Immunological profile of patients with skeletal dysplasia and disproportionate short stature

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Introduction
Skeletal dysplasia is a clinically diverse and genetically heterogeneous group of disorders affecting skeletal development among which several phenotypes could be associated with manifestations of immunodeficiency (ID). In many cases, these manifestations may be attributed to the original anatomical and physiological derangement caused by the genetic abnormality, but in other cases, there could be a concurrent primary defect in the immune system. These cases are called to have syndromic ID.

Aim
This study aimed to unravel syndromic ID among cases with skeletal dysplasias and disproportionate short stature and to specify a set of laboratory investigations that could be used as a panel for initial evaluation of suspected cases.

Patients and methods
This is an observational case–control single-center study, in which 25 patients with disproportionate dwarfism owing to skeletal dysplasia, along with 20 healthy participants matched for age and sex, were included. Both patients and controls were categorized into two age groups: from 0 to 3 years and from more than 3 to 15 years.

Results
Results of the study revealed the presence of nine cases of syndromic ID in the studied patients, that is, five in the younger age group (from 0 to 3 years) and four in the older age group (from >3 to 15 years), with a prevalence rate that equals 36% among the studied population.

Conclusion
The present work emphasizes the importance of screening for syndromic IDs in providing proper genetic counseling for patients and optimizing clinical care given by providing early and appropriate treatment. The implementation of flow cytometric measurement of lymphocyte subsets and immunoglobulin (Ig) quantification in cases suspected of having syndromic ID have shown to be useful as an initial panel for immunological evaluation of patients.

Keywords:
immune defects, primary immunodeficiency, skeletal dysplasia, syndromic immunodeficiency

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Introduction

Skeletal dysplasia is a term that is used to describe generalized disorders of cartilage and bone (Krakow and Rimoin, 2010). It comprises a heterogeneous group of more than 450 disorders commonly associated with orthopedic complications and variable degrees of dwarfism or short stature (Mortier *et al.*, 2019). Although each skeletal dysplasia is relatively rare, the overall incidence of these disorders is almost one case per 4000–5000 births (Martin *et al.*, 2011). The disorders occur owing to the inheritance of mutated genes, and most of them are diagnosed in children Chaudhary and Bano, 2012.

Syndromic ID can be defined as primary defects in the immune system components that accompany phenotypic abnormalities or laboratory findings implicating other system involvement. In these cases, the immune defect is usually not the presenting manifestation as it could be discovered later in the course of illness. It occasionally occurs only in some patients unlike the primary immunodeficiency (PID) syndromes where the immunological defects and their subsequent symptoms and complications are both fundamental and consistent in most patients (Ming and Stiehm, 2008).

Syndromic ID may be associated with defects in various processes including chromosomal abnormalities, teratogenic disorders, metabolic

abnormalities, or faulty embryogenesis (Kersseboom *et al.*, 2011). The importance of detecting immune defects in patients with genetic disorders in general, and skeletal dysplasia patients in particular, lies in accurate diagnosis by combining the phenotypic characteristics with the type of immune defect present and determining whether their assembly would fit into a recognizable syndrome or not. More importantly, the detection of primary defect in the immune system would optimize clinical care by giving the patient appropriate treatment, especially in cases with skeletal dysplasia where immunodeficiency (ID) symptoms like repeated respiratory tract infections are often attributed as being secondary to the anatomical malformations present (Helwa, 2018).

The immune defect that has been described accompanying most skeletal dysplasias is mainly a defect in the adaptive immune response either B-cell defect (humoral), T-cell defect (cell mediated) or a combination of both. Phagocytic cell defects have been also described in some skeletal dysplasia syndromes (Ming and Stiehm, 2008). There are many examples of ID syndromes associated with skeletal dysplasia such as short-limb skeletal dysplasia with combined immune deficiency, McDermid syndrome, kyphomelic dysplasia, spondylo-mesomelic-acrodysplasia, cartilage-hair hypoplasia, short-limb skeletal dysplasia with humoral immune defect, Schimke immuno-osseous dysplasia, Roifman syndrome, SPENCDI syndrome, and Kenny-Caffey syndrome (Ming and Graham, 2014).

According to the latest report of the International Union of Immunological Societies PID expert committee, PIDs have been classified into ten major groups according to the immunologic defect in different components (Tangye *et al.*, 2020). The second category namely 'combined immune deficiencies with associated or syndromic features' includes immuno-osseous dysplasias; however, other skeletal dysplasia syndromes belong to other categories like SPENCDI syndrome, which lie in the category of 'diseases of immune dysregulation.'

Contrary to the prevailing belief, PIDs are not rare diseases. In an epidemiological review that was based on data obtained from registries and two epidemiologic surveys, Bousfiha *et al.* (2013) estimated that 1/1200 people worldwide are potentially living with a PID. PIDs are remarkably underreported in developing countries owing to several factors like attribution of infections to malnutrition and diagnostic difficulties in limited resource settings with shortage of needed investigations (Galal *et al.*, 2016). Moreover, bearing in mind that most of skeletal dysplasia cases include anatomic defect which is largely associated with recurrent infections, the lack of familiarity with these disorders and lack of guidance regarding the appropriate recruitment of immunological investigations also represent major reasons for the delay in diagnosis (De Vries, 2006).

In an attempt to assist early diagnosis of PIDs, the 'National Primary Immunodeficiency Resource Center' adopted a list of '10 warning signs of PID' (Arkwright and Gennery, 2011). Regarding laboratory investigations, although it is logical reasoning and further cost-effective to acknowledge that 'the usage of laboratory tests in evaluating the immune system should not follow a shotgun approach but rather should be a focused evaluation using specific testing in an orderly process based on the clinical history' (Oliveira and Fleisher, 2010), the implementation of this wise approach is very difficult when dealing with PIDs as a part of syndromic IDs in which other organ systems could be primarily affected. In these cases, the pattern of immune deficiency may not have been clearly determined and the extent to which recurrent infections are a concern vary within individuals with the same syndrome (O'brien *et al.*, 2017). That is why we used a fixed set of laboratory tests with rapid turnaround time for evaluating suspected patients rather than adopting highly sophisticated algorithm of immune evaluation laboratory tests.

Quantification of T-cell receptor excision circles (TRECs) as a biomarker for normal T-cell development and its role in identifying naïve T-cell population recently generated from thymus has been used in several clinical applications in the field of PIDs starting from its adoption in newborn screening program for severe combined ID in the United States (Puck, 2019) to its utilization in the detection of T-cell lymphopenia in a wide spectrum of various other diseases (Kwan *et al.*, 2013). TRECs are formed as byproducts from T-cell antigen receptor (TCR) gene rearrangements during T-cell development in the thymus. It is produced late in maturation and is found in 70% of all thymocytes that express αβ-TCR (Puck, 2012). Flow cytometric quantification of recent thymic emigrants, through quantifying $CD45RA^+$ $CD27^+$ and $CD45RA^+$ $CD31^+$ T cells, has been proposed as a comparable alternative to TREC assay for assessment of naïve T-cell populations, but still it has not been established as a routine diagnostic test demanding more research in this matter (Ravkov *et al.*, 2017; Adams *et al.*, 2018).

Patients and methods

Patients

This is a case–control study that was conducted in the period from November 2015 to August 2017. It included 25 patients with disproportionate dwarfism owing to skeletal dysplasia. In addition, 20 healthy participants, matched for age and sex, were included as a control group. Both patients and controls were categorized into two age groups: from 0 to 3 years and from more than 3 to 15 years. Informed consent from the parents or guardians of patients included in our study was obtained according to the rules of the Medical Ethics Committees of both the National Research Center (ethical no. 15132) and Ain Shams University. The inclusion criteria for patients enrolled initially included presence of skeletal dysplasia with disproportionate short stature whether short limb and/or short trunk conditions, clinical manifestations suggesting the presence of immunological defect corresponding with warning signs (Supplementary Material 1), and patients are within age range from birth to 15 years old. However, we have encountered two patients through the study for whom laboratory immunological evaluation was incidentally done despite the absence of warning signs and revealed abnormal results which drove us to condone this criterion as a prerequisite for accepting inclusion of subsequent patients.

Methods

All the patients were subjected to clinical evaluation including medical history, pedigree analysis to three generations taking in consideration consanguinity, and history of symptoms of immunologic deficiency such as recurrent chest infections. Moreover, anthropometric measurements and radiographic evaluation were performed.

Sampling

Overall, 5 ml of peripheral venous blood samples was aseptically withdrawn from each patient and control and was divided as follows: 2.5 ml was collected in a sterile plain tube. Blood was left to clot then centrifuged at 4000–5000 rpm for 10 min, then serum was separated and used for measurement of immunoglobulins (Igs). The other 2.5 ml was divided into two EDTA tubes at a final concentration of 1.5 mg/ml as follows: 1 ml of blood taken for complete blood count. The remaining amount was used for flow cytometric analysis which was done within 24 h of collection as well as DNA extraction, which was done simultaneously or on refrigerated samples stored at 2–8°C within 1 week of collection.

Determination of immunoglobulins (IgA, IgM, and IgG)

Measurement of serum Ig was performed by the method of immunonephelometry (Aksu *et al.*, 2006) using Minineph (The Binding Site Ltd, Birmingham, UK), as the manufacturer's protocol.

Flow cytometric assessment of the lymphocyte subsets

Lymphocyte subset immunophenotyping was done using flow cytometry (Becton, Dickinson and Company, 1 Becton Drive, Franklin Lakes, NJ 07417-1880.). Analysis of lymphocyte surface markers was done on lysed whole blood using CD3 FITC labeled MoAbs for T lymphocytes, $CD16^{+}$ CD56 labeled with PE for NK cells, and FITC labelled CD19 for B lymphocytes. CD4 FITC and CD8 FITC (Becton, Dickinson and Company, BD Biosciences 2350 Qume Drive San Jose, CA 95131, USA) were used for further quantitation of T helper and T cytotoxic lymphocyte populations, respectively, in most of patient samples and all control samples. The assay was done on fresh samples within 24 h of collection with samples kept in 2–8°C when transported.

Real-time PCR for T-cell receptor excision circle measurement

Total DNA was extracted from blood samples using a DNA extraction kit (QIAamp) (QIAGEN Incorporation, 28159 Avenue, Stanford Valencia, CA91355, USA)

The assay was run on 7500 Fast Real-Time PCR (Applied Biosystems, 850 Lincoln Centre Drive | Foster City, CA 94404 USA) using the following sequences for primers and probes:

TRECs: Forward primer 5′-CAC ATC CCT TTC AAC CAT GCT-3'. Reverse primer 5'-GCC AGC TGC AGG GTT TAG G-3'. Probe FAM-5'-ACA CCT CTG GTT TTT GTA AAG GTG CCC ACT-TAMRA-3′.

β-actin: forward primer 5′-TCA CCC ACA CTG TGC CCA TCT ACG AG-3'. Reverse primer 5'-CAG CGA ACC GCT CAT TGC CAT GG-3' Probe FAM-5'-ATG CCC TCC CCC ATG CCA TCC TGC GT-TAMRA-3′.

RT-PCR was carried out using TaqMan Universal PCR Master Mix II. Overall, 10 μl of PCR reaction mix was added to 5-μl DNA samples, 1-μl forward primer (Applied Biosystems, USA), 1-μl reverse primer (Applied Biosystems, USA), 1-μl Taqman TAMRA probe, and 2-μl H2O in a sterile 48-well PCR plate using real-time cycler conditions of initial activation stage at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 15 s, and a combined primer/probe annealing and elongation

at 60°C for 1 min TRECs, and β-actin copy number has been obtained by extrapolating the respective sample quantities from the standard curve obtained by serial dilutions of human genomic DNA (Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711, USA). The initial copy numbers of both TRECs and β-actin was calculated from the following equation:

Copies of gene of interest = mass of gDNA/mass of haploid genome

The number of TRECs in each sample was calculated per μl of extracted DNA. β-actin copy number was used to judge successful amplification of each sample.

Statistical analysis

Data were collected, revised, verified, then edited on a personal computer. Data were then analyzed using IBM SPSS version 16.0 program. Statistical tests used in the study were as follows: description of qualitative data was carried out by using frequency and percentage, and description of quantitative data was carried out by using minimum, maximum, mean, and SD for normally distributed results or median, range, 5th percentile, and 95th percentile for skewed results (Sottini *et al.*, 2010). Comparison between two quantitative, nonparametric variables was carried out by using Mann–Whitney's *U* test. Comparison between normally distributed qualitative variables was carried out by using χ^2 test. Comparison between two small size independent groups regarding the categorized data was carried out using Fisher's exact test.

Results

Our results showed that patients (n = 25) experienced one or more of the following manifestations (warning signs): two or more pneumonias within 1 year (in 24% of patients); need for intravenous antibiotics to clear infections (4%); four or more new ear infections within 1 year (4%); recurrent, deep skin or organ abscesses (4%); and family history of PID (4%). Table 1 indicates their frequency in relation to each age group.

In a trial to assess the effect of age on the studied laboratory parameters, comparisons were held between the median values of examined laboratory parameters among controls of both age groups, as shown in Table 2. Comparisons were done only in control groups to be able to judge etiology of variations in the studied laboratory parameters based on the effect of age only in healthy participants without the possible interfering effect of ID that could be present in patients.

As previously reported, patients were diagnosed as having humoral ID when having diseases associated with impaired antibody production which may be caused by either a molecular defect intrinsic to B cells or a failure of interactions between B and T cells while cellular immunity is mostly intact (Notarangelo *et al.*, 2004). Combined ID is diagnosed in patients where immune defects were found affecting both cellular and humoral immunity (Roifman *et al.*, 2012). Based on the range obtained from control population of each age group [Table 2] and the clinical manifestations seen on suspected patients, nine of the 25 patients examined were diagnosed of having syndromic ID, that is, five in the younger age group (from 0 to 3 years) and four in the older age group (from >3 to 15 years), with a prevalence rate that equals 36% among the studied population. Four patients had findings indicative of humoral ID and five showed a picture suggestive of combined ID. Among

Table 1 Clinical characteristics among study groups

Variables	Groups		Result	*P*
Age (*n*/median) (min-max)	Group 1 (0-3 years)	Controls	9/2 (0.11-3)	0.093
		Patients	14/1.04 (0.06-3)	NS
	Group 2 (>3-15 years)	Controls	11/7 (3.33-15)	0.411
		Patients	11/6.17 (3.5-10)	NS
Sex [*n* (%)]	Patients	Male	16 (64)	
		Female	9 (36)	0.783
	Controls	Male	12 (60)	NS
		Female	8 (40)	
Immune Manifest of PID[a]	Group 1 (0-3 years) No		7 (53.8)	
	Yes		6 (46.2)	
	Group 2 (>3-15 years) No		6 (60)	
	Yes		4 (40)	
Consanguinity [*n* (%)]	Controls		2 (10)	
	Patients with skeletal dysplasia		16 (64)	0.0003 S
	Patients with syndromic immunodeficiency		5 (55.56)	0.0164 S

Analysis using Mann-Whitney test for age, χ^2 test for sex, and Fisher's exact test for consanguinity. Max, maximum; min, minimum; NS, nonsignificant; PID, primary immunodeficiency; S, significant. [a]The data are missing in two patients, one in each group, where immune manifestations could not be properly evaluated after initial visit due to incorrect patient's contact information.

Table 2 Comparison between controls of group 1 and group 2 regarding the different laboratory parameters

Parameters	Group 1 (*n*=9)	Group 2 (*n*=11)	*P*
Absolute lymphocyte count (×10^9/l)	3.2 (1-8.2)	2.5 (1.8-5.2)	0.191 NS
CD3%	61.1 (50.4-74.6)	59.55 (46.6-70.6)	0.528 NS
Absolute CD3 (×10^3/µl)	2.17 (1.56-4.89)	1.75 (0.97-3.67)	0.172 NS
CD4%	32.8 (11.7-50)	36.8 (26.3-48.6)	0.192 NS
Absolute CD4 (×10^3/µl)	1.07 (0.49-3.09)	0.87 (0.47-2.53)	0.725 NS
CD8%	13.9 (5-22)	20.4 (14.3-26.6)	0.008 S
Absolute CD8 (×10^3/µl)	0.43 (0.12-1.15)	0.57 (0.32-1.07)	0.374 NS
CD16%	7.8 (3.4-16.3)	8.7 (4.4-21.3)	0.466 NS
Absolute CD16 (×10^3/µl)	0.25 (0.05-0.54)	0.28 (0.13-0.38)	0.86 NS
CD19%	21.65 (19.4-34.2)	14.1 (9.4-24.9)	0.017 S
Absolute CD19 (×10^3/µl)	0.84 (0.67-2.57)	0.35 (0.18-0.75)	0.007 S
IgG (g/l)	8.1 (5.1-19)	10.75 (7.5-14)	0.022 S
IgM (g/l)	1.04 (0.4-3.99)	1.05 (0.7-1.7)	0.872 NS
IgA (g/l)	0.5 (0.38-3.3)	0.95 (0.6-1.7)	0.048 S
TRECs/µl DNA	19000 (12527-72729)	10831 (2774-83563)	0.305 NS

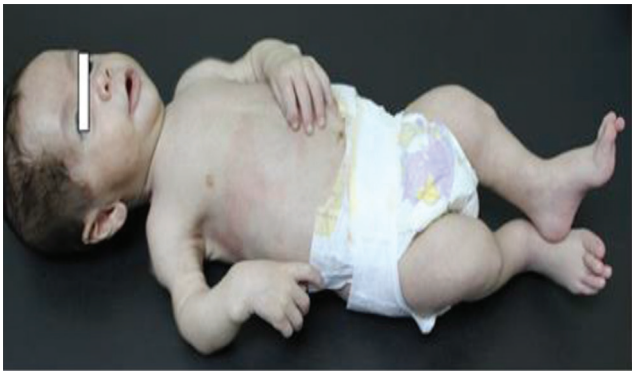
Data were presented as median and range (5th-95th percentile) and compared using Mann-Whitney test. Ig, immunoglobulin; NS, nonsignificant; S, significant; TREC, T-cell receptor excision circle.

the patients who were diagnosed with humoral ID, two received intravenous immunoglobulins (IVIG) with dramatic improvement in their clinical immune manifestation either through completely aborting incidence of infectious episodes or by significantly reducing the frequency and severity of them.

The results of the five patients who were diagnosed with syndromic ID in group 1 (0–3 years) were as follows: (only abnormal laboratory results are described. Other laboratory results and clinical data are shown in Supplementary Material Table 2a, b). The lower body segment is the measurement of the length from the pubic symphysis to the floor, and the upper body segment is the height minus the lower body segment. The U/L ratio (upper body segment: lower body segment) at birth is about 1.7, at age 3 years it is 1.3, and at more than 7 years, it is 1.0, with the upper body segment and lower body segment being about equal.

Patient A was an 11-month-old boy whose skeletal findings were suggestive of metatropic dysplasia (MIM: 156530) TRPV4 gene (MIM: 605427). His parents were nonconsanguineous. He had short-limbed dwarfism. His height was 68 cm which is −2.8 SD less than normal for his age. He had relatively shorter lower limbs (upper/lower segment=2.2) (Fig. 1). Radiological evaluation showed epiphyseal dysplasia with dumbbell-shaped (mushroom like) metaphysis, absent carpal bones, thin narrow ribs, and platyspondyly (Fig. 2). The patient had a history of recurrent chest infections with episodes recurring every 1 or 2 months. Immunological evaluation revealed mild decrease in relative and absolute CD19 count (13.9% and 0.57×10^3/µl; respectively). Ig measurement showed marked decrease of IgM (<0.26 g/l). No abnormality was detected either in the absolute lymphocyte count or in relative and absolute

Figure 1

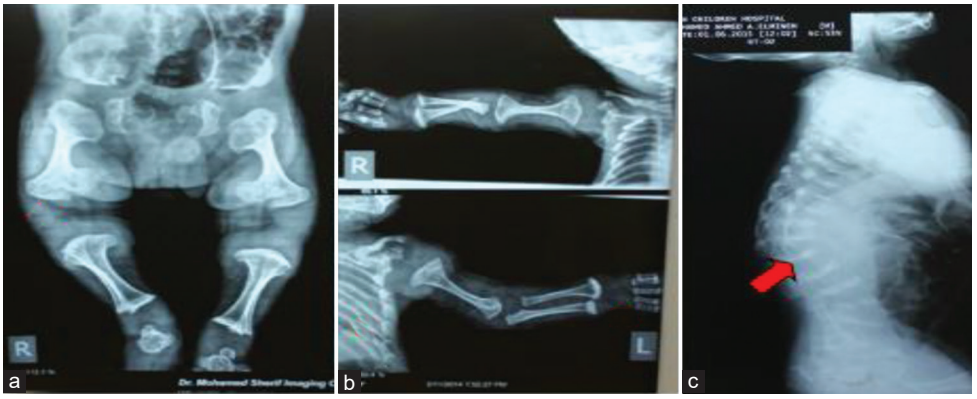


Patient A showing short-limbed dwarfism with relatively shorter lower limbs.

CD3 count. The patient was diagnosed as having IgM deficiency and referred to the regional health insurance authority. The patient received trial therapy with IVIG based on previous promising outcomes with such patients (Yel *et al.*, 2009) and failure of other antibiotic regimen to abort infectious episodes. On follow-up of the patient, his parents reported marked improvement after receiving IVIG treatment. They stated that their child remained 12 months free from infectious episodes and that they did not have to seek medical attention during that period, and then he markedly worsened after its stoppage.

Patient B was an 18-month-old girl who had dysmorphic features: depressed nasal bridge, bilateral epicanthal folds, and long philtrum. Her parents were remotely consanguineous. She had a notable perinatal history as she was incubated for 3.5 months after birth owing to respiratory distress during which she was mechanically ventilated for 2 months. Anthropometric measurements showed disproportionate short-limbed dwarfism with relatively shorter lower limbs (Fig. 3). Height was

Figure 2

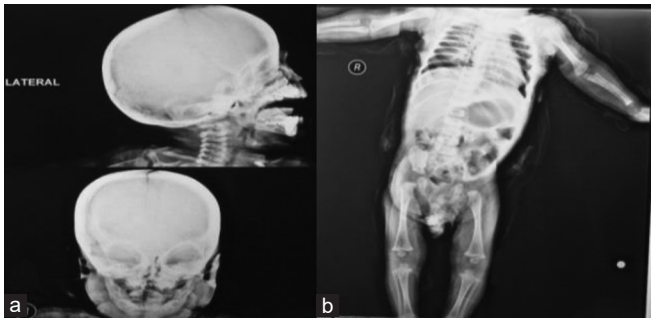


Radiography showing mushroom-shape appearance of metaphysis (a), absent carpal bones (b), thin narrow ribs (c), and platyspondyly (arrow).

Figure 3

Patient B showing depressed nasal bridge, bilateral epicanthal folds, and long philtrum (a). Disproportionate dwarfism is also illustrated (b).

Figure 4



Radiography showing Wormian skull bones (a), and metaphyseal cupping and widening (b).

63.5 cm (−3.8 SD) and upper/lower segment=1.9. Radiological investigations showed Wormian skull bones along with metaphyseal cupping and widening (Fig. 4). Clinical and radiographic findings were suggestive of SMD (undiagnosed type SMD variant of craniometaphyseal dysplasia early stage). The patient had remarkable history suggestive of ID. At the age of 9 months, she presented with bronchopneumonia, and since then, she was admitted to hospital five times with pneumonia which did not respond well to antibiotics. The last time pneumonia lasted for one and half month, and the patient was treated with multiple antibiotics including ampicillin/sulbactam, cefotaxime, meropenem, vancomycin, and lastly itraconazole. The pneumonia improved for only 1 week and recurred shortly after. Immunological investigations showed marked decrease of relative count of CD19 (3.9%) and moderate decrease of its absolute count ($0.36 \times 10^3/\mu l$). However, Ig quantification that was done at that setting was normal. Two weeks later, the patient was admitted again to hospital with severe pneumonia and counseling was given to repeat Ig measurement, which revealed low IgA (<0.07 g/l) with normal IgG and IgM. TREC values were within range of age-matched controls. The patient was diagnosed of having humoral ID, mostly selective IgA deficiency, and referred to the regional health insurance authority where she received IVIG treatment. On follow-up, the patient attacks had markedly improved in terms of frequency (one episode of chest infection in 6 months) and severity (treated as an outpatient with oral antibiotics at home which were effective in clearing infection).

Patient F was a 20-day-old male baby of consanguineous parents. Physical examination showed camptodactyly and arachnodactyly in upper and lower limbs. He had short-limbed dwarfism with significantly shorter lower limbs. Anthropometric measurements were as follows: height was 42.4 cm (−3.7 SD) and upper/lower segment=3.6 (Fig. 5). Radiological investigations were strongly suggestive of kyphomelic dysplasia (Fig. 6). Although the perinatal history was unremarkable, and the parents denied presence of immune manifestations, immunological evaluation was done based on geneticist recommendation, which revealed mild decrease of both CD19% and IgA (19.3% and 0.3 g/l, respectively).

Patient M was a 2-month-old girl of a nonconsanguineous marriage who was clinically diagnosed as having kyphomelic dysplasia. She had disproportionate short-limbed dwarfism with relatively shorter lower limbs. Height was 46 cm (−4.6 SD)

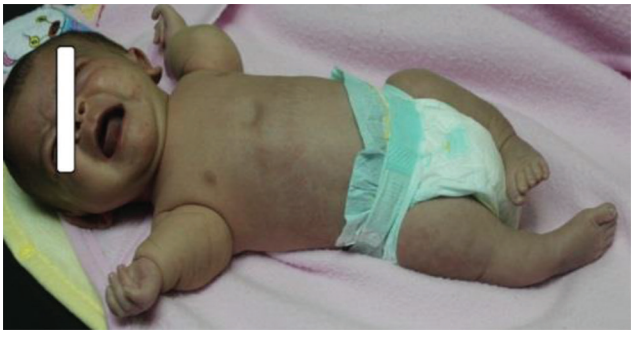
and upper/lower segment=2.3 (Fig. 7). Radiography showed bilateral hip dislocation and bilateral bowed femur (Fig. 8). Immunological evaluation was done on geneticist recommendation based on the presence of a diagnosis that was previously accidently found to be associated with immunological defects despite absence of history of immune manifestations of PID. Results showed mild decrease of relative and absolute CD3 count (32.8% and 1.5×10^3/µl; respectively). IgG showed mild decrease (4.3 g/l) and IgA showed moderate decrease (0.18 g/l). The patient had a remarkable decline of TRECs/µl DNA (3474) when compared with controls of the same age group.

Patient J was an almost 2-year-old girl whose skeletal findings were suggestive of opsismodysplasia (MIM: 258480) INPPL1 gene (MIM: 600829). Her parents were consanguineous. She had short-limbed dwarfism with relatively shorter lower limbs. Her height was 66.2 cm (–3 SD) and upper/lower segment=2.3 (Fig. 9a). Radiography showed decreased height of vertebral bodies with abnormal pear-shaped configuration (Fig. 9b). It also showed epiphyseal dysplasia with mushroom like metaphysis and absent carpal bones (Fig. 9c, d). Immunological evaluation was done despite absence of history of immune manifestations of PID, denied by parents, and as immune defects were also found in another patient with similar diagnosis (patient O). Results showed mild decrease of absolute count of CD3 (0.95×10^3/µl) and CD19% (13.4%) along with moderate decrease of absolute CD19 count (0.241×10^3/µl). Value of TRECs/µl DNA was decreased when compared with controls of the same age group. Findings were suggestive of combined ID, and subsequently, a recommendation was given to clinically follow-up patient for development of symptoms of combined ID.

The results of the four patients who were diagnosed with syndromic ID in group 2 (>3–15 years) were as follows: (Only abnormal laboratory results are described. Other laboratory results and clinical data are shown in Supplementary Material Table 3).

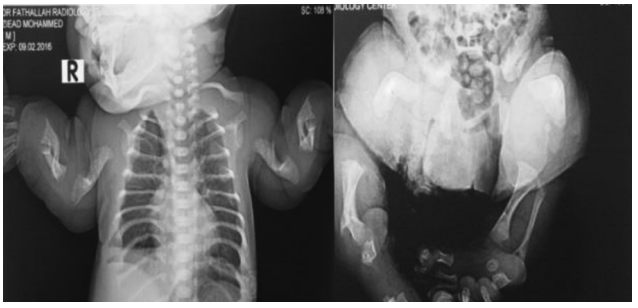
Patient O was a 4-year-old boy with disproportionate short-limbed dwarfism with slightly shorter lower limbs. Clinical and radiographic findings were suggestive of metaphyseal chondrodysplasia, Schmid type (MIM: 156500) Col10A1 (MIM: 120110). His parents were nonconsanguineous. His height was 70 cm (–5 SD) and upper/lower segment=1.5 (Fig. 10a). Radiography showed bilateral bowing of radii and femora with metaphyseal dysplasia which manifested as widening of distal metaphyseal endplates of long bones (Fig. 10b, c). Physician stated presence of history of repeated viral infection during winter periods; however, parents disclaimed presence of significant immune manifestations. Immunological evaluation showed mild decrease of relative CD3 and CD4 count

Figure 5



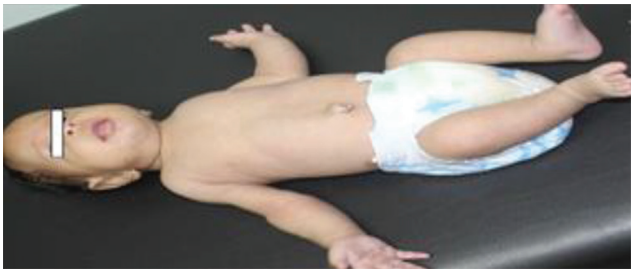
Patient F showing short-limbed dwarfism with significantly shorter lower limbs, camptodactyly, and arachnodactyly.

Figure 6



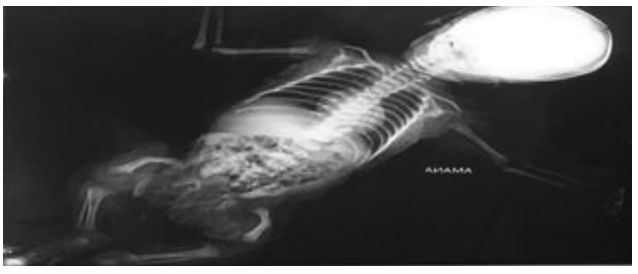
Radiography of patient F showing characteristic bowing of upper and lower limbs.

Figure 7



Patient M showing disproportionate short-limbed dwarfism with relatively shorter lower limbs.

Figure 8



Radiography showing bilateral hip dislocation and bilateral bowed femur.

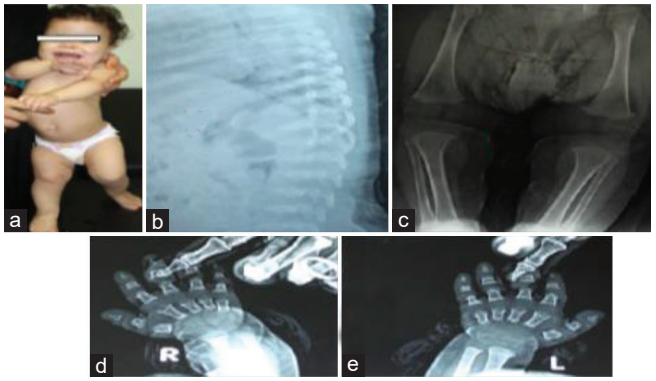
(34 and 25.6%) with mild decrease of IgG (6.9 g/l). TREC value was within range of age-matched controls.

Patient P was a 9-year-old girl of positively consanguineous parents. She was complaining of waddling gait and bilateral knee swelling. On physical examination, the patient showed facial asymmetry in the form of midfacial hypoplasia, left ptosis, depressed nasal root, bilateral mesomelia and bowing in forearm, left scoliosis, and exaggerated lumber lordosis (Fig. 11). Her height was 106 cm (–4 SD). Radiography showed metaphyseal widening and bowing in left femur, as well as bilateral bowing of both radii (Fig. 12). The patient was clinically diagnosed of having kyphomelic dysplasia (MIM: 211350). Immunologically, the patient had history of recurrent chest infections during early childhood. Laboratory evaluation showed mild absolute neutropenia, moderate decrease of relative CD19% (4.3%), mild decrease of absolute CD19 ($0.15\times10^3/\mu l$), mild decrease of CD4% (23.2%), and mild decrease of IgG (7.4 g/l). TREC value was within the range of age-matched controls.

Patient V was a 3.5-year-old boy who had disproportionate short-limbed dwarfism and genu valgus on right side (Fig. 13a). His parents were nonconsanguineous. His height was 82 cm (–3.2 SD). Radiographic survey showed bowing of both tibia and fibula with metaphyseal widening, irregularity, and flaring. Moreover, there was mild epiphyseal irregularity (Fig. 13b). Hand radiograph showed cupping and fraying of carpal and metacarpal bones (Fig. 13c). Mild dysplastic changes were also noticed in lower thoracic and lumbar vertebrae (Fig. 13d). Skull radiograph showed presence of Wormian bones (Fig. 13e). The patient was clinically diagnosed of having hypophosphatemic rickets AD (MIM: 193100). Positive immune manifestations were reported during evaluation described as increasingly severe common cold attacks, but symptoms are recently improving during follow-up of patient. Laboratory evaluation showed moderate decrease of relative CD16 (2%), mild decrease of absolute CD16 ($0.104 \times 10^3/\mu l$), and mild decrease of IgA (0.5 g/l). TREC value was within range of age-matched controls. Follow-up was recommended for suspicion of combined ID.

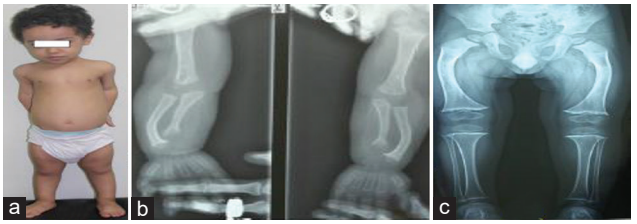
Patient Y was a 7-year-old boy who was clinically diagnosed as having SEMD short limb-hand type

Figure 9



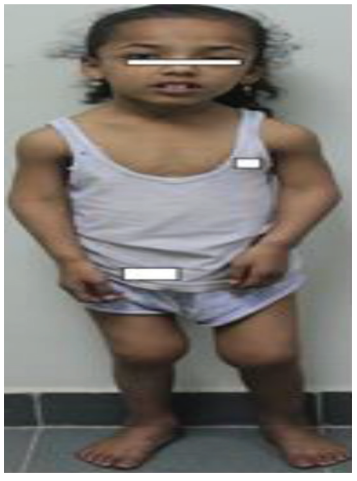
Patient J showing disproportionate short-limbed dwarfism (a), decreased height of vertebral bodies (b), epiphyseal dysplasia with mushroom like metaphysis (c and d), and absent carpal bones (d).

Figure 10



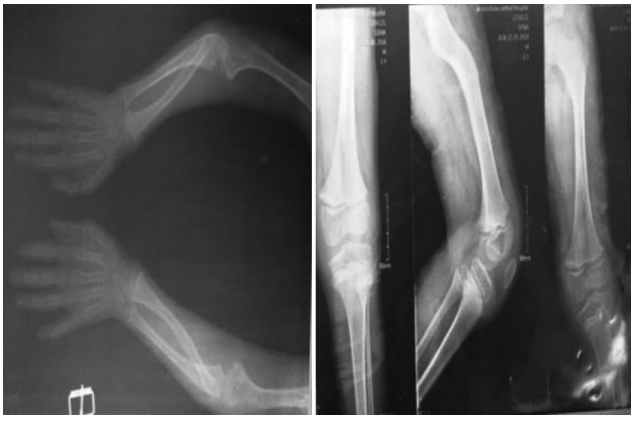
Patient O showing disproportionate short-limbed dwarfism (a) and bowing of radii and femora with metaphyseal dysplasia (b and c).

Figure 11



Patient P showing disproportionate dwarfism, facial asymmetry, and bilateral mesomelia.

Figure 12



Radiography of patient P showing metaphyseal widening and bowing in left femur, bilateral bowing of both radii.

abnormal calcification (MIM: 271665) DDR2 gene (MIM: 191311). His parents are consanguineous. The patient has disproportionate dwarfism with relatively shorter lower limbs (Fig. 14a). Height was 67 cm (–8 SD) and upper/lower segment=1.5. Radiographic survey showed shortening of long bones with metaphyseal widening, flaring, and presence of spurs (Fig. 14b, c). Platyspondyly was also seen (Fig. 14d). The patient has recurrent ear infections and skin abscesses. Laboratory evaluation showed moderate decrease of IgA (0.25 g/l). Result is highly suggestive of partial IgA deficiency.

Comparative statistics were done between the different immunological parameters in patients who were found to have syndromic ID and controls of the corresponding age group (Tables 3 and 4). Table 3 revealed that patients in the age group from 0 to 3 years showed significantly higher values than controls in CD16% and absolute CD16, whereas they had significantly lower values of CD19% and IgM. No such significance was found in the other parameters.

Table 4 shows the statistical difference in the studied parameters between patients with syndromic ID and controls in the age group more than 3–15 years. Only CD8% was significantly lower in patients when compared to controls.

Table 5 shows the correlation between age and different laboratory parameters among control population. Significant negative correlations were found between age and the following laboratory parameters: absolute lymphocyte count, absolute CD3, CD19%, absolute CD19, absolute CD4, and TRECs/μl DNA, whereas a significant positive correlation was found between age and each of the following: CD16%, IgG, and IgA.

Table 3 Laboratory parameters in patients with syndromic immunodeficiency in comparison with controls of matched age (0-3 years)

Parameters	Controls (*n*=9)	Patients (*n*=5)	*P*
Absolute lymphocyte count (×10^9/l)	3.2 (1-8.2)	4.5 (1.8-9.3)	0.126 NS
CD3%	61.1 (50.4-74.6)	56.2 (32.8-64.2)	0.306 NS
Absolute CD3 (×10^3/μl)	2.165 (1.56-4.89)	2.3 (0.95-5.65)	1 NS
CD4%	32.8 (11.7-50)	28.85 (25.4-32.3)	0.507 NS
Absolute CD4 (×10^3/μl)	1.07 (0.49-3.09)	1.925 (1.49-2.36)	0.111 NS
CD8%	13.9 (5-22)	8.1 (5-11.2)	0.182 NS
Absolute CD8 (×10^3/μl)	0.425 (0.12-1.15)	0.635 (0.23-1.04)	0.779 NS
CD16%	7.8 (3.4-16.3)	11 (8.6-22.6)	0.031 S
Absolute CD16 (×10^3/μl)	0.25 (0.05-0.54)	0.51 (0.15-2.1)	0.05 S
CD19%	21.65 (19.4-34.2)	13.9 (3.9-29.4)	0.045 S
Absolute CD19 (×10^3/μl)	0.84 (0.67-2.57)	0.57 (0.24-1.35)	0.201 NS
IgG (g/l)	8.1 (5.1-19)	6.5 (4.3-14.1)	0.088 NS
IgM (g/l)	1.04 (0.4-3.99)	0.7 (0.26-1.1)	0.039 S
IgA (g/l)	0.5) 0.38-3.3)	0.46 (0.18-0.55)	0.108 NS
TRECs/μl DNA	19000.96 (12527.5-72729.63)	58086.71 (3473.55-72729.63)	0.764 NS

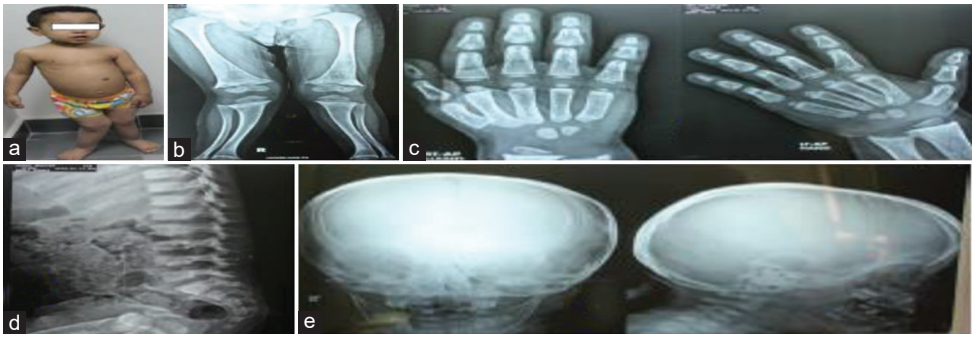
Data were presented as median and range (5th-95th percentile) and compared using Mann-Whitney test. Ig, immunoglobulin; NS, nonsignificant; S, significant; TREC, T-cell receptor excision circle.

Table 4 Laboratory parameters in patients with syndromic immunodeficiency in comparison with controls of matched age (>3-15 years)

Parameters	Controls (*n*=11)	Patients (*n*=4)	*P*
Absolute lymphocyte count (×10^9/l)	2.5 (1.8-5.2)	4.35 (3.5-10.7)	0.055 NS
CD3%	59.55 (46.6-70.6)	57.4 (34-58.4)	0.173 NS
Absolute CD3 (×10^3/μl)	1.75 (0.97-3.67)	2.53 (1.98-3.63)	0.174 NS
CD4%	36.8 (26.3-48.6)	27.7 (23.2-38.5)	0.103 NS
Absolute CD4 (×10^3/μl)	0.87 (0.47-2.53)	1.52 (0.81-2.74)	0.203 NS
CD8%	20.4 (14.3-26.6)	12.25 (8.9-18.2)	0.042 S
Absolute CD8 (×10^3/μl)	0.57 (0.32-1.07)	0.73 (0.31-0.96)	0.865 NS
CD16%	8.7 (4.3-21.3)	6.25 (2-18.9)	0.322 NS
Absolute CD16 (×10^3/μl)	0.28 (0.13-0.38)	0.42 (0.1-0.81)	0.569 NS
CD19%	14.1 (9.4-24.9)	15.4 (4.3-30)	1 NS
Absolute CD19 (×10^3/μl)	0.35 (0.18-0.75)	1.02 (0.15-1.86)	0.322 NS
IgG (g/l)	10.75 (7.5-14)	7.55 (6.9-15.2)	0.203 NS
IgM (g/l)	1.05 (0.7-1.7)	1.29 (0.71-2)	0.723 NS
IgA (g/l)	0.95 (0.6-1)	0.85 (0.25-1.3)	0.569 NS
TRECs/μl DNA	10831 (2774-83563)	16940 (11803-27882)	0.257 NS

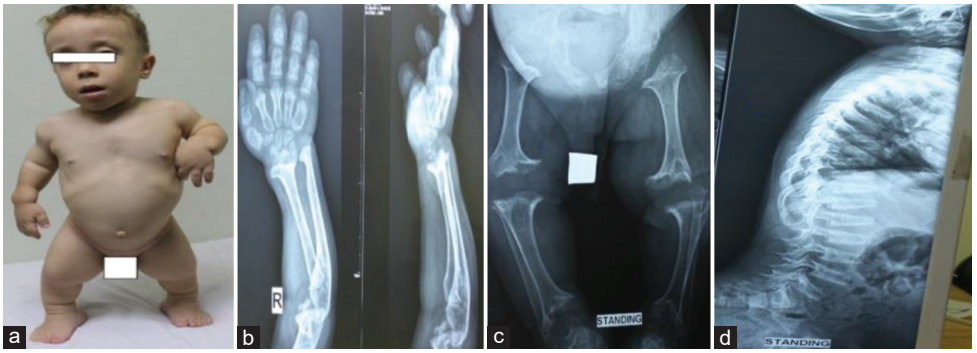
Data were presented as median and range (5th-95th percentile) and compared using Mann-Whitney test. Ig, immunoglobulin; NS, nonsignificant; S, significant; TREC, T-cell receptor excision circle.

Figure 13



Patient V showing disproportionate short-limbed dwarfism and right-sided genu valgus (a), radiography showing bilateral bowing of tibia and fibula with metaphyseal widening and mild epiphyseal irregularity (b), hand radiograph showing cupping and fraying of carpal and metacarpal bones (c), dysplastic changes in lower thoracic and lumber vertebrae, and (d) and skull radiograph showing Wormian bones (e).

Figure 14



Patient Y showing disproportionate dwarfism (a). Radiographic survey showed shortening of long bones with metaphyseal widening (b and c) and platyspondyly (d).

Table 5 Correlation between age and measured laboratory parameters in controls

Correlation between age (years)	*r*	*P*	Significance
Absolute lymphocyte count (×10⁹/l)	−0.739**	0.001	S
CD3%	−0.161	0.599	NS
Absolute CD3 (×10³/μl)	−0.671*	0.012	S
CD4%	−0.231	0.373	NS
Absolute CD4 (×10³/μl)	−0.725**	0.001	S
CD8%	0.462	0.112	NS
Absolute CD8 (×10³/μl)	−0.454	0.119	NS
CD16%	0.535*	0.033	S
Absolute CD16 (×10³/μl)	0.003	0.991	NS
CD19%	−0.673**	0.004	S
Absolute CD19 (×10³/μl)	−0.865**	0.001	S
IgG (g/l)	0.782**	0.001	S
IgM (g/l)	0.144	0.580	NS
IgA (g/l)	0.810**	0.001	S
TRECs/μl DNA	−0.673*	0.033	S

Ig, immunoglobulin; NS, nonsignificant; *r*, Spearman's correlation coefficient; S, significant; TREC, T-cell receptor excision circle. *Correlation is significant at the 0.05 level **Correlation is significant at the 0.01 level.

Discussion

The immune function defects present in syndromic deficiencies may include B-cell, T-cell, phagocytic, complement, innate defects, or a combination therefrom (Picard *et al.*, 2015).

The patients included in this study had disproportionate short stature owing to different skeletal dysplasias. Of these cases, nine (36%) were found to have syndromic IDs based on their clinical manifestations and laboratory assessment: five in group 1 (from 0 to 3 years) and four in group 2 (from >3 to 15 years). Results of the laboratory parameters in these patients were checked against controls of the same age group. The rate of ID was previously reported to differ according to the type of skeletal dysplasia (Ming and Graham, 2014). Seven of 25 patients included in our study were diagnosed as having kyphomelic dysplasia, of whom two (28.5%) had associated ID. This rate was higher than reported by Ming and Graham (2014) (<5%). From patients included in this work, 15 were diagnosed as having metaphyseal dysplasia either isolated, SMD, or SEMD. Six (40%) out of these 15 patients had ID. This rate was lower than that reported by Ming and Graham (2014) (>65%). The diagnosis of the last patient diagnosed with syndromic ID in this study was uncertain whether kyphomelic dysplasia or metaphyseal dysplasia.

The categorization of patients into age groups was done based on several findings in previous studies which concluded that immune parameters vary according to age (Shearer *et al.*, 2003; Lorenzi *et al.*,

2008; Kardar *et al*., 2012). An age interval was established to obtain sufficient number of both cases and controls in each group that would give statistically meaningful results and was done after observing the results of the studied parameters in different ages and finding that values differ significantly in children below the age of 3 years and those who are older than 3. This finding goes in line with previous finding of Sottini *et al*. (2010) who found that TRECs for instance were significantly different in children aged 2 months to 3 years with respect to those aged 3–16 years. Moreover, Tosato *et al*. (2015) stated that immunophenotyping of blood lymphocyte subsets is a basic tool in the diagnostic process of PIDs and its use is becoming more and more widespread as the knowledge about these illnesses increases. They also reported that the availability of reliable reference values, which need to be age matched for the pediatric population, is a prerequisite for the reliable interpretation of immunophenotyping data.

The median values of CD19% and absolute CD19 were significantly higher in group 1 controls (from 0 to 3 years) than in group 2 controls, whereas median values of CD8%, IgG, and IgA were significantly lower in group 1 controls. These results go in accordance with age-adjusted reference ranges for flow cytometric T-cell subsets reported by Shearer *et al*. (2003) and Ig quantification reported by Kardar *et al*. (2012). Although the values of other parameters did not reach a statistically significant difference, possibly owing to small sample size in each group. The median values of absolute lymphocyte count, CD3%, absolute CD3, CD4%, absolute CD4, and TRECs/µl DNA were higher in the younger age group (group 1). Contrarily, the values of CD16%, absolute CD16, and IgM were higher in the older age group. These findings also are in agreement with the reference ranges of flow cytometric T-cell subsets and Ig quantification mentioned by Shearer *et al*. (2003) and Lentner *et al*. (1984) and also with TREC age-adjusted ranges reported by Lorenzi *et al*. (2008) and absolute lymphocyte count mentioned in Bates and Lewis (2011).

In the same context and in agreement with some authors, correlation statistics between age and different laboratory parameters measured in the control group showed that the following parameters exhibited significant negative correlation with age: absolute lymphocyte count (Bates and Lewis, 2011), absolute CD3, CD19%, absolute CD19, and absolute CD4 (Shearer *et al*., 2003). On the contrary, each of the following parameters showed significant positive correlation with age: CD16% (Shearer *et al*., 2003), IgG, and IgA (Lentner *et al*., 1984).

Regarding consanguinity rates, there was a statistically significant rate in patients with skeletal dysplasias (64%) in comparison with controls (10%). Moreover, the rate was significantly higher when patients with syndromic IDs (55.56%) were compared with controls (10%). These results agree with what was previously found by Temtamy and Aglan (2012) who concluded that parental consanguinity rates in Egyptian patients with genetic diseases affecting various systems and organs, especially autosomal recessive and polygenic disorders, are statistically higher than that in the general Egyptian population.

Comparative statistics of the studied immunological parameters among cases with syndromic ID and controls in the younger age group from 0 to 3 years showed a statistically significant increase among cases in the following parameters: CD16% and absolute CD16, whereas the following parameters were significantly decreased: CD19% and IgM. In the older age group from more than 3 to 15 years, only CD8% showed statistically significant decrease among cases when compared with controls. No such difference could be found in the remaining parameters studied in both age groups. However, our study design was not originally intended to measure the diagnostic performance of the measured parameters whether flow cytometric measurement of different T-cell subsets, Igs quantification, or even the newly presented TRECs assay, as these parameters are already used in conventional routine assessment of the immune system (Folds and Schmitz, 2003; Oliveira and Fleisher, 2010; Locke *et al*., 2014).

TREC values were decreased in three of nine patients diagnosed with syndromic ID; all were in the younger age group from 0 to 3 years. In two of them (patient M and J) who were diagnosed of having combined ID, decreased TREC values were in accordance with the decreased CD3 counts (both relative and absolute CD3 in patient M and only with absolute CD3 in patient J). However, in patient A who was diagnosed of having humoral ID, low TREC levels did not go along with the satisfactory number of CD3 lymphocytes. This finding was previously noted by Sottini *et al*. (2010) who found low levels of TRECs that were inconsistent with normal values for CD3 lymphocytes in two of their patients diagnosed with severe combined ID and WAS. They justified this outcome by the presence of evidence of peripheral T-cell expansion with the presence of restricted TCR repertoires.

On the contrary, values of TRECs were not lowered in the three patients (patients O, P, and V) diagnosed with combined ID in the older age group from >3 to 15 years, although their CD3, CD4, and CD16 were below the

5th percentile of age-matched controls. Such a result was thoroughly investigated and discussed before by Sottini *et al.* (2014), and they attributed it to the longevity of naïve T cells which leads to biased overestimates of TRECs. This occurs owing to persistence of some long-lived naïve T lymphocytes where TRECs are still detected. In this case, the number of circulating naive T cells represents a composite of rate of production, apoptosis, memory cell generation, and naive T-cell homing to lymphoid tissues rather than a real-time measurement of thymic function. Our findings about the inconsistencies in its values among combined ID patients of the older age group and the discrepancy it showed with other immunological parameters in those patients support the findings previously mentioned by other studies about its limitations in the older age group (Hazenberg *et al.*, 2003; Sottini *et al.*, 2014).

In fact, several previous studies have discussed the effect of the previously mentioned biological parameters, namely, peripheral T-cell expansion and longevity of naïve T cells, as two important factors that affect the interpretation of TREC result, making it a more complex process and possibly limit its potential being an estimate of recent thymic output (Hazenberg *et al.*, 2003). Several complex mathematical models have been developed to compensate for variability in apoptosis rate of T cells to be able to use TREC assay in different applications (e.g. assessment of thymic output in HIV-infected patients, monitoring response after antiretroviral therapy, as a marker for senility, and to assess T-cell reconstitution after hematopoietic stem cell transplantation) (Ribeiro and Perelson, 2007; De Boer and Perelson, 2013).

Igs were decreased in eight of nine patients who were diagnosed of having syndromic ID: patient A showed decreased IgM, patients F, M, V, and Y had decreased IgA, and patients M, O, and P had decreased IgG. In one patient (patient B), Igs were measured in between attacks of respiratory tract infections when the patient was routinely checked in outpatient clinic and they were normal, although her relative and absolute CD19 counts were decreased. IgA level in this patient was decreased under stress of infection when patient was reinvestigated after admission to hospital. This could highlight the importance of additional testing for functional antibody levels and IgG subclasses as previously suggested by Oliveira and Fleisher (2010). This finding could be further seen when reviewing the results of patient J, whose relative and absolute CD19 levels are decreased with normal levels of Igs.

In this study, we could detect that there was a significant correlation between the presence of immune manifestations during initial clinical evaluation and the presence of immune defects after laboratory workup in cases with syndromic ID. This finding could additionally emphasize the importance of thoroughly investigating the patient for various immune manifestations and the possible beneficial role of using a screening paradigm like the 10 warning signs to be able to pick up those who are likely to have PIDs (Subbarayan *et al.*, 2011). This finding had been observed before by Reda *et al.* (2009) who found the warning signs being elicited in all their patients with variable frequencies, minimally two and maximally six. However, according to our observations, we could conclude that the absence of manifestations does not exclude the presence of immune defects (as noticed in patients F, M, J, and O), and this conclusion is supported by what was found by Arkwright and Gennery (2011), who applied the ten warning signs in their patients and found that they could have missed 20% of patients with PID in whom the clinical presentation did not involve infectious diseases and subsequently, they stated that 'the delay in considering the diagnosis of PID for the appearance of subsequent infections as suggested by the 10 warning signs may be courting disaster.' We have first questioned the validity of ten warning signs as a screening paradigm when we accidently encountered two patients whose immunological laboratory parameters were abnormal despite absence of warning signs which discouraged us to depend on it as a tool to pick up those who are likely to have syndromic ID.

Conclusion

The work done in our study emphasizes the importance of screening for syndromic IDs and its importance in providing the proper genetic counseling for patients. It also illustrates its vital effect in optimizing clinical care given to the patients by providing the early and appropriate treatment and the effect that this does on improving the quality of life of both patient and his family. So, we recommend routine implementation of the immunological profile used in this work including flow cytometric measurement of lymphocyte subsets and Igs quantification in other clinics of clinical genetics subspecialties in cases suspected of having syndromic ID in order to offer optimal care for patients and proper counseling. Our findings regarding TREC assay led us to a final conclusion that newborn screening would be the only application that would bypass limitations found in older ages and that applying it for older age groups is of questionable efficacy.

Our results in Supplementary Materials 2 and 3 were in agreement with what was previously reported in the following table by Ming and Stiehm (2017) who concluded that short-limb skeletal dysplasia is associated with combined ID.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary Material 1 The 10 warning signs of primary immune deficiency in children (Arkwright and Gennery, 2011)

1. Four or more new ear infections within 1 year
2. Two or more serious sinus infections within 1 year
3. Two or more months on antibiotic with little effect
4. Two or more pneumonias within 1 year
5. Failure of an infant to gain weight or grow normally
6. Recurrent, deep skin or organ abscesses
7. Persistent thrush in mouth or fungal infection on skin
8. Need for intravenous antibiotics to clear infections
9. Two or more deep-seated infections including septicemia
10. A family history of primary immunodeficiency

Supplementary Material 2 Results of patients with confirmed syndromic immunodeficiency from 0 to 3 years (patients were given letter coding)

Patients	Diagnosis	Age at analysis	Inheritance	Immune manifest of 1ry ID	Absolute neutrophil count (RR from literature) ($\times 10^9$/l)	Absolute lymphocyte count ($\times 10^9$/l)	CD3%	Absolute CD3 ($\times 10^3$/μl)	CD4%	Absolute CD4 ($\times 10^3$/μl)
A	Metatropic dysplasia (MIM: 156530) TRPV4 gene (MIM: 605427)	11 m	AD De novo mutation	yes	3.2 (1-7)	4.1	56.2	2.30		
B	Undiagnosed type SMD? variant of craniometaphyseal dysplasia early stage	18 m	Remote cons.	yes	3.3 (1-7)	9.3	60.8	5.65	25.4	2.362
F	kyphomelic dysplasia	20 days	AR (+ve cons., 2nd cousin)	no	3.6 (3-7)	4.5	64.2	2.88		
M	kyphomelic dysplasia	2 m 10 days	AR (+ve cons., 1st cousin)	no	2.6 (1-5)	4.6	32.8*	1.5*	32.3	1.485
J	Opsismodysplasia (MIM: 258480) INPPL1 gene	1 yr 11 m	AR (+ve cons., 1st cousin)	no	1.4 (1-7)	1.8	53.1	0.95*		
Controls median (5th- 95th) percentile		2 years (0.1-3)				3.2 (1-8.2)	61.1 (50.4-74.6)	2.166 (1.56-4.89)	32.8 (11.7-50)	0.23

Patients	Absolute CD4 ($\times 10^3$/μl)	CD8%	Absolute CD8 ($\times 10^3$/μl)	CD16%	Absolute CD16 ($\times 10^3$/μl)	CD19%	Absolute CD19 ($\times 10^3$/μl)	IgG (g/l)	IgM (g/l)	IgA (g/l)	TRECs	Type of immune defect detected
A				10.9	0.44	13.9*	0.569*	6.8	<0.26*	0.46	72,730	Humoral
B	2.362	11.2	1.0416	22.6	2.1	3.9*	0.362*	14.1	1.1	0.55	62,883	Humoral
F				13.5	0.6	19.3*	0.868	5.1	0.45	0.3*	58,087	Humoral
M	1.485	5	0.23	11	0.5	29.4	1.352	4.3*	0.7	0.18*	3,474*	Combined
J				8.6	0.155	13.4*	0.241*	6.5	0.7	0.5	10,410*	Combined
Controls median (5th- 95th) percentile	0.23	13.9 (5-22)	0.426 (0.115-1.148)	7.8 (3.4-16.3)	0.25 (0.05-0.54)	21.65 (19.4-34.2)	0.84 (0.669-2.566)	8.1 (5.1-19)	1.04 (0.4-3.99)	0.5 (0.38-3.3)	19,000 (12,527-72,729)	

*Values that are below 5th percentile of controls, cons.: consanguinity m: months, yr: year, RR from literature: reference ranges adopted from literature (Bates and Lewis, 2011). CD4 and CD8 were not included in our initial protocol and were only done for further evaluation in case of decreased CD3 counts or when initial manifestations were suggestive of combined immunodeficiency.

Supplementary Material 3 Results of patients with confirmed syndromic immunodeficiency from more than 3 to 15 years (patients were given letter coding)

Patients	Diagnosis	Age at analysis	Inheritance	Immune manifest of 1ry ID	Absolute neutrophil count (RR from literature) ($\times 10^9$/l)	Absolute lymphocyte count ($\times 10^9$/l)	CD3%	Absolute CD3 ($\times 10^3$/μl)	CD4%	Absolute CD4 ($\times 10^3$/μl)	CD8%
O	Metaphyseal chondrodysplasia schmid type (MIM: 156500) Col10A1(MIM: 120110)	3 yrs 11 m	AD	no	5.4 (1.5-8)	10.7	34*	3.63	25.6*	2.739	9
P	kyphomelic dysplasia (MIM: 211350)	9 yrs	AR (+ve cons., 1st cousin)	yes	1.7 (2-8)	3.5	58.4	2.04	23.2*	0.812	8.9
V	Hypophosphatemic rickets (MIM: 193100)	3.5 yrs	AD	yes	4.9 (1.5-8)	5.2	58.2	3.02	38.5	2.002	15.5
Y	SEMD short limb-hand type abnormal calcification (MIM: 271665) DDR2 gene	7 yrs	AR (+ve cons, one similarly affected sibs was aborted after intrauterine diagnosis)	yes	5.8 (2-8)	3.5	56.6	1.98	29.8	1.043	18.2
Controls median (5th- 95th) percentile		7 years (3.3-15)				2.5 (1.8-5.2)	59.55 (46.6-70.6)	1.75 (0.97-3.67)	36.8 (26.3-48.6)	0.866 (0.473-2.527)	20.4 (14.3-26.6)

Patients	Absolute CD8 ($\times 10^3$/μl)	CD16%	Absolute CD16 ($\times 10^3$/μl)	CD19%	Absolute CD19 ($\times 10^3$/μl)	IgG (g/l)	IgM (g/l)	IgA (g/l)	TRECs	Type of immune defect detected
O	0.963	7.6	0.813	17.4	1.86	6.9*	0.88	1.3	27,882	Combined
P	0.3115	4.9	0.171	4.3*	0.15*	7.4*	1.7	1.2	16,213	Combined
V	0.806	2*	0.104*	30	1.56	7.7	0.71	0.5*	11,804	Combined
Y	0.637	18.9	0.661	13.4	0.469	15.2	2	0.25*	17,668	Humoral
Controls median (5th- 95th) percentile	0.57 (0.316-1.066)	8.7 (4.4-21.3)	0.27 (0.132-0.383)	14.1 (9.4-24.9)	0.35 (0.18-0.75)	10.75 (7.5-14)	1.05 (0.7-1.7)	0.95 (0.6-1.7)	10,831 (2,774-83,563)	

SEMD: spondyloepimetaphyseal dysplasia, RR from literature: reference ranges adopted from literature (Bates and Lewis, 2011). *Values that are below 5th percentile of controls, cons.: consanguinity m: months, yr: year.

Name	Inheritance (chromosome)	Associated features	Immune defect	Frequency of ID
Short-limb skeletal dysplasia with immunodeficiency	AR	Short-limb skeletal dysplasia, metaphyseal dysplasia, may be associated with adenosine deaminase deficiency or Omenn syndrome; heterogeneous	T, B	++++

AR, autosomal recessive; B, B-cell defect; ID, immunodeficiency; T, T-cell defect; frequency of ID, ++++ $\geq$65%.