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ORIGINAL PAPER

Purine nucleoside phosphorylase deficiency with fatal course in two sisters

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Abstract Purine nucleoside phosphorylase (PNP) deficiency is a rare combined immunodeficiency disorder presenting with clinically recurrent infections, failure to thrive, various neurological disorders, malignancies, and autoimmune diseases. Here, we report two sisters with a fatal course of PNP deficiency due to delay in diagnosis. The first patient developed a liver abscess by *Aspergillus fumigatus* and the second patient developed *Mycobacterium tuberculosis* complex lymphadenitis and probable pulmonary tuberculosis due to disseminated BCG infection. The patients also suffered from sclerosing cholangitis. Mutation analysis of the *PNP* gene from both sisters revealed a homozygous mutation for a G>A at nucleotide 349 (349 G>A transition), which changes alanine 117 to theronine in exon 4 (A117T). An increased awareness of early signs, symptoms, and abnormal laboratory findings of PNP deficiency will establish the early prognosis and treatment.

Keywords Purine nucleoside phosphorylase deficiency · *Aspergillus fumigatus* · Tuberculosis · Sclerosing cholangitis

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Introduction

Purine nucleoside phosphorylase (PNP) deficiency is a rare combined immunodeficiency (CID) disorder with autosomal recessive inheritance. Patients with a PNP deficiency typically have profound T cell deficiency with variable B cell functions [6, 12]. Clinically, PNP-deficient patients suffer from recurrent bacterial, viral, and fungal infections, failure to thrive, various neurological disorders, malignancies, and autoimmune diseases [17]. The only curative treatment is hematopoietic stem cell transplantation (HSCT) [4].

PNP is an enzyme in the purine salvage pathway that reversibly converts inosine to hypoxanthine and guanosine to guanine. PNP is highly expressed in lymphoid tissues. Abnormal PNP activity results in deoxyguanosine triphosphate accumulation in the mitochondria which, inhibits mitochondrial DNA repair. This leads to increased sensitivity of T lymphocytes to DNA damage and apoptosis during the selection in thymus [8].

Here, we present a 7-year-old girl and her 3-year-old sister with PNP deficiency both having liver abscess due to *Aspergillus fumigatus* and axillary *Mycobacterium tuberculosis* complex lymphadenitis and probable pulmonary tuberculosis due to disseminated BCG infection, respectively. Both patients also suffered from sclerosing cholangitis.

Patient reports

Patient 1

A 7-year-old girl referred with recurrent episodes of upper and lower respiratory tract infections since the first year of her life. She was the first child of consanguineous parents.

From the first year of life, she has shown recurrent respiratory tract infections. The patient had received all regular vaccinations including live vaccination against BCG and polio without complications. Her weight was 14.5 kg (third percentile), height was 100 cm (third percentile), and head circumstance was 47.5 cm (third percentile). She had perioral granulomatous skin lesions and crusted wounds. She had no palpable lymph nodes but her tonsils were present. Auscultation of the lungs revealed diffuse crackles. Musculoskeletal evaluation showed genu valgus, talipes valgus deformities, and drop foot. Neuromuscular examination showed lower extremity spasticity, ataxia, and delayed psychomotor development.

Laboratory investigations showed anemia, lymphopenia, and neutropenia. Immunological examination revealed a CID. Serum uric acid level was low. Liver function tests were increased (Tables 1 and 2). Antinuclear (ANA), anti-double-stranded DNA (anti-dsDNA), and anti-smooth muscle antibodies were negative. HIV antibody was undetectable. Stool specimens for ova and parasite examination and antigens for *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum* were negative. Dilated bile ducts in abdominal ultrasonography and elevated GGT and ALP levels suggested possible sclerosing cholangitis. Chest computed-tomography (CT) scan showed an evidence of bronchiectasis. PNP enzymatic activity was very low in hemolysate (Table 2). Consequently, the final diagnosis of PNP deficiency is achieved. Subsequently, DNA sequencing of the *PNP* gene revealed homozygous for a G>A mutation at nucleotide 349 (349G>A transition), which changes alanine 117 to theronine in exon 4 (A117T).

Despite monthly intravenous immunoglobulin (IVIG; 400 mg/kg) treatment, *Pneumocystis carinii* prophylaxis and ursodeoxycholic acid (20 mg/kg/day), she experienced recurrent pneumonia and cholangitis attacks. HSCT could not be performed as there was no HLA-matched donor. Seven months later, she was admitted with a severe pneumonia and cholangitis attack. Chest CT scan showed a circular area within a dense consolidation region in the right lower lobe and a cavitary lesion in the left lower lobe of lungs. Abdominal CT showed a hypodense solitary lesion in the liver. According to the clinical presentation accompanied by the presence of neutropenia and radiological findings, broad-spectrum antibiotics, acyclovir (1,500 mg/m^2/day), G-CSF (5 μg/kg/day), and liposomal amphotericin B (5 mg/kg/day) therapies were started. The number of neutrophils did not increase despite the G-CSF treatment. Liver biopsy revealed necrotic material but *A. fumigatus* was identified. The tuberculin skin test was negative. Family screening was also negative. Gastric aspirates and liver biopsy materials were negative for acid-fast bacilli, as well as culture and PCR for the *M. tuberculosis* complex. As her little sister suffered from mycobacterial infection, she also received an anti-mycobacterial chemotherapy regimen composed of four agents [isoniazid (INH) 10 mg/kg/day, rifampicin (RIF) 15 mg/kg/day, streptomycin (SM) 40 mg/kg/day, and ethambutol (ETM) 15 mg/kg/day] for a possible mycobacterial infection. Anti-mycobacterial therapy could not be given on a regular base due to the intermittent increase in liver enzyme levels. Despite broad-spectrum antimicrobials including combined antifungal, antiviral therapy, IVIG infusions, G-CSF, and other intensive treatment for 5 months, she died from presumed systemic aspergillosis.

Patient 2

A 3-year-old girl was the sister of first patient. She was referred to us with similar complaints. She also diagnosed as PNP deficiency together with sclerosing cholangitis after detailed laboratory investigation and liver biopsy and put on monthly IVIG (400 mg/kg), *P. carinii* prophylaxis and ursodeoxycholic acid (20 mg/kg/day), but continued to deteriorate clinically despite these treatments.

Seven months later, the patient suffered from severe pneumonia, severe neutropenia, cholangitis attack, and a 2.5×2.5 cm diameter left axillary lymphadenitis. Chest CT scan showed a regular-shaped mass lesion in the right lower lobe and irregular consolidation area in the lingular lobe of lungs. Abdominal CT revealed distinct hypodense region around the periportal spaces. Broad-spectrum antibiotics, acyclovir (1,500 mg/m^2/day), liposomal amphotericin B (5 mg/kg/day), and G-CSF (5 μg/kg/day) therapy were started. However, no increase in the number of neutrophils was achieved following G-CSF treatment. Lymph node biopsy material was positive for acid-fast bacilli, *M. tuberculosis* complex PCR and culture. The tuberculin skin test was negative. Chest radiography and CT scan strongly suggested a disseminated mycobacterial infection. Anti-mycobacterial chemotherapy regimen comprising five agents [INH 10 mg/kg/day, RIF 15 mg/kg/day, SM 40 mg/kg/day, ETM 15 mg/kg/day, and morphazinamide 50 mg/kg/day] were started. Despite intensive treatment for 3 months, she died from disseminated mycobacterial infection.

Discussion

PNP deficiency is a rare inherited disease accounting for approximately 4% of patients with severe combined immunodeficiency (SCID) [17]. PNP deficiency is a disorder of purine metabolism. The defect causes accumulation of toxic metabolites due to the absence of PNP

Table 1 Immunological and laboratory investigations of the patients

	Patient 1		Patient 2	
		Normal range		Normal range
Hb (mg/dl)	9.2	11–16	9.3	11–16
Leukocyte count (mm^3)	2,000	5,000–14,500	6,700	6,000–17,000
Absolute neutrophil count (mm^3)	620	1,500–8,000	5,800	1,500–8,500
Absolute lymphocyte count (mm^3)	200	1,500–7,000	600	3,000–9,500
IgG (mg/dl)[a]	1,530	837–1,243[b]	756	722–1,037[b]
IgA (mg/dl)	83	66–150	37	46–91
IgM (mg/dl)	119	54–140	32	50–121
Blood group	AB Rh (−)		AB Rh (−)	
Isohemagglutinin titers	Not determined		Not determined	
HIV	–		–	

[a] Prior to IVIG

[b] See Ref. [3]

enzyme. PNP deficiency is characterized by recurrent infections, neurological disorders, malignancies, and autoimmune diseases [6, 8]. Clinical manifestation of the disease usually begins during the first year of life, but the onset may be delayed beyond infancy. The diagnosis of PNP deficiency is often suspected when lymphopenia is associated with low-serum uric acid and reduced PNP enzymatic activity in red blood cells [11].

Immunological examination revealed a CID in our patients and lymphopenia, neurological abnormalities and low-serum uric acid levels strongly suggested a PNP deficiency. Diagnosis of PNP deficiency was confirmed by the detection of abnormal PNP enzymatic activity in hemolysates. Sequence analysis showed a previously described [11] mutation within *PNP* gene.

PNP-deficient patients are associated with megaloblastic and dysplastic marrows. Our patients developed neutropenia that was unresponsive to G-CSF treatment. Neutropenia might be caused by the accumulation of deoxyguanosine and other metabolites [8]. Bone marrow morphology could not be examined as the parents did not agree to bone marrow aspiration. Sclerosing chol-

Table 2 Immunological and laboratory investigations of the patients

	Patient 1				Patient 2			
		Normal range	Absolute number (mm^3)	Normal range		Normal range	Absolute number (mm^3)	Normal range
CD3+CD16−56−, %	23	57–81[a]	46	1,000–4,900[a]	22	55–79[a]	132	1,900–3,600[a]
CD3+CD4+, %	12.2	24–47	24	500–2,700	5	26–49	30	600–2,000
CD3+CD8+, %	16.8	17–37	33	300–2,100	10	9–35	60	300–1,300
CD3−CD16+56+, %	69.1	8–28	138	200–900	73	5–28	438	200–1,200
CD19+, %	6	10–27	12	200–2,200	1.9	11–31	11	300–1,200
CD20+, %	4.1	11–25	8	200–2,000	0.6	11–29	3	300–1,100
Lymphoproliferative response to PHA, %	22	65±9.2			14	65±9.2		
Uric acid (mg/dl)	0.6	2.3–6.2			0.4	2.3–6.2		
PNP activity (nmol/h/mg)	42.4	2,382±183			2.0	2,382±183		
ADA activity (nmol/h/mg)	31.0	32.5±14			52.5	32.5±14		
AST (U/L)	334	15–55			147	15–55		
ALT (U/L)	162	5–45			114	5–45		
GGT (U/L)	287	5–32			1,041	5–32		
ALP (U/L)	1,529	145–420			5,136	145–420		

PHA phytohemagglutinin, *PNP* purine nucleoside phosphorylase, *ADA* adenosine deaminase, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma-glutamyltransferase, *ALP* alkaline phosphatase

[a] See Ref. [14]

angitis (SC) represents a spectrum of chronic, variably progressive cholestatic diseases of the intrahepatic and/or extrahepatic biliary system characterized by patchy inflammation, fibrosis, and stricturing [1]. SC can be due to primary or secondary abnormalities biliary system. Primary sclerosing cholangitis (PSC) is considered to be an immune-mediated cholestatic liver disease of multifactorial and polygenic etiology [16]. Secondary sclerosing cholangitis (SSC) is a disease that is morphologically similar to PSC but that originates from a known pathological process [1]. Most common causes of SSC include infectious, toxic, or ischemic injuries of the biliary tree [9]. Sclerosing cholangitis might be caused by the accumulation of toxic purine metabolites or infectious agents in our cases. Sclerosing cholangitis has not been previously reported among PNP-deficient patients.

Neurological abnormalities develop in more than one-half of PNP-deficient children [13]. Our patients showed marked neurological manifestations. The pathogenesis of neurological dysfunction in PNP deficiency is poorly understood.

Aspergillus species infections are rare in combined immunodeficiencies and are responsible for significant morbidity and potentially fatal outcome [2]. To date, only two infants with X-linked SCID were reported as having pulmonary aspergillosis [15, 19]. Invasive *Aspergillus* infection is facilitated by T cell immunodeficiency with neutropenia in our first patient. To our knowledge, this case is the first reported invasive *Aspergillus* infection in a patient with PNP deficiency.

Protective anti-mycobacterial immune response involves mainly T lymphocytes activating the macrophages and their microbicidal functions through the release of interferon γ [10]. Lack of T cells obviously accounts for mycobacterial disease in CID patients. BCG is administered to all children in Turkey to prevent severe tuberculosis. The literature includes >200 cases of disseminated BCG infection in patients with primary immunodeficiency [5]. Infection with *M. tuberculosis* is rare in SCID patients, reported so far in only one patient [7]. This is probably due to the fact that SCID is usually fatal before the age of 1 year [18]. In the second patient, the identification of *M. tuberculosis* complex through PCR and culture, together with compatible clinical findings increase the probability of BCG infection [5]. She died from disseminated *M. tuberculosis* infection despite intensive anti-mycobacterial treatment. To the best of our knowledge, this patient is the second report of mycobacterial disease in PNP deficiency [4].

In conclusion, unless a functioning immune system is established by HSCT, ultimately, death occurs in these patients. An increased awareness of early signs, symptoms, and abnormal laboratory findings of PNP deficiency can establish early diagnosis and treatment.

Conflicts of interest The authors declare that they have no conflict of interest and financial relationship with any organization or drug industry.

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