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Combined immunodeficiencies: twenty years experience from a single center in Turkey

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Abstract

Combined immunodeficiencies (CIDs) include a group of inherited monogenic disorders. CIDs are characterized by defective cellular and humoral immunities that lead to severe infections. CIDs can be classified according to immunologic phenotypes as $T^-B^-NK^-$ CID, $T^-B^-NK^+$ CID, $T^-B^+NK^-$ CID and $T^-B^+NK^+$ CID. In a 20-year period, from 1994 to 2014, a total of 40 CID patients were diagnosed at the Pediatric Immunology of Erciyes University Medical Faculty in Kayseri, Turkey. The gender ratio (F/M) was 3/5. The median age at the onset of symptoms was 2 months (range, 15 days – 15 years). Of the 14 $T^-B^-NK^-$ CIDs, 6, 2 (siblings), 1, 1 and 4 had a mutation in the ADA, PNP, Artemis, RAG1 genes and unknown genetic diagnosis respectively. Of the 15 $T^-B^-NK^+$ CIDs, 3, 2 (siblings) and 10 had a mutation in the RAG1, XLF/Cernunnos genes and unknown genetic diagnosis respectively. Of the 9 $T^-B^+NK^-$ CIDs, 2 siblings, 1, 1 and 5 had a mutation in the ZAP70, IL2RG, DOCK8 genes and unknown genetic diagnosis respectively. Of the 2 $T^-B^+NK^+$ CIDs, 2 had a mutation in the MAGT1 and ZAP70 genes respectively. Of the 40 CIDs, 26 (65%) were died and 14 (35%) are alive. Eight patients received HSCT (hematopoietic stem cell transplantation) with 62.5% survival rate. As a result, patients presented with severe infections in the first months of life have to be examined for CIDs. Shortening time of diagnosis would increase chance of HSCT as life-saving treatment in the CID patients.

Key words: *CID, XLF, MAGT1, ZAP70, JAK3, childhood.*

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Introduction

Defects in the adaptive immune system lead to humoral immune deficiency syndromes and CIDs (combined immunodeficiencies). Combined immunodeficiencies consist of a heterogeneous group of inherited disorders result from different monogenic defects. In recent years, advances in the diagnostic methods helped us in diagnosis of CID patients who presented with early-onset severe infections and leaded to selection of appropriate treatment [1]. In 2014, IUIS (International Union of Immunological Societies) Expert Committee on PIDs (primary immunodeficiencies) reported last updated CIDs classification as 29 categories [2]. The most frequent forms of CIDs are associated with genetic defects in development of T and/or B lymphocytes. These defects are classified according to presence or absence T, B and natural killer cells (NK cells) and are categorized as $T^-B^-NK^-$ CID, $T^-B^-NK^+$ CID, $T^-B^+NK^-$ CID and $T^-B^+NK^+$ CID phenotypes [3, 4]. Mutations in well-known genes such as *IL2RG, Artemis, RAG1–2, ADA, CD45, JAK3, IL7RA, CD3 (D,E and Z) XLF/Cernunnos and AK2* result in SCID (severe combined immunodeficiency). For example, while mutations in the *ADA* and *RAG1-2* genes cause $T^-B^-NK^-$ SCID and $T^-B^-NK^+$ SCID phenotypes, defects in the *JAK3* and *IL7RA* genes lead to $T^-B^+NK^-$ SCID and $T^-B^+NK^+$ SCID phenotypes, respectively [4, 5]. According to the newborn screening programs, overall incidence of SCIDs is considered to be as 1/50,000–100,000 live births [4, 6]. Classic SCID patients are characterized by recurrent, severe or opportunistic infections such as *Pneumocystis jiroveci*, *Cytomegalovirus* and *Mycobacterial* infections, chronic diarrhea, recurrent oral thrush, and FTT (failure to thrive). In addition to the classic SCID patients, some of SCID forms which are called Omenn syndrome and leaky/atypical SCID result from hypomorphic mutations in SCID associated genes. Other CIDs with dysfunctional T-lymphocytes are associated with mutations in some other genes such as *PNP, ZAP70, MAGT1* and *DOCK8*. These patients can be presented with different clinical manifestations such as autoimmunity, inflammatory disease, lymphoproliferation, and had increased risk of malignancies [2, 4, 5, 7-10]. In this retrospective study, we report clinical presentations and molecular defects of the 40 CID patients at the Pedi-

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atric Immunology, Erciyes University Medical Faculty, in Kayseri, Turkey as single center experience.

Material and methods

Patients and diagnostic criteria

Between 1994 and 2014 years, 40 patients (females: 15, males: 25) from 31 unrelated families were clinically diagnosed as CID. The diagnosis of CIDs was based on PAGID (Pan American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies) diagnostic criteria [11]. The definitive diagnosis of CIDs was performed with genetic confirmation in the causative genes. The normal values of lymphocyte subsets and immunoglobulins were based on previously described in the medical literature for Turkish children [12, 13]. This retrospective study was approved by Erciyes University Medical Faculty Ethics Committee. Also, signed consent form was obtained from parents.

Immunological investigations

The concentrations of Ig (immunoglobulin) G, IgA and IgM were determined with nephelometric method. T-cell subsets, B-cells and NK-cells were evaluated with flow cytometry using monoclonal antibodies (CD3, CD4, CD8, CD19, CD16/56; Becton Dickinson, San Jose, USA). The proliferation functions of T-cells were evaluated with using PHA (Phytohemagglutinin) as *in vitro*. When the PHA proliferation responses less than 10% of control it was accepted as decreased. Activity of ADA (adenosine deaminase) and PNP (purine nucleoside phosphorylase) enzymes were evaluated by tandem mass spectrometric screening with elevated level of ADA or PNP substrates [14]. The phenotypic classification of CID patients was performed based on absolute count of peripheral T^-, B^- and NK-cells.

Molecular diagnosis

Molecular tests to detect mutations in known CID associated genes were performed in two specialized laboratories on PIDs [(Erasmus MC, in Rotterdam, Netherland and Duke University, Durham City in North Carolina, United States of America (USA)]. According to the clinical manifestations, immunologic evaluations, and phenotypic groups of CID patients, mutations in known CID associated genes were sequenced [15].

Results

Demographic data

Forty patients were included in this retrospective study (Table 1). The ratio of female/male was 3/5. The median age at the onset of clinical manifestations was 2 months (range: 15 days – 15 years) while the median age of diagnosis was 4 months (range: 1 month – 17 years). Parental consanguinity was observed in 32 (80%) of patients. Only 2 patients were referred prior to clinical manifestation due to positive family history (P5, P10). Cumulative, of the 40 CID patients, 26 were lost (including 3 patients who received HSCT) by the end of 2014. The median age of death was 6 months (range, 2.5 months – 18 years). Most of deaths were observed within 12 months of life (20 of 26 lost patients, 77%). Of the 9 survived patients who did not receive HSCT, the median age was 4 years (range: 9 months – 17 years).

Family history

Of the CID 40 patients, 10 (25%) had a positive family history for suspected or confirmed PIDs. Of the 10 patients (females: 3, males: 7), 6 were confirmed by sequencing including 2 siblings with ADA deficiency (P4 and P5). The positive family history group includes 3, 1, 1, 1 and 4 patients with mutation in the *ADA, PNP, XLF/Cernnunos, ZAP70* genes and unknown molecular diagnosis respectively. Patients with positive family history had died sibling or siblings in their family. All patients in this group had history of parental consanguinity. In this group, 6 mothers were carrier for CIDs.

Presenting symptoms

Thirty eight patients displayed clinical symptoms when diagnosed. Only 2 of the CID 40 patients were diagnosed before onset symptoms because of positive family history (P5, P10). However, they had symptoms in their follow-up. The most frequent infection types were respiratory tract infections, chronic diarrhea, and oral thrush. Respiratory tract infections (33/40 cases, 82.5%) were the most common infections with pneumonia (27 cases, 67.75%) and upper respiratory infections (6 cases, 15%). Gastrointestinal infections and oral thrush were observed in 19 (19/40, 47.5%) and in 10 (25%) patients respectively.

Also, multiple infection combinations were observed in follow-up of patients as follows; (10/40, 25%) with combined pneumonia/diarrhea, (17.5%) with combined pneumonia/oral thrush, and 3 (7.5%) with combined diarrhea/oral thrush. Thirteen patients (13/40, 32.5%) had severe infections, including sepsis in 11 (27.5%), fungal infection as abscess formation in 2 (5%) and disseminated tuberculosis in 2 (5%). Disseminated *Cytomegalovirus* infection was observed in 6 (15%) patients. Also, non-infectious symptoms and signs were observed in this study as follows; FTT in 27 (27/40, 67.5%), hepatosplenomegaly in 16 (40%), hypouricemia in 7 (17.5%), autoimmunity in 4 (10%), and pancytopenia in 4 (10%), skin rash in 3 (7.5%), spastic paraparesis in 1 (2.5%), silent brain infarcts in 2 (5%), bird-like face in 2 (5%), intrauterine growth failure in 2 (5%), microcephaly in 2 (5%), congenital nephrotic syndrome in 1 (2.5%), and aorta aneurism in 1 (2.5%). In

Table 1. Clinical characteristics of patients

Patients	Gender	Consanguinity/ Family history	Age of onset	Age of diagnosis	Genetic defect	Infectious complications	Non-infectious complications	Outcome	Age of death
P1	male	+/–	1 mo	3.5 mo	ADA	diarrhea, oral thrush	FTT	alive (IVIG + AMP + ERT)	alive
P2	male	+/+	7 mo	1.5 y	PNP	pneumonia	FTT, spastic paraparesis, hypouricemia	alive (HSCT)	alive
P3	male	+/–	9 mo	2 y	PNP	pneumonia, diarrhea	hypouricemia	died	1.5 y
P4	male	+/+	1 mo	2 mo	ADA	pneumonia, diarrhea, sepsis	hypouricemia	died	8 mo
P5	female	+/+	15 days	1 mo	ADA	oral thrush, pneumonia	hypouricemia	died	2.5 mo
P6	male	+/–	1.5 mo	3 mo	ADA	diarrhea, pneumonia, sepsis	hypouricemia, hepatomegaly	died	5 mo
P7	male	+/+	1.5 mo	4 mo	unknown	oral thrush, pneumonia	FTT, hepatosplenomegaly	died	5 mo
P8	female	+/–	2 mo	3 mo	unknown	pneumonia, diarrhea, sepsis	FTT, hepatomegaly	died	4 mo
P9	male	+/–	2 mo	3 mo	ADA	pneumonia, diarrhea, sepsis	FTT, hypouricemia	died	6 mo
P10	female	+/+	1.5 mo	2 mo	ADA	oral thrush, CMV pneumonia	FTT, hepatomegaly, hypouricemia	alive (HSCT)	alive
P11	male	–/–	2 mo	5 mo	artemis	oral thrush, pneumonia, TBCitis	FTT	alive (HSCT)	alive
P12	female	–/–	2 mo	3 mo	unknown	diarrhea, sepsis	FTT, microcephaly	died	5 mo
P13	female	+/–	10 y	15 y	RAG1	URTI, pyoderma gangrenosm	AHA, pancytopenia, splenomegaly	alive (IVIG + AMP)	alive
P14	male	+/–	2 mo	3 mo	unknown	pneumonia, CMV, sepsis	low set ears, pancytopenia	died	4 mo
P15	female	–/–	1 mo	2 mo	unknown	diarrhea, oral thrush, sepsis	FTT, hepatomegaly	died	4 mo
P16	female	+/+	1.5 mo	3 mo	unknown	diarrhea, sepsis	hepatomegaly, skin rash	died	5 mo
P17	female	+/–	15 days	2 mo	unknown	oral thrush, pneumonia	FTT	died	4 mo
P18	male	+/–	1 mo	3 mo	unknown	pneumonia	hepatomegaly	died	5 mo
P19	male	+/+	3 mo	7 mo	XLF	oral thrush, pneumonia, diarrhea, fungal abscess	FTT, BLF, IUGR, NHL, microcephaly, pancytopenia	alive (IVIG + AMP)	alive
P20	female	+/–	10 mo	3.5 y	XLF	URTI, diarrhea	FTT, BLF, IUGR, microcephaly, pancytopenia	alive (IVIG + AMP)	alive
P21	female	–/–	1 mo	2.5 mo	unknown	oral thrush, pneumonia	FTT, hepatomegaly	died	5 mo
P22	male	+/–	1 mo	3.5 mo	unknown	pneumonia	FTT	alive (HSCT)	alive
P23	male	+/–	10 mo	1.5 y	unknown	URTI, diarrhea	FTT, AHA	alive (IVIG + AMP)	alive

Table 1. Cont.

Patients	Gender	Consanguinity/ Family history	Age of onset	Age of diagnosis	Genetic defect	Infectious complications	Non-infectious complications	Outcome	Age of death
P24	male	+/+	2 mo	4 mo	unknown	pneumonia	FTT, hepatomegaly	alive (IVIG + AMP)	alive
P25	female	+/–	5 mo	7 mo	unknown	pneumonia, diarrhea	FTT	died	8 mo
P26	female	+/–	4 mo	12 mo	RAG1	diarrhea, CMV, dermatitis	FTT, hepatosplenomegaly	died (HSCT)	1.5 y
P27	male	+/–	3 mo	5 mo	RAG1	pneumonia, sepsis	FTT	died (HSCT)	7 mo
P28	female	+/–	1.5 mo	5 mo	RAG1	pneumonia, diarrhea, lung tbc	FTT, hepatomegaly	died	7 mo
P29	male	+/–	2 mo	4 mo	JAK3	pneumonia, diarrhea, sepsis	FTT, HLH	died	6 mo
P30	female	+/–	2 mo	3.5 mo	unknown	pneumonia	FTT, skin rash	died	6 mo
P31	male	+/+	3 mo	8 mo	ZAP70	HSV, CMV pneumonia, fungal abscess	AHA, SBI, CNS, NHL	died	15 mo
P32	male	+/–	6 mo	1.5 y	ZAP70	diarrhea, URTI	hepatosplenomegaly	died	1.5 y
P33	female	+/+	3 mo	5 mo	unknown	pneumonia	FTT, hepatomegaly	died	7 mo
P34	male	–/–	3 mo	5 mo	unknown	pneumonia	FTT	died	8 mo
P35	male	–/–	4 mo	6 mo	unknown	oral thrush	FTT, hepatomegaly	alive (HSCT)	alive
P36	male	–/–	2 mo	3 mo	IL2RD	pneumonia, CMV, sepsis	FTT, HLH	died	5 mo
P37	male	–/–	4 mo	9 mo	unknown	URTI, diarrhea	FTT	alive (IVIG + AMP)	alive
P38	male	+/–	2 y	9 y	DOCK8	otitis media	aorta aneurism, viral dermatitis	alive (IVIG + AMP)	alive
P39	male	+/–	11 mo	2 y	ZAP70	pneumonia, lung TBC, diarrhea	FTT, SBI, hepatomegaly	alive (IVIG + AMP)	alive
P40	male	+/–	15 y	17 y	MAGT1	URTI, CMV, EBV	AHA, HL, GBS, AH	died (HSCT)	18 y

AH – autoimmune hepatitis; AHA – autoimmune hemolytic anemia; AMP – antimicrobial prophylaxis; BLF – bird like face; CMV – Cytomegalovirus; CNS – congenital nephrotic syndrome; ERT – enzyme replacement treatment; FTT – failure to thrive, GBS – Guillain-Barré syndrome; HSCT – hematopoietic stem cell transplantation; HL – Hodgkin lymphoma; HLH – hemophagocytic lymphohistiocytosis; HSV – Herpes simplex virus; IVIG – intravenous immunoglobulin; IUGR – intrauterine growth retardation; mo – month; NHL – non-Hodgkin lymphoma; URTI – upper respiratory tract infections; y – year

addition to these data, 3 patients had lymphoproliferative malignancies. Two patients had non-Hodgkin lymphoma (P19, P31) and 1 had Hodgkin lymphoma (P40). Of 27 patients (27/40, 67.5%) with FTT, 13 (32.5%), 7 (17.5%) and 4 (10%) had anemia, hypoalbuminemia and combined anemia/hypoalbuminemia respectively. Of the 40 CID patients, 14 (14/40, 35%) had BCG vaccination prior to diagnosis, 21 patients did not received BCG vaccination, and remaining 5 patients were unclear for BCG vaccination. Three patients had BCG complications, including lung TBC in 2 (P28, P39) and BCG it is in 1 (P11).

Immunological characteristics

Immunological data were showed in Table 2. Thirty six (90%) patients had lymphopenia with decreased absolute lymphocyte counts (ALC) (for < 2 years; < 3000/mm^3, for > 2 years; < 1500/mm^3) at the time of diagnosis. Only 4 patients (10%) had normal lymphocyte counts (P31, P32, P39 and P40). Of the CID 40 patients, 36 (90%) and 31 (77.5%) had significantly lower IgA and IgM levels respectively. The IgG levels were significantly lower (< 300 md/dl) in 21 (52.5%) patients. Some patients had normal or elevated serum IgG concentrations may reflect maternal IG levels at diagnosis of CID. Another 5 patients had also normal or elevated IgG levels with mutation in the *RAG1* gene in 1 (P13), ZAP70 deficiency in 2 (P31, P32), DOCK8 deficiency in 1 (P38), and mutation in the *MAGT1* gene in 1 (P40). Serum IgE levels were elevated in 3 patients with mutation in the *JAK3* (P29), *RAG1* (P26) and *DOCK8* (P38) genes, respectively (data not shown). The lymphocyte proliferative responses with PHA stimulation were absent or significantly decreased in all presented patients. Patients dived to 4 phenotypic groups based on T$^-$, B$^-$ and NK-cells as follows; T$^-$B$^-$NK$^-$ CID, T$^-$B$^-$NK$^+$ CID, T$^-$B$^+$NK$^-$ CID and T$^-$B$^+$NK$^+$ CID. Of the 40 CID patients, 14, 15, 9 and 2 had T$^-$B$^-$NK$^-$ CID, T$^-$B$^-$NK$^+$ CID, T$^-$B$^+$NK$^-$ CID and T$^-$B$^+$NK$^+$ CID phenotypes respectively. Of the 14 T$^-$B$^-$NK$^-$ CIDs, 6, 2 (siblings), 1 and 4 had a mutation in the *ADA, PNP, Artemis*, *RAG1* genes and unknown genetic diagnosis respectively. Of the 15 T$^-$B$^-$NK$^+$ CIDs, 3, 2 (siblings) and 10 had a mutation in the *RAG1*, *XLF/Cernunnos* genes and unknown genetic diagnosis respectively. Of the 9 T$^-$B$^+$NK$^-$ CIDs, 2 siblings, 1, 1 and 5 had a mutation in the *ZAP70, IL2RG, DOCK8* and unknown genetic diagnosis respectively. Of the 2 T$^-$B$^+$NK$^+$ CIDs, 2 had a mutation in the *MAGT1* and *ZAP70* genes respectively.

Outcomes and hematopoietic stem cell transplantation

Overall survival of patients was estimated as 35% (14 of the CID 40 patients) in this study. Of 32 patients who did not receive HSCT, 17 died soon after diagnosis (range: 15 days – 3 months) whilst 3 survived longer than 6 months with PNP deficiency in 1 (P3) and ZAP70 deficiency in 2 siblings (P31, P32). The cause of deaths was severe infections in all patients as follows; sepsis in 10, severe pneumonia in 4, and disseminated BCG in 1.

Of the CID 40 patients, only 8 (8/40, 20%) received HSCT by the end of 2014. Of the 8 transplanted patients, 6 received reduced intensity conditioning (RIC) regimen (anti-thymocyte globulin-based protocols with rituximab, fludarabine, thiotepa, etoposide and melphalan) and 2 (P26, P40) received myeloablative conditioning (MAT) regimen (busulfan plus cyclophosphamide based protocols). Two transplanted patients (P1, P40) experienced graft versus host disease (GVHD). GVHD was observed in the gut and skin as Stage 4 and Stage 3 in P1 and P40 respectively. Procedure was successful in 5 (P2, P10, P11, P22 and P35) patients, leading to full immune reconstitution and survival in 4 patients, IVIG replacement is needed in 1 patient. Two patients (P26, P27) died due to infectious complications after then post-transplant 6th month and 1 patient (P40) died due to early post-transplant complication (intracranial hemorrhage).

Discussion

CIDs should be thought in children who experienced early onset severe infections, recurrent oral thrush, manifestations of immune dysregulation, malignancy, chronic diarrhea, FTT, and BCG infections [16]. The SCID patients present with severe infections in the first months of life. In this study, the types of infections were consistent with previously published case series in the medical literature with the most common respiratory tract infections [5, 17]. When considering data associated with CIDs, the onset age of symptoms in patients with CID can be more heterogeneous. Especially, the onset of symptoms in some CID associated genes such as *IL2RG, JAK3, RAG1/2, ADA* defects can be significant heterogeneous from infancy to adulthood [17, 18]. In this study, we observed also symptom onset heterogeneities in the CID patients. The median age at the onset of infections was 2 months. However, the onset of symptoms was observed in the 4 CID patients after the first year (P13, P20, P38 and P40). In the presented study, the median age of diagnosis was 4 months (range: 15 days – 15 years). For the median age of diagnosis, when we compared our results with previously published studies, our data were consistent with 5 months in 44 patients in China, 6.2 months 30 patients in Greece, and 4.6 months in patients in France [17, 19, 20]. Severe infections such as pneumonia, sepsis and *Cytomegalovirus* infections are important mortality and morbidity reasons in the CID patients. In addition to the severe infections, FTT is another significant common symptom in patients with CID patients, especially in the SCID patients. Of the 40 CID patients, 27 (67.5%) had FTT. Although the ratio of FTT was higher than in some

Table 2. Immunological and genetic data of patients

Patients	Genetic defect	Mutations	ALC (mm^3)	CD3 (mm^3)	CD19 (mm^3)	NK (mm^3)	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)	
			$T^-B^-NK^-$							
P1	ADA	del [GAAGA] c.955-959	210	102	48	64	342	6.2	15.3	
P2	PNP	(c.700 C>T, p.R234X)	320	110	150	58	342	17.6	66.9	
P3	PNP	(c.700 C>T, p.R234X)	380	206	80	98	450	12.5	34	
P4	ADA	(c.736C>T, p.Q246X)	230	60	96	75	310	5.3	16.9	
P5	ADA	(c.736C>T, p.Q246X)	520	286	150	88	420	6.1	17.3	
P6	ADA	(c.736C>T, p.Q246X)	430	214	78	144	110	6.6	5.6	
P7	unknown	unknown	520	352	94	72	230	6.2	12.8	
P8	unknown	unknown	410	240	89	80	324	6.1	17.3	
P9	ADA	(c.736C>T, p.Q246X)	350	213	96	42	72.7	4.7	6.6	
P10	ADA	(c.736C>T, p.Q246X)	230	40	112	78	254	6.2	7.9	
P11	artemis	biallelic homozygous deletion in the first 3 exons	120	34	50	38	156	6.7	9.4	
P12	unknown	unknown	530	218	180	130	150	29	40	
P13	RAG1	(c.2290C>T, p.Arg764Cys)	750	412	184	158	2900	903	71	
P14	unknown	unknown	100	20	18	60	39	5.7	17	**T^-B^- CIDs**
			$T^-B^-NK^+$							
P15	unknown	unknown	840	468	89	262	148	8.1	12.7	
P16	unknown	unknown	480	203	48	230	130	5.8	7.8	
P17	unknown	unknown	1800	597	216	984	252	6.7	18.2	
P18	unknown	unknown	600	197	98	312	253	7.8	4.1	
P19	XLF	(c.622C>T, p.R178X)	1950	543	201	1210	784	60	190	
P20	XLF	(c.622C>T, p.R178X)	1070	300	253	526	134	6.2	138	
P21	unknown	unknown	700	263	98	434	160	13	8	
P22	unknown	unknown	950	321	198	428	168	8.6	10.2	
P23	unknown	unknown	690	112	248	341	377	6.7	34	
P24	unknown	unknown	780	284	279	288	378	8.2	24	
P25	unknown	unknown	1950	510	253	1183	145	6.3	22.6	
P26	RAG1	(c.1229 G>A, p.Arg410Gln)	1770	516	378	866	134	6.5	18	
P27	RAG1	(c.1767C>G, p.Tyr589Stp)	440	120	58	243	298	6.1	17.5	
P28	RAG1	(c.2209C>T, p.Arg737Cys)	630	125	137	371	210	6.4	17.1	
P29	JAK3	c.2324G>A	1660	598	297	775	320	24	106	

Table 2. Cont.

Patients	Genetic defect	Mutations	ALC (mm^3)	CD3 (mm^3)	CD19 (mm^3)	NK (mm^3)	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)	
			$T^-B^+NK^-$							
P30	unknown	unknown	920	252	580	88	137	6.1	20.8	
P31	ZAP70	(c.1193C>T, p.IIe398Ser)	4700	1680	2870	142	1680	38	114	
P32	ZAP70	(c.1193C>T, p.IIe398Ser)	3200	1425	1626	139	890	27	81	
P33	unknown	unknown	800	137	547	117	416	6.2	28	
P34	unknown	unknown	850	171	552	124	154	4.2	12.7	
P35	unknown	unknown	1210	160	743	118	69	7	6.5	**T^-B^+CIDs**
P36	IL2RD	c.595-1G>T	890	240	524	135	145	6.5	17.3	
P37	unknown	unknown	1060	214	712	132	318	25	43	
P38	DOCK8	large homozygous deletion between exon 18 and 48	1200	720	250	180	1600	120	79	
			$T^-B^+NK^+$							
P39	ZAP70	(c.1504_1505insGC, p.Pro502ArgfsX43)	3460	472	2310	696	1060	152	210	
P40	MAGT1	(c.555dup, p.Tyr186Ilefs*2)	1560	502	898	256	880	50	52	

ADA – adenosine deaminase; ALC – absolute lymphocyte count; CIDs – combined immunodeficiencies; DOCK8 – dedicator of cytokinesis 8; IgA – immunoglobulin A; IgG – immunoglobulin G; IgM – immunoglobulin M; JAK3 – Janus kinase 3; MAGT1 – magnesium transporter 1; NK – natural killer cell; PNP – purine nucleoside phosphorylase; RAG1 – recombination-activating gene 1; ZAP70 – zeta-chain associated protein 70 kDa; XLF – XRCC4-like factor

published studies with approximately 45% in Serbia/Montenegro and 38.64% in China, lower than in other reported studies with 88% that in United Kingdom and 100% in Egypt [5, 17, 21]. Also, BCG infections either localized or disseminated after vaccination are important mortality and morbidity reasons in patients with CID patients, especially in the SCID patients [8, 17]. In this study, 14 had BCG vaccination prior to diagnosis. Of the 14 vaccinated patients, 3 (21.3%) had BCG infections (2 had lung BCG infection as disseminated and 1 had localized infection). This ratio of BCG infections was significantly lower than that in Brazil with 65% and China with 41.18% [8, 17]. These differences in the BCG infections may result from the preventive health care policies in these countries. Also, lymphopenia is one of the most important findings in patients with CID, especially in the SCID patients [5, 7, 17]. In the presented study, of 40 patients, 36 (90%) had lymphopenia. Lymphopenia was reported as 90% by Gossage *et al.* [22] which was consistent with our data. Also, lymphopenia was reported as approximately 60% in the CID patients in a previously published study in Turkey. In addition to lymphopenia, some patients with CID may have normal or elevated ALC [1]. In this study, only 4 (P31, P32, P39 and P40) patients had normal ALC according to the aged normal values [12]. Excluding detection of immunoglobulins, subgroups of lymphocytes and lymphocyte proliferative responses other specific immunological tests such as sequencing have not been performed in Turkey for all known CID genes. So, we are having difficulty in diagnosis of patients with CID especially in patients with normal or elevated ALC in Turkey.

In this study, sequencing showed interesting genetic results. Although the X linked CIDs were reported as a most common group with approximately 45% among the CID groups, only 1 patient (2.5%) had a mutation the IL2GR gene in the presented study [20, 23, 24]. As the nearest ratio of X linked patients in the CID patients was reported as 14% in Serbia and Montenegro and approximately 9% in Greece [5, 19].

In the 29 T^-B^- CID phenotypic patients, 6 (6/40, 15%), 4 (10%), 2 (5%), 2 (5%, siblings), 1 (2.5%), 1 (2.5%) and 13 (32.5%) had a mutation in the *ADA, RAG1, PNP, XLF/Cernnunos*, *Artemis, JAK3* genes and 13 unknown genetic defect respectively. The ADA deficiency was reported as 9.4 % in the SCID patients by Stephan *et al.* [20]. Also, Buckley *et al.* [23] reported ADA deficiency as 14.8% which was consistent with our results. Mutations in the *RAG1/2* genes represent approximately 6-10% of all CID and 25% of atypical SCID cases [1]. In this study, we had 4 patients (4/40, 10%) with RAG1 deficiency. Of the 4 RAG1 defects, 1 (P13) had atypical presentation with pyoderma gangrenosum as leaky/atypical SCID.

Of the 11 T^-B^+ CID patients, 3 (3/40, 7.5%), 1 (2.5%), 1 (2.5%), 1 (2.5%) and 5 had a mutation in the *ZAP70,*

IL2RD, DOCK8, MAGT1 genes and unknown genetic defect respectively. Three patients (P31, P32 and P39) had ZAP70 deficiency in this study. Two of them (P31, P39) had silent brain infarcts as a common feature [25]. All patients with ZAP70 deficiency had SCID presentation with severe infection in their first year of life. The DOCK8 deficiency was found in 1 patient (P38) in the presented study. The patient presented with recurrent dermatitis and had a giant aortic aneurism.

In this study, symptoms were observed (P13, P38 and P40) after age of 1 year in 3 patients with mutations in the *RAG1, DOCK8* and *MAGT1* genes respectively. These patients did not meet the SCID criteria. MAGT1 deficiency has been reported in 10 patients in the medical literature so far. Our patient with MAGT1 deficiency (P40, called as XMEN syndrome) presented with severe autoimmunity and also had a novel mutation [26].

The overall risk of developing malignancy in children with PIDs is 4-25% [27]. Of the 40 CID patients, 3 had (3/40, 7.5%) malignancies. Two patients had (P19 and P31) diffuse large B cell lymphoma in the brain and in the liver in patients with XLF and ZAP70 deficiency respectively [28]. Another malignancy, Hodgkin lymphoma was observed in the MAGT1 deficient patient (P40) in this study [29].

Irrespective of genetic diagnosis, HSCT is the main curative treatment in patients with SCID. However, only 8 patients received HSCT in this study. Majority of patients died due to severe infections prior to HSCT. In contrast to our data, in France, of the 117 SCID patients, 95 received HSCT between 1970 and 1991, 166 patients received HSCT in almost last 28 years by the end of 2010 in USA [7, 30]. Of the 8 transplanted patients, 5 survived by the end of 2014. In this study, although the transplantation rate was much more lower than in France and USA [7, 30], higher than in China [17]. Also, although survival rate much lower than in France and USA [7, 30] higher than in China [17]. Most SCID patients cannot be diagnosed early enough and the most patients miss the window opportunity of transplantation prior to the development of severe infection. We can speculate that delayed diagnosis and active infections before transplantation can be principle reasons in lower rate of transplantation and survival in Turkey as in observed other developing countries.

In conclusion, there is no national registry center in Turkey for CIDs yet. Also, we can say that CIDs are more common in Turkey than in the developed countries due to high rate of consanguinity [1]. With this study, it has been confirmed once again CID is a pediatric emergency condition with high mortality rate (65%) in Turkey. Also, we can conclude that the patients presented with severe infections onset in the first months of life have to be examined for CIDs. In addition, leaky/atypical SCID should be considered in patients presented with atypical and late-onset of symptoms such as observed in P13, P38 and P40. In recent years, patients with CID have good opportunities with improvement in supportive care and conditioning protocols for HSCT as curative treatment. In these patients, diagnosis can be performed at the early age, infective mortalities/morbidities can be minimized and HSCT can be performed at the early ages. For early diagnosis, the awareness programs should be performed in Turkey for physicians. Also, new diagnostic methods such as whole exome/genome sequencing (WES/WGS) should be used in order to enhance the quality of diagnosis in Turkey. Also, population-based newborn screening programs with tandem mass spectrometry and/or T-cell receptor excision circles (TRECs) should be considered as general health policy for patients with CIDs and national practical treatment protocols should be established in Turkey.

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