Immunodeficiency, Motor Delay, and Hypouricemia Caused by a Novel Mutation of Purine Nucleoside Phosphorylase Gene in an Indian Infant

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Abstract

We describe an 11-month-old boy who presented with recurrent respiratory infections from 6 months of age. His elder sister died at 10 months with severe septicemia and meningitis. The boy had a mild motor delay. Investigations revealed T cell deficiency and very low serum uric acid suggestive of purine nucleoside phosphorylase (PNP) deficiency – a rare variant of severe combined immunodeficiency disease. A novel homozygous missense mutation of c.597C>G(p. S199R) of exon 5 on PNP gene confirmed the diagnosis. We suggest that uric acid should be a part of investigation profile for unidentified motor delay, as recurrent infections can be late presentation.

Keywords: Adenosine deaminase deficiency, hypouricemia, purine nucleoside phosphorylase deficiency, severe combined immunodeficiency

Introduction

Recurrent serious infections in infancy are usually secondary to immunodeficiency disorders. The common conditions presenting in infancy are Bruton's hypogammaglobulinemia, severe combined immunodeficiency, and chronic granulomatous disease which are followed by Wiskott–Aldrich syndrome and leukocyte adhesion defects. They have varied inheritance pattern and associated other system involvement which in itself would be the diagnostic handle for appropriate diagnoses. Here, we report a child with genetically confirmed purine nucleoside phosphorylase (PNP) deficiency, who presented with early-onset recurrent infections, motor delay, and seizures.

Case Report

An 11-month-old boy born to nonconsanguineous couple had the first episode of pneumonia at 6 months of age requiring intravenous antibiotics for 5 days, with complete recovery. The investigations revealed neutrophilic leukocytosis (white blood cell [WBC] count: 9700 cells/cumm with neutrophils 78%); chest X-ray demonstrated right midzone haziness and absent thymic shadow. He had recurrent episodes of acute suppurative otitis media requiring on/off oral antibiotics and an episode of severe gastroenteritis at 9 months of age requiring hospitalization.

Best-attained milestones at 11 months were as follows: gross motor: able to sit without support unable to pull to sit and stand, visuomotor: able to reach for objects and grab it, speech: babbling bisyllables, and sociocognitive: had stranger anxiety and used to wave "bye." There was no regression of milestones. His earlier neurological examination by one of the authors (LL) had shown the presence of dystonia of hands and hypotonia. Dystonia was transient but hypotonia persisted even at 2nd presentation to us. At 11 months of age, he had one episode generalized clonic seizure, fever, and dullness for 2 days before admission. Weight was on 3rd centile; height and head circumferences were appropriate for age. He had encephalopathy (posturing to painful stimulus), normal extraocular eye movements, briskly reacting, symmetric pupils, normal fundus, hypotonia, brisk deep tendon reflexes, and extensor plantars. No signs of meningeal irritation were present.

His sister evaluated elsewhere died at 10 months of age with severe sepsis and meningitis (cerebrospinal fluid [CSF]: 25 cells 90% L, protein: 489 mg/dl, and sugar: 53 mg%). Her WBC count was 8700 cells/cumm (absolute lymphocyte count: 2000 cells/cumm). Thymic shadow was absent on chest X-ray. She had severe failure to thrive and mild motor delay. There was no Bacillus Calmette–Guérin (BCG) scar in both siblings despite immunizing twice at 6-month interval.

The index child's investigations revealed hyponatremia (Na + 115 meq/L) – attributed to syndrome of inappropriate

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antidiuretic hormone with low urine output with normal renal parameters and low urinary sodium excretion, normal calcium, magnesium, and WBC count of 6900 cells/cumm (absolute lymphocyte count of 1800 cells/cumm). Diffuse cerebral edema was noted on CT brain. The magnetic resonance imaging brain (done 5 days later) and CSF analysis (two cells, protein: 15 mg/dl, sugar: 83 mg%) were normal but the CSF-polymerase chain reaction (PCR) was positive for pseudomonas and candida (species not specified). History and investigations suggested the possibility of primary immunodeficiency syndrome. He was treated with meropenem, colistin, amphotericin B, and voriconazole with strong clinical suspicion of immunodeficiency and organisms on PCR. There was persistent lymphopenia, and all lymphocyte subsets were significantly low including NK cells with normal immunoglobulins [Table 1]. Serum uric acid was "zero" on two occasions 48 h apart. The child died after 7 days of treatment due to worsening sepsis with multiorgan dysfunction and worsening encephalopathy. Gene sequencing for severe combined immunodeficiency disease (SCID) and variants was performed after the selective capture and sequencing of the protein-coding regions of the genome using SureSelect V5 (Agilent Technologies, USA) Exome sequencing kit. The DNA libraries were sequenced to mean >80–100× coverage on illumina next-generation sequencing platform. The sequences obtained were aligned to the GRCh37/hg19 human reference genome using Burrows-Wheeler-Aligner (BWA) program and analyzed using Picard and GATK-Lite toolkit to identify variants in the exome relevant to clinical indication. Annotation of the variant was performed against the Ensembl release 75 gene model. Clinically, relevant mutations were annotated using published variants in the literature and a set of variant databases including ClinVar, OMIM, GWAS, HGMD, SwissVar, and Exac. Only nonsynonymous and splice site variants found in the genes relevant to the clinical symptoms were used for clinical interpretation. A pathogenic homozygous missense variation in exon 5 of the *PNP* gene (Chr14: 20943356; C > G) which results in the amino acid substitution of arginine for serine at codon 199 (p. S199R; NM_000270) was detected. This variation has not been reported in the literature to cause PNP deficiency. However, this S199R variation has been predicted to be damaging by SIFT and possibly damaging by Polyphen and is conserved across species. This has not been reported in the 1000 genomes and Exac database. Parents of the affected child were heterozygous for this S199R variant.

Table 1: Immunological profile of patient

	Patient	Normal
Absolute CD3	15	1900-5000
Absolute CD4	12	1200-3500
Absolute CD8	2	350-2500
Absolute CD 16/56	92	129-614
g/L		
IgG	4.57	3.5-16.2
IgA	0.4	0.01-0.91

Discussion

PNP deficiency is a rare autosomal recessive immunodeficiency disorder, and variant of SCID, presenting with recurrent infections and neurological features in infancy. It accounts for around 4% of all SCID cases, with around 67 cases and 24 disease-causing mutations reported in the literature,[1] and ours is the second genetically confirmed case reported from India.[2] The missense mutation c.597C>G(p. S199R) in exon 5 of the PNP gene in our patient has not been reported previously.

This condition was first reported in 1975.[3] Patients with PNP deficiency present with failure to thrive, recurrent infections, neurologic dysfunction, and autoimmunity.[2]

In humans, PNP gene mutation can cause extensive loss of enzymatic activity leading to severe T cell deficiency with variable B cell defects.[4] Knockout model of mice has revealed that mutation in PNP gene causes severe phenotype with impaired thymocyte differentiation, reduced mitogenic and allogenic response, and reduced number of maturing thymocytes and circulating T cells. The missense mutation causes more gradual postnatal reduction in number and function of T cells.[4]

Among SCID variants, adenosine deaminase (ADA) and PNP deficiency manifest with central nervous system (CNS) manifestations. These children are prone to recurrent multisystem infections such as recurrent sinopulmonary infections, opportunistic infections such as candidiasis, *Pneumocystis jiroveci*, and disseminated infections with live vaccines (Varicella/Rotavirus/Measles).[5] PNP deficiency generally presents before 1 year of age. It can present as late as 5 years of age.[6]

The neurological manifestations not attributed to CNS infections or their complications are an important clue for this fatal disorder and are seen in nearly two-thirds of cases. The neurological manifestations may predate the onset of infections**,** including developmental delay, hypertonia, spastic diplegia, tremors, ataxia, motor delay, and intellectual disability.[2,5,7,8]

Approximately, one-quarter come to medical attention for neurological problems.[5]Other rare neurologic presentations associated with PNP deficiency are subcortical stroke[9] and progressive multifocal leukoencephalopathy.[10] In our case, mild motor delay and dystonia were noted.

Hypouricemia is common finding in PNP deficiency as noted in our case and not in ADA deficiency which has neurologic manifestations in common with PNP deficiency although uric acid levels can be normal in PNP deficiency.[1,11] Absent thymic shadow and absence of BCG mark can be one of the primary indicators to suggest T cell immunodeficiency in resource-limited countries as seen in both siblings. The diagnosis of PNP deficiency is suggested by low PNP activity in erythrocytes, lymphocytes, and fibroblasts. The definitive diagnosis is performed using PNP gene analysis.

As suggested by la Marca *et al*., dried blood spot subjected to TMS to measure nucleotide can be used for the diagnosis of PNP deficiency in preclinical state[12] so that therapies can be initiated at earliest.[13]

There is increased risk of autoimmune disorders such as autoimmune hemolytic anemia, immune thrombocytopenia, neutropenia, thyroiditis, and lupus among the survivors.[5,14] It can also present as lymphoma which is unusual for T cell deficiencies.[15] The index child died very young, and hence, no other manifestations were noted.

Definitive management is stem cell or bone marrow transplantation. The marrow and stem cell transplantation reverses immunodeficiency but neurological symptoms persist.[14,16] Currently, the facilities to perform this exist in India, albeit the prohibitive cost interferes with universal access to this procedure. Outcomes with currently available treatment options are poor. Newer strategies including intracellular enzyme replacement, gene therapy, and innovative protocols for stem cell transplantations hold great promise for improved outcomes in ADA and PNP deficiency.[13]

Learning objective

1. Investigations for unidentified motor delay in an infant should include uric acid as part of profile with other investigations, as recurrent infections can be delayed feature of PNPdeficiency
2. Absent BCG scar should raise suspicion about underlying cellular immunodeficiency and should be investigated appropriately (at least lymphocyte count and chest X ray for thymus)
3. Dried blood spot can be subjected to TMS for screening of PNP deficiency as it ispotentially treatable condition with an early bone marrow transplant.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest

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