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BAZI PARAZİTOZLARDA TNF- $\alpha$ , IL-1 $\beta$ , IL-2, IL-2R,  
IL-4, IL-6, IL-8, IL-10, MALONDİALDEHİT,  
SÜPEROKSİT DİSMUTAZ, KATALAZ VE  
GLUTATYON PEROKSİDAZ  
DÜZEYLERİNİN ARAŞTIRILMASI

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**THE INVESTIGATION OF SERUM LEVELS OF  
TNF- $\alpha$ , IL-1 $\beta$ , IL-2, IL-2R, IL-4, IL-6, IL-8, IL-10,  
SUPROXIDE DISMUTAZ, GLUTATHIONE  
PEROXIDASE, CATALASE AND  
MALONDIALDEHYDE  
IN SOME PARASITIC DISEASES**

**Ph.D. THESIS**

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## 1. INTRODUCTION

The role of cytokines in some parasitic diseases was studied and elucidated either by administration of cytokine to infected animals or neutralising it with specific monoclonal antibody. Most data were also obtained from the analysis the results of experimental studies on mice made transgenic for a particular cytokine or from mice in which a special cytokine gene were inactivated 'knockout mice' (6,95,143).

It was reported that cytokines secreted particularly from T helper CD4+ cells and other cells play a pivotal role in the regulation of the immune mechanism during infections. For different parasites, different cytokines responses are of primary importance. For example, cytokines originated from Th2 cells were thought to be associated with expulsion of intestinal helminths (137,180). While those of Th1 origin was associated with chronic worm infections (180).

In addition to cytokines, free radicals produced as a response of infections have implicated in pathology of many parasitic diseases. They contain one or more unpaired electrons in their outermost orbitals and they are formed predominantly by activated phagocytes and as results of side effects of chemicals (70-73,185). As a part of host defence mechanism, enzymatic and non-enzymatic defence system remove them. Failure or inhibition the activity of such system might lead to a marked increase of lipid peroxides (malondialdehyde). Nevertheless, it is well established the role of free radicals and antioxidant enzymes defence in some parasitic infections like malaria, leishmaniasis (49,131,184).

*Giardia lamblia* is one of common parasite that predominantly infects children in Malatya (135). It causes metabolic disorders associated with retardation of growth (4,60,115). *Ascaris lumbricoides* (a large roundworm nematode) is important from a clinical and epidemiological view, since this parasite causes complications and the percentage of human harboring the parasite is high throughout the world (60,100). Cystic echinococcosis is an important cestode infection endemic in Turkey (7). It is a potentially dangerous disease and may involve vital organs such as brain (60).

There is very little knowledge about the role of cytokines and antioxidant enzymes in such parasitic diseases. Hence, the objective of this study is to determine the serum levels of some cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-2, IL-2R, IL-4, IL-6, IL-8 and IL-10), IgE, antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) and lipid peroxides (malondialdehyde) in patients infected with either



*Giardia lamblia*, or *Ascaris lumbricoides*, and or larval stage of *Echinococcus granulosus* (cystic echinococcosis).

## 2. LITERATURE REVIEW

### 2.1. Cytokines:

They include a diverse group of proteins or glycoproteins that regulate not only innate and specific immunity but also inflammatory responses, wound healing, haematopoiesis, cancers development, and other biological processes. Their molecular weights range between 6.000-60.000 daltons. They are very potent substances and exhibit their activity to specific surface receptors on target cells. They differ from endocrine hormones in that they are not produced by specialised glands (1,18,35,164).

According to type of cells that secrete them, they are called lymphokines when they are secreted from lymphocytes. Monokines are those secreted by monocytes and macrophages. They also include a group called chemokines that are defined as cytokines with chemotactic functions. Cytokines can be also secreted by neutrophils, mast cells, eosinophils, fibroblasts, and endothelial cells (6,35,82).

According to their functions on the target cells, they can be classified into two groups: first group exhibits their actions on the same cells that produce them, it is called autocrine. While a second group showing activity on adjacent cells, is named paracrine. The action of cytokine may be also described as pleiotropic when individual cytokines have capability to exert many different effects. Conversely, different cytokines exhibit the same biological activity, so the action is called redundant (6,35)

Cytokines also exert action in synergistic manner (one cytokine interacts with the other cytokine producing augmentory effects) or in antagonistic way (one cytokine inhibits the action of other cytokine) (6).

On the basis of cytokine profile production, T cells can be classified into Th1 and Th2 cells (6,32,82,111,145). T cells that produce predominantly IL-2, IFN- $\gamma$  and tumour necrosis factor- $\beta$  are called Th1 cells, while cells that predominantly produce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 are referred to as Th2 cells (Figure 1). Th1 cells induce cell-mediated immune response (6,32) whereas Th2 cells induce humoral and an allergic immune response (77,111).

### 2.1.1. Common characteristics of cytokines (1,6,18,77):

1. They are non-specific substances, which are rapidly secreted in response to stimulus, and they are not stored within cells that produce them.
2. Most cytokines have short half life span.
3. Their binding to specific receptors is required for their efforts.

Cytokines include interleukins, interferons, colony stimulating factor, and chemokines (143).

### 2.2. Chemokines:

They are a group of structurally related peptides with potent leukocyte activation and /or chemotactic activity. They include at least 25 cytokines and all can bind to heparin receptors. They are divided into two groups:  $\alpha$ -chemokines which are also known as CXC chemokines characterised by presence of a single amino acid between the first and second cysteine residues;  $\beta$  or CC chemokines have adjacent cysteine residues. The CXC chemokines are encoded on chromosome 4 and primarily activate neutrophils. While CC chemokines activate monocytes, lymphocytes, basophils, and eosinophils and are encoded on chromosome 17 (35).

Interleukin-8 is the most studied CXC chemokines released by monocytes after stimulation with lipopolysaccharides (LPS) as well as IL- $1\alpha$ , IL- $1\beta$  and tumour necrosis factor  $\alpha$ . It is also released by a number of other cells including fibroblasts, epithelial cells, astrocytes, keratinocytes, synovial cells and various tumor cells. (77,18,35,143).

CC Chemokines include macrophage inflammatory protein-1 (MIP- $1\alpha$  , MIP- $1\beta$ ), regulated on activation normal T expressed and secreted (RANTES), monocyte chemotactic protein-1 (MCP-1, MCP-2, and MCP-3)(6). MCP-1 is mainly produced by monocytes after stimulation by inflammatory cytokines and LPS. MIP- $1\alpha$  and RANTES are chemotactic for eosinophils and stimulate basophils to release histamine. MIP- $\alpha$  is also chemotactic for B lymphocytes, activated CD8+cells, and natural killer (NK) cells. MIP- $\beta$  is chemotactic for CD4 + cells (6,35). Thus, chemokines play an important role in inflammatory response by inducing leukocyte population to migrate into areas of infection (18).

### 2.3. Tumor necrosis factor (TNF):

It is an inflammatory cytokine. William Coley first observed it in 1975 by observation of haemorrhagic necrosis in cancer cells injected with Coley toxin (LPS). LPS not itself induces necrosis but induces production of a cytokine, which is now

called TNF (77,169). It has pleiotropic function and plays an important role in host defence mechanism by involvement in inflammatory response

Two distinct forms of TNF have been described. TNF- $\alpha$  is produced predominantly by monocytes, activated macrophages, and less by other cells like B cells and somatic cells. TNF- $\beta$  (LT- $\beta$ ) is primarily a product of activated T lymphocytes. TNF- $\alpha$  and TNF- $\beta$  have overlapping biological activities since both bind to the same receptors on target cells (164). The major functions of TNF- $\alpha$  are involvement in the inflammatory response, activation of endothelial cells, and induction of fever and acute phase proteins. While the functions of TNF- $\beta$  are involvement in inflammatory response, and playing a role in killing the target cells by CD8 + cells (18).

TNF- $\alpha$  has synergism with gamma interferon (IFN- $\gamma$ ) for activation of macrophages and cytotoxicity to parasites and tumor cells (6,98,181). TNF- $\alpha$  also upmodulates the production of IL-10 which in turn downmodulates TNF- $\alpha$  production from monocytes (187).

TNF- $\alpha$  is also known as cachectin because it inhibits lipoprotein lipase in adipose tissues and thus reduces utilisation of fatty acids (77). TNF- $\alpha$  also enhances the growth and maturation of stimulated B cells (86).

TNF- $\alpha$  also causes disseminated intravascular coagulation by increasing in endothelial cell surface expression molecules such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule (ICAM-1) which mediate the adhesion of blood leukocytes (98).

Furthermore, TNF- $\alpha$  and TNF- $\beta$  are encoded by two separate genes which are located within the MHC complex on chromosome 6, 50 respectively. They are also called class III MHC proteins. TNF- $\alpha$  and TNF- $\beta$  share only 28% homology at the amino acid level (164).

TNF- $\alpha$  serum levels may be increased in sepsis, autoimmune diseases, various infectious diseases, and transplant rejection (28,75,84,106). It could be also detected in stool as marker of intestinal inflammation (21).

There are interactions between TNF and some interleukines as follow:

1. IL-6 causes a reduction in TNF production by inhibiting gamma interferon, which normally stimulates TNF production by increasing the transcription of TNF mRNA (191).

2. There is synergism between TNF- $\alpha$  and IL-8 for neutrophils mediated platelet activation (77).

3. In mice, IL-1 potentiates the lethal effect of TNF- $\alpha$  (6).

#### 2.4. Interleukins:

They constitute the majority of cytokines (IL-1 to IL-18). They are low molecular weight glycoproteins produced by leukocytes mainly T cells. Other cells like phagocytic and tissue cells can also produce them.

##### 2.4.1. Interleukin-1 (IL-1):

All nucleated cell types including monocytes, macrophages, B lymphocytes, NK cells, T-lymphocytes, keratinocytes, dendritic cells, astrocytes, fibroblasts, neutrophils, endothelial cells, and smooth muscle cells (6,40,143,164) produce IL-1 (also known haematopoietin). It has various actions on cells of the immune system (Figure 2).

It is a proinflammatory cytokine. Biologically, it is more closely related to TNF than any other cytokine even though their structures and receptors are clearly distinct. It consists of two distinct proteins encoded by two genes. They are called IL-1 $\alpha$  and IL-1 $\beta$  and they are polypeptides 159 and 153 amino acids long. They share about 22% amino acid homology and bind to the same receptor. IL-1 $\alpha$  is the acidic form and IL-1 $\beta$  is the neutral form (35,164).

Cells vary in their expression of IL-1 $\alpha$  and IL-1 $\beta$  genes. For instance, IL-1 $\alpha$  is produced mainly by keratinocytes, whereas IL-1 $\beta$  is predominantly produced by human monocytes. Another gene codes a protein known as IL-1 receptor antagonist (IL-1RAcP). The latter competes with IL-1 $\alpha$  and IL-1 $\beta$  for binding to IL-1 receptor. So, it is a competitive inhibitor of IL-1 $\alpha$  and IL-1 $\beta$  although it is biologically inactive (77,82).

IL-1 production by macrophages can be stimulated by TNF, LPS or IL-1 itself and contact with CD4+ cells (1,164).

IL-1 $\alpha$  and IL-1 $\beta$  share various biological activities including lymphocyte activation, macrophage stimulation, increased bone resorption, enhanced procoagulant activity of endothelial cells, stimulation of fibroblast growth and enhanced expression of adhesion molecules on endothelial cells (35,41,143)

IL-1 also involves in maturation T and B cell precursors and stimulates the production of other cytokines such as IL-2, IL-3, IL-6, interferons, colony stimulating

factor, and haematopoietic growth factor (6). Activation of IL-1 is increased by IL-6 and both are synergistic in IL-2 production (41). It also induces expression of IL-2R on T cells (41). IL-1 also augments binding NK cells to tumor cells and has synergistic property with IL-2 and IFN- $\gamma$  for NK activity. Synergism of this interleukin can also be seen with IL-3, IL-8 and TNF- $\alpha$ . IL-1 is predominantly constituted of IL-1  $\beta$ , which is mostly circulating, and IL-1 $\alpha$  is mostly membrane bound. IL-1 $\alpha$  and IL- $\beta$  along with TNF- $\alpha$  are endogenous pyrogenes via their action on hypothalamus (35). IL-1 also induces liver hepatocytes to produce acute phase proteins (C-Reactive Proteins). IL-1 production by macrophages is also enhanced by leukotrienes but is suppressed by prostaglandin (164).

The increased in IL-1 $\beta$  is observed during the luteal phase of menstruation and during the exercises (164). IL-1 $\beta$  is also increased in bone diseases, inflammatory diseases, cancers, and pneumonia (27,77).



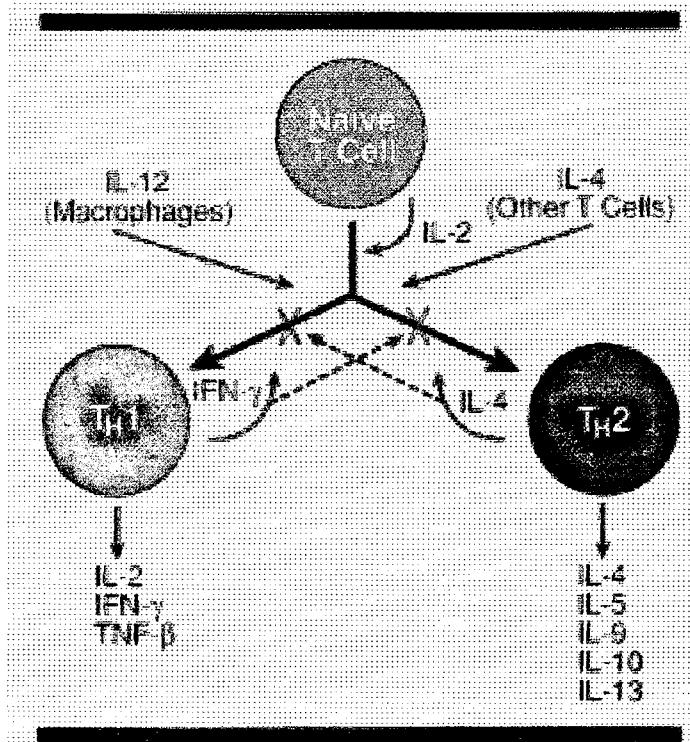


Figure 1. Shows the classification of Th cells and their relevant cytokines (82).

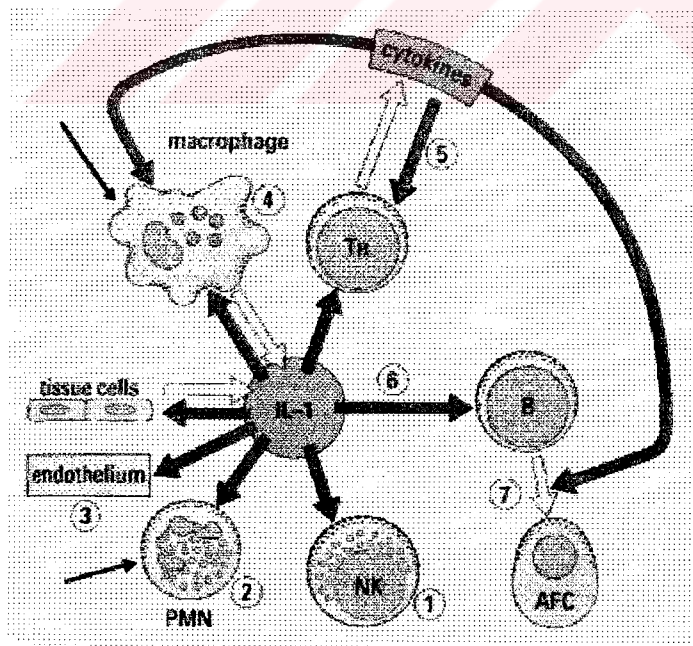


Figure 2. Shows the actions of IL-1 on the cells of the immune system (143).



### 2.4.2. Interleukin 2 (IL-2):

It was originally called T cell growth factor (TCGF). CD4<sup>+</sup> cells secrete IL-2 and in less quantity by CD8<sup>+</sup> cells and some NK cells (1,6,77). It is 15.4 kDa polypeptides, 133 amino acid long and encoded by a single gene on human chromosome 4. If it is glycosylated, it will have higher molecular weight. The glycosylate part is an inactive while its amino acid sequence is active and has no similarity to that of other cytokines (77).

Normally, resting T cells do not synthesise or secrete IL-2 unless they are stimulated by antigens or after exposure to mitogens. Consequently, IL-2 mRNA expression becomes detectable within few hours reaching peak at 12 hours (164). Because of very short half-life of IL-2, it acts locally on the cells that produced it and on the adjacent cells (164).

#### Functions of IL-2 (Figure 3):

1. It induces activation and proliferation of T-cells in an autocrine and a paracrine fashions.

2. IL-2 stimulates second round of IL-2 synthesis from activated T cells. Failure to produce sufficient amount of IL-2 may be the cause of antigen specific T cell anergy.

3. IL-2 stimulates the growth of NK cells and increases their cytolytic activity and produces the so-called lymphokine activated killer cells (LAK). Since NK cells express only P70 and not P55 that reduces the requirements of NK cells to be activated by IL-2, so higher amounts of IL-2 needed to stimulate NK cells and LAK formation (6,35,143).

4. IL-2 acts on human B cells and induces their proliferation and antibody production. Nevertheless, it does not cause isotype switching.

5. It causes activation and proliferation of tumor infiltrating lymphocytes (TIL) which are effective when combined with IL-2 therapy for management of melanoma (77).

#### IL-2 Receptor (IL-2R):

It is a novel marker of the immune system activation. Normally IL-2 binds to its receptor (IL-2R) on the surface of T cells resulting a series of intracellular signals that leads to the activation and proliferation of such cells and ultimately generation of cytokines that modulate immune reaction (143,147).

This high affinity IL-2 receptor is not expressed on resting T cells unless they are activated (6). It consists of three distinct polypeptide chains designated  $\alpha$ ,  $\beta$ , and  $\gamma$

(Figure 4). Combination of these 3 chains forms high affinity IL-2R (143,186) The IL-2R- $\alpha$  chain (also called Tac) binds IL-2 with low affinity but without signal. Tac chain is absent on resting T cells and it is important for normal T-cell hemotasis. It was found that in IL-2R  $\alpha$  knockout mice there is a marked enlargement of lymphoid organs and existence of autoimmunity disease. (77,143). IL-2R  $\beta$  binds IL-2 with intermediate affinity and IL-2R  $\beta$  knockout mice suffers from hyperactivation of T cells and autoimmunity. While IL-2R  $\gamma$  can not bind to IL-2 by itself. It is worthy to note that receptors for IL-4, IL-7, IL-9, IL-13, and IL-15 also contain IL-2R  $\gamma$  chain. Recently, it was found that the etiology of X-linked severe combined immuno deficiency (X-SCID) is mutation in the IL-2R $\gamma$  chain which causes loss of functions of these interleukins, (164).

IL-2R can be released upon action from B cells, monocytes, and granular lymphocytes. (143). It is present in low levels in healthy individuals and significantly increases in broad range of diseases (133,148,186,194) as shown in Table 1.



Table (1). Human diseases with increased IL-2R (148).

Malignancy

Adult T-cell leukemia

Hodgkin lymphoma

Non-Hodgkin lymphoma

Liver, breast, and lung cancers

Autoimmune or inflammatory disease

Rheumatoid arthritis

Systemic lupus erythematosus

Progressive systemic sclerosis

Polymyositis

Kawasaki disease

Multiple sclerosis

Diabetes (type I)

Infections

Viral Hepatitis

HIV-I (AIDS)

Infectious mononucleosis

Measles

Mycobacterial

Leprosy

Tuberculosis

Others

Malaria

Transplantation or rejection of

Kidney

Lung

Liver

Miscellaneous

Thermal injury

Chronic renal failure or dialysis

Sarcoidosis

Schizophrenia

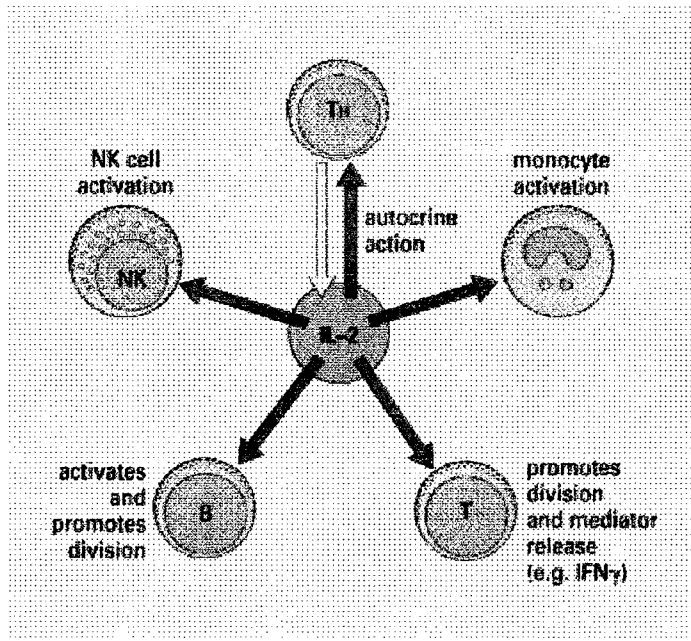


Figure 3. Shows the actions of IL-2 (143).

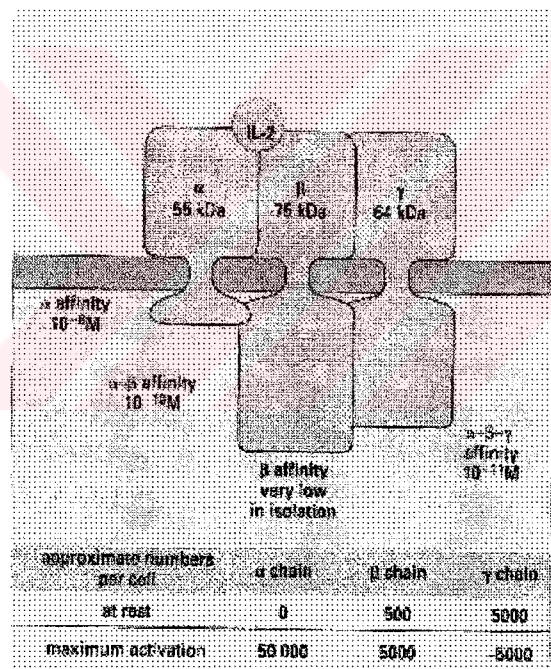


Figure 4. Shows the structure of IL-2R (143).

**2.4.3.(IL-3):**

It is a 28 kDa glycoprotein, previously known as multi-specific haemopoietin. It is secreted by two subsets of activated Th cells. It stimulates the growth and differentiation of various haematopoietic cells (red cells, granulocytes, macrophages and lymphocytes). It also causes localized inflammatory reactions by stimulation of mast cells (1,18,77,102).

Experimentally, IL-3 can indirectly enhance the activity of cytotoxic T cells against tumors by stimulating the release of T cell growth factor (134)

**2.4.4.(IL-4):**

It is a 15 kDa glycoprotein first described in 1982 as a B cell growth factor 'BCGF' (80). It is primarily secreted by Th2 cells and mast cells (18,77). It has several pleiotropic biological effects. It stimulates growth and differentiation of B cells to IgG<sub>1</sub> and IgE isotypes so it acts as an isotype switching factor. IL-4 exerts its effects by interacting with the particular high affinity receptor (IL-4R) presented on the surface of haematopoietic and non-haematopoietic cells. IL-4 also increases the expression of class II MHC antigen, CD23 receptor for IgE, IL-4R on surface of B cells, so it was previously called B-cell stimulatory factor-1 (77,134). On macrophages, it can activate macrophage cytotoxic functions and increase expression of class II MHC protein (164). It inhibits Th1 cells; in other words it means inhibition of production of inflammatory cytokines such as IL-1 and TNF- $\alpha$ . It also suppresses the synthesis of proinflammatory cytokines such as TNF- $\alpha$ , IL-1, IL-6, and IL8 by activated monocytes (143).

It has a central role in allergic diseases because of its direct effect on IgE production and indirect effect on eosinophils and mast cell activation and proliferation (Figure 6). Moreover, experimental studies showed that IL-4 knockout mice exhibited only deficiencies of IgE and Th2 cytokines production (77,164).

**2.4.5.Interleukin(IL-5):**

It is a 45 kDa glycoprotein secreted mainly by Th2 cells and less degree by mast cells (77,35,164). It has stimulatory effect on murine B cells but not on human B cells (7).

The major functions of IL-5 in humans is to stimulate growth and differentiation of eosinophils and to activate mature eosinophils to kill helminths (1). In mice, administration neutralizing antibodies to IL-5 inhibits the eosinophilia seen in helminth

infections (1,143). IL-5 also has synergistic effect with IL-4 to induce IgE secretion and enhances IgA secretion (29).

#### **2.4.6. Interleukin(IL-6):**

It is a 21 to 28 kDa glycoprotein owing to variation in the degree of glycosylation. The gene for IL-6 is located on human chromosome 7(6). It is produced by many cell types including T cells, B cells, macrophages, fibroblasts and endothelial cells (35,143). Its expression is induced by a variety of stimuli including TNF, IL-1, platelet derived growth factor and agents that activate T and B lymphocytes and macrophages (164). It is detected in serum in response to TNF or IL-1. It does not cause vascular thrombosis(1).

IL-6 has pleiotropic functions. It increases the mitogenic effect of IL-1 and TNF on T helper cells by its ability to increase IL-2R expressions (164). It also enhances TNF induced cachexia. It also causes hepatocytes to synthesize acute phase proteins. It plays a role in final maturation of activated B cells into antibody forming cells since IL-6R is only expressed by activated B cells but not by resting B cells (77). IL-6 synergistically enhances the activity of IL-3 on haematopoietic stem cells and maturation of megakaryocytes (78). IL-6 can also activate NK cells but can not induce LAK formation (158) .

IL-6 enhances the activity of malignant plasma cells to produce IL-6, therefore serum levels of IL-6 increases in multiple myeloma. Elevated levels of IL-6 has been observed in other pathological conditions such as bacterial and viral infections, human immunodeficiency virus infection (HIV), autoimmune diseases, lymphoma, alcoholic liver diseases, transplant rejections (27,65,78,149,165).

#### **IL-6R**

It is a high affinity binding site for IL-6 presented on surface of a variety of cells including macrophages, resting T cells, activated or Epstein-Barr virus infected B cells, plasma cells and hepatocytes. The receptor consists of two glycoprotein chains  $\alpha$  and  $\beta$ . IL-6R- $\alpha$  binds IL-6 and IL-6R- $\beta$  transduces signal into cytoplasm (164).

#### **2.4.7. Interleukin(IL-7):**

It is a 25 kDa glycoprotein which was initially described as pre B cell factor and called lymphopoietin. It is secreted by thymus, spleen, and bone marrow stromal cells. It causes proliferation of B cell precursors but has no effects on mature B cells and

plasma cells(10). However, it also affects the activity of T lymphocytes and NK cells (77,164).The study by Borger et al (20) showed that IL-7 at 5mg/ml concentration resulted in the accumulation of IFN- $\gamma$  (eight folds) and IL-4 (5 folds) from activated T cells which could be blocked by cyclosporin A. Thus, IL-7 upregulates IFN- $\gamma$  and IL-4 expression from Th1 and Th2 cells at transcriptional and post transcriptional levels (20). It can also induce LAK activity, but with less activity than IL-2 (116,164).

At higher concentration, IL-7 can increase macrophage cytotoxic activity and induces cytokine secretion by monocytes. Moreover, it was observed that IL-7 knockout mice suffered from lymphoid hypoplasia due to failure of T and B cells development (164).

#### **2.4.8.Interleukin(IL-8):**

It is non glycosylated CXC chemokine with a molecular weight of 8 kDa. Previously, it was believed that monocytes were the major sources of this interleukin, later on, it was found that other cells such as endothelial cells, epithelial cells, hepatocytes, fibroblasts, and others can also produce IL-8 (77,143,164).

A wide variety of stimuli (LPS, IL-1, TNF, viruses, lectins, urate crystals) can trigger the initiation the production of IL-8 (158). IL-2 and anti CD16 monoclonal antibodies can synergistically induce expression of IL-8 from NK cells. Contrarily, IL4, transforming growth factor-B (TGF-B) and glucocorticoides inhibit IL-8 release (77).

Since IL-8 has chemotactic activity towards neutrophils, it was previously called neutrophil activating protein (NAP-1), neutrophil activating factor (NAF) and monocyte derived neutrophil chemotactic factor (77). In addition to an inducer function of IL-8 in neutrophil chemotaxis, it is also responsible for the release of lysosomal enzymes by neutrophils. IL-8 binds to target cells via specific receptors. It was found that T lymphocytes normally have nearly 300 binding sites per each cell and respond 100 times less than neutrophils to IL-8 (114)

IL-8 has a crucial role in many inflammatory diseases. IL-8 was found to be increased in psoriasis, cystic fibrosis, septic shock, neoplasms, pleural diseases, idiopathic pulmonary fibrosis and rheumatoid arthritis (125,143,164).

#### **2.4.9.Interleukin(IL-9):**

Previously it was called P40 mouse T cell growth factor. It is a glycoprotein secreted by Th cells. This interleukin is expressed by peripheral blood T cells after

activation by lectin, and IL-2 is necessary for expression of IL-9 since anti-IL-2 blocks the induction of IL-9 (138). IL-4 has synergistic property with IL-9 to induce IgE and IgG production (6). IL-9 has also a role in activation and proliferation of mast cells and it may be involved in allergic response (18,138,143). It also enhances the host resistance against *Trichuris muris* infection (52).

#### **2.4.10. Interleukin (IL-10):**

Originally it was called cytokine synthesis inhibitory factor (CSIF) because of its ability to inhibit cytokine production by activated Th1 cells. It is a 18 kDa polypeptide secreted mainly by Th2 subsets of T cells (82,143). Other cells like macrophages, monocytes, keratinocytes and activated B cells can also produce IL-10 (18).

It is an anti inflammatory cytokine and suppresses Th1 cytokines production (Figure 5). It also inhibits antigen presentation and production of IL-1, IL-6, and TNF- $\alpha$  by macrophage (143). It also suppresses the macrophage production of reactive oxygen species and nitric oxide (164) The inhibitory mechanism of IL-10 may be explained by its suppression effect on expression of MHC class II and subsequent functions of macrophage (164). IL-10 also inhibits the secretion of IFN- $\gamma$  and TNF- $\alpha$  by NK cells stimulated by IL-2, but it does inhibit LAK activity induced by IL-2 (81).

In addition to inhibitory effect of IL-10, it acts with IL-4 to increase the proliferation of B cells (110,112,143). IL-10 also shows differential effect on activated B cells causing large quantity of IgM, IgG, and IgA production. This activity can be antagonised by IL-4 (77). It was also found that T cells in the lamina propria is the major source of IL-10 which controls intestinal immunity but does not suppress basic immunity (23,152). Therefore, this interleukin can be used for therapy of inflammatory bowel disease (23).

Circulating IL-10 is increased in many diseases such as malaria, visceral leishmaniasis (19,128).

#### **2.4.11. Interleukin 11 (IL-11):**

It is produced by bone marrow stromal cells. Similar to IL-1, IL6, and TNF, it also induces production of acute phase protein. It also has stimulatory effects on haematopoiesis (164). IL-11 has synergistic effect with IL-3 and IL-4 for the proliferation of haematopoietic progenitors (77).



#### **2.4.12. Interleukin 12(IL-12):**

It was previously known as NK cell stimulatory factor or cytotoxic lymphocyte maturation factor (93). It is a heterodimeric cytokine composed of covalently linked disulfide P35, P40 chains. It is produced by monocytes, macrophages, and less degree by B cells (77). Production of IL-12 by activated macrophages is suppressed by IL-4 and IL-10 (164). It induces IFN- $\gamma$  production by T cells and NK cells. It also induces the differentiation of Th0 cells into Th1 cells and antagonizes the differentiation into Th2 cells (Figures 1, 6).

It induces IL-2 with which it has synergistic capability in promoting cytotoxic T cell response (164). IL-10 is considered a physiological antagonist of IL-12 (22).

Recently, it was found that this interleukin promotes survival of intestinal worms and establishes a chronic intestinal nematode infection (14) On the other hand, it induces cell-mediated immunity and plays an important role in resistance to intracellular parasites such as *Leishmania* and *Toxoplasma* spp. (22).

#### **2.4.13. Interleukin 13(IL-13):**

It is a protein secreted by activated T cells and it is similar both in structure and function to IL-4 but does not affect T cells (15,18,35,77,109,143,195). It induces B cell growth and differentiation and inhibits pro-inflammatory cytokine production. Like, IL-4, it acts as switch factor on synthesis of IgE (18,35,77,143). Its serum value increases in allergic patients (77).

#### **2.4.14. Interleukin 14(IL-14):**

It is a 50-60 kDa glycosylated protein. It is produced by malignant B cell and normal cells. Its activity is to induce proliferation of activated B cell but not resting B cell, thus it participates mainly in secondary immune response (77,143).

#### **2.4.15. Interleukin 15(IL-15):**

This interleukin is produced by epithelial cells and monocytes. It stimulates B and T cells and activates LAK formation. It has biological similarity to IL-2 as T cell growth factor. It can interact with components of IL-2R ( $\beta$  &  $\gamma$  chains). IL-15 is widely expressed in placenta, skeletal muscles, kidney, lung, liver, heart and bone marrow stroma (77,164).

**2.4.16. Interleukin 16(IL-16):**

It is previously known as lymphocyte chemo-attractant factor. It is produced by eosinophils and CD8+ T cells. It induces migration of CD4+ cells, eosinophils, and monocytes (164).

**2.4.17. Interleukin 17(IL-17):**

It is produced by CD4+ cells. Its principle function is to induce the release of IL-6 and IL-8, and enhances ICAM-1 expression (143).

**2.4.18. Interleukin 18(IL-18):**

It is produced by hepatocytes. It induces IFN- $\gamma$  production and enhances NK cell activity (16).





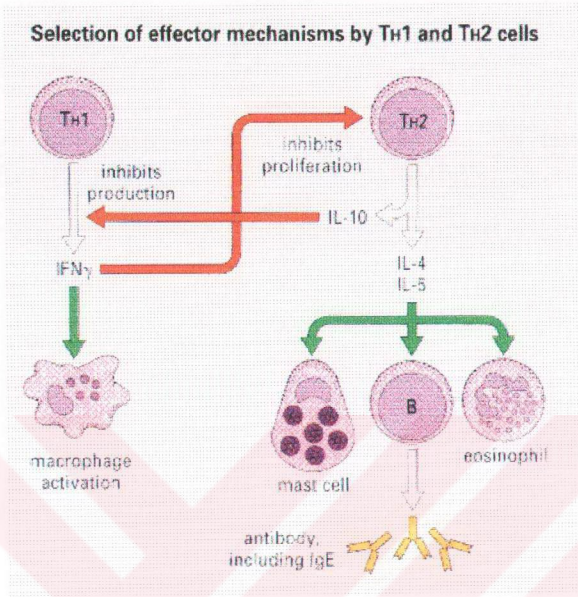


Figure 5. Shows effector mechanisms of Th1 and Th2 (143).

## 2.5. The role of cytokines in parasitic infections:

Recently, the nature of host immunity against parasites and adaptation of parasites to host immunity have been elucidated through the studies and researches performed on what is called immunobiology of parasites (143). As a result, the significance of dichotomy in cytokines production by Th1 and Th2 in some parasitic diseases is well understood (33,36,46,47,137,153,156).

Cytokine cascade plays a central role in in pathology of some parasitic diseases. Since the parasites inhabit different enviromental conditions in the body, there are obvious differences in the evolution of host protective mechanism, states of parasite survival, and the pathology associated with these infections (95).

The ultimate consequence of the parasitic infection whether disease or resistance depends on the balance of cytokines produced by the host in response to parasite stimulation.

Although there is limited literature on cytokine regulation in some parasitic diseases, the cytokine pattern in other parasitic infections such as malaria, leishmaniasis, toxoplasmosis and schistosomiasis is well studied (38,95,141,143).

In an experimental study on *Leishmania major* in mice, it was found that localized lesions were developed in the skin of resistant mice C57/ BL6. These animals expressed IFN- $\gamma$  but little IL-4. In contrast, progressive disseminated disease was developed in susceptible mice (BALBL/C) and these animals expressed IL-4 and IL-6 but not IFN- $\gamma$  (153). These data clearly indicate that protection from leishmaniasis correlated with the development of Th1 cytokines while susceptibility with the production of Th2 cytokines (95,143).

In other studies, it has been also demonstrated that TNF- $\alpha$  and IFN- $\gamma$  induce nitric oxide production by activated macrophages and this has parasiticidal activity (98,99). Whereas IL-4 downregulate IFN- $\gamma$  induced the macrophage activity and thus increase the probability of surviving the parasite (95,143).

In localized cutaneous leishmaniasis in man, Th1 cytokines such as IFN- $\gamma$  and IL-2 are predominant, whereas in chronic severe cases of mucocutaneous lesions both type 1 and type 2 cytokines with relative increase in IL-4 are demonstrated (130).

The same conclusion can be drawn in human leishmaniasis similar to that in animals, that is to say IL-4 production correlated with susceptibility to severe leishmaniasis and IFN- $\gamma$  production with resistance to disease (95).

Experimental studies on *Giardia muris* mouse model has demonstrated the importance of both cellular and humoral mechanisms in the development of protective immunity and resistance to giardiasis. The cellular immune response includes IL-2, whereas secretory IgA and IgE antibodies are involved in humoral immune response (8,42,50,51,61,76,157,175).

Th1 and Th2 cells play a role in immune response against helminth infections with particular emphasis on Th2 cells which are necessary for elimination of intestinal worms (6,56,57,180). Mast cells, eosinophils have also a role by interaction of their products with components of parasite (18,143).

The immune response in helminth infections generally depends on the particular parasite, stage of infection, the worm burdens, and nature of the host immune system and genetic makeup (95,142,143).

In intestinal helminthic infection, it was reported that Th1 cytokines were associated with chronic infection and Th2 cytokines conferred protective immunity and resistance to nematodes (15,56,57,63,180). In ascariasis, there was less detrimental effects than systemic nematode infections and mucosal macrophages were responsible for the output of cytokines that had systemic effects (36).

The immune response to cystic echinococcosis is complex (142). Little is known about cytokine production in pre-encystment (establishing phase) and post-encystment (establishment phase) (142). Nevertheless, serum IgE, IgG1, and IgG4 responses may involve in cystic echinococcosis (101,142,155).

Macrophages, neutrophils, eosinophils, and platelets can kill worms as well as protozoa by secreting cytotoxic molecules such as reactive oxygen species and nitric oxide (54,98,99,143). These cells become more effective functionally when activated by cytokines such as TNF- $\alpha$  which activates macrophages, eosinophils to kill several species of protozoa and helminths. Neutrophils can kill parasites in similar manner of macrophages, but they produce more intense respiratory burst than macrophages and their secretory granules contain highly toxic proteins (143).

Eosinophils play an important role in controlling the worm infection by limiting the migration of helminths through tissues. Nevertheless, they are less phagocytic than neutrophils and their activities are enhanced by cytokines such as TNF- $\alpha$ . They degranulated when activated and their binding to larva of worms coated with IgE resulted in the increase of the release their granular contents (such as major basic protein) (18,143). Thus, they can kill helminths by oxygen dependent (respiratory burst)



and oxygen independent (nitric oxide). The killing effect can be also increased by mast cell mediators (18,143).

Platelets can kill many types of parasites including helminths and protozoa and their cytotoxic activity is enhanced by cytokines such as TNF- $\alpha$  and IFN- $\gamma$  (95,143).

The different immune mechanisms may also vary according to different anatomical sites that parasites inhabit, and at different stages of life cycle. Although, the exact immune response against parasites is unknown, experimental studies and few clinical works have shown the importance of Th1 and Th2. cytokines in intracellular protozoa and worm infections respectively (95,143).

## 2.6. IgE

It is a monomer immunoglobulin composed of glycoprotein with carbohydrate content 12%. Its half life span is 2-3 days. It plays an important role in immunity to parasites (particularly helminths). In developed countries, it is more commonly associated with allergic diseases such as asthma and hay fever (143).

IgE has two different receptors Fc  $\epsilon$ RI and Fc  $\epsilon$ RII. The former is classical IgE receptor with high affinity. High levels of these receptors are found on the surface of basophils, mast cells, and in low level on the surface of eosinophils. The latter is characterized by low affinity and found on the surface of macrophages, monocytes, lymphocytes, eosinophils and platelets (37,143).

IgE can interact with eosinophils leading to production of eosinophil peroxidase and eosinophil cationic protein. These activated eosinophils play an important role in protective immunity against parasites (30).

IgE production is under the control of IL-4 and IL-13 (77,55,143,) as shown in figure 6. It is present in low concentration (0.05 ng/ml) in the serum of normal individuals. It is elevated in parasitic diseases and allergic conditions (41,42,58,64,66-69,104,143,182). High IgE levels may be found in patients with immunological disorders such as Hyper-IgE syndrome "Job syndrome" (132). Although IgE plays an important role in IgE mediated hypersensitivity. The latter may be rare in chronic parasitic infection due to presence of blocking antibodies IgG4 (182). The increased production of IgE was also observed in atopic patients (35).

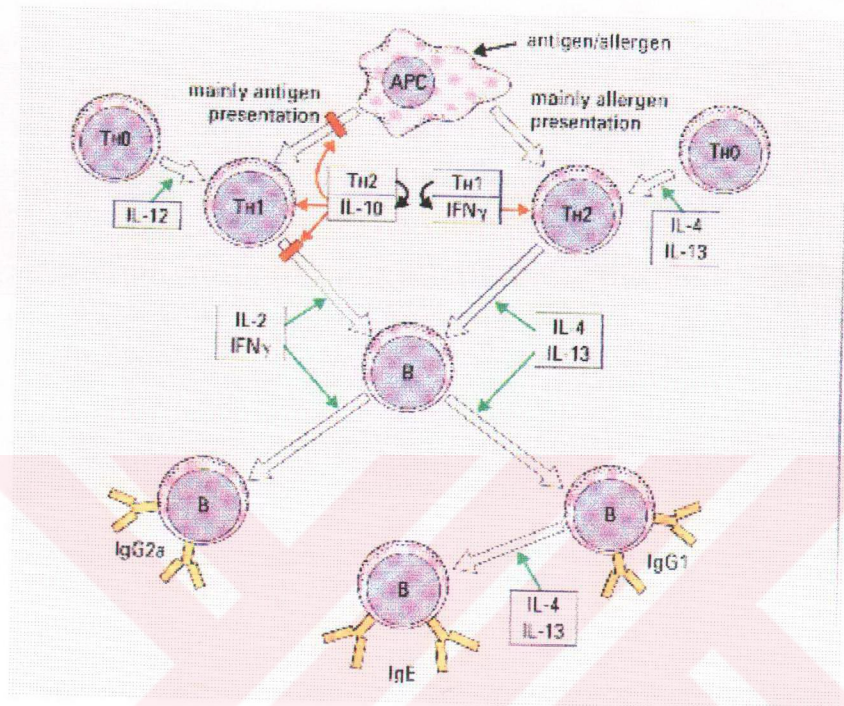
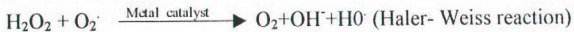


Figure 6. Shows the cytokines control of IgE (143).

## 2.7.Free radicals:

They are toxic reactives that differ from other compounds in that they have unpaired electrons in their outer orbitals (160,185). These radicals were resulted from utilization of oxygen in a variety of biochemical reactions. Of these, hydroxyl radical is the most potent one that causes oxidative damage to tissues, it is generated by the interaction of the superoxide anion and hydrogen peroxide in the presence of metal catalyst (70,71,85,160,185).



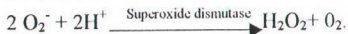
Experimental studies demonstrated that some cytokines such as TNF- $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IFN- $\gamma$  and granulocyte macrophage colony-stimulating factor(GM-CSF) stimulate reactive oxygen species (ROS) from a variety of phagocytes (185). On other hand, IL-4 inhibit ROS release (185).

Reactive oxygen species can cause damage to DNA, inactivate enzymes, oxidize hormones and kill several types of cells. Moreover, they may play an important role in various degenerative and inflammatory processes including microbial and non-microbial pathogenesis (70,72,73).

The adverse biological effects of these radicals can be controlled in body by a wide spectrum of antioxidant defense mechanisms such as vitamin C and E, carotenoid metabolites (glutathione, uric acid), and antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase)(11,70,118,185).

## 2.8.Superoxide dismutase (SOD):

It is a metalloenzyme that plays a first line of defence against superoxide ion radical ( $\text{O}_2^-$ ). It converts the superoxide anion to hydrogen peroxide and water (85,160).



The superoxide anion is yielded by single reduction of oxygen. There are 3 distinct types of SOD; copper and zinc containing enzyme (Cu Zn SOD) manganese containing enzyme (Mn SOD), and iron containing enzyme (Fe SOD)(85).

The iron and manganese superoxide dismutases are highly homologous to each other but quite distinct from copper-zinc SOD. Obligate anaerobes contain only Fe SOD (26). Parasitic protozoa including *Entamoeba histolytica*, *Trichomonas vaginalis*, and *Trypanosoma* spp. contain iron superoxide dismutase (107).

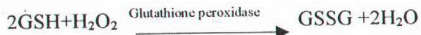
Contrarily, *Giardia lamblia* not only lacks superoxide dismutase but also glutathione peroxidase. Instead, it contains NADH oxidase which reduces oxygen to



water and this circumvents the production of superoxide anion and hydrogen peroxide (24,25). The enzyme (Zn-Cu SOD) has been reported to be abundant in muscles, nerve trunks, esophagus and uterine and accessory cells of *Ascaris lumbricades* and *Ascaris suum* (85,150).

### 2.9. Glutathione peroxidase (GSH-px):

It is a seleno-protein. It is an antioxidant enzyme that protects tissue from oxidative damage. At low concentration of hydrogen peroxide, it catalyzes the breakdown of  $H_2O_2$  with the existence of glutathione (GSH) to form glutathione (GSSG) and water (122,160,185).



The oxidized glutathione (GSSG) is converted to the reduced form (GSH) by an enzyme glutathione reductase using NADPH formed by the pentose phosphate pathway (122,160,185). Glutathione peroxidase also catalyzes the reduction of lipid peroxides by glutathione and hence it prevents propagation of lipid peroxidation (70,71).

Parasitic protozoa such as *Giardia lamblia*, *Entamoeba histolytica*, *Trichomonas vaginalis*, *Trichomonas foetus*, *Leishmania* spp and trypanosomes are deficient in glutathione and substitute GSH with an alternative cysteine (a low molecular weight thiol) which is involved in similar mechanism of detoxification (26).

### 2.10. Catalase:

It is an old enzyme and it is called hydrogen peroxide oxido-reductase. It has two enzymatic functions. First, it catalyzes the breakdown of hydrogen peroxide into water and oxygen. Second, it catalyzes the oxidation of electron donors such as ethanols and phenols. Its activity starts when hydrogen peroxide presented in the high concentration. It consists of four subunits. Each unit has about 60.000 molecular weight and contains one ferriprotoporphyrin (126).

Its mode of action consists of two steps. First, the native ferric haemoprotein (free catalase) reacts with  $H_2O_2$  to form a green primary complex called compound I. and in the second step, two electrons are transferred from an electron donor to form an oxidized product and water. The electron donor can be hydrogen peroxide (catalactic mode) or other substances such as methanol, ethanol, or formic acid (peroxidatic mode). However, the two electrons may not be transferred simultaneously as with phenol, this

results in accumulation of a red one-electron reduced form of compound I called compound II which is active form of enzyme (126).

### **2.11. Malondialdehyde (MDA):**

It is a hydroperoxide product formed as a result of oxidation of unsaturated fatty acids by oxygen reactive species. The process involves either controlled enzymatic steps or uncontrolled radical chain reaction (17). This hydroperoxide may decompose to form free radical and carbonyl compound. The formed free radical interacts with other lipid molecules containing unsaturated fatty acids. These processes are collectively known as lipid peroxidation (17,34). The free radicals, superoxide anion and hydroxyl stimulate lipid peroxidation. Both peroxides and aldehyde molecules possess potent biological activities and causes cell membrane damage. In an experimental animal study, it was reported that enterocytes injury in giardiasis was mediated by superoxide radicals and there was high MDA concentrations in such enterocytes (62).

Malondialdehyde is increased in non pathological conditions such as pregnancy and ageing (31,105,192), and in diverse pathological conditions such as diabetes, rheumatic diseases, atherosclerosis, gastric ulcers, thalassemia, and others (31,97,121,151,190). Lipid peroxide activity has been also implicated in parasitic diseases such as cutaneous leishmaniasis. The MDA serum level is increased in that disease and decreased after successful treatment (49,131,184).



### 3. MATERIALS AND METHODS

#### 3.1. Patients:

A total of 86 patients with giardiasis (40 males and 46 females), 34 patients with ascariasis (13 males and 21 females), 32 patients with cystic echinococcosis (10 males and 22 females), and 30 healthy controls (12 males and 18 females) were recruited for this study.

Patients were selected among the individuals attending Clinical Microbiology Laboratory, Turgut Özal Medical Centre for parasitological examination. The diagnosis of *Giardia lamblia* and *Ascaris lumbricoides* was made by stool examination. While cystic echinococcosis was diagnosed by microscopic examination of cyst fluid for scolices or hooklets and results of radiographic examination, indirect haemagglutination test, histopathological evaluation, and clinical examinations. Stool examination was made on cystic echinococcosis and controls to exclude intestinal parasites.

The ages of the patients with giardiasis ranged from 2.5 to 59 years with a mean age of  $12.34 \pm 11.32$  years (median=7 years). The ascariasis patients were between 3 and 68 years old, with a mean age of  $26.25 \pm 20.86$  years (median=22 years). The age range of the patients with cystic echinococcosis was 12-74 years with a mean  $46.52 \pm 19.62$  years (median=42 years). Healthy control subjects were between 16 and 47 years old with a mean age of  $27.77 \pm 9.74$  years (median=24.5 years) (Table 2).

Decision was made whether control subjects were healthy according to results of blood examination (routine biochemical and haematological tests) in addition to apparent healthy status with no history of current illness and pathological condition. Venous fasting blood samples were collected in sterile tubes. Blood samples were centrifuged as soon as possible at  $1500 \times g$  for 15 minutes to obtain the serum. The lipemic or haemolysed sera were discarded and other sera were divided into 4 tubes for each subject and stored immediately at  $-20^\circ C$  until measurement. For each determination, a new aliquot was used since freezing and thawing of a once or used sample may partially destroy its biological actions.

### 3.2 Materials:

#### 3.2.1 Chemicals:

Ammonium sulphate (Merck)  
 Bovine serum albumin essentially free fatty acid (Merck)  
 n-butanol (Merck)  
 Copper chloride (Merck)  
 Ethylene diamine tetra acetate sodium salt "EDTA"  
 Glutathione reduced form (Sigma)  
 Glutathione reductase (Sigma)  
 Hydrochloric acid (Merck)  
 Hydrogen peroxide (Merck)  
 tris Hydroxy methyl amino methane (Merck)  
 NADPH (Sigma)  
 Nitro blue tetrazolium "NBT" (Sigma)  
 Phosphoric acid (Merck)  
 Potassium dihydrogen phosphate  
 Sodium azide  
 Sodium carbonate (Merck)  
 Sodium chloride (Merck)  
 Sodium citrate (Merck)  
 Sodium hydrogen phosphate (Merck)  
 Sodium molybdate (Merck)  
 Sodium tungstate (Merck)  
 Sulphuric acid (Merck)  
 1,1,3,3-tetra-methoxy propan (Sigma)  
 Thiobarbituric acid (Sigma)  
 Triton x-100 (Merck)  
 Xanthine (Sigma)  
 Xanthine oxidase (Sigma)

#### 3.2.2. Kits:

Cellognost Echinococcosis IHA(Boehring)  
 Cytoscreen Immuno assay human IL-2 kit(Biosource International Inc.)

The Pelikine human IL-4 ELISA kit (Central Laboratory of the Netherlands, Red Cross Blood Transfusion)

The Pelikine Compact human IL-10 ELISA (Central Laboratory of the Netherlands, Red Cross Blood Transfusion)

Solid-Phase two site chemiluminescent enzyme immunoassay for TNF- $\alpha$ , IL-1 $\beta$ , IL-2R, IL-6, IL-8 (Immulite automated analyzer, EURO / DPC Ltd)

Total IgE ELISA (Clone System, Biochem ImmunoSystems S.P.A.).

### **3.3. Methods.**

#### **3.3.1. Cytokines measurement:**

Serum levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-2 R, IL-6 and IL8 were determined by a solid-phase two site chemiluminescent enzyme immunometric assay with the immulite automated analyzer. DPC IMMULITE was manufactured by EURO DPC Ltd under a Quality System approved by the British Standards Institute

#### Principle of the procedure:

The solid phase, a polystyrene bead enclosed within an IMMULITE test unit which is coated with an anti-ligand (binding molecule). While patient's sample, alkaline-conjugated monoclonal antibody, and ligand-labeled antibody are incubated for 60 minutes at 37 °C in the test unit with agitation. If a specific cytokine presents in the sample, it will form a sandwich with the two antibodies which recognize different epitopes of the cytokine molecule. Then the formed sandwich will bind to the solid phase by ligand-anti-ligand bridge. Then washing to remove unbound conjugate, after which a substrate is added and incubated for 10 minutes. The chemiluminescent substrate (a phosphate ester adamantyl dioxetane) undergoes hydrolysis in the presence of the alkaline phosphatase to produce an unstable intermediate, the continuous production of this intermediate results in the emission of light (photon output). The photon output is measured by the luminometer and it is proportional to the concentration of cytokine (the bound sandwich complex).

**IL-2:**

IL-2 was measured by an ELISA with 5 pg/ml detection limit (Cytoscreen Immunoassay human IL-2 kit, Biosource International Inc., USA) following the manufacturer's instructions.

**IL-4:**

IL-4 was measured by an ELISA with 0.2 pg/ml detection limit (The Pelikine human IL-4 ELISA kit, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, The Netherlands) following the manufacturer's instructions.

**IL-10:**

It was measured by an ELISA with 5 pg /ml detection limit (The Pelikine Compact human IL-10 ELISA Kit, Central laboratory of the Netherlands Red Cross Blood Transfusion Service, The Netherlands) following the manufacturer's instructions.

**Principle of ELISA kit assays:**

They are based on a sandwich type of Enzyme Linked Immuno Sorbent Assay (ELISA). A specific monoclonal antibody for each interleukin is coated on polystyrene microwell strips. If a specific interleukin is present in the serum or standard, it will bind to monoclonal antibody. Non-bound material is washed. The latter step is followed by the addition of a biotin-conjugated antibody which binds to specific interleukin. After removal of excess biotinylated antibody by washing, polymerized horseradish peroxidase (HRP) conjugated to streptavidin is added. This binds to the biotinylated antibody complex. After an incubation at 25 °C for 1hr, excess enzyme is removed by washing. Then, a substrate solution tetramethylbenzidine (TMB) is added, its interactions with the bound enzyme yields a coloured product in direct proportion to the amount of interleukin present in the sample or standard. The reaction is terminated by the addition of a stop solution (HCl or H<sub>2</sub>SO<sub>4</sub>). Absorbance is read at 450 nm for each well. Calculations is made from the standard curve. All cytokines are expressed in pg/ml except IL-2R (U /ml).

### 3.3.2. Total IgE:

It is measured by an ELISA with the minimal detectable concentration of 5 IU/ML (Clone System, Biochem Immuno Systems, ITALTA S.P.A., Italy ) following the manufacturer's direction.

#### The principle of the test:

It is a solid phase sandwich ELISA. IgE molecules is sandwiched between the solid phase coated monoclonal antibody and an enzyme (horseradish peroxidase) conjugated antibody differing from a previous ELISA procedure in that absence of biotin- conjugated antibody. After subsequent additions of substrate and stop solutions, the result is measured spectrophotometrically at 450 nm. The total IgE concentration of unknown serum is calculated from the standard curve. The obtained values are directly proportional to the colour intensity in well.

### 3.3.3. Eosinophil count:

Eosinophils were counted in Coulter STKS Analyzer (Miami Florida 33 196).

### 3.3.4. Antioxidant enzymes:

#### a. Superoxide dismutase:

This enzyme is measured in serum and hydatid cyst fluid according to a method developed by Sun et al (197).

#### The principle of the assay.

The method for assay of SOD activity is indirect assay based on a competition between SOD and an indicator substance, nitroblue tetrazolium (NBT) for superoxide radical. First it was described by Beanchamp and Fridovich. Xanthine-anthine oxidase was utilized to generate superoxide anion ( $O_2^-$ ) which reduced nitroblue tetrazolium to form blue formosan. The produced colour was read at 560 nm in LKB spectrophotometer (Biochrom marka ultra spec plus 40 54 model) As the amount of SOD increased in sample the rate of NBT reduction is decreased. That is to say the NBT reduction is inhibited, hence the SOD activity is calculated as follows

$$\% \text{ inhibition} = \frac{\text{Absorbance (Blank)} - \text{Absorbance (sample)}}{\text{Absorbance (Blank)}} \times 100$$

$$\text{SOD activity in serum (U/ml)} = \frac{\% \text{ inhibition}}{50 \times 0.1}$$

$$\text{SOD activity in cystic echinococcosis fluid (U/mg protein)} = \frac{\text{U/ml}}{\text{mg/ml protein}}$$

Note: one unit SOD activity is defined as 5 % inhibition of NBT reduction.

**b. Catalase activity:**

The catalase activity was measured by method of Aebi, H. (5). Five readings for each sample at 240 nm with intervals of 15 seconds were recorded.

The principle of the test:

The ultraviolet absorption of  $H_2O_2$  is read at 240 nm. On decomposition of  $H_2O_2$  with catalase, the absorption decreases with time, and from this decrease the enzyme activity can be calculated. Catalase activity is measured according as follows:

	Blank Cuvette	Sample cuvette
Phosphate buffer*	2.99 ml	-
$H_2O_2$ solution *	10 $\mu$ l	2.99 ml
Sample	-	10 $\mu$ l

\* Previously prepared according to specifications of method.

Catalase activity

$$K = \frac{2.3}{At} \times \log \frac{A_1}{A_2}$$

$$At = t_2 - t_1 = 15 \text{ seconds}$$

2.3 = Factor to convert from ln to log,

$A_1$  = initial absorbance

$A_2$  = final absorbance

Catalase activity in cyst fluid:

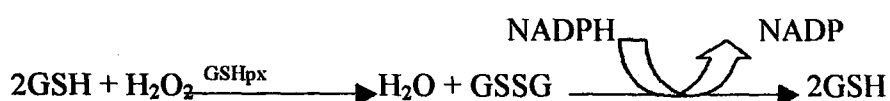
$$K / \text{gram protein} = \frac{K \times \text{sec}^{-1}}{\text{mg protein}}$$

**c. Glutathione peroxidase:**

This enzyme was determined in serum and cyst fluid by method of Paglia and Valentine (122).

The principle of test:

Glutathione peroxide catalyzes the decomposition of  $H_2O_2$  to water and singlet oxygen that converts the reduced form of glutathione to oxidized form. The latter is reconverted to reduced form by means of NADPH (118).





The conversion of NADPH to NADP decreases the optical density of solution, which is read at 340 nm in spectrophotometer utilized for SOD and catalase.

Calculation of Glutathione peroxidase

$$\mu/L = (\Delta OD / \text{Minute}) \times 24.1 \times 10^3$$

$$\Delta OD = \text{Initial optical density} - \text{final optical density}$$

For cyst fluid

$$\text{Glutathione activity (U/mg protein)} = \frac{U/L}{\text{mg protein}}$$

In all three enzymes, protein assay in cyst fluid was made by Lowry's method (103).

**3.3.5. Malondialdehyde assay (MDA):**

This assay was performed in accordance with the method of Wasowicz and his colleagues (188). To 50  $\mu$ l of serum, one milliliter of thiobarbituric acid was added. The mixture was heated at 96 °C for 60 minutes in water bath. After cooling to room temperature, 25  $\mu$ l of 6 M HCl was added, followed by 3.5 ml of n- butanol and agitated in a vortex for 5 minutes. After centrifugation at 1500xg for 10 minutes at 4 °C, the absorbance of the supernatant was read at 525 nm for excitation and 547 nm for emission using 4010 model Hitachi fluorescent spectrophotometer. The calibration curve was prepared with standards of 0.2-8  $\mu$ mol/ L. The results of all biochemical parameters are expressed in terms of mean  $\pm$  SD in each parasitic and control groups.

**3.4. Statistical analysis:**

Statistical analysis of the obtained data was performed using one way analysis of variance (ANOVA), Duncan, Chi-square, and Student's t tests in the microsoft computer programme package (170). P values below 0.05 were considered significant.

## 4. RESULTS

The study covered 182 subjects attending Turgut Özal Medical Centre/Malatya. They were 86 individuals infected with *Giardia lamblia*, 34 with *Ascaris lumbricoides*, 32 with larval stage *Echinococcus granulosus*, and 30 healthy controls. Of the patients with giardiasis, 3(3.48%) had only trophozoites, 8(9.30%) had both trophozoites and cysts, and 75(87.22%) had only cysts. *Trichuris trichiura* was seen in 7 out of 34(20.58%) patients with *Ascaris lumbricoides*.

The ratio of males to females in the groups of giardiasis, ascariasis, cystic echinococcosis, and controls were 0.87, 0.62, 0.45 and 0.66 respectively (Table 2). Most of subjects with giardiasis were children (mean  $\pm$ SD=12.28 $\pm$ 11.98, median=7 years) while those with ascariasis were children and adults (mean  $\pm$ SD= 26.25 $\pm$ 20.86 median 22 years). Cystic echinococcosis occurred in all age groups; the youngest case was 12 years and the oldest case was 74 years old (Table 2).

The frequency of hydatid cysts localized in the liver was (68.75%) followed by lungs (15.64 %) and kidney (6.25%). There were multiple cysts in liver and omentum in one woman (35 years old) (Table 3). In another woman (74 years old), there were cysts in both liver and lung. Cysts located in both lung and brain were observed in an old man (68 years old). All cysts were found to be surrounded by fibrous layer except that of brain. Few cases of sterile kidney cysts were excluded from this study since they were identified as simple epithelial cysts on the basis of histopathological examination.

### 4.1.TNF- $\alpha$ :

It is increased in sera of most cases of giardiasis, ascariasis, and cystic echinococcosis. The highest percentage of increase was seen in ascariasis (85.29 %) followed by giardiasis (82.56 %) and cystic echinococcosis (65.63 %). The statistical test indicated no significant difference of TNF- $\alpha$  among parasitic groups ( $p>0.05$ ) (Table-4).

It is also increased in sera of patients suffering from parasitic infections and concomitant diseases such as typhoid fever, brucellosis, group A streptococcal throat infections and others (Table 13).



The normal serum range of TNF- $\alpha$  in our control group was 5.1-9.8 pg/ml while its mean serum level was  $8.59 \pm 1.12$  (Table 12). According to kit instructions the values between 4-10 pg/ml were considered normal (Table 4).

#### 4.2. IL-1 $\beta$ :

This interleukin was increased in 5.81 % and 14.71 % of giardiasis and ascariasis cases respectively. No increase was observed in the sera of the patients with cystic echinococcosis (Table 5). The normal serum concentration of IL-1 $\beta$  in healthy subjects was less than 5 pg/ml (Table 12).

#### 4.3. IL-2:

The highest increase in IL-2 serum concentration was seen in the patients with *Ascaris lumbricoides* (85.29 %). In giardiasis and cystic echinococcosis cases, the increase was nearly the same 46.51 % and 46.88 % respectively (Table 6). Because of the high rate in ascariasis group, differences among the tested groups were found to be significant ( $P < 0.001$ ). In comparison the giardiasis group with cystic echinococcosis group, there was no significant difference ( $P > 0.05$ ). The normal serum concentration of IL-2 was less than 5 pg/ml (Table 12).

#### 4.4. IL-2R:

As with IL-2, the highest concentration of IL-2R was found in ascariasis group 88.23 %, followed by 79.1 % in giardiasis and 50 % in cystic echinococcosis group (Table 7). There was significant differences ( $P < 0.01$ ) among the three parasitic groups, that is due to low percentage of increased serum IL-2R in cystic echinococcosis group. These results clearly showed that the serum IL-2R levels were increased in most cases of ascariasis and giardiasis and less degree in cystic echinococcosis patients. The increased IL-2R was also seen in the subjects with both parasitic infections and other diseases such as hairy cell leukemia (Table 13). In our healthy controls, the normal serum range of IL-2R was 334-906 pg/ml and its mean level was 633.03 pg/ml (Table 12).

**4.5.IL-4:**

This interleukin was significantly increased in cystic echinococcosis group 68.75 % (P < 0.001) in comparison to the percentages in giardiasis group (2.32 %) and ascariasis group (14.71 %). On comparison the giardiasis group versus ascariasis one, there was also significant differences (P < 0.001). This clearly indicated the importance of IL-4 in immune response to cystic echinococcosis and ascariasis. The serum level of IL-4 in control subjects was less than 0.4 pg /ml (Table 12).

**4.6.IL-6:**

It was characteristically increased nearly in all cases of cystic echinococcosis (31 out of 32 cases 96.87 %, P < 0.001). Undoubtedly, this interleukin was involved in the immune response of host to the larval stage of *Echinococcus granulosus*. Regarding both giardiasis and ascariasis groups, there was no significant difference between these two groups (P > 0.05)(Table 9). The maximum serum level of IL-6 (>2000 pg/ml) was observed in many patients, of these, one had both *Giardia lamblia* and group A streptococcal infections (Table 13).

The standard serum concentration ranges from 2 to 15 pg /ml. In the current study, the normal serum range in healthy controls was between 3.2 and 13.8 pg /ml and the mean serum level  $\pm$  SD was  $7.62 \pm 2.89$  pg /ml (Table 12).

**4.7.IL-8:**

This interleukin was increased in 5/86 (5.81%) giardiasis group, 7/34 (20.58%) ascariasis group and 5/32 (15.62 %) cystic echinococcosis group (Table 10 ).

This may indicate little significance of IL-8 in the current parasitic groups and such increase may be due to other diseases (Table 13). The standard normal serum range of IL-8 is 0-70 pg /ml. In healthy controls, the range and the mean serum levels were 5-47.7 pg /ml,  $12.95 \pm 11.35$  pg /ml, respectively (Table 12).

**4.8.IL-10:**

This interleukin was not increased in any patients with *Giardia lamblia*. In both ascariasis and cystic echinococcosis groups, the percentages of increased IL-10 were 2.94 % and 40.63 % respectively (Table 11). The greatest increase of IL-10 was

found in cystic echinococcosis in the current work. The mean serum level of IL-10 in healthy controls was as same as the normal standard level ( $< 5.0$  pg/ml )(Table 12).

#### **4.9.Total IgE:**

Most patients within the three parasitic groups had increased total IgE levels (65.1 % in giardiasis, 70.5 % in ascariasis and in 89.4 % cystic echinococcosis). Statistically, there was no significant differences among the three groups ( $P>0.05$ ) as shown in Table 15.

#### **4.10.Eosinophilia:**

Eosinophilia was seen in few cases of giardiasis 5/86 (5.81 %) while it was relatively higher in both ascariasis (44.12 %) and cystic echinococcosis groups (56.25 %) as shown in Table 16. Thus, the statistical analysis indicated significant differences among the three groups ( $P<0.001$ ) and no differences between ascariasis and cystic echinococcosis groups ( $P>0.05$ ).

The ranges of eosinophil percentages in giardiasis, ascariasis, and cystic echinococcosis were found to be 0.7-11.9%, 2.1-42.3%, 4.6-17.9% respectively, while in control healthy group, the range was 0.2-1.8 % .

#### **4.11.Antioxidant enzymes and malondialdehyde :**

Table 17 presented antioxidant enzymes activities (superoxide dismutase, glutathione peroxidase, and catalase) and malondialdehyde levels in the serum of patients with giardiasis, ascariasis, and cystic echinococcosis.

##### **4.11.1. Superoxide dismutase (SOD):**

The means  $\pm$  standard deviations of SOD in serum of subjects in giardiasis, ascariasis, cystic echinococcosis and healthy control groups were  $5.305 \pm 0.0981$  U/ml,  $5.405 \pm 0.918$  U/ml,  $5.553 \pm 1.104$  U/ml, and  $2.93 \pm 0.654$  U/ml respectively (Table 17).

Among the four groups, there was statistically significant differences ( $P < 0.05$ ). Significant statistical differences were also obtained in comparison each parasitic group with healthy control group ( $P < 0.05$ ). These results clearly indicated significant increases in SOD activity in three parasitic diseases.

#### **4.11.2. Glutathione peroxidase (GSH-px):**

In contrast to SOD, there was a decrease in glutathione peroxidase activity in the sera of patients with giardiasis, ascariasis and cystic echinococcosis but this decrease was not statistically significant ( $P > 0.05$ ).

Significant differences using Duncan test were also obtained in comparison each parasitic category with controls ( $P < 0.05$ ). The means + standard deviations values were:  $0.306 \pm 0.086$  U/ml (giardiasis group),  $0.484 \pm 0.130$  U/ml (ascariasis group),  $0.364 \pm 0.114$  U/ml (cystic echinococcosis), and  $1.025 \pm 0.181$  U/ml (control group) (Table 17).

#### **4.11.3. Catalase:**

The lowest serum catalase level was found in giardiasis group (mean  $\pm$  SD =  $31.196 \pm 16.873$  K/ml). , 6 out of 86 giardiasis subjects (6.97 %) showed no catalase activity at all (zero value). The catalase activity was also lower in ascariasis and cystic echinococcosis ( $103.595 \pm 15.503$  K/ml,  $87.541 \pm 16.817$  K/ml respectively) than the controls ( $156.483 \pm 25.797$  K/ml). Statistical analysis showed no significant differences among the four groups ( $P > 0.05$ ) but, there was significant differences in each giardiasis, ascariasis, and cystic echinococcosis group when compared to controls ( $P < 0.05$ ).

#### **4.11.4. Malondialdehyde (MDA):**

Table 17 also demonstrated that there was significantly increased lipid peroxidation in cystic echinococcosis group compared to controls ( $4.031 \pm 999$   $\mu$ mol/L,  $2.253 \pm 0.355$   $\mu$ mol/L). Significant increase of MDA was also observed in ascariasis

group( $P<0.05$ ). However, malondialdehyde levels in giardiasis group were not significantly changed from the control levels ( $P>0.05$ ).

### 3.11.5. Other findings:

The results of the antioxidants enzymes and MDA assays of human and sheep hydatid cyst fluids were shown in table (18). Statistical analysis by the student t test indicated no significant differences in any enzyme activity between the two groups. Increased MDA value was found in human cyst fluid compared to that of sheep ( $P<0.01$ ).



Table (2). Distribution of the parasitic patients and healthy controls according to sex and age.

Subjects With	Sex				Age (years)		
	Male (M)	Female (F)	Ratio M/F	Total	Mean±SD	Median	Range
Giardiasis	40	46	0.87	86	12.28 ± 11.98	7	2.5-59
Ascariasis	13	21	0.62	34	26.25 ± 20.86	22	3-68
Cystic echinococcosis	10	22	0.45	32	46.52 ± 19.62	42	12-74
Healthy controls	12	18	0.66	30	27.77 ± 9.74	24.5	16-47

Table (3). Location sites of hydatid cysts .

Location sites	Number	Percent
Liver only	22	68.75
Lung only	5	15.64
Kidney only	2	6.25
Both liver & lung	1	3.12
Both liver & omentum	1	3.12
Both lung & brain	1	3.12
<b>Total</b>	<b>32</b>	<b>100</b>

Table (4). The comparison serum TNF- $\alpha$  in parasitic groups.

Parasitic groups	TNF $\alpha$					
	Increased level		Normal level		Total	
	Number	Percent	Number	Percent	Number	Percent
Giardiasis	71	82.56	15	17.44	86	100.0
Ascariasis	29	85.29	5	14.71	34	100.0
Cystic Echinococcosis	21	65.63	11	34.37	32	100.0

$\chi^2 = 4.94$ , no significant differences ( $P > 0.05$ ).

Table (5). The comparison serum IL-1 $\beta$  in parasitic groups.

Parasitic groups	IL-1 $\beta$					
	Increased level		Normal level		Total	
	Number	Percent	Number	Percent	Number	Percent
Giardiasis	5	5.81	81	94.19	86	100.0
Ascariasis	5	14.71	29	85.29	34	100.0
Cy.echinococcosis	0	0	32	100.0	32	100.0



Table (6). The comparison serum IL-2 in parasitic groups.

Parasitic groups	IL-2					
	Increased serum level		Normal serum level		Total	
	Number	Percent	Number	Percent	Number	Percent
Giardiasis	40	46.51	46	53.49	86	100.0
Ascariasis	29	85.29	5	14.71	34	100.0
Cy.echinococcosis	15	46.88	17	53.12	32	100.0

$\chi^2 = 15.97$ ,  $P < 0.001$ . Statistical significance was resulted from the value of ascariasis group.

Table (7). The comparison serum IL-2R in parasitic groups.

Parasitic groups	IL-2R					
	Increased level		Normal level		Total	
	Number	Percent	Number	Percent	Number	Percent
Giardiasis	68	79.06	18	20.94	86	100.0
Ascariasis	30	88.23	4	11.77	34	100.0
Cy.echinococcosis	16	50.0	16	50.0	32	100.0

$\chi^2 = 9.59$ ,  $P < 0.01$ . Statistical significance was resulted from the value of cystic echinococcosis group.

Table (8). The comparison serum IL-4 in parasitic groups.

Parasitic groups	IL-4					
	Increased level		Normal level		Total	
	Number	Percent	Number	Percent	Number	Percent
Giardiasis	2	2.32	84	97.68	86	100.0
Ascariasis	5	14.71	29	85.29	34	100.0
Cy.echinococcosis	22	68.75	10	31.25	32	100.0

$\chi^2 = 67.19$ ,  $P < 0.001$ . Statistical significance was resulted from the value of cystic echinococcosis group.

Table (9). The comparison serum IL-6 in parasitic groups.

Parasitic groups	IL-6					
	Increased level		Normal level		Total	
	Number	Percent	Number	Percent	Number	Percent
Giardiasis	28	32.56	58	67.44	86	100.0
Ascariasis	11	32.35	23	67.65	34	100.0
Cy.echinococcosis	31	96.87	1	3.13	32	100.0

$\chi^2 = 21.63$ ,  $P < 0.001$ . Statistical significance was resulted from the value of cystic echinococcosis group.

Table (10). The comparison serum IL-8 in parasitic groups.

Parasitic groups	IL-8					
	Increased level		Normal level		Total	
	Number	Percent	Number	Percent	Number	Percent
Giardiasis	5	5.81	81	94.19	86	100.0
Ascariasis	7	20.58	27	79.42	34	100.0
Cy.echinococcosis	5	15.62	27	84.18	32	100.0

Table (11). The comparison serum IL-10 in parasitic groups.

Parasitic groups	IL-10					
	Increased level		Normal level		Total	
	Number	Percent	Number	Percent	Number	Percent
Giardiasis	0	0	86	100.0	86	100.0
Ascariasis	2	5.88	32	94.12	34	100.0
Cy.echinococcosis	13	40.63	19	59.37	32	100.0

Table (12). Cytokines levels in the sera of thirty healthy controls.

Cytokines	Mean±SD*	Range*
TNF- $\alpha$	8.59±1.12	5.1-9.8
IL-1B	< 5.0	< 5.0
IL-2	< 5.0	< 5.0
IL-2R	633.03±136.58	334-906
IL-4	< 0.4	< 0.4
IL-6	7.62±2.89	3.2-13.8
IL-8	12.95±11.35	5-47.7
IL-10	< 5.0	< 5.0

\*All values are expressed in pg/ml except for IL-2R (U/ml).

Table (13). Cytokines levels in parasitic patients suffering concomitantly from other diseases.

Diseases	TNF- $\alpha$	IL-1B	IL-2R	IL-6	IL-8
Giardiasis+typhoid fever	40.5*	17.1*	1815*	40.8*	21.2
Giardiasis+salmonellosis	150*	11.3*	2422*	4.3	**
Giardiasis+brucellosis	19.2*	<5.0	1550*	26.3*	8.2
Giardiasis+sore throat	>1000.0*	62.1*	>7200*	>2000*	9.7
Giardiasis+unidentifiable disease	>1000.0*	>1000.0*	1851*	>2000*	6.7
Ascariasis+hairy cell leukemia	12.5*	<5.0	>7200*	23.1*	27.8*
Ascariasis+duodenal ulcer	199*	16.8*	479	201*	2518*
Ascariasis+heart & skin diseases	37.7*	6.4	3568*	94.0*	175*

\* Indicates increased values.

\*\* Not calculated.

All values are expressed in pg/ml except for IL-2R (U/ml).

Table (14). Increased cytokines levels in apparently healthy individuals.

Control Cases	TNF- $\alpha$	IL- $\beta$	IL-2R	IL-6	IL-8
1	12.7*	< 5.00	668	5.00	7.3
2	67.5*	5.2	742	47.7*	709*
3	12.0*	< 5.00	1012*	17.2*	25.2
4	197*	< 5.00	1099*	165*	5.00
N.S.L.**	4-10	0-10	200-1000	2-15	0-70

\* Indicates above the normal standard level.

\*\* Indicates the normal standard level (N.S.L.).

All values are expressed in pg/ml except for IL-2R (U/ml).

Table (15) The comparison serum IgE in parasitic groups.

Parasitic Groups	IgE					
	Increased level		Normal level		Total	
	Number	Percent	Number	Percent	Number	Percent
Giardiasis	56	65.1	30	34.9	86	100.0
Ascariasis	24	70.5	10	29.5	34	100.0
Cy.echinococcosis	27	84.4	5	15.6	32	100.0
Total	107	70.4	45	29.6	152	100.0

$\chi^2 = 4.15$ , no significant differences ( $P > 0.05$ ).

Table (16). Distribution of eosinophilia in parasitic groups.

Parasitic groups	Eosinophilia					
	Present		Absent		Total	
	Number	Percent	Number	Percent	Number	Percent
Giardiasis	5	5.81	81	94.19	86	100.0
Ascariasis	15	44.12	19	55.88	34	100.0
Cy.echinococcosis	18	56.25	14	43.75	32	100.0
Total	38	25	114	75	152	100.0

$\chi^2 = 40.17$ ,  $P < 0.001$ . Statistical significance was resulted from the value of giardiasis group.

Table (17). The means  $\pm$  standard deviations of serum levels of superoxide dismutase, glutathione peroxidase, catalase, and malondialdehyde in healthy controls and parasitic patients.

Subjects	SOD (U/ml)	GSHpx (U/ml)	Catalase(K/ml)	MDA ( $\mu\text{mol/L}$ )
Giardiasis	5.305 $\pm$ 0.981	0.306 $\pm$ 0.086	31.196 $\pm$ 16.873	2.443 $\pm$ 0.420
Ascariasis	5.405 $\pm$ 0.918	0.484 $\pm$ 0.129	103.595 $\pm$ 15.502	2.867 $\pm$ 0.386
Cy.echinococcosis	5.553 $\pm$ 1.104	0.364 $\pm$ 0.113	87.541 $\pm$ 16.817	4.031 $\pm$ 0.999
Controls	2.930 $\pm$ 0.654	1.025 $\pm$ 0.181	156.483 $\pm$ 25.797	2.253 $\pm$ 0.355
ANOVA, P-value	3.58, <0.05	2.12, >0.05	2.08, >0.05	17.91, <0.001
Duncan test 1	*	*	*	NS
Duncan test 2	*	*	*	*
Duncan test 3	*	*	*	*

1 : Difference between giardiasis & control groups.

2 : Difference between ascariasis & control groups.

3 : Difference between cystic echinococcosis & control groups.

\*:significant, NS: not significant

Table (18 ). The means  $\pm$  standard deviations of serum levels of superoxide dismutase, glutathione peroxidase, catalase, and malondialdehyde in human and sheep hydatid cyst fluids.

Cyst fluids	SOD*	GSH-px*	Catalase**	MDA***
Human n=(32)	0.70 $\pm$ 0.74	0.134 $\pm$ 0.105	20.55 $\pm$ 13.50	0.84 $\pm$ 0.25
Sheep n=(32)	0.61 $\pm$ 0.38	0.09 $\pm$ 0.07	26.38 $\pm$ 16.49	0.60 $\pm$ 0.15
T-test	0.79	1.73	1.55	4.45
P-value	>0.05	>0.05	>0.05	<0.01

\*=U/mg protein, \*\*=K/mg protein, \*\*\*= $\mu\text{mol/L}$ .

## 5. DISCUSSION

The pathophysiology of parasitic diseases generally depends on many factors. First, the direct effects of the parasites on host via their size and location site within the host (host cell degeneration and lysis or parasite adherence). Second; indirect effects via some parasitic molecules (toxins, enzymes and antigens) which exert effects on host cells that in turn produce a cascade of biological events including the production of inflammatory cytokines, antibodies, prostaglandins, free radicals, lipid peroxides, and nitric oxide. Third; the role of host itself, its genetic background, nutritional and immunological states that will influence the outcome of the infection such a symptomatic infection in some individuals and mild or severe illness in others (79,189).

### 5.1. Cytokines:

Most previous studies on the role of cytokines in parasitic infections have been performed on laboratory animals and there were generally few studies on serum cytokine profile in certain human parasitic infections such as giardiasis, ascariasis, and cystic echinococcosis (91,95,99).

In giardiasis, much knowledge on the immune response originated from ; 1. In vitro studies involving the challenge of immune cells removed from a variety of hosts with *Giardia duodenalis* trophozoites 2. An animal model involving mice or gerbils infected with *Giardia duodenalis* and 3. Animal model involving mice infected with *Giardia lamblia* (50,62,162,183). An ideal assay about the immune response to human giardiasis can not be made on the basis of above points. Since the laboratory conditions (medium, property of lymphoid tissues), strain of parasites, susceptibility, the immune status and strain of hosts, and existence of natural pathogens in laboratory animals (e.g. *Giardia muris* and others), all these factors may affect the research results and cause problems in interpretation of the assays (13,50,51).

Generally speaking, the immune response to human giardiasis consists of humoral and cellular immunity. Both confer the protective immunity and resistance to *Giardia* infection (61,76). Another factor that may affect the type and amount of

cytokine response is whether the infecting parasite is invasive or non-invasive. In an experimental study by Jung et al (88), it was reported that colon epithelial cells challenged with non-invasive *Giardia lamblia* showed no expressed mRNA for IL-1, IL-2, IL-4, IL-6, IL-10, IL-12, and INF- $\gamma$  but showed TNF- $\alpha$  and IL-8 expression which appears the general property of colon epithelial cells.

In the present study, both TNF-  $\alpha$  and IL-2R were increased in majority of giardiasis (82.56 %, 79.1% respectively) but IL-2 only in 46.51%. The lower percentage of IL-2 compared to IL-2R may be due to following reasons : First; IL-2R is more stable and resistant than IL-2 (108,148,186). Second; IL-2 has very short life span (164). In giardiasis, most of these cytokines are produced by CD4+ but not CD 8+ cells of peyer's patches, or generated from the mucosa associated lymphoid tissue (MALT) as a result of long duration antigenic stimulation via trophozoites or cystic stage of *Giardia lamblia* that exists in intestine during the entire life cycle (61,76,129). The increase of IL-4 in few cases and no increase in IL-10 serum level in giardia infected individuals may indicate a minor role of Th2 cytokines in immune response to such infections and they may have no influence on immunity to giardiasis (183). Thus, the immune response to giardiasis mainly consists of Th1 cytokines and cell- mediated immunity in addition to local secretory IgA (76,129,183).

Moreover, although pyrogens such as TNF- $\alpha$  and IL-6 are involved in many cases of giardiasis, no signs of fever were observed in such cases. This may be due to the concentration of these cytokines is not enough to induce pyrexia.

Other cytokines such as IL-1 $\beta$  (pro-inflammatory interleukin) and IL-8 (a chemokine that attracts neutrophils to the site of infection) were rarely elevated in giardiasis (Tables 3,8 ). Such elevations may be resulted from exogenous or endogenous factors other than giardiasis. Nevertheless, the exact immunological mechanisms in combating giardiasis particularly self limited cases is not completely understood (162).

In regard to helminth infections, the mechanisms that control the release of cytokines are still unclear. The roles of these cytokines in host immunity seem to be quite complex and may differ with genera and species of helminth, its size and location within the host, its metabolic products, and species of host (56,95,120,143,176). It was

reported that susceptibility and resistance to the intestinal worms has been related to the dominance of Th1 and Th2 cytokines respectively (15,56,57,63,143,180). It has been found prominent Th2 response in *Ascaris lumbricoides* infected individuals who expelled this parasite and an inappropriate Th2 response in those harbouring high numbers of large round worms (63). It was also noted production of high levels of Th2 cytokines in mice infected with gut worm which were expelled more rapidly (171).

Again, it is not yet known that the results of laboratory model studies are similar to situations in human beings (63). Since many factors may influence the immune response in the clinical field (63) including differences in exposure to the parasite over time, nutritional status of man, and presence of other concurrent diseases (63). It was also reported that the immune response is not only different among mice with different helminths but also among different hosts infected with the same parasite (56).

Results of this current study indicated significant elevations in the level of TNF- $\alpha$ , IL-2, and IL-2R in the persons infected with ascaris (Tables 4,6,7). The increased level of IL-2R contradicts the results of Josimovic -Alasevic and his coworkers (87) who reported that patients with helminthic infections such as ascariasis and trichuriasis had IL-2R levels similar to those of healthy controls. The exact explanation for this discrepancy can not be made, but it may result from the existence of underlying or concurrent diseases in our patients who may be also susceptible hosts or sequelae of continuous stimulation of B and T lymphocytes by ascaris antigen (56,113). Another fact taken into consideration that may affect the results of this study is nearly 20% of patients with ascariasis had also *Trichuris trichiura* infection. Moreover, the results of previous studies in Turkey on IL-2R level in patients with malaria and visceral leishmaniasis were higher than those obtained in current study on the patients with giardiasis and ascariasis (94,178). This may be resulted from the systemic localisation of these parasites. Both *Giardia lamblia* and *Ascaris lumbricoides* inhabit the enteric region and subsequently stimulate the gut-associated lymphoid tissues and they cause little changes in systemic lymphoid tissues. The migration of larval stage of *Ascaris lumbricoides* in the early stage of infection may lead to more



exposure and accessibility of ascaris antigens to B and T lymphocytes than giardia antigens (171).

In spite of known protective effects of Th2 response to *Ascaris*, Th2 cytokines such as IL-4, IL-6, and IL-10 were found to be increased at lower values than TNF- $\alpha$ , IL-2, and IL-2R in ascariasis. This may be explained by cooperative effects of Th1 cytokines in building the immune mechanism against *Ascaris lumbricoides* infection. However, to better understanding, analysis of intestinal fluids and affected intestinal mucosa for IL-2R and other cytokines is necessary.

With regards to cystic echinococcosis, there is no detailed information about relevant cytokines production, but it was suggested dominance of Th2 immune response represented by significant elevations of IgG4 and IgE (142,155). In addition, there is cellular infiltration at the cyst area consisting of neutrophils, macrophages, eosinophils, and fibroblasts (142).

There is no severe inflammatory processes and cysts are surrounded by a fibrous layer which separates the laminated layer from the host tissues. The exception is brain where no fibrous layer surrounding the cyst as observed in one brain case during this study. Till now, it is unknown whether such cellular infiltration is due to innate host response and parasite releasing chemotactic substances or due to cytokine's effects of Th0 / Th1 subsets (142).

The experimental studies in mouse-model echinococcosis and human echinococcosis demonstrated that cytokine profile varies with the age of cyst. Generally speaking, there is Th0 or Th1 response in early infection and Th2 response in late and chronic infections (2,142), even though the situation in human infections is somewhat promiscuous (2). Rigano et al (139) reported elevated levels of IFN- $\gamma$ , IL4, and IL-10 after 129 days post infection. In another study, it was reported that IFN- $\gamma$  and IL-10 were elevated in the first three months of infection (74). It is also important to note that Th1 cell activity may co-exist with Th2 cell activity in some cases (142).

In early cystic echinococcosis, there is predominant Th0/Th1 immune response. Later on Th2 cells play a major role in immune response. Parasite biomass and antigen

/mitogen release may determine Th1 or Th2 immune response through out the infection ( 2,142).

In assessing the results of this study, IL-6 increased in 96.87 % of patients. This result coincided with previous study by Touil Boukoffa et al (177) who also reported an increased level of IL-6 in Algerian patients who had liver, lung, or both liver and lung cystic echinococcosis and contributed such increase to host defence mechanism. IL-2R was elevated in half of patients (Table 7). The percentages of elevated IL-4 and IL-10 in cystic echinococcosis were higher than in giardiasis and ascariasis. TNF- $\alpha$  was increased in 65.63% of cases. In comparing with ascariasis and giardiasis such lower TNF- $\alpha$  percentage in cystic echinococcosis may be contributed to IL-6 which decreases the level of TNF- $\alpha$  (35,152). Another important factor in cystic echinococcosis that may affect the cytokine profile is the site of location of cyst within liver. Torcal et al (176) showed significant increase in values of TNF - $\alpha$ , IL-1, IL-2, IL-4, and IgE in patients having cysts located in the central area of liver than peripheral location. However, our results indicated a bias towards Th2 response (IL-4, IL-6, IL-10). In a previous study, Fauser and Kern(53) obtained the similar results.

One explanation for variations in cytokine responses in different parasitic infections may be differences in mode of antigen processing, presentation, and recognition (77,143). It is worthy to note other factors such as the the time period till serum separation, sera whether hemolyzed or lipemic, the number of freez-thaw cycles, storage conditions of sera and type of kits, using methods , and the expertise of performing persons may have effects on the sensitivity and specificity of the cytokine results in general (89).

It should be noted that within the control group, few individuals whose health conditions were well, and routine biochemical and haematological tests results within the normal values but their some cytokines values were increased (Table 14). These subjects were excluded from the control group. These individuals may have subclinical or asymptomatic infections or an underlying disease. The results corroborated the fact that the co-existence of subclinical infections or underlying diseases with parasitic diseases undoubtedly affect the test results. I think an attempt to introduce the technique

of serum interleukin determination in routine check up may be of great value to assess the general health state of any individual.

After this debate, one can postulate that induction of Th1 or Th2 immune responses is substantially dependent on type of parasite although such responses are not pure. Thus, there is some degree of shifting towards from Th1 to Th2 or from Th2 to Th1 in the studied parasitic diseases (giardiasis, ascariasis, and cystic echinococcosis).

Finally, the question remains to be answered why such variations and inappropriate phenotype immune responses do mount within each group of parasitized patients. The answer needs further studies.

## 5.2.IgE:

Table 15 showed that most parasitic patients had an elevated IgE levels. This finding is compatible with classical and theoretical background that most parasitic infections are characterized by having features of elevated serum IgE levels, eosinophilia and intestinal mastocytosis (hallmarks of parasitic infections)(56,143,180 ). As it is well known that IgE is under the control of interleukin 4, so the high level of IgE in parasitic diseases may be results of overproduction of IL-4 or deficiency IL2 /or gamma interferon production (161).

In contrast to cytokines, high IgE level may not be detected in early infection with primary giardiasis, but cross-reaction of Th1 and Th2 cells may lead to increased total IgE (41,42,127). The high IgE serum level in giardiasis was also reported in many previous studies (41,42,45,50,51,127) except in one recent study by Kazmi & Qershi (91) who reported similar IgE results in both giardiasis and control groups. Such increase is incriminated to the fact that *Giardia lamblia* produces intestinal mucosal damage so greater amount of intestinally absorbed antigens in addition to parasite's allergen called giardine can initiate allergic reactions and may result in high IgE level (9,41). It was also observed allergic skin disorders in few *Giardia lamblia* infected persons who were dermatology outpatients (data not shown). An elevated total IgE level and its association with allergic skin disease was also previously demonstrated (43)

Although high level of IgE is under control of IL-4, the latter was rarely increased in giardiasis (Table 8). The reason is unknown. It may be said that other cytokines such as IL-13 may be responsible for overproduction of IgE.

The total serum IgE level was found to be higher than healthy ones in a study performed on individuals infected with ascaris (69). In a similar study in Bangladesh, Pulmer et al (123) found the positive correlation between IgE level and ascaris burden; being higher in heavily infected children than lightly infected ones. In another study, a significant elevation in IgE level ( $458.90 \pm 273.64$  U/ml) was also observed in pulmonary ascariasis patients (168). As in previous studies (66-69), the high level of serum IgE was observed in 24 of 34 (70%) ascaris infected individuals (Table 15). The most widely accepted hypothesis about such elevation in parasitic infection (helminth infection in particular) is that IgE response evolved as part of host defence mechanism against parasites (56). It also depended on the nutritional status of hosts since malnutrition potentiates the polyclonal stimulation of IgE possibly via an enhanced production of specific interleukins (68,69).

Again, the serum IgE level has been found to be increased in cystic echinococcosis since such infection induces IgE production (3,64,139,140). Rigano et al (139) demonstrated high levels of IL-4, IL-10, and IgG4 associated with high IgE but with low or lack of IFN- $\gamma$ . Conversely, high IFN- $\gamma$  was associated with lack of IL-4, low IL-10, low IgE but high IgG3 in patients completely recovered from the disease (120).

In the present study, 84.4 % of hydatid patients had increased IgE level. In a study performed in India, this value was 60 % (136). The author attributed the high IgE level to many reasons such as the vitality of cysts, secondary bacterial infection, smoking habit, and existence of other helminth infection.

### **5.3. Eosinophilia :**

It is another hallmark of parasitic infection (56). It was observed in many helminthic and few giardial infections in this study. Detection of eosinophilia in giardiasis was also reported by Ortiz et al (119) and Di Prisco et al (51). Nevertheless,

the percentage of eosinophilia may depend on the level of IL-5 since it is under control of such interleukin (18,143,164)..

#### 5.4. Antioxidant enzymes:

Antioxidant enzymes have protective functions against oxygen toxicity. Absence of these enzymes can lead to elevated levels of reactive oxygen species. These reactive oxygen species have dual functions in that they can eliminate infectious agents and parasites, and they can also induce tissue damage thereby leading to an inflammation (146).

Information on the role and changes of antioxidant enzymes levels in these three parasitic diseases is little and limited.

The significant increase in activity of superoxide dismutase in all parasitic patients may be due to a result of primary defence mechanism against a higher concentration of superoxide anion radical produced by phagocytic and inflammatory cells. However, Delibaş et al (39) reported a decrease erythrocyte SOD activity and contributed such decrease in cytoplasmic SOD to a decrease in serum levels of zinc and copper as consequences of disorders in metabolic intestinal absorption.

It was also demonstrated higher production of superoxide anion to specific hydatid cyst antigen in unfestered cysts than festered ones (90). This may explain variations in SOD levels in different cases of cystic echinococcosis .

The increased concentration of SOD may convert the superoxide anion to hydrogen peroxide. The overproduced  $H_2O_2$  may decrease the levels of catalase and glutathione peroxidase that in turn convert  $H_2O_2$  to water (122,126,160) . This may explain the reduced levels of catalase and glutathione peroxidase in the parasitic group (Table 17). Taneli et al (173) reported a decreased glutathione peroxidase in giardiasis patients. A more recent study by Öksel and his colleagues (117) reported absence of catalase activity in hundred percent of blood samples of children with acute giardiasis by the method of putting their blood in contact with hydrogen peroxide and naked eye observation of presence bubbles or not. By using spectrophotometric assay, catalase activity was absent in 6.97% of sera of giardiasis patients. Low levels of serum trace elements as consequences of malabsorption in parasitic diseases ( giardiasis in particular) or low intake of such elements by people with low socio-economic status .may affect the antioxidant levels (172,173)

### **5.5. Malondialdehyde:**

Malondialdehyde level is an index of the extent of lipid peroxidation . It is significantly increased in cystic echinococcosis patients group (Table 17). Our knowledge is poor concerning the biological events which may lead to such increase. Generally, abnormal increase in the MDA level may be due to following reasons. First, the results of influences that promote autooxidation. Second, availability of high MDA substrates (polyunsaturated fatty acids). Third, much free radical production and impaired antioxidant protective response (165). In cystic echinococcosis , the third reason may be the most probable explanation for MDA elevation . Additionally, the cystic echinococcosis is clinically more severe and degenerative disease than ascariasis in which it was found statically non significant MDA increase (Table 17) . In regard to giardiasis, the malabsorption of fat soluble vitamins such as A and E which have antioxidant activity in addition to the increased polyunsaturated lipids may affect the MDA level (118,173). An important factor should be deemed that MDA normally increases with age being highest in 50-70 years old due to reduced levels of antioxidants enzymes (31,59,92) and some patients in current study particularly in cystic echinococcosis are aged with a median of 42 years that may affect their MDA level .

### **5.6. Cyst fluids:**

Results of table 18 indicates low levels of antioxidant enzymes and MDA levels in both human and sheep hydatid cyst fluids since such fluids are usually devoid of inflammatory and host somatic cells which may be their sources. Leaking of blood or other body fluids and cells including bacteria into hydatid cyst fluids in complicated or festered cysts may affect the level of such biochemical parameters (90,136).



## 6. CONCLUSIONS

1. Taken together, our results indicated that analysis of some cytokines alone or a few number of cytokines was not conclusive and did not seem sufficient to explain the nature of the immune response in any parasitic disease.

2. The current study has demonstrated differences in percentages of increased cytokines levels in various parasitic diseases.

3. Our results also indicates that there is no pure Th1 or Th2 cytokine patterns in parasitic diseases but some degree of shifting towards Th1 or Th2 depending substantially on type of parasite. Additional studies will be required to establish the actual reasons or mechanisms for cytokine changes in parasitic diseases.

4. An increase in levels of some cytokines in apparently healthy individuals may indicate existence of an underlying or asymptomatic diseases.

5. Abnormal changes in the serum levels of total IgE, antioxidant enzymes, and MDA may indicate their involvement in the pathology and /or outcome of parasitic diseases.

6. Although IL-4 is responsible for IgE production, our results indicate the percentage of an increased IgE is not necessarily associated with of an increased IL-4.

7. It can also be concluded that the elevated IgE level is mostly associated with giardiasis, ascariasis, and cystic echinococcosis irrespective of co-existence with eosinophilia.

## 7. SUMMARY

The predominant human immune responses to protozoal and helminthic infections are generally Th1 and Th2 types, respectively. Our objective in this study is to investigate the serum levels of some cytokines, total IgE, antioxidant enzymes, and malondialdehyde (MDA) in giardiasis (n=86), ascariasis (n=34), and cystic echinococcosis (n=32). An ELISA method was used for measuring IL-2, IL-4, and IL-10, and total IgE, while chemiluminescent enzyme immunometric assay was used for TNF- $\alpha$ , IL-1 $\beta$ , IL-2R, IL-6, and IL-8. Antioxidant enzymes and MDA were determined by different biochemical techniques. Results showed that TNF- $\alpha$  was significantly increased in most parasitic patients ( $P < 0.001$ ) with no statistical differences among the three parasitic groups ( $P > 0.05$ ). IL-1 $\beta$  was not increased at all in cystic echinococcosis patients and increased at low percentages in ascariasis (14.71%), and giardiasis (5.81%). The highest percentages of elevated IL-2 (85.29%) and IL-2R (88.23%) were found in ascariasis, while the highest percentages of IL-4, IL-6, and IL-10 were found in cystic echinococcosis 68.75%, 96.87%, 40.63% respectively. IL-1 $\beta$  as IL-8 was found to be of less importance in current parasitic groups since it was increased at low percentages. In regard to IgE, most patients showed an elevation of this immunoglobulin. Eosinophilia was mostly seen in ascariasis (44.12%) and cystic echinococcosis patients (56.25%) but at low percentages in giardiasis (5.81%). Concerning antioxidant enzymes, the mean serum SOD level was found to be higher in parasitic groups than controls ( $P < 0.05$ ) whereas glutathione peroxidase and catalase enzymes were significantly lower than controls ( $P < 0.05$ ). MDA was significantly increased in ascariasis and cystic echinococcosis ( $P < 0.05$ ). It was also found that the liver was the most common site for hydatid cyst (68.75%). It can be concluded from this study that cytokines responses vary among different parasitic infections and analysis of certain cytokines alone or a few number of cytokines was not sufficient for better understanding and interpretation of such cytokines patterns in parasitic diseases.

## 8.ÖZET

İnsanlardaki protozoal infeksiyonlarında genellikle Th1, helment infeksiyonlarında ise Th2 hücre yanıtı predominant olarak rol oynamaktadır. Bu çalışmanın amacı, giyardiya (n=86), askariya (n=34) ve kist hidatikli (n=32) kişilerde bazı sitokinler, total IgE, antioksidan enzimler ve malondyaldehit (MDA)'nın serum düzeylerini araştırmaktır. IL-2, IL-4 ve IL-10 ile total IgE ölçümünde ELISA metodu; TNF- $\alpha$ , IL-1 $\beta$ , IL-2R, IL-6 ve IL-8 düzeylerinin tespitinde ise chemiluminescent enzim immunometrik yöntem kullanıldı. Antioksidan enzimler ve MDA farklı biyokimyasal tekniklerle tespit edildi. Sonuçlar TNF- $\alpha$  düzeyinin parazitik hastaların pek çoğunda belirgin olarak arttığını (P<0.001), üç parazitik grup arasında ise istatistiksel bir fark olmadığını (p>0.05) gösterdi. Kist hidatik hastalarının hiçbirinde IL-1 $\beta$  düzeyinde artış olmadı. Askariya (%14.71) ve giyardiya (%5.81) de ise düşük oranda artış oldu. Askariya da en fazla artış IL-2 (% 85.29) ve IL-2R (% 88.23) de gözlenirken, kist hidatikte IL-4 (%68.75), IL-6 (% 96.87) ve IL-10 (%40.63) de görülmüştür. IL-1 $\beta$  gibi IL-8 de düşük yüzde arttığı için bu parazitik grupta daha az önemli olduğu bulundu. IgE hastaların çoğunda artmış olarak gözlemlendi. Eozinofili kist hidatik hastalar pek çoğunda görüldü (%56.25). Bu oran askariya vakalarının için (%44.12) idi. Fakat giyardiya da düşük oranda bulundu (% 5.81). Antioksidan enzimlerden ortalama serum SOD (süperoksit dismutaz) düzeyleri parazitik grupta kontrol grubundan daha yüksek bulundu ( P<0.05). Oysa glutatyon peroksidaz ve katalaz enzim düzeyleri kontrol grubundan belirgin olarak daha düşüktü (P<0.05). MDA düzeyi ise askariya ve kist hidatikte belirgin şekilde artmıştı (p<0.05). Karaciğerin hidatik kistin en çok yerleştiği bölge olduğu bulundu (%68.75). Bu çalışmadan, sitokin yanıtının farklı parazitik infeksiyonlar arasında değişken olduğu, sitokin paternlerinin daha iyi anlaşılması ve yorumlanması belirli sitokinlerin için tek başına veya az sayıda sitokin analizinin yeterli olmadığı sonucuna varılabilir.

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