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 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 distinguishes self from non self and eliminates potentially harmful non self molecules and cells from the body. The immune system also 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)*.

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)*.

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)*.

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)*.

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 and are also secretory *(Langermans et al., 1994)*.

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 are the major types of lymphocytes and are derived from hematopoietic stem cells in the bone marrow *(Holtmeier and Kabelitz, 2005)*.

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 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)*.

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)*.

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)*.

Helper T-cells:

Helper T 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. The stimulation of B cells and macrophages succeeds a proliferation of T helper cells (figure 1) *(McHeyzer et al., 2006)*.

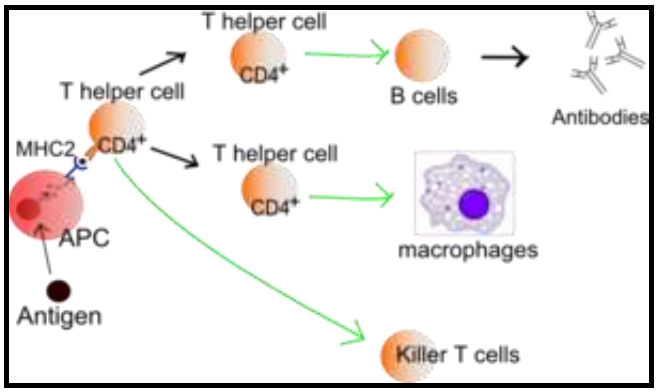

Fig. (1): Activation of helper T-cell.

Helper T-cells express T-cell receptors (TCR) that recognize antigen bound to class II MHC molecules. The MHC: antigen complex is also recognized by the helper cell's CD4 co-receptor, which recruits molecules inside the T cells and is responsible for T cell activation *(Abbas et al., 1998)*.

γδ T-cells:

γδ 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 CD1d-restricted NK T cells, γδ T-cells straddle the border between innate and adaptive immunity *(Girardi, 2006)*.

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 heterogenous 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)*. 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)*.

Classifications of PIDs:

PID disorders are classified into main 8 groups based on the type of cells affected *(Geha et al., 2007)*. 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): Classification of primary immunodeficiency disorders

1- Combined B - T cell immunodeficiency						
Disease	**Circulating T cell**	**Circulating B cell**	**Serum immunoglobulin**	**Associated features**	**Inheritance**	**Gene defects/presumed pathogenesis**
1. T-B1 SCID*						
a. gc deficiency	Markedly decreased	Normal or increased	Decreased	Markedly decreased NK cells	XL	Defect in g chain of receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21
b. Jak3 deficiency	Markedly decreased	Normal or increased	Decreased	Markedly decreased NK cells	AR	Defect in JAK3 signaling kinase
c. IL7Ra deficiency	Markedly decreased	Normal or increased	Decreased	Normal NK cells	AR	Defect in IL-7 receptor a chain
d. CD45 deficiency	Markedly decreased	Normal	Decreased	Normal g/d T cells	AR	Defect in CD45
e. CD3d deficiency	Markedly decreased	Normal	Decreased	Normal NK cells	AR	Defect in CD3d or CD3e chains of T-cell antigen receptor
2. T-B2 SCID						
a. RAG ½ deficiency	Markedly decreased	Markedly decreased	Decreased	Defective VDJ recombination	AR	Complete defect of Recombinase activating gene (RAG) 1 or 2
b. Artemis deficiency	Markedly decreased	Markedly decreased	Decreased	Defective VDJ recombination	AR	Defect in Artemis DNA recombinase repair protein
c. ADA deficiency	Absent from birth (null mutations) or progressive decrease	Absent from birth or progressive decrease	Progressive decrease	Costochondral junction flaring	AR	Absent ADA, increased lymphotoxic metabolites (dATP, S-adenosyl homocysteine)
d. Reticular dysgenesis	Markedly decreased	Decreased or normal	Decreased	Granulocytopenia, thrombocytopenia (deafness)	AR	Defective maturation of T, B, and myeloid cells (stem cell defect)
3. Omenn syndrome	Present; restricted heterogenei	Normal or decreased	Decreased, except increased IgE	Erythroderma, eosinophilia, adenopathy, hepatosplenomegaly	AR	Missense mutations allowing residual

	ty					activity, usually in RAG1 or RAG2 genes but also in Artemis and IL-7Ra genes
4. DNA ligase IV	Decreased	Decreased	Decreased	Microcephaly, facial dystrophy, radiation sensitivity	AR	DNA ligase IV defect, impaired non homologous end joining
5. Cermunnos/ decreased XLF deficiency	Decreased	Decreased	Decreased	Microcephaly,in utero growth retardation, radiation sensitivity		Cermunnos defect impaired NHEJ
CD 40 ligand deficiency	Normal	IgM and IgD B memory cells present, but others absent	IgM increased or normal, other isotypes decrease	Neutropenia, thrombocytopenia; hemolytic anemia, (biliary tract and liver disease, opportunistic infections	XL	Defects in CD40 ligand (CD40L), defective B- and dendritic cell signaling
6. CD40 deficiency	Normal	IgM andIgD B memory cells present, but others absent	IgM increased or normal, other isotypes decrease	Neutropenia, gastrointestinal and liver disease, opportunistic infections	AR	Defects in CD40, defective B- and dendritic cell signaling
7. PNP deficiency	Progressive decrease	Normal	Normal or decreased	Autoimmune haemolytic anemia, neurologic impairment	AR	Absent PNP, T-cell, and neurologic defects from increased toxic metabolites (eg, dGTP)
9. CD3g and CD3e deficiency	Normal (reduced TCR expression)	Normal	Normal		AR	Defect in CD3g
10. CD8 deficiency	Absent CD8, normal CD4 cells	Normal	Normal		AR	Defects of CD8 a chain
11. ZAP-70 deficiency	Decreased CD8, normal CD4 cells	Normal	Normal		AR	Defects in ZAP-70 signaling kinase
12.Ca ++ channel deficiency	Normal count, defective TCR mediated activation	Normal count	normal	Autoimmunity, anhydrotic ectodermic dysplasia,non progressive myopathy	AR	Defect in Orai-1 Ca++ channel component
13. MHC class I deficiency	Decreased CD8,normal CD4	Normal	Normal	Vasculitis	AR	Mutation in TAP1,TAP2 or TRAP(tapasin)genes giving MCH class1 deficiency
14. MHC class II deficiency	Normal number, decreased CD4 cells	Normal	Normal or decreased		AR	Mutation in transcription factors for MHC class II proteins (C2TA, RFX5, RFXAP, RFXANK genes
15-Winged helix	Markedly	Normal	decreased	Alopecia, abnormal thymic	AR	Defects in fork

deficiency	decreased			epithelium (resembles nude mouse)		head box N1transcription factor encoded by FOXN1, the gene mutated in nude mice
16-CD 25 deficiency	Normal to modestly decreased	Normal	Normal	Lymphoproliferation (lymphadenopathy, hepatosplenomegaly),autoimmunity	AR	Defect in IL-2R chain
17-STAT5b deficiency	modestly decreased	Normal	Normal	Growth hormoneinsensitive dwarfism, dysmorphic feature, eczema, lymphocytic interstitial pneumonitis	AR	Defects of STAT5B gene, impaired development and function of g T-cell, T-regulatory and NK cells, impaired T-cell proliferation

2- Predominantly antibody deficiencies

Diseases	B-cell number Ig	Associated features	inheritance	Gene defect
1. Severe reduction in all serum Ig isotypes with profoundly decreased or absent B cells				
(a)Btk deficiency	All isotypes decreased	Severe bacterial infections	XL	Mutations in BTK
(b) m Heavy chain deficiency	All isotypes decreased	Severe bacterial infections	AR	Mutations in m heavy chain
(c) 5 Deficiency	All isotypes decreased	Severe bacterial Infections	AR	Mutations in l5
(d) Iga deficiency	All isotypes decreased	Severe bacterial infections	AR	Mutations in Iga
(e)Igb deficiency	All isotypes decreased	Severe bacterial infections	AR	
(f)BLNK deficiency	All isotypes decreased	Severe bacterial infections	AR	Mutations in BLNK
(g) Thymoma with immunodeficiency	All isotypes decreased	Severe bacterial infections	none	Unknown
(h) Myelodysplasia	All isotypes decreased	Severe bacterial infections	variable	May have monosomy 7, trisomy 8or dyskeratosis congenita
2. Severe reduction in serum IgG, IgA with normal, low or very low numbers of B cells				
Common variable immunodeficiency	Low IgG and IgA; variable IgM	Bacterial infection might have autoimmune, lymphoproliferative, and/or granulomatous disease	AR or AD	
(a) ICOS deficiency	Low IgG and IgA; normal IgM	Might have autoimmune, lymphoproliferative, and/or granulomatous disease	AR	Mutation in ICOS
(b) CD19 deficiency	Low IgG, IgA and IgM	Might have autoimmune, lymphoproliferative, and/or granulomatous disease	AR	Mutation in CD19
(c) X-linked lymphoproliferative syndrome 1	All isotypes decreased	Some patients have antibody deficiency, although most present with fulminant EBV infection	XL	Mutation in SH2DIA

		or lymphoma		
3. Severe reduction in serum IgG and IgA with normal/ increased IgM and normal numbers of B cells				
(a)CD40L deficiency	IgG and IgA decreased, IgM may be normal or increased; B cell number may be normal or increased	Opportunistic infection, neutropenia, autoimmune disease	XL	Mutation in CD40L (also called TNFSF5 or CD154)
(b) CD40 deficiency	Low IgG and IgA , normal or increased IgM	Opportunistic infection, neutropenia	AR	Mutation in CD40 (also called TNFSF5)
(c) AID deficiency_	Low IgG and IgA , increased IgM	Enlarged lymph nodes and germinal centers	AR	Mutation in AICDA gene
(d) UNG deficiency_	Low IgG and IgA , increased IgM	Enlarged lymph nodes and germinal centers	AR	Mutation in UNG gene
4. Isotype or light chain deficiencies with normal numbers of B cells				
(a) Ig heavy chain deletions	IgG1, IgG2, or IgG4 absent; IgA1 and IgE might be absent	Might be asymptomatic Asymptomatic	AR	Chromosomal deletion at 14q32
(b) k Chain deficiency	All immunoglobulins have lambda light chain	Might be asymptomatic or have recurrent viral-bacterial infections	AR	Mutation in Kappa constant gene
(c) Isolated IgG subclass deficiency	Reduction in one or more IgG subclass	Recurrent bacterial infections	Variable	Unknown
(d) IgA with IgG subclass deficiency	Reduced IgA with decrease in one or more IgG subclass;	Might be asymptomatic, have recurrent infections with or without poor antibody response to carbohydrate antigens, allergies or autoimmune disease	Variable	Unknown
(e) Selective IgA deficiency	IgA decreased	Some cases progress to CVID, others coexist with CVID in the same family	Variable	Unknown
5. Specific antibody deficiency with normal Ig concentrations and numbers of B cells	Normal	Inability to make antibodies to specific antigens	Variable	Unknown
6. Transient hypogammaglobulinemia of infancy	IgG and IgA decreased	Recurrent moderate bacterial infections	Variable	Unknown