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Determination of humoral and cellular immune status in children with clinically suspected primary immunodeficiency disorders

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Abstract:

Primary immunodeficiency is a cluster of disorders resulting in impaired function and/or development of the immune system that

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share a common theme of excessive susceptibility to infections. These serious episodes of infection markedly impair patient's ability to lead a normal life. Diagnosis is essential to treat patients at early stage and reduce morbidity and mortality rate while increasing the quality of life in affected population. This study was done to detect different categories of primary immunodeficiency disorders (PIDD) from clinically suspected PIDD patients of child age group. Blood samples were collected from 50 clinically suspected children (<18 years) with repetitive attack of infection and refractory to antibiotic treatment and following tests were done: complete blood count with differential count on the leucocytes, serum immunoglobulin level, lymphocyte subset analysis and nitro blue tetrazolium dye test. PIDD was diagnosed in patients according to European Society for Immunodeficiency (ESID) diagnostic criteria. The diagnosed patients were classified into eight different sub groups according to the International Union of Immunological Societies (IUIS) classification criteria. Ten patients (20%) were diagnosed as primary immune deficiency disorders. The mean age of the patients at the time of diagnosis was 2.89 ± 2.61 years (range: 2 months -11yrs). The majority (60%) had predominantly antibody deficiency disorders followed by combined immune deficiency (30%) and phagocytic disorder (10%). Transient hypogammaglobulinemia of infancy (33.33%) was the commonest antibody deficiency disorder followed by 16.66% in each case of common variable immune deficiency, agammaglobulinaemia, selective Immunoglobulin M deficiency and Immunoglobulin A deficiency. Three patients had severe combined immune deficiency (SCID), including two with T-B- SCID and one with T-B+SCID. Congenital neutropenia was diagnosed in one patient. Common mode of presentations were recurrent pneumonia in 80% patients, otitis media in 20% patients, deep organ abscess in 10% patients and oral thrush in 10% patients. Family history of previous sibling death due to infections was elicited in 40% (4/10) patients. Predominantly antibody deficiency disorders are common among PIDD cases. In a resource constrained country like us, understanding the clinical clues and using a small set of basic tests may enable diagnosis of most PIDD, so that appropriate treatment can be sought.

Key words: Primary immunodeficiency, Common variable immune deficiency, agammaglobulinemia, Severe combined immune deficiency, Congenital neutropenia

INTRODUCTION

Primary immune deficiency disorders (PIDD) are a group of diseases caused by inherited defects of the immune system, in which the common hallmark is susceptibility to infection. Nowadays, more than 220 types of PIDD have been identified [1]. Common PIDD include disorders of humoral immunity, T lymphocytes defects, combined B and T lymphocytes defects, phagocytic disorders and complement deficiencies [2]. During the last 20 years the number of known PIDD has increased considerably in Japan [3]. The estimated number of patients with PIDD 2,900 with a prevalence of 2.3 per 100,000 people [4]. The European Society for Immunodeficiency (ESID) has suggested 10 warning signs for suspicion of PIDD [5]. Children showing these signs must be evaluated further for an underlying PIDD. In low-income countries where limited information is available regarding PIDD, the 10 warning signs provide relatively sensitive criteria for identifying patients with suspected PIDD. The presence of lymphocytopenia on complete blood count suggests T-lymphocyte deficiency because T lymphocytes comprise the majority (50-70%) of peripheral blood mononuclear cells, whereas a finding of neutropenia suggests a phagocytic disorder. Disorders, such as congenital neutropenia or cyclic neutropenia, can be easily detected by using absolute neutrophil count [6]. PIDD are still under-diagnosed entities in Bangladesh. Relatively uncommon, these illnesses are easily missed in a child with too many infection and poor response to conventional treatment. In Bangladesh, the literature on these disease is limited that suggest majority of child may be dying

due to infection without diagnosis. The reason for missing the diagnosis may be low index of suspicion and non-availability of diagnostic facility. Immunoglobulin replacement, judicious use of prophylactic antibiotics and hematopoietic stem cell transplantation can prevent the significant end organ damage and improve long-term outcome, also quality of life, in patients with PIDD if diagnosed early. Prompt and accurate diagnosis of PIDD not only helps to direct the most appropriate treatment, and predict prognosis, but also it is important for further genetic counseling for the family.

MATERIALS AND METHODS

Fifty patients (age<18 years) with recurrent infections and features suggestive of an underlying immune deficiency referred to the Department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU) were investigated for underlying primary immunodeficiency disorders (PIDD). Patients were referred from Pediatrics department of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka Medical College Hospital (DMCH), Sir Salimullah Medical College Hospital (SSMCH) and Dhaka Shishu Hospital (DSH). Infants and children presenting at inpatient departments at the study hospitals who met the clinical case definition of suspected PIDD [7] were eligible for participation in the study. Diagnosed HIV infected child, child getting cancer chemotherapy and corticosteroid therapy were excluded from the study. A total of 05 ml blood was taken for investigations and the following tests were done; the complete blood count, flowcytometry (to quantify T lymphocytes and subsets (CD 3, CD 4 and CD 8), B lymphocytes (CD 19), and Natural Killer cells (CD 16/56) (using BD FACS Verse machine, the monoclonal antibodies were from Becton Dickinson, BD), serum immunoglobulin (IgG, IgA, IgM) levels by nephelometry.

The nitro blue tetrazolium assay (NBT) was performed on freshly collected blood sample to detect neutrophil dysfunction. PIDD was diagnosed in patients according to the European Society of Immunodeficiencies (ESID) diagnostic criteria for primary immune deficiency disorders [8] and grouped according to the updated classification of PIDD introduced by the Expert Committee of International Union of Immunological Societies (IUIS) on Primary Immunodeficiency. Ethics approval was granted by the Institutional Review Board (IRB) of BSMMU. Written informed consent was obtained from the patients parents.

RESULT

Out of 50 suspected patients, 10 (20%) cases were confirmed PIDD. Out of 10 confirmed PIDD, Predominantly antibody deficiency disorder was diagnosed in 6(60%) patients, combined immune deficiency was diagnosed in 3(30%) patients and congenital defects of phagocyte number, function or both was diagnosed in 1(10%) patient. The mean age of the patients at the time of diagnosis was 2.89 ± 2.61years (range: 2month-11yrs). Male female ratio was 9:1. Among predominantly antibody deficiency patients, transient hypogammaglobulinaemia of infancy was found in 33.33% (2/6) followed by 16.66% (1/6) in each cases of common variable immune deficiency, agammaglobulinaemia, selective Immunoglobulin M deficiency and Immunoglobulin A deficiency. Three patients had severe combined immune deficiency (SCID), including two patients with T-B- SCID and one patient with T-B+ SCID. All three patients were below 1 year age along with decreased age matched T cell numbers. Congenital neutropenia was diagnosed in a 2 months male infant with age of onset of symptoms at one month and history of previous sibling death. Absolute neutrophil count was 980/µl

of blood. Pneumonia (80%) was the most common presenting feature in primary immune deficiency patients. 2(20%) patients with PIDD had otitis media and 2(20%) patients had other infection included chronic diarrhea and umbilical infection. 1(10%) patient had deep organ abscess and 1(10%) patient had oral thrush. 4(40%) patients had family history of previous sibling death due to similar infections.

Table - I: Diagnostic profile of PIDD patients (n=10)

PIDD	Age/Sex	Clinical feature	Findings
Humoral/antibody deficiency (n=6) Common variable immunodeficiency	5yr/M	Bronchiactasis, otitis media	Low IgG, IgM and IgA with normal number of B cells.
Agamaglobulinemia	11yr/M	Recurrent pneumonia, H/O sib death	Severe reduction in all serum immunoglobulin isotypes with profoundly decreased B cells.
Selective IgM deficiency	5yr/F	Recurrent pneumonia, otitis media	IgM decreased with normal IgG & IgA.
Transient hypogamaglobulinemia of infancy	13m/M	Recurrent pneumonia	IgG below age-related normal value detected in the first two years of life with normal number of B cells.
Transient hypogamaglobulinemia of infancy	1yr/M	Recurrent pneumonia	IgG below age-related normal value detected in the first two years of life with normal number of B cells.
IgA deficiency	5yr/M	Lung abscess	IgA decreased
Combined immunodeficiencies (n=3)			Decreased numbers of lymphocytes and immunoglobulins levels associated with opportunistic infections
T-B+ SCID	7 m/M	Recurrent pneumonia, oral thrush, H/O sib death	CD4+T cell & CD8+ T cel count decreased
T-B-SCID	3m/M	Recurrent pneumonia, H/O sib death	Decreased both T & B cells
T-B-SCID	3m/M	Recurrent pneumonia, umbilical infection	Decreased both T & B cells.
Congenital neutropenia	2m/M	Chronic diarrhea, H/O sib death	Decreased absolute neutrophil count

DISCUSSION

In this study primary immune deficiency disorder was confirmed in 10 (20%) patients among 50 clinically suspected cases. This finding correlates with the previous study in

Bangladesh where confirmed PIDD were found in 24% cases. A study in Srilanka [9] reported PIDD in 7.75% (73 out of 942) cases. A study in Egypt [10] reported a higher rate (45%) of PIDD among 204 patients who presented with recurrent, severe or unusual infection .The similarities or dissimilarities of findings in various studies may vary according to the size of study population, geographical location and genetic status. Whatever may be the reason this study revealed that PIDD cases in our country is not negligible and need attention of the concern authority and physician. In this study among the different category of PIDD, the majority (60%) had antibody deficiency disorders. Similar finding was reported in Srilanka where the majority (60.27%) had antibody deficiency. In Mexico [11] predominantly antibody deficiency was 65.37%. The most frequent type of PIDD was antibody deficiency (71%) in Australia [12]. Finding of our study is consistent with the above studies. Among predominantly antibody deficiency disorders, the most frequent was the transient hypogamaglobulinemia of infancy (2/6, 33.33%) in this study. Transient haypogammaglobulinemia of infancy (THI) was the most common variant in antibody deficiency disorder in Turkey [13] and in Eastern Europe [14]. On the other hand, common variable immune deficiency (CVID) was reported as the commonest antibody deficiency disorder (28.7%) in Srilanka, as in Europe (21.01%) and in Mexico (47%).The major cause of this discrepancy with the present study is that the current study population was in Pediatric age group. No adult patient was included in the present study. On the other hand, mentioned above studies included both adult and children patients in their study. More than two thirds of patients with CVID are diagnosed after second decade of life [15]. Among the 10 PIDD patients in this study, recurrent pneumonia was the most common infection (80%). This is similar to that of Egypt and Mexico where the most common infection was pneumonia in

PIDD patients. In the present study 40% (4 out of 10) patients had family history of previous sibling death due to similar infections. This is consistent with that of Egypt where family history of sibling death was elicited in 21.73% PIDD patients. As PIDD are usually inherited in an autosomal recessive or X-linked recessive fashion, it is not surprising that four of our patients had a family history of previous sibling death due to similar infection. The mean age of the patients at the time of diagnosis was 2.89 ± 2.61years (range: 2month-11yrs). This study showed that all (100%) combined immune deficiency patients were at ages less than 1 year. Most of the (50%) predominantly antibody deficiency patients were in the 5- <9 years age group. This findings are consistent with that of the study in Srilanka that showed all SCID patients are in <1 year age and predominantly antibody deficiency disorders are predominant in 5-< 12 years age group. Male to female ratio of PIDD cases in the present study population was 9:1. Since the pattern of inheritance of some primary immunodeficiency diseases is gender-related, the overall incidence of primary immunodeficiency diseases was reported 1.4 to 2.3 times more in males than females in other studies. Relatively higher male patients in our study may be due to a bias in seeking better medical care for male children that still exists in our society.

CONCLUSION

Primary immune deficiency cases are not rare in our country which results in mortality and morbidity among children. Increased knowledge and awareness regarding PIDD, in addition to accurate diagnostic tests, will result in better recognition of more undiagnosed PIDD cases as well as reduced diagnostic delay. Diagnosis of PIDD is a challenge under any circumstance. Understanding the clinical clues and using a

small set of basic tests enable diagnosis of most PIDD, so that appropriate treatment can be sought.

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