INTRODUCTION

Primary immunodeficiency diseases (PID) represent a class of disorders in which there is an intrinsic defect in the human immune system (rather than immune disorders that are secondary to infection, chemotherapy or some other external agent). In some cases, the body fails to produce any or enough antibodies to fight infection. In other cases, the cellular defenses against infection fail to work properly. There are more than 150 different PID currently recognized by the World Health Organization ***(Boyle and Buckley, 2007).***

PIDs are a divers group of rare genetic disorders that affect the development and / or function of the immune system. Affected individuals are predisposed to increased rate and severity of infections, allergy, autoimmunity, and malignancy ***(Conley et al., 1999; Bonilla et al., 2003).***

During the last decade, expansive increase in the knowledge of basic immunology and human genetics has led to recognition of several distinct immunodeficiency disorders and their underlying genetic causes ***(Notarangelo et al., 2004, 2005).***

Epidemiological studies have shown wide geographical and racial variations in terms of prevalence and pattern of immunodeficiency. Many countries worldwide have developed registries to estimate the prevalence and

characteristic of different PID phenotypes among their populations ***(Kirkpatrick and Riminton, 2007).***

PIDs can affect components of the adaptive immune system, namely T cells and B cells, as well as components of the innate immune system, namely neutrophils, phagocytes, complement, and natural killer cells ***(Notarangelo et al., 2006)***.

The principal clinical manifestation of immunodeficiency is increased susceptibility to infection. The pattern of organ systems affected and characteristic pathogen vary with the type of immune defects. Therefore, it is important to look for primary immunodeficiency in any infant or child with recurrent infections ***(Chaple, 2005).***

The reason for missing the diagnosis may be many and include: low index of suspicion, very high rates of infections in the general population and non-availability of diagnostic facilities at most centers ***(Verma et al., 2008).***

AIM OF THE WORK

The purpose of this study is to evaluate the lymphocyte subsets among infants and children with recurrent infection, especially among those with lymphopenia in order to recognize patients with primary immunodeficiency diseases.

THE IMMUNE SYSTEM

The immune system protects the body from potentially harmful substances by recognizing and responding to antigens. Antigens are molecules (usually proteins) on the surface of cells, viruses, fungi, or bacteria. Nonliving substances such as toxins, chemicals, drugs, and foreign particles (such as a splinter) can also be antigens. The immune system recognizes and destroys substances that contain these antigens ***(Firestein, 2007).***

Even the body's cells have proteins that are antigens. These include a group of antigens called HLA antigens. The immune system learns to see these antigens as normal and usually does not react against them ***(Goronzy and Weyand, 2007).***

The immune system has the capacity to recognize and destroy abnormal cells from host tissues. The skin, cornea, and mucosa of the respiratory, gastrointestinal, and genitourinary tracts form a physical barrier that is the body's first line of defense ***(Naik, 2003).***

Classification of the Immune system

1- The innate immune system:

The innate immune system provides a rapid first line of defense, to keep early infection in check, giving the adaptive immune system time to build up a more specific response. Components include phagocytic cells (neutrophils and monocytes in blood, macrophages and dendritic cells in tissues), antigen-

presenting cells (APC), natural killer (NK) cells, polymorphonuclear leukocytes (PNL) ***(Pankin and Cohen, 2001).***

a. Natural killer cells (NK)

NK cells are important effector lymphocytes of innate immunity. Functionally, they exhibit cytolytic activity against a variety of allogeneic targets in a non-specific, contact-dependent, non-phagocytotic process which does not require prior sensitization to an antigen. These cells also have a regulatory role in the immune system through the release of cytokines which in turn stimulate other immune functions. However, NK cells can be distinguished from T lymphocytes by the expression of distinct phenotypic markers such as $CD16^{+}$, $CD56^{+}$ (human NK cells only) and lack of rearranged T cell receptor gene products ***(Medzhitov, 2007).***

b. Dendritic Cells

Dendritic cells, which originate in the bone marrow, function as antigen presenting cells (APC). In fact, the dendritic cells are more efficient APCs than macrophages. These cells are usually found in the structural compartment of the lymphoid organs such as the thymus, lymph nodes and spleen. However, they are also found in the bloodstream and other tissues of the body. It is believed that they capture antigen or bring it to the lymphoid organs where an immune response is initiated ***(Guermonprez, 2002).***

c. Granulocytes or Polymorphonuclear (PMN) Leukocytes

They are group of white blood cells is collectively referred to as granulocytes or polymorphonuclear leukocytes (PMNs). Granulocytes are composed of three cell types identified as neutrophils, eosinophils and basophils, based on their staining characteristics with certain dyes. These cells are predominantly important in the removal of bacteria and parasites from the body. They engulf these foreign bodies and degrade them using their powerful enzymes ***(Martin and Leibovich, 2005).***

d. Macrophages

Macrophages are important in the regulation of immune responses. They are often referred to as scavengers or antigen-presenting cells (APC) because they pick up and ingest foreign materials and present these antigens to other cells of the immune system such as T cells and B cells. This is one of the important first steps in the initiation of an immune response. Stimulated macrophages exhibit increased levels of phagocytosis ***(Langermans et al., 1994).***

e. Mast cells

Mast cells reside in connective tissues and mucous membranes, and regulate the inflammatory response ***(Krishnaswamy et al., 2006).*** They are most often associated with allergy and anaphylaxis ***(Stvrtinová et al., 2007).***

2- Adaptive (specific) immunity:

The adaptive immune response is antigen-specific and requires the recognition of specific "non-self" antigens during a process called antigen presentation. Antigen specificity allows for the generation of responses that are tailored to specific pathogens or pathogen-infected cells. The ability to mount these tailored responses is maintained in the body by "memory" cells ***(Pancer and Cooper, 2006).*** Cells of the adaptive immune system are special types of leukocytes, called lymphocytes, B cells and T cells ***(Holtmeier and Kabelitz, 2005).***

a. B-lymphocytes and antibodies

The B-cell identifies pathogens when antibodies on its surface bind to a specific foreign antigen ***(Sproul et al., 2000).*** This antigen/antibody complex is taken up by the B cell and processed by proteolysis into peptides. The B cell then displays these antigenic peptides on its surface with MHC class II molecules. This combination of MHC and antigen attracts a matching helper T-cell, which releases lymphokines and activates the B-cell ***(Kehry and Hodgkin, 1994).*** As the activated B-cell then begins to divide, its offspring (plasma cells) secrete millions of copies of the antibody that recognizes this antigen. These antibodies circulate in blood and bind to pathogens expressing the antigen and mark them for destruction by complement activation or for uptake and destruction by phagocytes. Antibodies can also neutralize challenges directly, by binding to bacterial toxins or by

interfering with the receptors those viruses and bacteria use to infect cells**.** These antibodies are: IgM, IgG, IgA, IgD and IgE ***(Saji et al., 1999).***

b. T-lymphocyte

T-cells recognize a "non-self" target, such as a pathogen, only after antigens (small fragments of the pathogen) have been processed and presented in combination with a "self" receptor called a major histocompatibility complex (MHC) molecule. There are two major subtypes of T cells: the killer T cell and the helper T-cell. Killer T-cells only recognize antigens coupled to class I MHC molecules, while helper T-cells only recognizes antigens coupled to class II MHC molecules. A third minor subtype is the γδ T-cells that recognize intact antigens that are not bound to MHC receptors ***(Holtmeier and Kabelitz, 2005).***

c. Killer T-cells

Killer T-cells directly attack other cells carrying foreign or abnormal antigens on their surfaces. Killer T-cell are a sub-group of T-cells that kill cells infected with viruses (and other pathogens), or are otherwise damaged or dysfunctional ***(Harty et al., 2000).***

Killer T cells are activated when their T-cell receptor (TCR) binds to this specific antigen in a complex with the MHC Class I receptor of another cell. Recognition of this MHC: antigen complex is aided by a co-receptor on the T-cell, called CD8. The

T-cell then travels throughout the body in search of cells where the MHC I receptors bear this antigen. When an activated T cell contacts such cells, it releases cytotoxins, such as perforin, which form pores in the target cell's plasma membrane, allowing ions, water and toxins to enter ***(Radoja et al., 2006).***

d. Helper T-cells

T helper cell (Th cells) recognize (APCs) by their class II MHC molecules together with the help of their expression of CD4 co-receptor (CD4+). The activation of a resting helper T cell causes it to release cytokines and other stimulatory signals that stimulate the activity of macrophages, killer T cells and B cells, the latter producing antibodies ***(McHeyzer et al., 2006).***

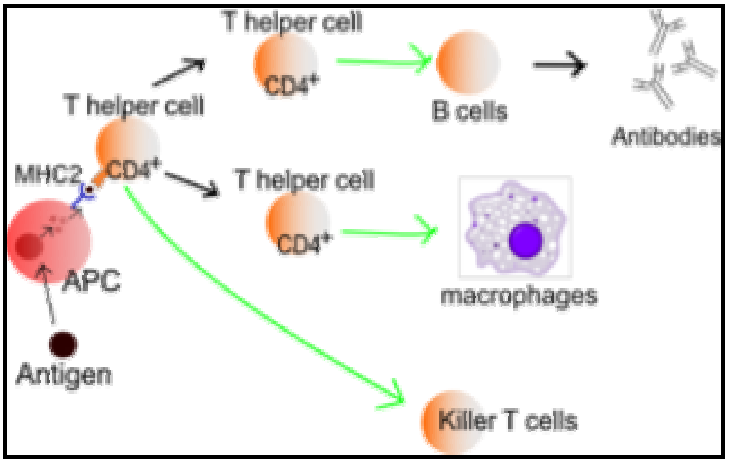

Figure (1): Activation of helper T-cell ***(Quoted from: McHeyzer et al., 2006).***

e. γδ T-cells:

γδ T-cells (gamma delta T-cells) possess an alternative T cell receptor (TCR) as opposed to CD4+ and CD8+ (αβ) T cells and share the characteristics of helper T cells, cytotoxic T cells and NK cells. The conditions that produce responses from γδ T-cells are not fully understood. Like other 'unconventional' T-cell subsets bearing invariant TCRs, such as CD1-restricted NK T cells, γδ T-cells straddle the border between innate and adaptive immunity ***(Girardi, 2006).***

f. Regulatory T-cells

These cells mediate suppression of immune responses. The process involves functional subsets of CD4 T-cells that either secrete cytokines with immunosuppressive properties or suppress the immune response by poorly defined mechanisms that require cell-to-cell contact. Some regulatory T cells express the CD8 T-cell phenotype ***(Harty et al., 2000).***

PRIMARY IMMUNODEFICIENCY DISEASES

Definition:

(PIDs) are a heterogeneous group of rare genetic disorders of immune system function resulting in a broad susceptibility to multiple and recurrent infections caused by weakly pathogenic and more virulent microorganisms ***(Casanova et al., 2005).***

Many are associated with single gene defects, whereas others may be polygenic or may represent interaction of genetically determined characteristics with environmental or infectious stresses ***(Bonilla and Geha, 2005).***

Prevalence:

Classical primary immunodeficiency disease (PID) is relatively rare approximately 1: 500-1:500.000 in the general population, with variable degrees of ascertainment in different countries ***(De Vries, 2006).***

Ten percent of children with recurrent infections have an immunodeficiency, with a defect in one or more components of the immune system ***(Bonilla et al., 2005).***

Classification of PID categories:

The classification of primary immunodeficiencies (PIDs) provides a framework to help in the diagnostic approach to patients. PIDs are classified into main 8 groups based on the type of cells affected ***(Bousfiha et al., 2011).***

These groups are compined B and T-cells immuno-deficiencies, predominantly antibody deficiencies, other well defined immunodeficiency syndromes, diseases of immune dysregulation, congenital defect of phagocyte (number, function, or both), defects in innate immunity, autoinflammatory disorders, and complement deficiencies. Associated features and mode of inheritance of PID disorders of these groups are shown in (table 1).

Table (1): Classifications of some PID categories:

1- Combined T and B- cell immunodeficiencies:

Disease	Circulating T cells	Circulating B cells	Serum Ig	Inheritance
1. T−B+ Severe combined immunodeficiency (SCID)				
(a) γc deficiency	Markedly decreased	Normal or increased	Decreased	XL
(b) JAK3 deficiency	Markedly decreased	Normal or increased	Decreased	AR
(c) IL7Rα deficiency	Markedly decreased	Normal or increased	Decreased	AR
2.T−B− SCID				
(a) RAG ½ deficiency	Markedly decreased	Markedly decreased	Decreased	AR
(b) DCLRE1C (Artemis) deficiency	Markedly decreased	Markedly decreased	Decreased	AR
(c) Adenosine deaminase (ADA) deficiency	Absent from birth (null mutations) or progressive decrease	Absent from birth of progressive decrease	Progressive decrease	AR
3. Omenn syndrome	Present; restricted heterogeneity	Normal or decreased	Decreased, except increased IgE	AR
4. CD3γ deficiency	Normal, but reduced TCR expression	Normal	Normal	AR
5. CD8 deficiency	Absent CD8, normal CD4 cells	Normal	Normal	AR
6. MHC class I deficiency	Decreased CD8, normal CD4	Normal	Normal	AR
7. MHC class II deficiency	Normal number, decreased CD4 cells	Normal	Normal or decreased	AR
8. Complete DiGeorge syndrome	Profoundly decreased	Low to normal	Decreased	AD
9. Cartilage hair hypoplasia	Decreased or normal; impaired lymphocyte proliferation	Normal	Normal or reduced. Antibodies variably decreased	AR

2- Well-defined syndromes with immunodeficiency:

Disease	Circulating T cells	Circulating B cells	Serum Ig	Inheritance
1.Wiskott–Aldrich syndrome (WAS)	Progressive decrease, abnormal lymphocyte responses to anti-CD3	Normal	Decreased IgM: antibody to polysaccharides particularly decreased; often increased IgA and IgE	XL
2. DNA repair defects (other than those in table 1)				
(a) Ataxia–telangiectasia	Progressive decrease	Normal	Often decreased IgA, IgE, and IgG subclasses; increased IgM monomers; antibodies variably decreased	AR
(b) Ataxia–telangiectasia-like disease (ATLD)	Progressive decrease	Normal	Antibodies variably decreased	AR
(c) Nijmegen breakage syndrome	Progressive decrease	Variably reduced	Often decreased IgA, IgE, and IgG subclasses; increased IgM antibodies variably decreased	AR
(d) Bloom syndrome	Normal	Normal	Reduced	AR
(e) Immunodefi- ciency with centromeric instability and facial anomalies (ICF)	Decreased or normal; Responses to PHA may be decreased	Decreased or normal	Hypogammaglobulinemia; variable antibody deficiency	AR
3.Thymic defects				
DiGeorge anomaly (chromosome 22q11.2 deletion syndrome	Decreased or normal	Normal	Normal or decreased	*De novo* defect or AD
4. Hyper-IgE syndromes (HIES)				
(a) AD-HIES (Job syndrome)	Normal Th-17 cells decreased	Normal (switched and non-switched memory B cells are reduced; BAFF level increased)	Elevated IgE; specific antibody production decreased	AD, often *de novo* defect
(b) AR-HIES				AR
(i) TYK2 deficiency	Normal, but multiple cytokine signaling defect	Normal	(±) Elevated IgE	
(ii) DOCK8 deficiency	Reduced	Reduced	(±) Elevated IgE, low IgM	
5. Hepatic veno-occlusive disease with immunodeficiency (VODI)	Normal (decreased memory T cells)	Normal (decreased memory B cells)	Decreased IgG, IgA, IgM absent germinal centers absent tissue plasma cells	AR

3- Predominantly antibody deficiencies:

Disease	Serum Ig	Inheritance
1. Severe reduction in all serum immunoglobulin isotypes with profoundly decreased or absent B cells		
(a) BTK deficiency	All isotypes decreased in majority of patients; some patients have detectable immunoglobulins	XL
(b) μ Heavychain deficiency	All isotypes decreased	AR
(c) Thymoma with immunodeficiency	One or more isotypes may be decreased	None
(d) Myelodysplasia with hypogammaglobulinemia	One or more isotypes may be decreased	Variable
2. Severe reduction in at least 2 serum immunoglobulin isotypes with normal or low number of B cells		
(a) Common variable immunodeficiency disorders	Low IgG and IgA and/or IgM	Variable
(b) CD19 deficiency	Low IgG and IgA and/or IgM	AR
(c) CD20 deficiency	Low IgG, normal or elevated IgM, and IgA	AR
3. Severe reduction in serum IgG and IgA with normal/elevated IgM and normal numbers of B cells		
(a) CD40L deficiency	IgG and IgA decreased; IgM may be normal or increased; B cell numbers may be normal or increased	XL
(b) CD40 deficiency	Low IgG and IgA; normal or raised IgM	AR
(c) AID deficiency	IgG and IgA decreased; IgM increased	AR
4. Isotype or light chain deficiencies with normal numbers of B cells		
(a) Ig heavy chain mutations and deletions	One or more IgG and/or IgA subclasses as well as IgE may be absent	AR
(b) κ chaindeficiency	All immunoglobulins have lambda light chain	AR
(c) Isolated IgG subclass deficiency	Reduction in one or more IgG subclass	Variable
(d) IgA with IgG subclass deficiency	Reduced IgA with decrease in one or more IgG subclass	Variable
(e) Selective IgA deficiency	IgA decreased/absent	Variable
5.Transient hypogam-maglobulinemia of infancy with normal numbers of B cells	IgG and IgA decreased	Variable

4- Diseases of immune dysregulation:

Disease	Circulating T cells	Circulating B cells	Serum Ig	Inheritance
1. Immunodeficiency with hypopigmentation				
(a) Chediak– Higashi syndrome	Normal	Normal	Normal	AR
(b) Griscelli syndrome, type2	Normal	Normal	Normal	AR
(c) Hermansky– Pudlak syndrome, type 2	Normal	Normal	Normal	AR
2. Familial hemophagocytic lymphohistiocytosis (FHL) syndromes				
(a) Perforin deficiency, FHL2	Normal	Normal	Normal	AR
(b) UNC13D (Munc13-4) deficiency, FHL3	Normal	Normal	Normal	AR
(c) Syntaxin 11 deficiency, FHL4	Normal	Normal	Normal	AR
3. Lymphoproliferative syndromes				
(a) SH2D1A deficiency, XLP1	Normal	Normal or reduced	Normal or low	XL
(b) XIAP deficiency, XLP2	Normal	Normal or reduced	Normal or low	XL
4. Syndromes with autoimmunity				
(a) Autoimmune lymphoprolifera- tive syndrome (ALPS)				
(i) ALPS-FAS	Increased CD4− CD8− double negative (DN) Tcells	Normal, but increased number of CD5+ B cells	Normal or increased	AD (AR cases are rare and severe)
(ii) ALPS- FASLG	Increased DNT cells	Normal	Normal	AD AR
(iii) ALPS- CASP10	Increased DNT cells	Normal	Normal	AD
(iv) Caspase 8 defect	Slightly increased DNT cells	Normal	Normal or decreased	AD
(v) Activating N-RAS defect, activating K-RAS defect	Increased or normal DNT cells	Elevation of CD5 B cells	Normal	Sporadic
(vi) FADD deficiency	Increased DNT cells	Normal	Normal	AR
(b) IPEX, immune dys-regulation, polyendo-crinopathy, enteropathy (X-linked)	Lackof (and/or impaired function of) CD4+ CD25+ FOXP3+ regulatoryT cells	Normal	Elevated IgA, IgE	XL
(c) CD25 deficiency	Normal to modestly decreased	Normal	Normal	AR
(d) ITCH deficiency	Not assessed (Th2skewingin *Itch*-deficient mice)	Not assessed (B cells are dysfunctional in *Itch*-deficient mice)	Not assessed (elevatedin *Itch*-deficient mice)	AR