mother was confirmed to be a heterozygous carrier of this mutation.

The presence of CGD may explain possible tuberculosis (TB) lymphadenitis; however, the prolonged and recurrent course upon adequate treatment of BCGitis is unusual (5). The differential diagnosis for one of the genes causing Mendelian susceptibility to mycobacterial diseases (MSMD) was considered. Thus, he was investigated in more detail by next-generation sequencing (NGS) approach. Whole-exome sequencing confirmed NCF2 c.229C>T homozygosity and demonstrated that there was not any mutated MSMD gene that can explain the BCGitis. At the same time, the presence of a second pathogenic gene variant causing KS I (OMIM; 147920, rs375635160, ENST00000301067) was detected with a heterozygous nucleotide change c.3983G>A in the KMT2D gene at chromosome 12q13.12 that caused a novel missense mutation (p.Arg1328Gln). To our knowledge, it is the first pathogenic variant regarding this missense mutation described in

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**Figure 1.** DHR-123 assay (dihidro-rhodamine with PMA stimulation) and MPO expression of neutrophil by flow cytometry
(a) A left=FS/SS; A right=MPO expression. (b) B left=plot PMA stimulated DHR; B right=histogram stimulated DHR. (c) C left=DHR control; C right=DHR histogram control. DHR SI: 1.4

**Figure 2.** Skeletal abnormality like scoliosis related to Kabuki syndrome I (left) and ventricle-peritoneal shunt for Arnold Chiari malformation I. Right; 1=Scoliosis at the thoracolumbar axis, 2=Ventricle-peritoneal shunt.
KS I. Parental transmission to offspring is rare in KS I and may occur in many patients sporadically (2).

The patient was treated with both antituberculosis treatment and itraconazole and co-trimoxazole prophylaxis for three years. Although the patient’s clinical condition has been well, he unexpectedly died due to high fever and sino-pulmonary infection at 24 years of age. No microbiological investigations or post-mortem studies were performed. Patient consent was obtained for this study.

**DISCUSSION**

The neurological and skeletal findings in Arnold-Chiari type I can be confused with many other diseases. Scoliosis with Arnold-Chiari type I may also vary but is generally considered to be rare, and the genetic etiology of this malformation cannot be established, usually (3, 6). Here, we presented a rare case of KS with scoliosis at the thoracolumbar axis and Arnold Chiari malformation I (Fig. 2). Neural axis abnormality (NAA) has been described in adolescent idiopathic scoliosis (AIS). Of 381 patients with AIS, NAA was observed in 34 (8.9%). The 32 patients had a synrix, one patient had an arachnoid cyst, and one patient had a Chiari malformation (6). Symptomatic Chiari I malformation is a very rare manifestation in KS I, which was detected in our case (3).

Our patient has recurrent skin abscess and a long term antituberculosis treatment in his childhood. NADPH oxidase activity diminished completely, and CGD diagnose delayed to 20-years old. Although he was living in a rural area, he did not admit to hospital with severe fungal infections or pyogenic bacterial infections at childhood based on the patient’s statements. The co-existence of mycobacterial disease and CGD, resulting in BCGitis, is also known and incidence depends on the exposure and as well as populations investigated (1, 5). BCG vaccination and mycobacterial diseases induce IFN-γ secretion and specific CD8+ T-cell responses, as Th1 immune responses and the ratio of Th1 and Th2 cells are reciprocally regulated (7, 8). The patient had elevated CD8 T cell percentage (70%); it might be due to persistent BCGitis. Repeated stimulation of the immune system may change the ratio of T cells subset and their capacity for the secretion of cytokines like IFN-gamma by altering the Th1-macrophage pathway (7). Hence, the patient might be protected from life-threatening bacterial and fungal infection in the childhood period due to endogenous IFN-gamma induced immunity (9). Bustamante et al. (5) suggested that BCG vaccination may be life-threatening in infancy and childhood, but in adolescence and adulthood, the immune system is mature and more effective to counter mycobacterial infection than in infancy.

BCG vaccination is routinely performed in infants in Turkey. A small group of children may develop BCGitis or BCGosis, especially immunodeficient children. Thus, BCG is contraindicated in CGD patient. The intracellular microbial activity in the phagosome was defective in CGD due to the lack of reactive oxygen species (ROS) production by NADPH oxidase, which is needed for the acidity of the phagosome. It is important to note that phagosome acidification is highly regulated. The optimal pH of the phagosome was between 5.0–5.5 for protease enzyme activity inside the phagosome (10). This raises the following issue: might be a long-time ongoing stimulation of the host with a persistent BCG strain alert phagocyte into killing pathways other than the NADPH oxidase? It should be clarified by further studies with more cases or by an experimental approach focused on the macrophage. We assume that the upregulation of macrophage pathways may only postpone microbial inoculation stage and the role is limited when infection becomes serious in CGD patient.

Another question from this case is as follows: Did molecular upregulation due to KS play a role in the late presentation of CGD? KS is associated with a modest immunodeficient in antibody formation, which may culminate in a common variable immunodeficiency like presentation (9, 11). Many KS patients show increased susceptibility to infections and have reduced immunoglobulin serum levels due to the defect in the B-cell pathway (11), while some patients may suffer from autoimmune manifestations, such as autoimmune thyroiditis, idiopathic thrombocytopenia or hemolytic anemia (9, 11). This was not the case in our patient. Instead, he might have benefitted from the concomitant CGD regarding the elevated Ig production. Additionally, KS causes reduced rates of somatic hypermutation of IgG and deficiency of IgA in nearly all patients due to the dysregulation in the B-cell terminal differentiation pathway (11). Our patient had increased IgA and IgG level and this was concordant with CGD. This might be a sign that immunoglobulin production is reciprocally affected in KS and CGD.

To our knowledge, this is the first report of a case with both CGD and Kabuki syndrome. The presence of both deficiencies, which has the possibility of bringing some advantages like the late presentation of CGD, but it was not sufficient to avoid severe sino-pulmonary infections detected just before the patient passed away. If underlying mechanisms are enlightened, the NADPH system and its regulatory pathways could be better investigated. CGD is always considered for the differential diagnosis during BCGitis history and recurrent skin abscesses.

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