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.

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 led 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 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.
atrie 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, 4 and 2 patients with mutation in the ADA, PNP, XL/CFMUNOS, 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%).
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, diarreha, 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, TBCTis | 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, pacytopenia   | 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       |
| 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           | pneumonia, diarrhea    | CMV, dermatitis              | died (HSCT) | 1.5 y        |
| P27      | male   | +/-                           | 3 mo         | 5 mo            | RAG1           | pneumonia, sepsis      | FTT                           | died    | 7 mo         |
| P28      | female | +/-                           | 1.5 mo       | 5 mo            | RAG1           | pneumonia, diarrhea    | Lung tbc                      | died    | 7 mo         |
| P29      | male   | +/-                           | 2 mo         | 4 mo            | JAK3           | pneumonia, diarrhea    | sepsis                        | died    | 7 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                           | 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, diarhrea | FTT, SBI, EBV, NHL | 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 receive 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/mm², for > 2 years; < 1500/mm²) 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 m/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 were unclear for BCG vaccination. Three patients had BCG complications, including lung TBC in 2 (P28, P39) and BCG it is in 1 (P11).

**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 complications (intracranial hemorrhage).

**Discussion**

CIDTs 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 CIDTs, 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) |
|----------|----------------|------------------------------------|--------------|--------------|--------------|-------------|-------------|-------------|-------------|
| 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          |
| 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         |
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, PNP, XL7F, 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 X-Men 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 compared to other developed 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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