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ORIGINAL ARTICLE

ADA Deficiency: Evaluation of the Clinical and Laboratory Features and the Outcome

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Abstract

Introduction Adenosine deaminase (ADA) deficiency is an autosomal recessive primary immunodeficiency. It results in the intracellular accumulation of toxic metabolites which have effects particularly on lymphocytes and the brain. The aim of this study was to evaluate the outcome of 13 ADA-deficient patients. We planned to evaluate their clinical and laboratory findings before and after enzyme replacement therapy (ERT), allogeneic hematopoietic stem cell transplantation (aHSCT), and hematopoietic stem cell gene therapy (HSCGT).

Methods Measurement of ADA enzyme activity and metabolites and sequencing of the ADA gene were performed in most of the patients with ADA deficiency. One of the patients with late-onset ADA deficiency was diagnosed by the help of primary immunodeficiency panel screening.

Results Ten out of 13 patients were diagnosed as SCID, while 3 out of 13 were diagnosed as delayed-/late-onset ADA deficiency. Late-onset ADA deficiency patients had clinical and laboratory findings of combined immunodeficiency (CID). Eight patients with ADA-SCID were found to have higher levels of ADA metabolite (dAXP%) (62.1% (34.6–71.9)) than 3 patients with delayed-/late-onset ADA deficiency (6.9% (2.1–8.9). All but one patient with SCID had T-B-NK− phenotype, one had T-B-NK+ phenotype. Genetic defect was documented in 11 patients. Four out of 11 patients had compound heterozygous defects. Three out of 4 patients with compound heterozygous defects had delayed-onset/late-onset ADA deficiency. Seven out of 11 patients with SCID had homozygous defects. Five out of 7 had the same homozygous indel frameshift mutation (c.955-959delGAAGA) showing a founder effect. There were two novel splice site defects: one (IVS10+2T>C) was heterozygous in a patient with late-onset ADA deficiency, and the other was homozygous (IVS2delT+2) in a SCID patient. Other defects were missense defects. Nine out of 13 patients were put on pegylated ADA ERT. Four out of six patients were transplanted without using a conditioning regimen. HSCGT was performed to one of the patients.

Conclusion The genetic diagnosis of SCID is utmost important. There is a chance to give ERT before the definitive therapy if the patient with SCID/CID has ADA deficiency. Although ERT was insufficient to restore a normal immune function in ADA-SCID patients, it was useful to improve and stabilize the clinical status before curative therapy (aHSCT/HSCGT). Enzyme replacement therapy was successful in patients with late-/delayed-onset ADA deficiency who presented with the features of combined immunodeficiency. Gastrointestinal polyposis in a patient with late-onset ADA deficiency may be an association or a coincidental finding. Intermittent neurodevelopmental evaluation especially for hearing impairment should be performed in most of the ADA-deficient patients. This may alleviate the speech delay and cognitive abnormalities which may be observed in the follow-up.

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Keywords Late-onset adenosine deaminase deficiency · SCID · ADA enzyme replacement therapy

Introduction

Adenosine deaminase (ADA) is a monomeric enzyme of purine metabolism which catalyzes the deamination of adenosine (Ado) and 2′-deoxyadenosine (dAdo) to inosine and 2′-deoxyinosine. Adenosine deaminase deficiency is an autosomal recessive primary immunodeficiency in which elevated levels of Ado and dAdo cause the intracellular accumulation of toxic metabolites, such as dATP. These metabolites disturb cellular functions in numerous tissues and particularly impair lymphocyte development, viability, and function. A correlation between the severity of the ADA gene defect and metabolic disturbance has been reported [1, 2].

Adenosine deaminase deficiency is a common form of severe combined immunodeficiency (SCID). It accounts for about 10–20% of all cases in USA [3]. Generally, ADA deficiency is diagnosed during infancy. More than 85% of the patients present in the first 6 months of life with manifestations of severe combined immunodeficiency: recurrent and opportunistic fungal, viral and bacterial infections, lymphopenia, and failure to thrive. The manifestations are milder in the late-onset form of the disease. The patients with the late-onset form generally have lymphopenia affecting T, B, and NK cells. Rachitic rosary, liver disease, and cognitive and behavioral abnormalities may be seen [3, 4].

In ADA deficiency, first-line treatment is hematopoietic stem cell transplantation (aHSCT). Preceding aHSCT, polyethylene glycol-conjugated bovine ADA (PEG-ADA) can be used as enzyme replacement therapy (ERT). If there is no HLA compatible donor, hematopoietic stem cell gene therapy (HSCGT) may be an alternative therapy to aHSCT. Success of HSCGT depends on residual thymic activity at the initiation of HSCGT and number of gene-corrected HSCs administered to the patient [5].

The aim of this study was to evaluate the outcome of 13 ADA-deficient patients. We planned to evaluate their clinical and laboratory findings before and after ERT, allogeneic hematopoietic stem cell transplantation (aHSCT), and hematopoietic stem cell gene therapy (HSCGT).

Patients

Thirteen patients who were diagnosed/followed up in 2000–2017 period with the diagnosis of ADA deficiency in Hacettepe University Immunology Unit were enrolled into the study. We grouped the patients according to their erythrocyte deoxyadenosine nucleotide (dAXP) levels, as it is reported that the clinical phenotypes correlate with this level [1]. The patients were also grouped according to their clinical phenotypes. The diagnosis of SCID was based on the criteria given by Pai et al. [6], and diagnosis of CID was based on PAGID&ESID diagnostic criteria [7]. Less severe "delayed" onset combined immune deficiency (CID) is usually diagnosed between age 1 and 10 years, and "late/adult onset" CID is diagnosed in the second to fourth decades [1]. Enzyme replacement was given (A first dose of 10U/kg, a second dose of 15U/kg, a third/maintenance dose of 20U/kg, then dose of 30U/kg i.m. twice weekly) to some of the patients. The clinical and laboratory manifestations, effects of ERT on these manifestations, and genotype–phenotype relation were evaluated.

Methods

Flow Cytometry The analysis of peripheral blood lymphocyte populations was performed by one laser three-color flow cytometry (BD Biosciences FACSCalibur, USA), using 100 μl of whole blood stained with 20 μl of the following monoclonal antibodies against lymphocyte subsets with fluorescein isothiocyanate (FITC), phycoerythrin (PE) (obtained from Beckton Dickinson, BD, USA) ((CD3 (FITC), CD4 (FITC), CD8 (PE), CD16+56 (PE), and CD19 (PE)), and incubated in the dark for 15 min at room temperature.

Measurement of ADA Enzyme Activity and Metabolites Cells (2 to 5 × 105 PBMCs) were washed with HBSS, pelleted, and frozen at − 80 °C until use. Pellets were thawed at 37 °C and resuspended at 5 × 106 /ml in M-Per Mammalian Protein Extraction Reagent (Pierce), vortexed, and left on a cell shaker for 30 to 40 min. Lysates were then cleared of cellular debris by centrifugation, and 10 μl aliquots were transferred to 0.5-ml tubes and incubated with 10 μl of 1 mM 14C-adenosine (50 mCi/ml) at 37 °C for 5 to 40 min. The products of the enzymatic reactions were then separated by thin-layer chromatography and the conversion of adenosine into inosine determined by using a phosphoimager (Fuji Medical Systems) and expressed as units of ADA (1 unit = 1 nmol of adenosine deaminated per 108 cells per min). ADA activity and the total Ado and d Ado nucleotides (AXP and d AXP expressed as a percentage of the total) were quantified in extracts of dried blood spots as described previously [8].

Genetic Analysis

Targeted Primary Immunodeficiency (PID) Panel Screening One of the patients with the delayed-/late-onset ADA deficiency (P9) was diagnosed by the help of panel screening.

HaloPlex™ probes which were designed to capture 356 PID-related genes were used previously [9].

Sanger Sequencing Genotype analysis of all but one (P9) of the patients were done in Duke University [10].

Results

Results of the ADA Enzyme Activity and ADA Metabolites ADA enzyme activity in 13 and ADA metabolites in 11 patients were tested. ADA enzyme activity was zero in 11, and it was very low (0.3–0.4 nmol/h/mg) in 2 patients (P4, P9). Eight patients with SCID were found to have higher blood ADA metabolite (dAXP%) levels (62.1% (34.6–71.9)) than 3 patients (P6, P7, P9) with delayed-/late-onset ADA deficiency (6.9% (2.1–8.9)).

Patients with SCID Due to ADA Deficiency There were 10 patients diagnosed with SCID (Table 1). M/F ratio was 5/5. The median age at onset of symptoms was 3 months (1 month-17 months). Median age at diagnosis was 4 months (2 months-18 months). P2 and P11 were diagnosed after they died.

The clinical features are summarized in Table 1. P4 had nodular skin lesions (Fig. 1) in which perivascular/perieccrine lymphocytic infiltration was seen pathologically. No microorganism was isolated and acid-fast bacteria was negative. P12 had lobular panniculitis (Fig. 2). Lymphopenia was prominent in all patients. Only P5 had T-B-NK+ SCID phenotype, others had T-B-NK− SCID phenotype (Table 2).

Patients with Delayed-/Late-Onset ADA Deficiency Patient 7 was diagnosed early, following the diagnosis of his brother. However, P7 was evaluated as delayed-/late-onset ADA deficiency as P6 and P9, because these three patients had less severe clinical features. Immunophenotypic analysis showed relatively higher lymphocyte and CD3 counts. Lower levels of ADA metabolites (dAXP) were present compared with ADA-SCID patients.

The median age at onset of symptoms was 21 months (13–108). Median age at diagnosis was 22 months (14–120).

P6 had hypoalbuminemia and hypogammaglobulinemia due to protein-losing enteropathy and needed bi-/tri-weekly albumin transfusions during his follow-up. Gastric polyps were detected by endoscopy in addition to colonic polyposis. The family did not have a history of polyposis coli, and his brother with the same compound heterozygous defect did not have symptoms such as mucoid/bloody stool. After the first aHSCT was performed to P6 without conditioning regimen, chimerism studies were found to be low. Then, second aHSCT with conditioning regimen was performed. The albumin infusion need decreased in the early post-transplant period under the conditioning regimen and wide-spectrum antimicrobials in the first months of second aHSCT. However, the albumin need increased again.

P9, who was 19 years old, presented with recurrent pneumonia, bronchiectasia, and hypogammaglobulinemia.

ADA Gene Defects in Patients and Genotype–Phenotype Correlation The defects of 11 patients are shown in Fig. 1. There were compound heterozygous defects in 4 out of 11 patients. Three out of 4 patients with compound heterozygous defects had delayed-onset/late-onset ADA deficiency. Seven out of 11 patients with SCID had homozygous defects. Five out of 7 had the same homozygous indel defect leading to frameshift mutation (c.955-959delGAAGA). Two patients had novel splice site defects. One of these defects (IVS10+2T>C) was heterozygous in a patient with late-onset ADA deficiency (P9). The other (IVS2delT+2) was homozygous in a SCID patient (P4). Other defects were missense defects.

Red cell ADA enzyme activity was undetectable in all patients with ADA deficiency regardless of their genotype. The correlation was seen between genotype and red cell dAXP% levels. All SCID patients having deletions, some missense and splice site mutations, had higher red cell dAXP% (48.5–71.9%). The patients having some missense and splice site mutations presented at an older age had lower red cell dAXP%.

Enzyme Replacement Therapy and Its Effects Nine out of 13 patients were put on ADA ERT. In total, four patients; P1 and P2 who were born before the date that ERT had become available, P11 who had died before diagnosis, and P13 who had been transplanted early, were not given ERT. Patients 4, 5, 8, and 10 were given ERT for less than 6 months (1 month-6 months) before aHSCT. Patients 3, 6, 7, 9, and 12 were given ERT for more than 12 months (12months-24months) (Fig. 3).

The patients' lymphocyte numbers increased mildly after ERT (Fig. 3). CD3, CD19, and CD16+56 levels did not increase significantly after ERT. The highest increase in lymphocyte counts and lymphocyte subsets with ERT among patients with SCID was in P2 (ALC increased from 100 to 300/mm^3 and CD3%/number increased from 2% (53–84)/2mm^3 (2500–5500) to 17% (53–84)/51/mm^3 (2500–5500)). Among the late-onset ADA deficiency patients, the highest increase with ERT in lymphocyte count and lymphocyte subsets is observed in P6; the lymphocytes increased from 2600/mm^3 (3600–8900) to 5400/mm^3 (3600–8900), and CD3%/number increased from 74% (53–75)/1924 (2100–6200) to 82% (53–75)/4400 (2100–6200). ERT was insufficient to restore normal immune function in ADA-SCID patients. It was only useful to improve and stabilize clinical status before curative therapy. So, all the patients on ERT were given IVIG therapy.

Table 1 Clinical characteristics of patients with ADA deficiency

Patients	Gender	Consanguinity/family history	Age at onset of symptoms	Diagnosis	Age at diagnosis	Clinical findings	Laboratory findings	Therapy	Outcome
P1	Female	−/+	12 months	SCID	17 months	Pneumonia, moniliasis	T-B-NK− SCID	IVIG-PAT	Died (18 months)
P2	Male	−/−	10 days	SCID	4.5 months	Sepsis, preseptal cellulitis, viral pneumonia	T-B-NK− SCID	IVIG-PAT-aHSCT (CR−)	Died (6 months)
P3*	Male	+/+	3 weeks	SCID	1 month	–	T-B-NK− SCID	IVIG-PAT-ERT+HSGT	Alive
P4*	Female	+/−	3 months	SCID	4 months	Diarrhea, pneumonia, pelvic left kidney	T-B-NK− SCID	IVIG-PAT-ERT+aHSCT (CR−)	Alive
P5*	Female	+/−	4 months	SCID	5 months	Granulomatous lesions on skin, pneumonia	T-B-NK+ SCID	IVIG-PAT-ERT+aHSCT (CR−)	Died (10 months)
P6	Male	−/−	13 months	SCID	14 months	GIS polyposis, PLE, pneumonia, GVA	CID	IVIG-PAT-ERT+aHSCT (CR−, CR+)	Alive
P7	Male	−/+	1 month	SCID	2 months	–	CID	IVIG-PAT-ERT	Alive
P8	Female	+/−	3 months	SCID	4 months	Pneumonia, moniliasis, hypercalcemia, erythroderma	T-B-NK− SCID	IVIG-PAT-ERT+aHSCT (CR−)	Alive
P9	Male	−/−	19 years	CID	19 years	Recurrent pneumonia, bronchiectasis	Low CD4, normal IgA, G, M, high IgE	IVIG-PAT-ERT	Alive
P10*	Male	+/+	1 month	SCID	2 months	Neonatal sepsis, moniliasis	T-B-NK− SCID	IVIG-PAT-ERT+aHSCT (CR+)	Alive
P11	Male	+/−	9 months	SCID	After death	Pneumonia, moniliasis, sepsis, neutropenia	T-B-NK− SCID	IVIG-PAT	Died (9 months)
P12*	Male	+/−	1 month	SCID	2 months	Diarrhea, moniliasis, pneumonia, panniculitis	T-B-NK− SCID	IVIG-PAT-ERT	Alive
P13*	Female	−/−	1 month	SCID	1.5 months	Pneumonia	T-B-NK− SCID	IVIG-PAT+aHSCT (CR−)	Died (16 months)

aHSCT, allogeneic hematopoietic stem cell transplantation; *CR*, conditioning regimen; *ERT*, enzyme replacement therapy; *GVA*, Galen vein aneurysm; *GIS*, gastrointestinal system; *IVIG*, intravenous immunoglobulin therapy; *HSCGT*, hematopoietic stem cell gene therapy; *PAT*, prophylactic antibiotherapy; *PLE*, protein-losing enteropathy

*Patients 3, 4, 5, 10, 12, and 13 have the same homozygous defect (c.955-959delGAAGA)

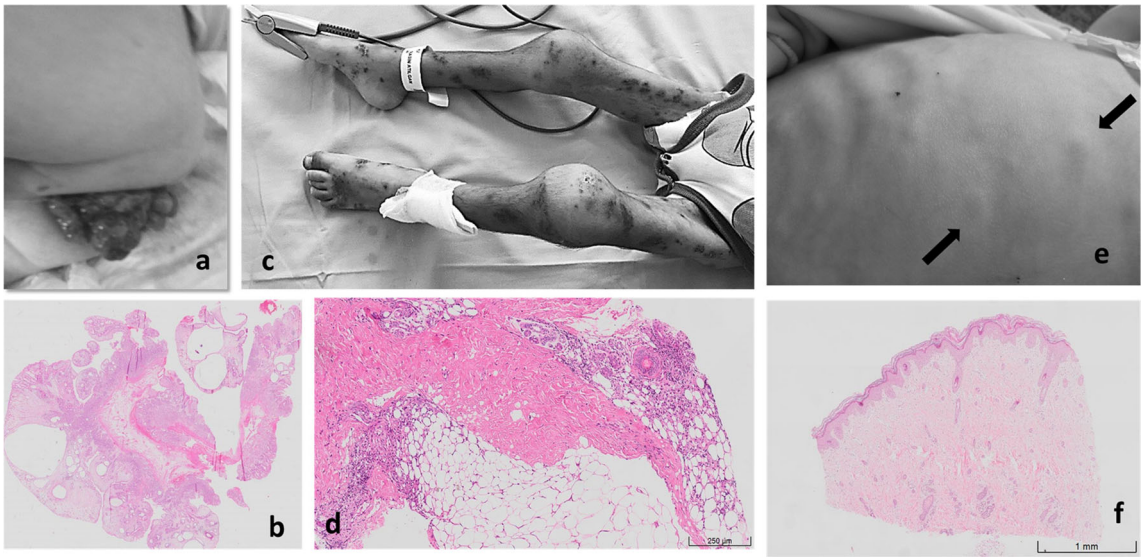

Fig. 1 Rectal polypoid lesions (P6) (a, b). panniculitis lesions (P11) (c, d); nodular skin lesions (P11) (e, f)

In two patients with late-onset ADA deficiency (P7, P9), ERT is still being used in addition to IVIG and prophylactic antibacterial therapy and is sufficient to control the symptoms.

Allogeneic Hematopoietic Stem Cell Transplantation and Hematopoietic Stem Cell Gene Therapy Six patients (P2, P4, P5, P6, P8, P10, P13) were transplanted (Table 1). Four of them (P2, P4, P8, P13) were not given conditioning regimen. Patient 6 (late-onset form) was transplanted firstly without conditioning therapy, but the chimerism was low and aHSCT was performed secondly with conditioning therapy. Patients 5, 6, and 10 were given conditioning regimen including busulfan (4.8–5.1 mg/kg/day for 4 days) and fludarabine (40 mg/m^2/day for 4 days). P2 and P5 died due to infectious complications soon after aHSCT. P13 died due to aspiration pneumonia 1 month after she discharged from hospital. Patients 4, 6, 8, and 10 are alive after aHSCT, at ages 9, 5, 4, and 1 respectively. Patient 6 had polyposis coli and needed IVIG and albumin therapy also after aHSCT. Other patients are of IVIG therapy after aHSCT. Acute GVHD was observed in P6 and P8, but controlled in first 3 months of aHSCT by steroids and cyclosporin A therapy.

HSCGT is performed to P3 at age 2 in San Raffaele Telethon Institute for Gene Therapy (SR-Tiget) with retroviral vector after conditioning therapy [11]. He is vaccinated approximately 6 months after IVIG therapy was stopped. He is now well despite lymphopenia (800–1000/mm^3). His

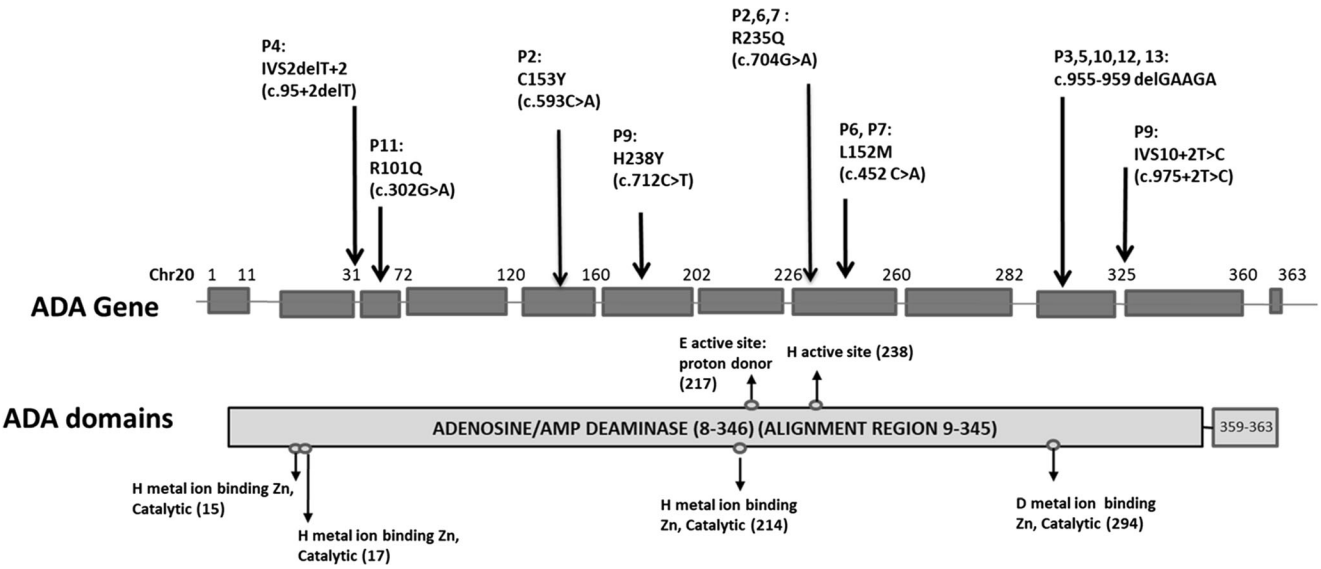

Fig. 2 ADA gene defects of the patients (P6 and P7 are from the same family)

Table 2 ADA activity, dAXP%, and other laboratory data of the patients with ADA deficiency

Patients	ADA activity	dAXP%	WBC (/mm^3)	ALC (/mm^3)	IgA (mg/dl)	IgG (mg/dl)	IgM (mg/dl)	IgE (IU/L)	CD3 (%–no.)	CD19 (%–no.)	CD16+56 (%–no.)	Gene defect
P1	Low	–	3800	980	21 (30–107)	52.4 (605–1430)	19 (66–228)	–	16 (53–75)/ 156 (2100–6200)	2 (16–35)/ 20 (720–2600)	2 (3–15)/ 20 (180–920)	NA
P2	Low	–	2900	700	22 (17–69)	120 (463–1006)	13 (46–159)	< 1	4 (51–77)/ 28 (2500–5600)	5 (11–41)/ 35 (430–3000)	9 (3–14)/ 63 (170–830)	c.593C>A, C153Y c.704G>A, R235Q
P3*	0	71.9	2500	100	< 6.67 (11–14.1)	396 (633–1466)	< 4 (22–87)	180	2 (53–84)/ 2 (2500–5500)	0 (6–32)/ 0 (300–2000)	0 (4–18)/ 0 (170–1100)	c.955-959delGAAGA
P4	0.3	67.7	4300	100	25 (13.5–72)	240 (294–1165)	10 (33–154)	–	0 (51–77) 0 (2500–5600)	3 (11–41)/ 3 (430–3000)	24 (3–14)/ 24 (170–830)	c.95+2delT, IVS2del T+2
P5*	0	48.5	11,600	300	< 6.67 (13.5–72)	107 (294–1165)	< 4 (33–154)	< 1	1 (51–77)/ 3 (2500–5600)	2 (11–41)/ 6 (430–3000)	52 (3–14)/ 156 (170–830)	c.955-959 delGAAGA
P6	0	6.9	10,100	5400	< 6.67 (30–107)	321 (605–1430)	20.2 (66–228)	20	82 (53–75)/ 4428 (2100–6200)	2 (16–35)/ 108 (720–2600)	7 (3–15)/ 378 (180–920)	c.452C> A L152M c.704G>A R235Q
P7	0	2.1	6700	3900	< 6.67 (13.5–72)	501 (294–1165)	15.2 (33–154)	318	77 (53–84)/ 3003 (2500–5500)	11 (6–32)/ 429 (300–2000)	3 (4–18)/ 117 (170–1100)	c.452C>A L152M c.704G>A R235Q
P8	0	62.1	3900	100	< 6.67 (13.5–72)	1670 (294–1165)	< 4 (33–154)	< 1	0 (51–77)/ 0 (2500–5600)	0 (11–41)/ 0 (430–3000)	0 (3–14)/ 0 (170–830)	NA
P9	0.4	8.9	14,900	1600	144 (139–378)	1450 (913–1884)	47 (88–322)	1942	81 (53–75)/ 1296 (2100–6200)	0 (16–35)/ 0 (720–2600)	15 (3–15)/ 240 (180–920)	c.712C>T, H238Y c.975+2T>C, IVS10+ 2T>C
P10*	0	63.9	2000	0	< 6.67 (13.5–72)	874 (294–1165)	< 4 (33–154)	< 1	0 (53–84)/ 0 (2500–5500)	0 (6–32)/ 0 (300–2000)	0 (4–18)/ 0 (170–1100)	c.955-959 delGAAGA
P11	0	34.6	4200	100	46.8 (17–69)	541 (463–1006)	65 (46–159)	1.35	0 (54–76)/ 0 (1600–6700)	0 (15–39)/ 0 (600–2700)	20 (3–17) /20 (200–1200)	c.302G>A, R101Q
P12*	0	58.6	780	50	< 6.67 (13.5–72)	461 (294–1165)	15.3 (33–154)	2.5	5 (53–78)/ 2.5 (2500–5500)	5 (6–32)/ 2.5 (300–2000)	20 (4–18)/ 10 (170–1100)	c.955-959 delGAAGA
P13*	0	67.1	1400	0	< 6.67 (13.5–72)	1060 (294–1165)	< 4 (33–154)	< 1	0 (53–84)/ 0 (2500–5500)	0 (6–32) 0 (300–2000)	0 (4–18)/ 0 (170–1100)	c.955-959 delGAAGA

ALC, absolute lymphocyte count; *dAXP*, deoxyadenosine nucleotides; *WBC*, white blood cell

* Patients 3, 5, 10, 12, and 13 have the same homozygous defect (c.955-959delGAAGA)

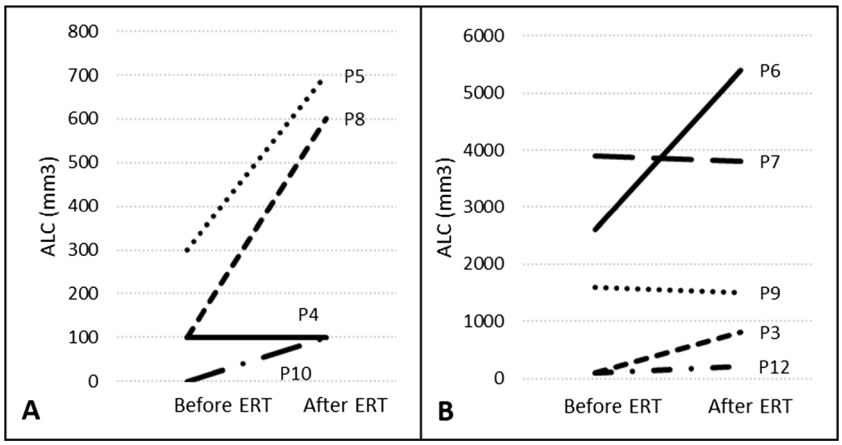

Fig. 3 ALC of the patients before and after PEG-ADA enzyme replacement therapy (< 6 month period (A), > 12 month (B))

immunoglobulin, lymphocyte subsets, and lymphocyte activation tests were normal at the fourth year of HSCGT.

Outcome In total, five patients (P1, P2, P5, P11, P13) died. Four patients, P1, P2, P11, and P13, had no chance of ERT and died due to infections. Patient 3 is well and alive following gene therapy. IVIG therapy was stopped. Patients 7, 9, and 12 are still under ERT. P12 has no donor, so gene therapy or haploidentical aHSCT is planned. P13 died due to aspiration pneumonia 1 month after HSCT was performed and discharged from hospital. Four out of 7 transplanted patients are alive. Three patients died soon after aHSCT due to infectious complications. P3, P4, P8, and P10 were subjected to regular vaccination in 6 months after IVIG therapy was stopped. P6 suffered from some serious complications of juvenile polyposis including recurrent hospitalizations, refractory hypoalbuminemia, and gastric outlet obstruction with duodenal intussusception necessitating emergent surgery. Mild/severe neurodevelopmental delay was documented in most of the patients with ADA deficiency (Table 3).

Discussion

ADA deficiency is a systemic metabolic disease which severely affects the immune system such as leukocyte adhesion deficiency type II and PGM3 deficiency. It is a systemic purine metabolism disorder which is caused by mutations in the gene encoding ADA. In patients with ADA deficiency, effects on different systems are reported [12]. These nonimmune abnormalities are related to brain, lung, kidney, liver, skin, and bone [12].

Neurodevelopmental delay and mild mental retardation are frequently observed in patients with ADA deficiency [12]. In our series, we saw that most of the patients had developmental delay and delay in speech (Table 3). It may be due to the adenosine receptors, their role in development of embryo and newborn, and their effects during perinatal stress [13]. The delay in speech may be partly due to sensorineural hearing impairment which is also commonly seen in ADA-deficient patients [14]. So, patients should be evaluated and treated for hearing impairment in an early convenience. This may alleviate the speech delay and cognitive abnormalities frequently observed in ADA-SCID patients.

In ADA deficiency, lymphopenia, hypogammaglobulinemia, defect in antigen dependent B cell maturation, poor vaccine response, functional spleen problems, disturbed thymus structure, and thymic atrophy are reported [15]. Thrombocytopenia and neutropenia in addition to lymphopenia observed in P12 were thought to be due to bone marrow suppression. These decreased the efficacy of ERT in P12. Hypocellular bone marrow, myeloid dysplasia, and other myeloid abnormalities, such as neutropenia may be seen in the course of ADA deficiency due to metabolic toxicity [3]. Antibiotic-related myelotoxicity is also seen in high ratio in ADA deficiency [16].

Wide spectrum of gastrointestinal signs and symptoms are observed in SCID. Oral, esophageal, and perianal candidiasis, viral and opportunistic infections of gastrointestinal tract, chronic diarrhea, inflammatory bowel disease, malabsorption, and villous atrophy have been described in SCID. Polyposis in a patient with primary immune deficiency is an uncommon finding. Data regarding this association are scarce [17]. Inflammatory duodenal polyposis in a patient with agammaglobulinemia and giardiasis has been very recently reported [18]. As far as we know, juvenile polyposis in a SCID patient as in P6 has not been described before. Juvenile polyposis is a rare syndrome characterized by multiple juvenile-type hamartomatous polyps in the gastrointestinal tract. Common clinical presentations include anemia, recurrent gastrointestinal bleeding, diarrhea, rectal prolapse, intussusception, protein-losing enteropathy, and malnutrition. Our patient also suffered from some serious complications of juvenile polyposis including recurrent hospitalizations due to

Table 3 Neurodevelopmental status of the patients with ADA deficiency

	Neurodevelopmental Status	Current Age
P1	Severe developmental delay (social, gross and fine motor, speech), spasticity	Died (18 months)
P2	Developmental delay (gross and fine motor, speech)	Died (6 months)
P3	Decreased school performance	Alive (9 years old)
P4	Sensorineural hearing loss, developmental delay (Fine motor, language (delayed speech), decreased school performance)	Alive (9 years old)
P5	Mild developmental delay (gross and fine motor)	Died (10 months)
P6	Severe developmental delay (social, gross and fine motor, speech)	Alive (6 years old)
P7	Normal	Alive (2 years old)
P8	Mild developmental delay (Fine motor)	Alive (3 years old)
P9	Decreased school performance	Alive (19 years old)
P10	Mild developmental delay (gross motor)	Alive (9 months)
P11	Mild developmental delay	Died (12 months)
P12	Developmental delay (gross and fine motor, speech)	Alive (3 years)
P13	Developmental delay (gross and fine motor), feeding/swallowing dysfunction	Died (16 months)

refractory hypoalbuminemia and gastric outlet obstruction due to duodenal intussusception. Juvenile polyposis syndrome (JPS) is inherited as an autosomal dominant manner, and about 50–60% of JPS patients have a germline mutation in the SMAD4 or BMPR1A gene. A specific antigen-triggered inflammatory polyposis was thought to be present in P6 before the final diagnosis of juvenile polyposis syndrome. Polypoid masses are defined with some viruses and opportunistic bacteria in non-human primates [19]. We thought that this clinical condition might be due to an immune dysregulation in ADA deficiency. A prominent decrease in the albumin need under conditioning therapy and wide-spectrum antimicrobials was observed, which we attributed to the decreased inflammatory status of the gut. However, persistence of polyps after aHSCT supported the presence of hamartomatous rather than inflammatory nature of polyps. The coexistence of juvenile polyposis with ADA deficiency may also be due to a second molecular defect causing polyposis. As ADA deficiency associates with bone deformities, neurological, pulmonary, and renal findings, ADA-deficient patients with intractable diarrhea and protein-losing enteropathy should be evaluated with endoscopy for polyposis.

Panniculitis is the inflammation of subcutaneous adipose tissue. This lesion is reported to be associated with immunodeficiency diseases [20–22]. Atypical mycobacteria, such as *Mycobacterium chelonae*, which sometimes may not be shown histopathologically, can cause lobular panniculitis [22]. Fungi, such as histoplasmosis, may cause skin lesions similar to lobular panniculitis and it is difficult to isolate them from skin [23]. So, panniculitis seems to be a vasculitis-like lesion triggered after infections in patients with immunodeficiency. Lobular panniculitis in P12 is possibly triggered by an unidentified infectious agent. Although it is difficult to isolate some microorganisms, screening for as many microorganisms as possible should be performed. But, we could not isolate any infectious agent and have given cyclosporine in addition to steroids [24]. The lesions partially resolved after this therapy.

There was no significant difference in ADA enzyme levels between ADA-SCID and late-onset ADA-deficient patients. They all had very low enzyme level. However, we noticed that the adenosine metabolite (dAXP%) level is an important parameter which correlates with the clinical status of the patient [1]. The delayed-/late-onset ADA-deficient patients prominently have lower red cell dAXP% levels compared with ADA-SCID patients.

The patients were evaluated according to their molecular defect to define the presence of genotype–phenotype correlation. Genotype–phenotype correlation seems to be present in ADA deficiency [25]. Six ADA-SCID patients having the same defect had similar clinical features. Same mutation found in some of the patients is an example of the founder effect [26]. We did not notice any difference in the clinical features between ADA-SCID patients with/without this defect. The SCID phenotype is usually T-B-NK− in all ADA-SCID patients. However, in our cohort, P5 carrying the same founder mutation homozygously, had T-B-NK+ SCID.

Patient 9 with chronic pulmonary complications had a compound heterozygous ADA defect. There were a missense mutation in exon 8 (c.712C>T, H238Y) and a 5′splice site mutation in IVS10 (c.975+2T>C, IVS10+2T>C). Hershfield et al. found that H238Y mutation severely reduces ADA activity by bacterial expression studies [1]. The patient's late-

onset phenotype is probably due to the low level of enzymatically active ADA.

It is difficult to differentiate late-onset ADA deficiency from other patients with combined immunodeficiency. P9 had low CD4 percentage and numbers (12%, 240/mm^3), and low CD19 (0%). Thus, uric acid level is a clue for ADA deficiency as in purine nucleoside phosphorylase deficiency [27]. The uric acid level was low in P9. However, the diagnosis in P9 is established with next-generation studies. ADA enzyme level was measured after the genetic diagnosis, and it was compatible with ADA deficiency.

ADA deficiency should be diagnosed and treated on time because it has a fatal course due to severe and overwhelming infections, and most patients die within the first year of life unless therapeutic intervention is applied. Current treatment options include ERT, aHSCT, and HSCGT. The genetic diagnosis of ADA deficiency in a patient with SCID is utmost important, as there is chance to give ERT before curative therapy. aHSCT and HSCGT are curative therapies and tend to be favored treatment options for patients with ADA deficiency. But, a definite time period is needed to do workup before aHSCT/HSCGT. A chance for an immediate intervention is provided to the patients by the help of ERT. Enzyme replacement therapy provides positive clinical effects on the follow-up of patients [28]. However, limited levels of immune reconstitution was achieved by ERT. The increases in absolute lymphocyte counts (Fig. 3) and percentages of CD3, CD19, and CD16+56 were very limited. Enzyme replacement therapy was used as a transition therapy in P4, P5, P6, P8, and P10 before aHSCT, and in P3 before HSCGT. It helped to prepare the patients for curative therapy. Although ERT was insufficient to completely control the symptoms in severe ADA deficiency, it was useful to gain time before aHSCT or gene therapy.

We saw in our study that the prognosis of patients with ADA-SCID has improved after the use of the ERT. Patients with ADA-SCID, unless diagnosed early in life, have very low chance to get the ERT. So, identification of the patients with ADA-SCID among SCID patients is utmost important. Enzyme replacement therapy is critically important in ADA-SCID, as it helps patients in the process of aHSCT/HSCGT. ERT alone seems to be efficient to control the symptoms in patients with delayed-/late-onset ADA deficiency. The only problem in these patients is the cost of the therapy as they should take the ERT lifelong. So, it should be used as a transition therapy in patients who have a chance for curative therapies.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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