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Systematic investigation for underlying causes of recurrent infections in children: surveillance of primary immunodeficiency

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KEY WORDS

recurrent infection; primary immunodeficiency; children; confirmatory diagnostic studies; clinical patterns, management

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Summary

Recurrent infections seem to be a common complaint in children who are referred to general practitioners' and pediatricians' offices. Detection of primary immunodeficiencies (PID) etiology is very important for achieving appropriate diagnosis and treatment of these patients. The absence of appropriate treatment could lead to subsequent complications, in a hospital inpatient and/or outpatient settings. This study was performed in a group of children with recurrent infections to identify patients with underlying PID. A cross-sectional study was designed to evaluate the final clinical diagnosis obtained in 100 pediatric patients with a history of recurrent infections referred to Children's Medical Center, Tehran, Iran, during one year (2011-2012). History taking and physical examination, complementary laboratory tests including immunological investigations were done to confirm the main causes of disease according to our previously published stepwise approach to recurrent infections. Among all studied patients, 21% (11 males and 10 females) were diagnosed to have PID. Parental consanguinity ($p = 0.001$) and soft tissue infections ($p = 0.004$) were significantly higher in PID group, comparing to other causes of recurrent infections. Gender and location of infections were also linked to the type of PID including antibody deficiency, combined immunodeficiency and phagocytosis disorders. The real rate of PID as a cause of recurrent infection appears to be much higher than what is generally considered in a selected group of pediatric patients; so, following the suggested stepwise guideline can improve timely diagnosis and appropriate treatment of these patients.

Introduction

Primary immunodeficiency diseases (PIDs) are a heterogeneous group of inherited disorders associated with infectious and non-infectious complications (1). Infectious diseases occur in children more frequently than adults (2-5). Normally, a child might suffer from infectious episodes between 6-15 times per year (6,7). This range of infectious events depends on many predisposing factors (2,8). The most important promoting elements are age, exposure to other children in school or daycare units (9,10), passive smoking, inadequate nutrition, living in an air-polluted area, atopy, anatomical defects and other chronic underlying disorders (11).

Therefore, a challenge exists for practitioners to verify the abnormal pattern and unusual type of the infection (12), and to make a decision about the necessity of further evaluation for finding more serious underlying conditions such as immunodeficiency (13-15). To achieve this goal, starting from 20 years ago, diagnostic aids such as "10 warning signs of PID" have been designed (16,17), that were based on patients' medical history and physical examination (7,8). Although these guidelines are so applicable and useful in the diagnosis of PID in both children and adults, further development and refinement of the suggested warning signs show that reliance on these signs cannot identify all patients with PID (3,15,16).

Since early diagnosis and treatment of PID are very important (18,19), it is necessary to know the proportional rate of PID among the children suffering from recurrent infections (20). However, this rate varies among different settings and different ethnicities. Early diagnosis can improve long-term quality of life and prevent secondary complications of PID (9,12). Improving the awareness of pediatricians about these facts could be helpful in better evaluation and management of the condition (21,22). Although there are some important reports on registries of PID patients in Iran and other countries, there is no clinical study on children with recurrent infectious diseases, to classify them into diagnostic subgroups and show the applicability of a stepwise approach to the recurrent infection. Furthermore, there are only a few similar articles in this field all over the world (16,20,23). This survey was designed to identify the frequency of underlying primary immunodeficiency among pediatric patients with recurrent infection.

Materials and methods

Patients

A cross-sectional prospective study was designed. Patients were selected at the main pediatric tertiary hospital, "Children's Medical Center affiliated to Tehran University of Medical Sciences, Tehran, Iran" from November 2011 to November 2012. Patients younger than 14 years old that were referred to the emergency unit, the general pediatric clinic, the immunology,

allergy and infectious diseases outpatient clinics, along with the patients admitted to the infectious, gastroenterology, intensive care unit (ICU) and immunology wards, were enrolled. Patients with a history of recurrent or chronic infection and also cases with a serious infectious complication were included to the next step. There is a family medicine for each patient in Iran, and each episode of the medical event should be documented by corresponding family physicians before referring to the third level hospital. Thus, we obtained data of all episodes of infections in the studied patients by reviewing these documents. The Ethics Committee of the Tehran University of Medical Sciences approved the project. Written informed consent was obtained from all the cases and/or their parent(s).

Clinical evaluation

Recurrent infection was defined as a history of at least two severe infections in a year, three or more bacterial respiratory infections (e.g., sinusitis, otitis media, and bronchitis) in one year, or the requirement of antibiotics for two months / year. Severe / serious infections were also considered as grave infections, including those with persistent fever or confinement to bed for a week or more, failure to respond to oral antibiotics and/or the need for intravenous antibiotics or hospitalization, infections with an unusual pathogen, unusual complications (e.g., mastoiditis, pleural effusion, abscesses), or persistent laboratory abnormalities (e.g., prolonged leukocytosis, elevated erythrocyte sedimentation rate [ESR] or C-reactive protein [CRP], persistent imaging abnormalities) (24).

A detailed itemized questionnaire, including whether or not there was a family history of recurrent infection or unexpected death, consanguinity of parents, age at the time of first infection and current age, physical examination details, the types and severity of infections, and reports of para-clinical evaluations (e.g. laboratory tests and imaging and pathological reports of patients), was filled for all enrolled patients.

Furthermore, based on the predominant presentation and infectious location of each patient, we grouped the infections into 6 main categories including upper respiratory infections (25), lower respiratory infections (26-28), gastrointestinal infections, skin infections, soft tissue infections, and severe or life-threatening infections (meningitis, sepsis, osteomyelitis) (23,29,30).

Laboratory tests

From all individuals, a 5 milliliter blood sample was taken for measuring complete blood count (CBC), ESR, CRP; then, according to each medical history and a previously published stepwise flowchart of approach to recurrent infections, complementary diagnostic laboratory tests were done (6), such as serum immunoglobulin levels, isohaemagglutinin levels, ni-

troblue-tetrazolium test (NBT), human immunodeficiency virus (HIV) antibody, the titer of specific antibodies for tetanus, diphtheria or pneumococcal antigens, purified protein derivative (PPD) test, and the skin prick test. The inhalant allergens for the later test include trees (elm, maple, oak), grasses (Bermuda grass, Johnson grass, June grass [Kentucky bluegrass]), weeds (common ragweed, cocklebur, rough pigweed), molds, and miscellaneous allergens (mite, dog dander, cat dander, cockroach) (31). In addition to inhalant allergens, we evaluated allergens that patients mentioned.

Moreover, serum complement components' levels, sweat test, spirometry, flow-cytometry analysis, complementary imaging and pathological investigation were also performed under some special conditions, as explained before in the literature (6,14,32). The final diagnosis of patients was recorded as follow: healthy children, PID, secondary or acquired immunodeficiency (SID), anatomical or functional disorders, and allergic conditions (33-36). Patients with a definite diagnosis of PID were classified based on the final reports of the International Union of Immunological Societies (IUIS) (33).

Statistical analysis

Statistical analysis was performed, using a commercially available software package (SPSS Statistics 17.0.0, SPSS, and Chicago, Illinois). For the comparison of the age of onset and diagnosis between different groups, analysis of variance (ANOVA) was used, while for categorical variables the chi-square test was used. A *p* value of less than 0.05 was considered statistically significant in our study.

Results

General information

We included 100 patients with recurrent infections according to criteria explained in Materials and methods section. Among these patients, 67% were male and 33% were female. The mean age of patients was 6.2 ± 4.0 years. Although all patients had a chief complaint of recurrent infections, the patients were categorized into 6 main groups according to main affected organs. We found out that the most frequent complaints were lower respiratory infections (31%) and upper respiratory infections (30%), followed by severe systemic infections (13%), gastrointestinal infections (11%), soft tissue infections or abscess (11%) and skin infections (4%).

Consanguineous marriage rate was 38% overall and there were only 2 patients with a family history of PID. One patient was a 9-year old girl with a history of common variable immunodeficiency (CVID) in her maternal uncle; she had recurrent

pneumonia due to her congenital heart disease and there was no serologic evidence of PID. The second patient was a 5-year old boy with hyper IgM syndrome (HIgM) with a history of death in his infant sibling and his maternal uncle, also diagnosed with the same syndrome.

Comparison of groups' characteristics

After careful laboratory tests and evaluation of clinical diagnostic criteria, we categorized patients with a history of recurrent infections into the following 5 subgroups: healthy children, PID, SID, anatomical or functional disorders and allergic conditions. Accordingly, we diagnosed 30 cases as healthy, 18 as allergy, 16 as SID, 15 as anatomical or functional disorders, and 21 as PID (**table I**). In PID group, the mean age of patients was 6.3 ± 4.6 years and there were 11 (52.3%) males in this group. Fifteen patients born of consanguineous marriages (71.4%, **figure 1**) were seen in the PID group. The mean age at time of the first manifestation was 4.6 ± 4.0 years and the mean diagnostic delay in this group was 2.8 ± 0.3 years. There was only 1 HIgM (4.5%) case with a family history of PID. The most frequent presentation in this group was soft tissue infections or abscess, in 7 (33.3%) patients.

The main diagnosis in allergy group was hyper-reactive airway disease (HRAD including small hyper-reactive airway) with the frequency of 15 (83%) of all 18 cases. The coincidence of HRAD and gastro-esophageal reflux disease (GERD) were seen in 1 (5.6%) case in allergy group, while the two remaining patients' diagnoses were asthma 2 (11%).

The main disease in anatomical or functional defects group was cystic fibrosis with a frequency of 5 (33.3%) cases, followed by congenital heart disease in 4 (26.7%), GERD in 2 (13.3%) and hypertrophic adenoids in 2 (13.3%). Interstitial lung disease and nasal septum deviation were each identified in one patient. In the SID group, HIV infection in 4 (25%) and corticosteroid side-effect in 3 (19%) cases were the main evidenced causes of recurrent infections. Regarding the other SID diseases, there was 1 (6.3%) each with cytomegalovirus infection, Crohn's disease, protein-losing enteropathy, fatty acid oxidation disorder, Gaucher's disease, juvenile polyposis, intestinal lymphangiectasia, lymphoma, and maple syrup urine disease.

Demographic and clinical data of other causative groups of recurrent infection are summarized in **table I**. PID group presented a significantly higher rate of parental consanguinity ($p = 0.001$) and soft tissue infections ($p = 0.004$). In contrast, healthy individuals significantly manifested upper respiratory infection ($p = 0.024$), while the lower respiratory infection was more frequent in cases with anatomical or functional defects ($p = 0.001$). Sex of patients did not influence the incidence of PID or anatomical disease, but there was a slightly higher rate of males in allergy and SID.

Table I - Classification of demographic and clinical data of 100 pediatric individuals with complaint of recurrent infection based on the final definite diagnosis.

Parameters	total (n = 100)	healthy (n = 30)	atopy (n = 18)	anatomical (n = 15)	SID (n = 16)	PID (n = 21)	p-value
sex (m/f)	67/33	22/8	14/4	7/8	13/3	11/10	0.099
onset age, years (SD)	3.7 (3.0)	2.1 (2.0)	3.9 (1.8)	4.4 (4.8)	2.8 (1.4)	4.6 (4.0)	0.321
diagnosis / current age, years (SD)	6.2 (4.0)	6.0 (3.5)	5.8 (3.0)	7.8 (5.2)	5.1 (3.8)	6.3 (4.6)	0.482
consanguinity (%)	37 (37.0)	5 (16.6)	4 (22.2)	8 (53.3)	5 (31.2)	15 (71.4) ^a	0.001
positive family history (%)	2 (2.0)	0	0	1 (6.6)	0	1 (4.5)	0.52
episodes of infection / year (SD) ¹	5.9 (4.7)	5.3 (2.7)	6.9 (3.2)	4.8 (2.0)	7.4 (3.6)	6.6 (4.0)	0.23
Major complications							
upper respiratory infection (%)	30 (30.0)	14 (46.7) ^c	8 (44.4)	3 (20.0)	2 (12.5)	3 (14.3)	0.024
lower respiratory infection (%)	31 (31.0)	2 (6.6) ^d	8 (44.4)	10 (66.6) ^d	6 (37.5)	5 (23.8)	0.001
gastrointestinal infection (%)	11 (11.0)	3 (10.0)	1 (5.5)	2 (13.3)	4 (25.0)	1 (4.8)	0.320
skin infection (%)	4 (4.0)	1 (3.3)	0	0	0	3 (14.2)	0.097
soft tissue infection or abscess (%)	11 (11.0)	3 (10.0)	0	0	1 (6.2)	7 (33.3) ^e	0.004
severe infection (%)	13 (13.0)	7 (23.3)	1 (5.5)	0	3 (18.8)	2 (9.5)	0.156
Laboratory tests							
white blood count (cell/ul)	13230 ± 7120	15229 ± 4330	13107 ± 4591	16211 ± 3060	11028 ± 3416	7023 ± 6028	0.07
lymphocytosis (%)	72 (72.0)	28 (93.3)	14 (77.8)	15 (100)	5 (31.2)	10 (47.6)	<0.001
leukopenia (%)	9 (9.0)	0	0	0	3 (18.7)	6 (28.5)	0.001
neutrophils (cell/ul)	7450 ± 7277	7952 ± 3171	8341 ± 2400	8591 ± 2803	5801 ± 2974	3704 ± 3380	0.05
neutropenia (%)	5 (5.0)	0	0	0	1 (6.2)	4 (19.0)	0.01
lymphocytes (cell/ul)	5200 ± 5147	5427 ± 2730	7379 ± 4100	5278 ± 3003	5914 ± 5774	3710 ± 3099	0.03
lymphopenia (%)	9 (9.0)	0	0	0	4 (25.0)	5 (23.8)	0.002
high erythrocyte sedimentation rate (%)	86 (86.0)	25 (83.3)	17 (94.4)	15 (100)	8 (50.0)	21 (100)	< 0.001
high C-reactive protein (%)	85 (85.0)	26 (86.6)	17 (94.4)	14 (93.3)	7 (47.7)	21 (100)	< 0.001
human immunodeficiency virus (%)	4 (4.0)	0	0	0	4 (25.0)	0	< 0.001
low serum IgM level (%)	7 (7.0)	0	0	0	1 (6.2)	6 (28.5)	< 0.001
higher serum IgM level (%)	29 (29.0)	5 (16.6)	7 (38.8)	9 (60.0)	5 (31.2)	3 (14.2)	0.01
low serum IgG level (%)	5 (5.0)	0	0	0	1 (6.2)	4 (19.0)	0.01
higher serum IgG level (%)	27 (27.0)	7 (23.3)	7 (38.8)	3 (20.0)	7 (47.7)	3 (14.2)	0.21
low serum IgA level (%)	8 (8.0)	0	0	0	1 (6.2)	7 (33.3)	< 0.001
low isohaemagglutinin titers (%)	8 (8.0)	0	0	0	0	8 (38.0)	< 0.001

Parameters	total (n = 100)	healthy (n = 30)	atopy (n = 18)	anatomical (n = 15)	SID (n = 16)	PID (n = 21)	p-value
low anti-P23 pneumococcal antibody (%)	5 (5.0)	0	0	0	0	5 (23.8)	< 0.001
low anti-tetanus antibody (%)	5 (5.0)	0	0	0	0	5 (23.8)	< 0.001
low anti- diphtheria antibody (%)	7 (7.0)	0	0	0	0	7 (33.3)	< 0.001
low CD3 ⁺ T-cells (%)	3 (8.0)	0	0	0	0	3 (14.2)	0.02
low CD4 ⁺ T-cells (%)	5 (5.0)	0	0	0	0	5 (23.8)	< 0.001
low CD8 ⁺ T-cells (%)	3 (3.0)	0	0	0	0	3 (14.2)	0.02
low CD19 ⁺ B-cells (%)	4 (4.0)	0	0	0	0	4 (19.0)	0.003
low CD16-56 ⁺ NK-cells (%)	3 (3.0)	0	0	0	0	3 (14.2)	0.02
negative nitroblue-tetrazolium test (%)	0	0	0	0	0	0	-
negative purified protein derivative test (%)	4 (4.0)	0	0	0	0	4 (19.0)	0.003
positive skin prick test (%)	19/26 (73.0)	NI	15/18 (83.3)	NI	NI	4/8 (50.0)	0.46
defective spirometry test-higher than 6 years (%)	16/48 (33.3)	0/11 (0)	7/7 (100)	2/9 (22.2)	2/8 (25.0)	5/13 (38.4)	0.06

Abbreviations: SID, secondary immunodeficiency; PID, primary immunodeficiency; not indicated.

¹Including episodes of viral infections, common cold and flu.

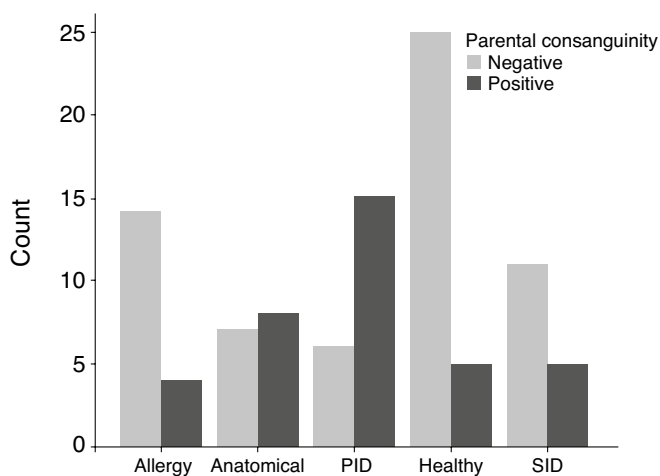
²p < 0.001 and p = 0.016 in comparison with healthy and anatomical groups, respectively.

³p = 0.016 and p = 0.02 in comparison to PID and SID groups, respectively.

⁴p < 0.00 in comparison to healthy group.

⁵p = 0.008, p = 0.042 and p = 0.045 in comparison to allergy, anatomical and healthy groups.

Figure 1 - Consanguinity frequency in different diagnostic groups of 100 pediatric individuals with complaint of recurrent infection. SID, secondary immunodeficiency; PID, primary immunodeficiency.



Intragroup comparison of PID

According to IUIS classification, we classified PID cases as following: 3 patients of combined immunodeficiency (all with severe combined immunodeficiency), 2 of well-defined syndromes with immunodeficiency (both with ataxia-telangiectasia), 6 of predominantly antibody deficiencies (X-linked agammaglobulinemia in 33.3% and CVID, HIGM, IgA deficiency and undefined hypogammaglobulinemia each in 16.6%), 8 of congenital defects of phagocytosis (Mendelian susceptibility to mycobacterial disease and severe congenital neutropenia each in 37.5% and leukocyte adhesion deficiency in 25%), one of immune dysregulation with hemophagocytic lymphohistiocytosis disease, and one of defects in innate immunity with anhidrotic ectodermal dysplasia. There were no patients with a diagnosis of autoinflammatory disorders or complement deficiencies in the present survey.

In antibody deficiencies group, the mean age of patients was 7.3 ± 5.7 years and the mean age of onset was 6.2 ± 6.0 years, which had a later onset and diagnosis comparing to phagocytosis disorders (6.9 ± 4.8 and 4.0 ± 3.2 years, respectively) and combined

Table II - Comparisons of main affected organ by infections in different types of PID patients.

Parameters	PID (n = 21)	Combined immunodeficiency (n = 3)	Antibody deficiencies (n = 6)	Defects of phagocytosis (n = 8)	Other PIDs (n = 4)
upper respiratory infection (%)	3 (14.3)	-	1 (16.6)	-	2 (50)
lower respiratory infection (%)	5 (23.8)	1 (33.3)	4 (66.7)	-	-
gastrointestinal infection (%)	1 (4.8)	1 (33.3)	-	-	-
skin infection (%)	3 (14.2)	-	1 (16.6)	-	2 (50)
soft tissue infection or abscess (%)	7 (33.3)	-	-	7 (87.5)	-
severe infection (%)	2 (9.5)	1 (33.3)	-	1 (12.5)	-

immunodeficiencies (2.7 ± 0.57 and 2.5 ± 0.38 years, respectively), but these differences were not statistically significant ($p = 0.37$ and $p = 0.17$, respectively).

There were 7 (87.5%) females with phagocytosis disorders, but there was a male dominance in the antibody deficiency (100%) and in the combined immunodeficiency (67%) groups; in which phagocyte disorders were significantly more frequent among females than males compared to antibody deficiencies ($p = 0.007$). Parental consanguinity was recorded in the 87.5% of phagocyte disorders and 100% of combined immunodeficiency groups. In the antibody deficient patients, however, there were 3 (50%) consanguineous marriages ($p = 0.124$).

Regarding to the first presentation and the main organ involvement, among phagocytosis disorders there were 7 (87.5%) patients with soft tissue infections, in antibody deficiency group there were 4 (66%) patients with lower respiratory infections, and in combined immunodeficiencies group, there was 1 (33.3%) patient with a lower respiratory infection, 1 (33.3%) with a gastrointestinal infection and 1 (33.3%) with severe systemic infections ($p = 0.015$, **table II**). Comparing antibody deficiency and phagocyte groups, systemic and soft tissue infections were significantly more frequent in phagocyte group ($p = 0.012$), while respiratory tract infections were the main presentation of in antibody deficiency group ($p = 0.04$).

Discussion

A considerable proportion of children with recurrent infections referred to the tertiary hospitals may have PID, and parental consanguinity was significantly higher in this group, comparing to other causes of recurrent infections. Moreover, presenting symptoms of children with recurrent infections may be helpful to determine the underlying causes of disease, as recurrent soft tissue infections strongly suggest a PID especially in phagocytosis disorders, and recurrent lower respiratory tract infection suggest anatomical / functional defects or an antibody immunodeficiency.

In our previous retrospective study on the rate of PID among children with recurrent infections in 2012 in the same tertiary hospital, we have shown that only 11% of 260 patients were labeled as PID (6), whilst by applying the recommended step-wise guideline to these patients in the current prospective study, this rate was higher (21%), suggesting a risk of lower-estimation and undiagnosed mild form of PID among patients underwent non-systematic approach to recurrent infections.

Parental consanguinity seems to be a key finding in the PID group, as there was about 72% of consanguinity in the family history of these patients. Although the consanguinity was also frequent in patients with anatomical disorders (53.3%, in 5 patients with cystic fibrosis, 2 patients with congenital heart disease and 1 patient with interstitial lung disease) this rate was lower than PID patients. In the PID subgroups section, 90% of phagocyte disorders group, 50% of antibody deficiency group, and 100% of combined immunodeficiency group had consanguineous parents. In a previous study in 2013, the mean proportion of consanguineous marriages was 65.6% among Iranian PID patients who were registered in the database, while the overall rate was 38.6% in general population of Iran. However, the rate of consanguinity was reported about 76% in combined immunodeficiency, 73% in defects of phagocytic function, and 54% in predominantly antibody deficiencies (37). Although genetic analysis was not the scope of this study, the findings of higher consanguinity and slightly higher rate of male gender in antibody and combined immunodeficiencies reflect the presence of autosomal recessive and X-linked inheritance pattern of PID in our cohort, respectively, as has been reported previously (38,39). These findings are in accordance with other reports from the Middle Eastern region (40) with a high rate of consanguineous marriage, and North of Africa countries (41,42). No patient with a final diagnosis of complement deficiencies or autoinflammatory diseases was recorded in this survey. As a fact, among PIDs, complement deficiencies are relatively rare

Table III - Comparisons of median delay in diagnosis of different types of PID patients diagnosed with and without systematic approach to recurrent infection.

Cause of recurrent infections	Patients diagnosed without systematic approach to recurrent infection, years (range)	Patients diagnosed with systematic approach to recurrent infection, years (range)	p-value
primary immunodeficiency	4 (0-33)	2.8 (0-5)	0.003
combined immunodeficiency	0.16 (0-12)	0.13 (0-1.2)	0.07
antibody deficiencies	2.13 (0-28)	1.3 (1-5)	0.01
defects of phagocytosis	0.5 (0-15)	0.2 (0.1-1.7)	0.04

(less than 2% of total PID registry in Iran [37]), and the majority of patients with complement deficiencies do not present with increased susceptibility to infections, and usually suffer from hereditary angioedema and autoimmune disorders such as systemic lupus erythematosus, glomerulonephritis, vasculitis and autoimmune cytopenia (43,44). Of course, this depends on which complements are missing, but terminal complement deficiencies presenting with recurrent *Neisseria* infections including meningitis, are very rare disorders.

Similarly, patients who have an autoinflammatory disease such as familial Mediterranean fever rarely complain from recurrent or chronic infections (45,46). Therefore, it would be noted that non-infectious warning signs of PID particularly associated with complement deficiencies and autoinflammatory diseases, should be combined in the current guideline of approach to recurrent infectious patients.

Moreover, the findings of this survey demonstrated that attention to presenting symptoms of children with recurrent infections may be helpful to target complementary para-clinical tests, and to determine the underlying causes of disease. Based on our findings, recurrent soft tissue infections strongly suggest a PID, especially in phagocytosis disorders. In contrast, recurrent upper respiratory tracts infections usually presented as a mild condition in healthy individuals, and lower respiratory tract infection presented in those patients with anatomical or functional defects.

It should be noted that this data was collected in a tertiary referral hospital; therefore, most of our patients in this study were referred from other primary / peripheral centers. Indeed, some of them had many prior admissions or visits with a chief complaint of recurrent infections; so, a higher incidence of PID would be expected than in general populations. In addition, the higher percentage of SID or anatomical disease and a lower rate of healthy patients in this study might reflect this notion.

Although the findings of unusual pathogens have been reported to be crucial for diagnosis of PID (6), infection due to enhanced

susceptibility to disease with a specific common germs even in a single organ can however be associated with PID (47). Moreover, most of the patients with recurrent infections were under different antibiotic therapies, which directly affect the microbiologic evaluation (48). Therefore, we did not aim to evaluate the pathogens of patients in this study.

One of the important results of this survey was the evidence of reduction of PID diagnostic delay through a stepwise approach that we previously elucidated (6). Based on that approach, in the present study we evaluated the distribution of recurrent infections among 100 patients. The mean diagnostic delay of PID in this study was 2.8 years, which was significantly lower than 4 years, indicated in our previously published registry report (37). **Table III** demonstrates the delay in diagnosis in each main PID disorder, in which antibody deficiency and phagocytosis showed significant lower delay in diagnosis prospectively compared to previously published patients (6). Intriguingly, the rate of long-term complication of these earlier discovered patients decreased significantly (particularly bronchiectasis in group of antibody deficiency decreased from 25% to 16.6%), and the chance of performing hematopoietic stem cell transplantation were increased (less than 5% to 66.6% in group of combined immunodeficiency), suggesting the importance of timely diagnosis by the established guideline on treatment and management of PID patients.

However, there is a meaningful lag to timely diagnosis and treatment of PID, even after hospital admissions as observed in the medical history of currently studied patients. This fact explains the necessity of attention to increasing awareness of physicians and the need for proper laboratory tests in peripheral centers other than referral hospitals (21). In a developing country like Iran, there is insufficient medical equipment, and also an unequal distribution of diagnostic facilities. For example, NBT is a common test for diagnosis of CGD in Iran, and the current diagnostic criteria were based on the result of this method. NBT

is an old test that is not considered the currently preferred methodology for the diagnosis of CGD, and we hope change NBT to flow cytometric method as a common test for diagnosis of CGD in future in Iran.

Regular and continuous education should be considered for pediatricians and general practitioners to inform them about updated screening steps and preliminary diagnostic tests to perform timely referral to a specialist when a chronic condition such as PID is suspected.

Conflict of interest

The authors declare that they have no conflict of interest.

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