Clinical and Laboratory Characteristics of Primary Immunodeficiency Patients from a Tertiary Care Center in Pakistan

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Abstract
Objective: The aim of this study was to describe and identify clinical presentation of primary immunodeficiency disorders (PIDs). Characteristic quantitative and qualitative immunological abnormalities have been described which help in establishing a definitive PID diagnosis.

Methods: This was a cross sectional study conducted in the Immunology department of the Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from Jan 2016 to Dec 2018. Sixty patients of different PIDs including humoral defects, combined immunodeficiency, phagocytic defects and other miscellaneous disorders, were diagnosed over a period of 3 years in our institute. Their clinical presentation and laboratory data are presented in this study.

Results: In 3 years, 40 (66%) males and 20 (33%) females were diagnosed, with 13 (21.6%) patients of humoral deficiency, 22 (36.6%) of severe combined immunodeficiency, 18 (30%) of phagocytic defects and 7 (11.6%) of other miscellaneous disorders. Maximum patients belonged to Punjab province, i.e., 23 (38.3%). Their mean age for initiation of symptoms was 7±12.6 months, while diagnosis was made at mean age of 26±39.28 months, in all groups combined. Respiratory infections were commonest presentation, in 46 (76.6%) patients. Also 46 (76.6%) patients had consanguineous parents. Presence of family history of PID in 27 (45%) patients was not associated with an earlier diagnosis (p 0.955). Each group of patients carried characteristic laboratory findings.

Conclusion: PIDs should be suspected in offsprings with warning signs coming from consanguineous parents. There is a need to introduce genetic diagnosis of PIDs in order to timely diagnose less characteristic PID presentations.

Keywords: Primary immunodeficiency, recurrent infections, immunology, consanguinity, family history, diagnostic lag

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Introduction
Primary immunodeficiency disorder (PIDs) is considered a group of over 250 disorders that are either because of defects in immune system development and/or function caused by defects of different components of the immune system. These disorders are characterized by an increased susceptibility to infections and a predisposition to autoimmunity and malignancy. PIDs are broadly classified as disorders of adaptive immunity (i.e., T-cell, B-cell or combined immunodeficiencies) or of innate immunity (e.g., phagocyte and complement disorders). Early diagnosis and treatment are imperative for preventing significant disease-associated morbidity and, therefore, consultation with a clinical immunologist is essential. PIDs should be suspected in patients with: recurrent sinus or ear infections or pneumonias within a 1-year period; failure to thrive; poor response to prolonged use of antibiotics; persistent thrush or skin abscesses; or a family history of PID. Patients with multiple autoimmune diseases should also be evaluated. Diagnostic testing often involves measurement of serum immunoglobulin (Ig) levels, assessment of serum specific antibody titers in response to vaccine antigens, neutrophil function assays, stimulation assays for cytokine responses, lymphocyte proliferation assays, flow cytometry and complement studies. The treatment of PIDs is complex and generally requires both supportive and definitive strategies. Ig replacement therapy is the mainstay of therapy for B-cell disorders, and is also an important supportive treatment for many patients with combined immunodeficiency disorders. The heterogeneous group of disorders involving the T-cell arm of the adaptive system, such as severe combined immunodeficiency (SCID), require immune reconstitution as soon as possible. The treatment of innate immunodeficiency disorders varies depending on the type of defect, but might involve antifungal and antibiotic prophylaxis, cytokine replacement, vaccinations and bone marrow transplantation.

All forms of PIDs are rare and worldwide incidence of PIDs is variable ranging from around 1 in 10,000 to 3 in 100,000 live births except IgA deficiency, which is comparatively common and incidence is 1 in 600 live births. PID registries are established in several countries to determine the incidence and prevalence of the disease. In Pakistan, no such registry has been established so far and no studies have been done regarding the incidence and prevalence of PIDs. Lack of awareness and consideration about PID,
led to an increased diagnosis lag and inappropriate treatment, which is the main cause of morbidity and mortality in these patients. A considerable delay in the diagnosis of antibody deficiency has been shown in patients from northwest England with a median delay of 5.5 years in adults and 2.5 years in children. Several other studies have confirmed this finding, based on the time of initial symptoms until the time of diagnosis. A UK national audit led to recommendations on early diagnosis that was distributed to all UK general medical practitioners and specialist clinicians to whom patients with antibody deficiency are most commonly referred. There is a quite dearth of knowledge regarding the incidence of PIDs in Pakistan. With the high rate of consanguineous marriages, the incidence is likely to be high. However, if children with PIDs are to be successfully treated with early antifungal and antibiotic prophylaxis, cytokine replacement, vaccinations and bone marrow transplantation, the health care community needs to be sensitized about the prevalence and early diagnosis of this disorder.

The aim of this study was to describe clinical presentation of PIDs. Characteristic quantitative and qualitative immunological abnormalities have been described which help in establishing a definitive PID diagnosis.

**Methods**

This cross-sectional study was carried out in Immunology department of Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from Jan 2016 to Feb 2019. Initial screening was done with thorough history on a pre designed pro-forma from all patients (or their parents) who were referred to us by paediatricians/physicians, for PIDs workup. Patients were investigated for having respiratory tract infections (tonsillitis, pharyngitis, laryngitis, sinusitis, otitis media, bronchitis or pneumonia), gastrointestinal tract (GIT) infections (diarrhoea), or skin infections (including omphalitis). Final inclusion criteria in the study was based on European Society for Immunodeficiencies (ESID) registry criteria, in the light of history findings and results of laboratory investigations performed, as mentioned in laboratory tests section of methods.

Informed consent was taken from patient’s parents/guardians that clinical information; sample and data will also be used for research purposes. Ethical committee of the institute approved the project because all the tests carried out were part of routine PID work up and did not include any other intervention.

Parent’s consanguinity was defined as father and mother of patient being second cousins or closer.

Family history of immunodeficiency was defined as patient having at least one sibling who died due to frequent respiratory, gastrointestinal or skin infections. The diagnosis may be correct or not for the expired sibling.

Normal umbilical cord separation time has been estimated to be approximately 6 days, whereas over two weeks separation was considered delayed.

Broadly, diseases were classified into humoral immunodeficiency (including agammaglobulinaemia, common variable immunodeficiency, hyper IgM syndrome and IgG subclass deficiency), combined immunodeficiency (including all T, B and NK lineages deficiency), phagocytic dysfunction defects (including chronic granulomatous disease and leukocyte adhesion deficiency type I) and miscellaneous (including CD4 and CD8 deficiency, Wiskott-Aldrich syndrome, Hyper IgE syndrome and autoimmune lymphoproliferative syndrome). Since facilities for genetic diagnosis of PIDs are not available in Pakistan, European Society for Immunodeficiencies (ESID) registry criteria was used to classify the patients into different PID disorders wherever possible.

After taking a thorough history and physical examination, a provisional PID diagnosis was made and patient was investigated accordingly. From all the patients suspected of having predominantly antibody/cellular/combined PID, 2-3 ml of potassium ethylene diamine tetra acetate (EDTA) and 2-3 ml of serum sample in a clot activator tube were taken. Serum immunoglobulin (Ig) levels (IgG, IgA and IgM) were measured by nephelometry using Binding site, UK kit on SPA Plus instrument. Serum IgE levels were estimated using Bioscience, USA ELISA kit. IgG subclasses where indicated were done using Binding site, UK radial immunodiffusion (RID) kit. Flow cytometry was carried out on either Becton Dickinson (BD) FACSCalibur or FACCanto II instrument, using anti CD45, CD3, CD4, CD8, CD19, CD16/56 monoclonal antibodies either in FITC or PE combinations from BD Biosciences, San Jose, CA, USA. Flow cytometry for suspected leukocyte adhesion deficiency type I (LAD I) cases was done using anti CD11b, CD11c and CD18 monoclonal antibodies from same the manufacturer. Diagnosis of neutrophil function defect (chronic granulomatous disease, CGD) was confirmed by dihydrorhodamine (DHR) assay by flow cytometry.

All the data was entered in statistical package for social sciences (SPSS) version 20.0 (IBM Corp, Armonk, NY) and analyzed for frequencies and statistical significance. Nominal variables like gender, clinical manifestations, parent’s consanguinity and delayed cord separation were analyzed for percentages and compared using chi square test. Numerical variable like mean age at diagnosis, mean age of presentation, and other laboratory parameters were
analyzed for mean and standard deviation and compared using one-way ANOVA and t test.

**Results**

Over 3 years, we made confirmed PID diagnosis in total 60 patients, out of which 40(66.6%) were males and 20(33.3%) were females. Broadly, we had 13(21.6%) patients of humoral immunodeficiency, 22(36.6%) patients of combined immunodeficiency, 18(30%) patients of phagocytic defects and 7(11.6%) patients of miscellaneous disorders. Detailed frequency distribution of different disorders is shown in figure. Twenty-three (38.3%) patients belonged to Punjab, 18 (30%) to Khyber Pakhtunkhwa, 8 (13.3%) to Sind, 4 (6.6%) to Northern areas, while province of 7(11.6%) patients was not known. Mean age at start of symptoms in all patients was 7±12.6 months (range 1-65), while mean age of diagnosis was 26±39.28 months (range 1-216). Detailed age distribution from start of symptoms till diagnosis is made in different PID groups is given in table 1. It is evident that humoral deficiencies are marked by maximum delay in diagnosis.

Frequency of respiratory, gastrointestinal tract and skin infections, parents' consanguinity and family history of PID, for all PID groups has been shown in table 2. Respiratory infections are commonest among all PID groups (76.6%). Rate of parent's consanguineous marriages and family history of immunodeficiency is fairly high among all PID patients (76.6% and 45% respectively). Chi square test determined p-value of 0.955, indicating that presence of family history of PID was not associated with diagnosis before mean age, i.e., 26 months.

Table 3 shows characteristic laboratory findings in humoral and combined immunodeficiency groups. T lymphocytes are significantly reduced in combined immunodeficiency as compared to humoral deficiency. With regards to CGD patients, all had absent neutrophil oxidative burst activity on DHR assay. Similarly, all LAD I patients had CD11b, CD11c and CD18 expression on less than 2% of their neutrophils.

**Discussion**

In three years' time, we were able to make confirmed diagnosis in 60 cases, with male: female 2:1. The spectrum was dominated by severe combined immunodeficiency disorders (36.6%) involving T, B and NK lineages in different combinations (Figure). Majority of patients (68.3%) were from Punjab and KPK, since our institute serves as catchment area mainly for Northern/middle part of Pakistan. In UK, PID disorders were predominantly antibody deficiencies, with almost equal gender distribution.10 In Iran, gender distribution was almost same as ours, with combined immunodeficiency/phagocytic defects (17% each) second to antibody deficiency disorders (30%).13 Similar pattern followed in India with X linked agammaglobulinaemia dominating combined immunodeficiency.12 The symptoms of humoral deficiency were latest to start (mean 19 months), causing delay in

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**Table 1:** Delay in diagnosis in different PID groups.

<table>
<thead>
<tr>
<th>S No</th>
<th>PID Group</th>
<th>Mean age in months at start of symptoms (range)</th>
<th>Mean age in months at diagnosis (range)</th>
<th>Mean delay (range) in diagnosis (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Humoral deficiency (n=13)</td>
<td>19.23±20.20 (1-65)</td>
<td>53.46±59.63 (2-216)</td>
<td>34.23±56.42 (1-204)</td>
</tr>
<tr>
<td>2</td>
<td>Combined immunodeficiency (n=22)</td>
<td>2.18±2.46 (1-12)</td>
<td>6.59±12.09 (1-60)</td>
<td>4.41±9.83 (0-48)</td>
</tr>
<tr>
<td>3</td>
<td>Phagocytic dysfunction (n=18)</td>
<td>3.22±4.78 (1-20)</td>
<td>23.67±34.82 (1-120)</td>
<td>20.44±30.51 (0-100)</td>
</tr>
<tr>
<td>4</td>
<td>Miscellaneous (n=7)</td>
<td>8.14±6.76 (1-20)</td>
<td>40.86±28.67 (3-72)</td>
<td>32.71±28.97 (2-69)</td>
</tr>
<tr>
<td>5</td>
<td>Combined (n=60, all groups)</td>
<td>6.88±11.93 (1-65)</td>
<td>25.87±39.28 (1-216)</td>
<td>18.98±34.48 (0-204)</td>
</tr>
</tbody>
</table>

One Way ANOVA <0.001 0.003 0.050

**Table 2:** Frequency of different clinical parameters among patients from different PID groups.

<table>
<thead>
<tr>
<th>S No</th>
<th>PID Group</th>
<th>Patients with respiratory infections (%)</th>
<th>Patients with gastrointestinal infections (%)</th>
<th>Patients with skin infections (%)</th>
<th>Patients with parent’s consanguinity (%)</th>
<th>Patients having family history of PID (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Humoral deficiency (n=13)</td>
<td>11 (84.6)</td>
<td>3 (23.0)</td>
<td>6 (46.1)</td>
<td>8 (61.5)</td>
<td>6 (46.1)</td>
</tr>
<tr>
<td>2</td>
<td>Combined immunodeficiency (n=22)</td>
<td>19 (86.3)</td>
<td>12 (54.5)</td>
<td>8 (36.3)</td>
<td>16 (72.7)</td>
<td>8 (36.3)</td>
</tr>
<tr>
<td>3</td>
<td>Phagocytic dysfunction (n=18)</td>
<td>10 (55.5)</td>
<td>1 (0.0)</td>
<td>12 (66.6)</td>
<td>17 (94.4)</td>
<td>10 (55.5)</td>
</tr>
<tr>
<td>4</td>
<td>Miscellaneous (n=7)</td>
<td>6 (85.7)</td>
<td>3 (42.8)</td>
<td>5 (71.4)</td>
<td>5 (71.4)</td>
<td>3 (42.8)</td>
</tr>
<tr>
<td>5</td>
<td>Combined (n=60, all groups)</td>
<td>46 (76.6)</td>
<td>19 (31.6)</td>
<td>31 (51.6)</td>
<td>46 (76.6)</td>
<td>27 (45)</td>
</tr>
</tbody>
</table>

Chi square test 0.093 0.008 0.176 0.162 0.684

**Table 3:** Serum immunoglobulin levels and lymphocyte subset analysis in humoral and combined immunodeficiency groups.

<table>
<thead>
<tr>
<th>PID Group</th>
<th>IgG concentration</th>
<th>IgA concentration</th>
<th>IgM concentration</th>
<th>Lymphocyte count (%)</th>
<th>CD3 count (%)</th>
<th>CD4 count (%)</th>
<th>CD8 count (%)</th>
<th>CD19 count (%)</th>
<th>CD16 count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humoral deficiency (n=13)</td>
<td>1.20±1.62</td>
<td>0.27±0.39</td>
<td>1.28±2.48</td>
<td>3260.38±1710.61 (42)</td>
<td>2608.00±1503.15 (75)</td>
<td>917.92±628.84 (25)</td>
<td>1610.69±1031.88 (47)</td>
<td>227.85±441.65 (8)</td>
<td>269.77±289.01 (11)</td>
</tr>
<tr>
<td>Combined immunodeficiency (n=22)</td>
<td>3.80±5.11</td>
<td>0.60±0.76</td>
<td>0.39±0.43</td>
<td>1403.09±1726.74 (19)</td>
<td>230.77±623.86 (10)</td>
<td>131.00±505.49 (5)</td>
<td>80.59±326.66 (4)</td>
<td>493.91±1438.49 (18)</td>
<td>531.73±517.93 (52)</td>
</tr>
<tr>
<td>T test</td>
<td>0.085</td>
<td>0.162</td>
<td>0.110</td>
<td>0.004</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.523</td>
<td>0.104</td>
</tr>
</tbody>
</table>
diagnosis age (mean 53 months) and subsequently maximum lag in diagnosis period (mean 34 months), when compared to other PID disorders (Table 1). Mean diagnostic delay in all groups combined was 19 months, compared to Iranian patients (10 months).13 For CVID patients, Chapel et al have reported a diagnostic delay of 5 years22 whereas in Mexico it is 2.17 years for all PID groups.23

Our data showed that respiratory infections were commonest type of infections in humoral deficiency, combined immunodeficiency and miscellaneous group of patients, while phagocyte defect patients were more frequently affected by skin infections. GIT infections were least common in all groups except combined immunodeficiency where skin infections were least found. This is in conjunction with Nima Rezaei findings where respiratory infections were commonest in PID patients except phagocytic defects who were more frequently affected with cutaneous infections. GIT infections were least common in all groups except combined immunodeficiency where skin infections were least found.

Limitations
Recent International Union of Immunological Societies (IUIS) classification of PIDs had introduced categories other than humoral, cellular and phagocytic defects also.29 These include less profound PIDs, those with syndromic features and immune dysregulation defects, autoinflammatory disorders and defects in intrinsic and innate immunity. Most of these require genetic diagnosis usually by next generation sequencing (NGS). We have started collaborations with centers of excellence in PID diagnosis in order to make genetic diagnosis in order to make genetic diagnosis and to collect data for common mutations here, until the time these technologies are available in Pakistan.

Conclusion
PIDs encompass a broad range of disorders that should be suspected in offspring with warning signs coming from consanguineous parents. Respiratory infections and presence of family history of PID are frequent findings. Characteristic laboratory findings help diagnose suspected cases. There is a need to introduce genetic diagnosis of PIDs in order to timely diagnose less characteristic PID presentations.

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Conflict of Interest: None.
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References