Rare disease

Purine nucleoside phosphorylase deficiency with a novel PNP gene mutation: a first case report from India

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Summary

The authors report a case of purine nucleoside phosphorylase (PNP) deficiency for the first time from India. The case presented with recurrent severe infections, developmental delays, seizures and progressive neurological deterioration. The diagnosis of primary immunodeficiency disorder was delayed in spite of recurrent infection due to predominant neurological symptoms. Sequencing of the PNP gene revealed a novel mutation resulting in a premature stop codon.

BACKGROUND

Purine nucleoside phosphorylase (PNP) is a ubiquitous enzyme essential for the degradation of purine nucleosides.¹ PNP is expressed at high levels in the thymus and lymph nodes, where it removes deoxyguanosine generated from DNA breakdown. PNP cleaves the purine-sugar bond of guanosine, inosine, deoxyguanosine and deoxyinosine, releasing guanine and hypoxanthine. These bases are either salvaged as precursors for ATP and guanosine triphosphate (GTP) or oxidised to uric acid. In the absence of PNP, its nucleoside substrates become elevated and uric acid production declines.²

Furthermore, deoxyguanosine is abnormally phosphorylated by deoxycytidine kinase, a nuclear enzyme, or deoxyguanosine kinase, a mitochondrial enzyme. The resulting expansion of deoxyguanosine triphosphate (dGTP) inhibits DNA synthesis and repair. These deleterious effects of dGTP are most apparent in lymphoid tissue undergoing rapid cell turnover, especially T lymphocytes undergoing thymic maturation. The neurologic abnormalities in PNP deficiency may result from defective mitochondrial function or depletion of GTP in neurons.^{2 3}

PNP deficiency (OMIM 164050) is a rare form of severe combined immunodeficiency disorder (SCID) with autosomal recessive inheritance. It results in profound T cell

defect with variable B cell dysfunction⁴ and was first reported in 1975 in a child with recurrent infection and anaemia.⁵ Patients with PNP deficiency usually present with failure to thrive, recurrent and severe infections, neurologic dysfunction and autoimmunity.

PNP deficiency accounts for only 4% of the SCID patients. Less than 50 cases have been reported with PNP deficiency and to best of our knowledge, none from India. We report clinical and immunological findings of an Indian girl with PNP deficiency and the molecular defect responsible for the same.

CASE PRESENTATION

A 22-month-old female fourth sibling, born of a third degree consanguineous marriage was referred to us with recurrent lower respiratory tract infections, failure to thrive and global neurologic dysfunction. She presented first time at 15 days of life with pneumonitis and oral candidiasis. She required hospitalisation for similar complaints three times in first year. She also had failure to thrive and global developmental delays since birth. However, the child every time received symptomatic treatment without any immunological investigations. At 12 months of age, the child was admitted with pneumonia involving posterior segment of the upper lobe on the right side with respiratory distress and also noticed to have involuntary movements. She also received antitubercular therapy on empirical basis for her chest infections and neurological problems. Finally at the age of 22 months she was referred to immunology department to rule out underlying immunodeficiency disorder. On examination, the patient was poorly nourished and growth parameters were below third percentile. She was immunised with OPV-1, BCG but did not show a BCG scar. She had oral thrush. Chest examination revealed bilateral coarse basal crepitations. A neurological examination revealed global developmental delay. She had axial hypotonia and

Table 1 Immunological investigations

		Patient	Normal range
(Lymphocyte subsets evaluation/mm ³)	CD3	116	1900–5900
	CD4	109	1400–4300
	CD8	7	500–1700
	CD19	153	610–2600
	CD56/16	62	160–950
Immunoglobulin levels g/l	IgG	8.19	4.0–15.9
	IgA	1.75	0.01–0.91
	IgM	2.17	0.34–2.06

Figure 1 Sequencing of the PNP gene coding region and flanking intronic sequences revealed a novel homozygous non-sense mutation c.199C>T, PNP p.67R>X mutation in the patient. This mutation results in a premature stop codon in exon 3 predicting a truncated protein. The parents were heterozygous for the mutation.

increased tone in both the lower limbs with brisk deep tendon reflexes.

INVESTIGATIONS

Investigations revealed Hg 9.2 gm%, white blood cell count $18\,200/\text{mm}^3$ with an absolute lymphocyte count of $364/\text{mm}^3$ and a platelet count of $546\,000/\text{mm}^3$. Due to the patient's significant neurological problems, she was investigated further with cerebrospinal fluid (CSF) studies and MRI scan of brain. CSF revealed a picture suggestive of meningitis with $14\text{ cells}/\text{mm}^3$ (11% neutrophil and 89% lymphocytes, glucose 44 mg%, proteins 21mg %). CSF culture and repeated sputum cultures did not grow any organism. MRI showed basal leptomeningial enhancement in the supratentorial region posteriorly, suggestive of an inflammatory process such as meningitis. Immunological investigation revealed lymphopenia with T, B and natural killer cell low phenotype summarised in table 1. In view of neurological symptoms, serum uric acid was performed which was low (1 mg%) (normal range 2–7 mg%). PNP activity in red cell lysate was undetectable in the patient whereas her parents and her brother showed carrier range activity (mother: 1328 nmol/h/mgHb, father: 1786 nmol/h/mgHb, brother: 2040 nmol/h/mgHb with normal control range 3000–7000 nmol/h/mgHb). Her sister was found to have normal PNP levels.

Analysis of the PNP gene revealed a novel homozygous non-sense mutation c.199C>T (p.67R>X) in the patient. This mutation in exon 3 results in a premature stop codon predicting a truncated protein. The parents and the brother were heterozygous for the mutation (figure 1) whereas the sister showed wild type sequence.

DIFFERENTIAL DIAGNOSIS

Other forms of SCID with T-B- phenotype like adenosine deaminase (ADA) deficiency remain a major differential diagnosis. However the child's ADA levels were within normal range.

TREATMENT

The child received symptomatic treatment with antibiotics and antifungal agents.

OUTCOME AND FOLLOW-UP

The patient developed progressive neurological deterioration with severe dystonic movements and a repeat MRI revealed cerebral cortical and cerebellar atrophy and loss of white matter myelination. There was also shrinkage of caudate nuclei. Finally, the patient died due to septicaemia.

DISCUSSION

PNP deficiency leads to a defect in T cells and thus makes the patients susceptible to various life-threatening infections with common opportunistic pathogens such as *Candida albicans* and *Pneumocystis jiroveci*. Disseminated varicella, persistent herpes simplex virus infections are very severe or even fatal in some cases. It is also associated with increased risk of autoimmune diseases. However, these symptoms are variable and may not be apparent in the first few months of life.^{1–6}

Neurological problems including developmental delay, hypertonia, spasticity, tremors, ataxia, retarded motor development, behavioural difficulties and varying degrees of mental retardation are seen in more than half of the patients.¹ Thus patients with neurological impairments

with recurrent infections should be thoroughly evaluated for immunodeficiency. Patients with lymphopenia and low absolute counts of T cells should also be considered for PNP deficiency. A low uric acid level is a hallmark of PNP deficiency. Therefore, a screening uric acid level on lymphopenic patients may permit earlier diagnosis of PNP deficiency, even before the syndrome is fully expressed. Undetectable level of PNP enzyme activity in red blood cell lysates confirms the diagnosis of PNP.⁷ The molecular characterisation of PNP deficiency helps in confirmation of diagnosis and genetic counselling and prenatal diagnosis. There are around 40 different mutations described in the patients with PNP deficiency. However the mutation c.199C>T (p.67R>X) found in this patient has never been described before. This mutation resulted in severe PNP deficiency with undetectable catalytic activity.

The prognosis of PNP deficiency if untreated is poor as most of the patients die of severe infections. The only available cure for PNP deficiency is haematopoietic stem cell transplantation (HSCT).⁸ Though HSCT restores the purine nucleoside metabolism in non-neuronal cell populations, reports suggest that it may also stabilise the neuronal dysfunction in these patients.⁹

Learning points

- ▶ Simple investigations such as lymphocyte immunophenotyping, immunoglobulin level and serum uric acid estimation which are widely available can give very important clues for diagnosis of PNP deficiency.
- ▶ Neurological symptoms may divert the focus of investigations and delay the diagnosis in some cases.
- ▶ A novel mutation in PNP gene was found in this case.

Competing interests None.

Patient consent Obtained.

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