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MEASUREMENF LEVEL CD4 AND CD8 ,CD44OF TOXOPLASMA GONDIIIMMNOCOMPROMISEDPATIENTS IN KIRKUK CITY

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Abstract

The current study is aimed to investigate the prevalence of toxoplasmosis in immunosuppressed people by using serological methods (ELISA) and genetic methods polymerase chain reaction (PCR) it also studies their effects on enzymes and liver functions. The study included the collection of 250 blood samples from disease groups (patients with rheumatism who are taking immunosuppressive therapy, patients with Patients with cancerous disease, patients with pulmonary tuberculosis, patients with diabetes and patients with thalassemia). The study also collected samples from healthy people.It was also noted that CD4 T cells recorded the highest significant value in patients with pulmonary tuberculosis, followed by the healthy group, then the rest of the other study groups.While the T cells recorded CD8 the highest significant value in the healthy group and its level did not differ from the group of patients with tuberculosis, while the significant values were low in the rest of the groups. While CD44 T cells recorded the highest significant value in the group of patients with pulmonary tuberculosis, followed by the healthy group and its value decreased significantly in the rest of the groups.finaly, It was also noted that the rheumatoid factor recorded the highest significant value in both of the groups of patients with rheumatism and cancer, and it decreased .significantly in the rest of the study groups

Introduction

Toxoplasmosis caused by the parasite Toxoplasma gondii is a disease of global spread in most population groups due to the ability of this parasite to cause infection and reproduction in almost all the nucleated cells of milk and birds. There are three infective stages of T. gondii, which are the tachyzoite, which divide strongly within all cells of the final and intermediate host except for the intestinal epithelial cells of the second host, and the bradyzoite stage, which reproduces slowly within the cyst of tissue formed inside the cells of the host body and which varies in size and shape Depending on the age and location of the infection, the oocyst is developed, which is excreted with the feces of infected cats, to form the infected spores later. Transmission of infection to humans occurs mainly through eating foods or drinks contaminated with infected sacs of the inoculum, or eating undercooked meat containing live tissue sacs, as Kasper (1998) showed that eating a single mature inoculum sac is sufficient to cause infection in humans. Blood is an important source of transmission of infection in the acute phase, and the same is true in transfusions of organs and tissues, which may be infectious to healthy people who are given immunosuppressive drugs, which play an important role in the occurrence of active infection (Slavin&Mayers., 1994). Acute toxoplasmosis is characterized by its spread And the rapid intracellular growth of the rapid alveoli from the intestine

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to the various organs of the host (Channon et al., 2000) causes necrosis and the formation of lesions resulting in an inflammatory reaction that leads to the emergence of symptoms that range from fever, headaches and anemia to complications that lead to Destruction of cells in the lungs, liver, heart, brain, eyes, and sometimes the involvement of the central nervous system (Simpson., 2002). However, in immunocompetent individuals, the infection becomes chronic, accompanied by a halt in the multiplication of rapid follicles and tissue cysts containing slow follicles that remain within the tissues for several years without causing any clinical effects. Toxoplasmosis is a common disease in the world, especially in hot, humid regions

Cluster differentiation CD

Immune protection against many intracellular pathogens including Toxoplasma gondii depends on the robust response of CD8 T cells. APC-presenting cells present pathogen-derived antigens and provide appropriate antigens to stimulate T cells, causing their activation and differentiation, stimulating secretion of the cytokines IFNγ and TNFα, and toxicity. cellular. (Hamid et al.,2021) The first place of entry for Toxoplasma gondii is the intestinal mucosa of the host body, and the effect of innate lymphoid cells (ILCs), which is a group of lymphocytes consisting of three groups on the basis of functional characteristics, appears, which are lymphocytes ILCs1, ILCs2 and conventional natural killer cells, and stimulation of first lymphocytes will cause the production of Th1 cytokines, such as IFN-γ and TNF-α1, and stimulation of second lymphocytes with Th2 cytokines such as IL-5, IL-9, and IL- 13 As for the third lymphocytes (ILCs 3), their stimulation will lead to the production of IL-17A and IL-22. (Zhu et al., 2021; (Mulas, et al., 2021 1

Clusters of heterogeneous CD or "CD" are defined as a group of protein expressed on the surface of the cells of the blood system. These proteins are used to label lymphocytes. There are approximately 300 CD heterogeneous clusters. These proteins are often associated with specificity and function of the cells. Cells with different functions express different heterogeneous clusters (eg: CD3+ cells are total T lymphocytes, CD4+ cells are T helper cells, CD8+ cells are cytotoxic T lymphocytes and CD19+ are B cells. (Enshaeieh) et al., 2021) Immunity against Toxoplasma gondii depends on IL-12, which is required for the production of interferon-gamma (IFN-γ) by natural killer cells early after infection, which is subsequently produced by lymphocytes with clusters of CD4 and CD8 heterodimers at later times. , and although early defense against Toxoplasmosis depends on CD8α, inflammatory monocytes and macrophages also produce IL-12, which enhances IFN-γ production and elevated levels of interferon are essential for controlling acute and chronic Toxoplasmosis infection. of hematopoietic cells and tissue cells.Interferon-gamma has numerous effects on cells, including induction of immune-related GTPases, and recent studies also suggest the existence of a second family of inducible GTPases. For interferons, they are called guanylate-binding proteins (GBPs), which are also important in controlling Toxoplasmosis infection. (Mahmoudzadeh et al., 2021; Mukhopadhyay et al., 2020 Mahmoudzadeh) Toxoplasmosis infection depends mainly on IL-12, which is secreted and stimulated by natural killer dendritic cells, CD4 + T helper T cells and CD8 + T cells, resulting in the production of large amounts of A large amount of granular necrosis factor, and when a defect in the immune system will occur, a decrease in the number of B and T lymphocytes will occur, so the role of natural killer cells emerges (Amro et al., 2021). Studies have

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indicated the high importance of toxic CD8 + T cells in the protection and control of Toxoplasmosis infections, especially during the acute stage and the latent stage, due to their ability to produce gamma-interferon. Perforin dependent cytotoxic ability of CD8 T by which it can control parasitic infections (Lutshumba et al., 2020). Toxoplasmosis infection causes strong stimulation of CD4 T cells, which are the main source of interferon-gamma during acute and chronic infections, and this behavior is seen in most intracellular pathogens

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detection of T cells(CD4)

Test principle

Cluster of differentiation4 (CD4) antibodies are quantified by the quantitative sandwich ELISA technique. The CD4-specific antibody was pre-coated on the titration plate. Then the samples are added to the titration plate tubes. If CD4 is present, it will bind to the fixed antibody. When the antibody (anti-biotin specific to CD4) bound to the enzyme is added, it will conjugate with CD4, then a substrate

solution is added to the pits and the color develops in proportion to the amount of CD4 bound in the initial step. The severity of the color change depends on the amount of bound antibodies, which depends on the amount of CD4 present

Table (3-1): Materials used to detect CD4

Substance	Quantitative
(Assay plate (12x 8 coated Microwells)	holes 96) 1
Standard solution	2
Biotin-antibody	1x 120 µl
HRP-avidin-linked enzyme	x 120 µl1
Biotin antibody dilution	x 120 µl1
HRP-avidin Diluent Enzyme Diluent	x 15µl1
Sample diluent	x 120 µl1
Substrate solution l	0 µl15x
Stop solution	x 12 µl1

Action Steps

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1- All reagents, working standards and samples were prepared according to the company's instructions

2= µl of buffer and sample were added to each hole, then the holes were covered with tape, and the plate was 100 .incubated for 2 hours at 37°C

-3The excess is removed without washing

4- µl of biotin antibody (1x) was added to each hole. Then the pits are covered with new masking tape. The dish 100 .is incubated for 1 hour at 37 °C

5- The excess liquid is poured out and washed three times (washing is done by filling the holes with 200 µl of washing solution) and then left for two minutes. The liquid is removed at each step necessary for good performance, the plate is turned over and dried with clean paper towels

6- µl of conjugated enzyme was added, the plate was covered with new tape and incubated for 1 hour at 37°C. 100 Then the washing process is carried out five times

7- µl of TMB substrate was added to each hole. The dish is incubated for 15-30 minutes at 37°C. Protected from 90 .light

-8µl of stop solution was added to each hole 50

9- Determination of the optical density of each hole within 5 minutes using an ELISA reader at a wavelength of 450 .nm

detection of T cells (CD8)

Test principle: The antibody quantity of the fourth cluster of differentiation8 (CD8) is measured using the quantitative sandwich ELISA technique. The CD8-specific antibody was pre-coated on the titration plate. Then the samples are added to the titration plate tubes. If CD8 is present, it will bind to the fixed antibody. When the antibody (anti-biotin specific to CD8) bound to the enzyme is added, it will conjugate with CD4, then a substrate solution is added to the pits and the color develops in proportion to the amount of CD8 bound in the initial step. The severity of the color change depends on the amount of bound antibodies, which depends on the amount of CD8 .present

Table (3-2): Materials used to detect CD8

Substance	Quantitative
(Assay plate (12x 8 coated Microwells)	holes 96) 1
Standard solution	2

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Biotin-antibody	**x 120 µl 1**
HRP-avidin-linked enzyme	**x 120 µl 1**
Biotin antibody dilution	**µl5x 1 1**
HRP-avidin Diluent Enzyme	**µl5x 1**
Sample diluent	**µl201 x**
Substrate solution	**µl50x 1**
Stop solution	1 x 10 µl

Action Steps

1- All reagents, working standards and samples were prepared according to the company's instructions

2- µl of buffer and sample were added to each hole, then the holes were covered with tape, and the plate was 100 .incubated for 2 hours at 37°C

-3Remove the excess without washing

4- µl of biotin antibody (1x) was added to each hole. Then the pits are covered with new masking tape. The dish is 10 .incubated for 1 hour at 37 °C

5- The excess liquid is poured out and washed three times (washing is done by filling the holes with 200 µl of washing solution) and then left for two minutes. The liquid is removed at each step necessary for good performance, the plate is turned over and dried with clean paper towels

6- µl of conjugated enzyme was added, the plate was covered with new tape and incubated for 1 hour at 37°C. 100 Then the washing process is carried out five times

7- µl of TMB substrate was added to each hole. The dish is incubated for 15-30 minutes at 37°C. Protected from 90 .light

-8µl of stop solution was added to each well 850

9- Determination of the optical density of each hole within 5 minutes using an ELISA reader at a wavelength of 450 .nm

detection of T cells (CD44)

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Test principle: The antibody quantity of cluster of differentiation4 (CD44) is measured using the quantitative sandwich ELISA technique. The CD44-specific antibody was pre-coated on the titration plate. Then the samples are added to the titration plate tubes. If CD44 is present, it will bind to the fixed antibody. When the antibody (anti-biotin specific to CD44) bound to the enzyme is added, it will conjugate with CD44, then a substrate solution is added to the pits and the color develops in proportion to the amount of CD44 bound in the initial step. The severity of the color change depends on the amount of bound antibodies, which depends on the amount of CD44 presen

Table (3-3): Materials used to detect CD44

Quantitative substance	
(Assay plate (12x 8 coated Microwells)	**holes 96) 1**
Standard solution	**2**
Biotin-antibody	**x 120 µl 1**
HRP-avidin-linked enzyme	**x 120 µl 1**
Biotin antibody dilution	**x 120 µl 1**
HRP-avidin Diluent Enzyme Diluent	**x 120 µl 1**
Sample diluent	**x 120 µl 1**
Substrate solution	**x 120 µl 1**
Stop solution	1 x 10 µl

Action Steps

Results and Discussion

The level of T cells (CD8, CD44 and CD4) in the study group

The results of our current study showed a decrease in CD4 T cells and CD8, CD44 T cells. I have rheumatoid patients as well as those with cancerous diseases, and perhaps the reason for this is due to their abuse of immunosuppressive drugs and chemical drugs, and this result agrees with what was indicated by (Knutson et al., 2007; Jørgensen et al., 2021 Sharika et al., 2021; Haycook et al., 2021;) when comparing the levels of cells with clusters of CD4 heterogeneity and infections predisposing to infection with toxoplasmosis, it was observed that they decreased for all disease cases, and this results are identical to the results reached by (Nissapatorn et al., 2001; 2002) in his study on people with toxoplasmosis. And the immunodeficiency disease. He pointed out that the immunodeficiency disease AIDS caused a decrease in the number of cells with heterogeneous clusters CD4, and this decrease is predisposing to infection with toxoplasmosis. pointed out

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Mariuz et al., 1997)) indicated that a decrease in the number of CD4 heterogeneous cells was an important and alarming indicator of the risk of infection with Toxoplasma gondii. CD4 and CD8 T-lymphocyte counts are used to measure an individual's immune strength and are an important indicator for monitoring an individual's immune function and as indicators to initiate prophylactic treatment against opportunistic infections (Yang, Wu et al., 2015). The difference in molecular and individual in the results obtained in our study from other studies may be caused by what was indicated by the scientific studies, which indicate that

Differences in CD4+ and CD8+ T-lymphocytes can depend on some important factors, namely environment, ethnicity, genetic differences, dietary patterns, as well as age and sex (Verma et al. 2003). The results of our current study concluded that an increase or decrease in the level of CD8 T cells was accompanied by an increase or decrease in CD4 T cells. Perhaps this explains the main role of CD4 cells in stimulating the production and differentiation of CD8 T cells, and CD4 T cells help the response of CD8 T cells mainly from By facilitating antigen presentation and regulating the action of molecules on dendritic cells and delivering them to optimal levels, which in turn stimulates a strong CD8 T-cell response, it also plays a key role in the production of memory CD8 T cell, and low levels of CD8 T (or Dysfunction in their work) was clearly observed in chronic injuries due to the consumption or preoccupation of T cells, or the lack of CD4 T cells production due to chronic injuries. Moreover, the cytokines produced by CD4 T cells have a major role in maintaining and endometriosis of CD8 T cells. Scheme (2-1) illustrates the process of association .(between CD4 T cells and CD8 T cells (Khan et al., 2019

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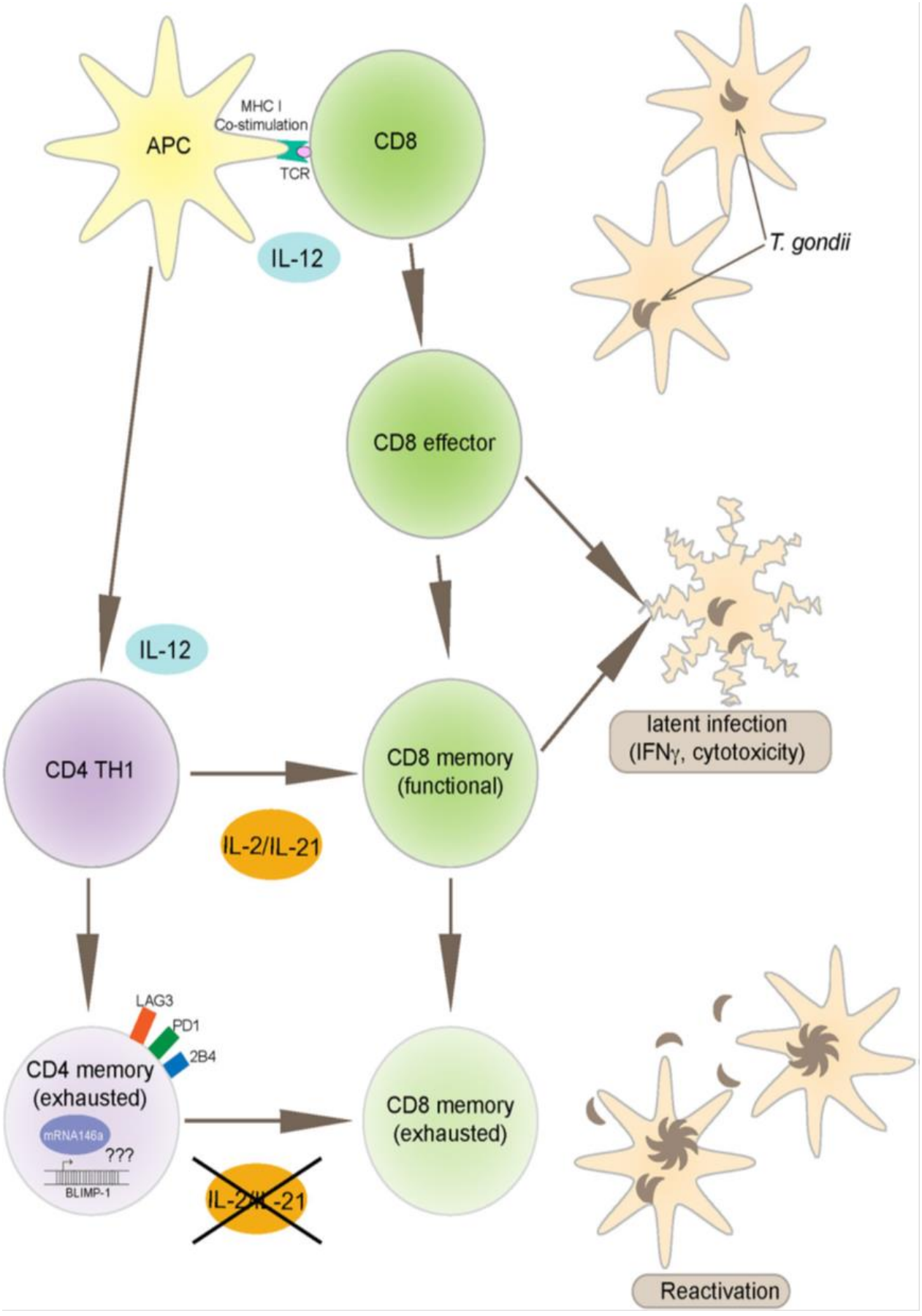

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(Chart (2-1): The process of association between CD4 T cells and CD8 T cells (Khan et al., 2019

The results of our current study showed a significant increase in CD4 T cells and an insignificant increase in CD8, CD44 T cells. Every year around the world, nearly 10 million new cases and about 1.5 million deaths related to tuberculosis are recorded

CD4 T cells are critical for the control of Mycobacterium tuberculosis. Any disease that causes a decrease in CD4 T cells greatly increases susceptibility to tuberculosis. Although IFNγ produces CD4 T cells that are key components of an immune response against tuberculosis, these cells alone Inadequate protection Studies have indicated that an increase in TB-specific CD4 lymphocytes is inversely correlated with the bacterial load in granulomas. (Michel et al., 2021 et al., 2021). Tuberculosis is detected by cells of the innate immunity that recognize pathogen-associated molecular patterns, via toll-like receptors (TLRs) and nucleotide-associated domain receptors, and initiate the inflammatory response by increasing numbers of Macrophages and dendritic cells (DCs) in infected lung tissues and drain lung lymph nodes, after which activation by cytokines and innate receptor agonists begins. Activation of both CD4 + and CD8 + T cells is observed in active TB disease in humans, and CD4 type 1 T helper (Th1) T lymphocytes are thought to be the most important and there is evidence to suggest that CD8 T cells as well as T cells Atypical such as CD1-restricted cells that recognize innate lipids contribute to optimal disease control, and it is believed that T cells The chemokines that are attracted to the affected lung control infection by producing interferon gamma, which works to stimulate the killing of intracellular bacteria through nitrogen and oxygen compounds (; Agarwal et al., 2020 Huang et al., 2020) The results of the current study showed a decrease in the helper and killer lymphocytes in Toxoplasmosis infection associated with diabetes, and this study was consistent with previous studies (Sanchez et al., 2000; Al-Aubaidi et al. .,2020; DeMasi et al.,2021) Diabetes affects the activity of the thymus gland, which is the main center of differentiationCD4, CD8 T cells, as well as affect the bone marrow, the main center for the production of these cells (Bachereau T,

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